LX.—*Invertase: a Contribution to the History of an Enzyme or Unorganised Ferment.*

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CONTENTS.

	PAGE
PART I.—General and Introductory.	
Objects of the Work	835
Literature referring to Invertase	836
Mode of Procedure	838
PART II.—The Action of Invertase on Cane-Sugar.	
Rapidity of the Reaction	843
Influence of the Proportion of Invertase present	847
Influence of the Concentration of the Solution	849
Influence of Temperature	850
Influence of Foreign Substances	852
Molecular Weight of Cane-Sugar undergoing Inversion	861
Extent of the Power of Invertase	864
Comparison of the Laws governing Inversion with Harcourt's Laws	864
Method of Estimating and Recording the Activity of Preparations of Invertase	865
PART III.—The Preparation of Invertase.	
Liquefaction of Yeast	869
Separation of Invertase from Yeast Liquor	875
Separation of the Ash	882
Purification of Invertase	884
Yeast Albuminoid	886
PART IV.—The Invertan Series.	
Splitting up of Invertase	887
Yeast Albuminoid derived from Invertase	892
α -Invertan	894
β „ or Invertase	896
γ „	901
δ „	903
ϵ „	904
ζ „	908
Molecular Weight of ζ -Invertan	911
Absorption Spectrum of ζ -Invertan	912
Action of Boiling Sulphuric Acid on Invertan	912
η -Invertan	913
Invertan Copper Compounds	914
Summary of the Invertan Series	916
PART V.—Theoretical.	
Theory of Inversion by Invertase	918
Constitution of the Invertan Series	921
PART VI.—Summary	926

PART I.—GENERAL AND INTRODUCTORY.

Objects of the Work.

THE group of bodies known as unorganised ferments or enzymes are, as far as the products of their action are concerned, well defined; but, notwithstanding much useful and valuable work, we know very little of their mode of action and far less of their chemical constitution. They are a highly interesting and important class of substances, and are named unorganised ferments in contradistinction to the living or organised ferments, because they possess a life function without life. The function or functions of the organised ferments are highly complicated, while the function of the unorganised ferments is more or less a simple breaking down of some organic compound. They may be properly called *the transforming* ferments, for their function is a transforming or breaking down of one substance into two or more others. They may be looked upon as the reagents of life, and they are all products of it. Their function is a life function. Is there anything in this which can be distinguished from ordinary chemical action? If so, what?

Roberts, in his admirable little work on "The Digestive Ferments," holds that the living organism by which they are produced imparts to them a certain definite amount of vital force. This force is stored up in the ferment until it comes in contact with the particular substance upon which it is intended to act, and then it is used in altering it. This it continues to do until the whole of the vital force is exhausted. When this happens the ferment is still left—but it is dead.

Other observers think that the action of the enzyme is an ordinary chemical reaction, the ferment being either destroyed in the process, or remaining uninjured as in catalytic reactions.

To throw some light on these points was one of the objects of this work; the other was to arrive at some knowledge of the chemical constitution of the active substance. The generally received idea is that they are the products of some change in albuminoids, but Hartley has shown that some of them at least do not act upon the spectrum in the same way as albuminoids; and the published analyses represent them as containing far less nitrogen than that generally yielded by those bodies.

Of the many members of this class of compounds there are few that can be easily prepared in any quantity, and very few the function of which can be followed with moderate ease and sufficient accuracy. Our choice of material fell upon *invertase*, or, as it is often called, *invertin*. We prefer the former name, because we consider the ending (*ase*) should be the distinguishing affix of the

names of this group. The reasons that we chose invertase were that we succeeded in discovering a method by which it is to be obtained in abundance, and because its function can be followed with ease and precision. We hope that the light thrown upon the mode of action and chemical constitution of one member of the group will be a key to a wider knowledge of the others.

Literature referring to Invertase.

Invertase has been the subject of a considerable amount of study, but the following may be taken as the principal published results on the subject:—

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 Béchamp. *Mém. Académie Sciences*, **28**, 269, 347, and 352.

We may briefly summarise these results.

Occurrence.—Beer yeast and, probably, all the saccharomycetes contain invertase. Hansen mentions several other micro-organisms in which it is present. Probably all organisms which have the power of assimilating cane-sugar contain invertase, though Hansen states that *Monilia candida* is an exception to this rule.* Bernard, and afterwards Brown and Heron, found it in the small intestine of the dog, pig, rabbit, rat, and various other animals, and the latter in the cold-water extract of malt. Kosman found it in the buds and leaves of young trees.

Preparation.—Beer yeast has always been the source of the preparation of invertase. The following are the principal methods used,

* We think it probable that if this organism were placed in a very slightly acid solution of cane-sugar and the whole heated to a temperature of 55° C., this apparent exception would disappear.

but it does not seem that any of them yield more than very small amounts of the ferment.

Yeast is killed with a little ether, and then the invertase is easily extracted by water and precipitated by alcohol (Hoppe-Seyler). It may be extracted from washed yeast by means of glycerine (Gunning). Yeast is washed with alcohol, dried, and powdered; then extracted with water, and the filtrate shaken up with ether, which causes the invertase to separate in a frog-spawn-like mass. This is extracted with water, and the ferment thrown out of solution by alcohol (Zulkowsky and König, and also Donath). Yeast, dried and powdered, is heated at 105° for six hours, then extracted with water, and precipitated by alcohol. Barth thus obtained about 0.4 per cent. of the yeast as *invertase*. Yeast is simply extracted with water, and sometimes the invertase is purified by precipitation with alcohol, which, however, weakens it (Kjeldahl). Yeast is exhausted with alcohol, then carefully pounded with water and fine sand, and the invertase precipitated from the filtered solution by alcohol (Mayer).

Constitution.—Most of the authorities (except Gunning and Béchamp) agree that it is not an albuminoid. Hartley concludes from the absorption spectra that invertase and diastase are very different from albumin, casein, and serin. Two analyses have been published, but no proof of the purity of the substances analysed is given.

Barth	C = 43.9	H = 8.4	N = 6.0	S = 0.63
Donath ...	C = 40.5	H = 6.6	N = 9.4	

Barth's preparation also contained 22 per cent. of ash, which was allowed for in the calculations.

Properties.—As precipitated by alcohol, it is when dry a white substance, soluble in water yielding a neutral solution. It precipitates lead, copper, and mercurous salts, but has no action on iron perchloride or potassium ferrocyanide, and it is not rendered turbid on boiling with acetic acid (Barth). It is without action on starch, dextrin (Donath), maltose, dextrose, lævulose, inulin, and gum (Kjeldahl). It is considered by Béchamp to have an optical activity $[\alpha]_D = +41^{\circ}$. For the action on cane-sugar, the most favourable temperature is $50-60^{\circ}$, and the most favourable degree of concentration of the sugar solution 2—20 per cent. (Kjeldahl, Müller). Until 40 per cent. of the sugar is inverted, the transformation is proportionate to the time (Kjeldahl); but the products of inversion are strongly opposed to the reaction (Müller). The amount of inversion is not proportionate in any way to the amount of invertase present, and the time reaction does not follow the course of catalytic

actions (Tamman). Invertase is not destroyed by its own action (Mayer), and it will invert about 750 times its own weight of sugar in 48 hours (Barth). The action is much hindered by alkalis and mercury salts, whilst acids in very small quantities augment it, hinder it in larger, and again augment it in still larger quantities; this last is, however, due to the inverting action of the acid itself (Kjeldahl).

Anthozymase.—Béchamp (*Mém. Acad. Sci.*, **28**, 347) claims to have isolated from the petals of *Robinia pseudoacacia* a ferment which he calls anthozymase, and which he says not only inverts cane-sugar but also saccharifies starch.

This substance is, like invertase, soluble in water and insoluble in alcohol. Its solutions are coagulated by heat. It has an optical activity $[\alpha]_D = -38^\circ$ (*circa*). He was only able to obtain it in extremely minute quantities.

M. Béchamp adduces no evidence whatever to show that this is not an imperfectly purified mixture of diastase and invertase.

These are the principal published facts with regard to invertase; and we have given them in this form to avoid any necessity of again referring to them in the body of the article; what we add to the knowledge will be clear to anyone who wishes to study the subject, and we shall put forward what we consider new and important in the summary at the end.

Mode of Procedure.

We have already stated that the two great questions we wished to solve by this work were, first, the nature of the inverting power or function of invertase; and, secondly, the chemical composition of the active agent. The function of invertase is to transform sucrose according to the equation—

Our first object was to determine if by the aid of what we could learn from the above-mentioned publications, and by any further experiments that might suggest themselves to us, we could find some quantitative expression for this function. We have so far no other factor which acts as a criterion of the purity of the substance, and no means of estimating it. We merely know that invertase is the only known substance which transforms cane-sugar, as described above, in the cold. We have no characteristic to guide us but this. Hence, when we have found that a certain product possessed this action, we have considered that invertase was present, whilst, on the

contrary, if it did not invert cane-sugar under suitable conditions, we always considered that invertase was absent. From this it will be seen that we discarded the idea of such a substance as *dead* invertase. If a solution which was formerly known to contain invertase was found, under suitable circumstances, to have no power of inverting sucrose, we concluded that invertase was no longer present, and that the chemical substance, invertase, had been in some manner changed into another compound, or compounds.

It was clear at starting that we had to establish a method of estimating a *function* and not a quantity, or rather we had to find a quantitative expression for a function.

For this purpose it was necessary to make a choice between two theories for a working hypothesis. If Roberts is right, it is obviously of no use for our purpose to estimate the strength of such a variable quantity as the unspent vital force. If, on the contrary, the chemical theory is correct, it is without doubt possible not only to prove the presence or absence of invertase, but also its relative amount.

Once the laws which govern the reaction properly understood, it would, by estimating the intensity of the function, or, in other words, the extent or speed of the reaction, be easy to calculate *relatively* the amount of invertase present, just as we might estimate the amount of free sulphuric acid by its action on a boiling solution of sucrose under standard conditions. And invertase has one great advantage over sulphuric acid, in that there is no other known substance that acts in the same manner, and therefore we are not misled by the presence of other inverting agents.

This necessitated a complete study of the action of invertase on sucrose.

Before describing our experiments, it is as well that we should draw attention to some of the general laws relating to the course of chemical change. For this purpose we have made the following extracts from A. Vernon Harcourt's paper "On the Observation of the Course of Chemical Change" (*Journ. Chem. Soc.*, **20**, 460—492). They refer to an experiment entitled "On the Reaction of Hydrogen Dioxide and Hydrogen Iodide." In this reaction the amount of hydrogen iodide is kept constant by means of an excess of sodium hyposulphite, whilst the hydrogen dioxide is of course constantly decreasing in amount by being converted into water.

The following extracts give his chief results:—

"This result probably represents a general law of chemistry, which may be thus stated: When any substance is undergoing a chemical change, of which no condition varies excepting the diminution of the changing substance, the amount of change occurring at any moment is directly proportional to the quantity of the substance. . . .

"If the course of the change observed has been determined by a single gradual reaction, and the law of proportionality holds good, we shall expect to find that the amount of change at any moment has varied directly with the quantity of dioxide in the solution; and, if this be the case, the quantities of dioxide at the end of a series of times taken in arithmetical progression will be in geometrical progression, and the curve, as before, a logarithmic curve. . . .

"The amount of chemical change occurring at any moment is proportionate to the amount of dioxide in the solution.

"We have made a large number of similar sets of experiments under very various conditions. . . . Under all these circumstances the reaction still conforms to the same law. . . .

"The amount of chemical change occurring in the solutions at any moment varies directly with the amount of iodide, if all the other conditions are the same. . . .

"The amount of change varies directly (1) with the amount of dioxide, and (2) with the amount of iodide in a unit volume of the solution; (3) with the time during which the change proceeds; (4) with the total volume of the solution; and, finally, with some function of each of the other conditions under which the change occurs. Of these other conditions the quantities of hydrogen sulphate . . . and the temperature of the solution have been made the subject of experiment. . . .

"On comparing the rates at which the change proceeds with successive increments of acid, we find that these rates are in arithmetical progression. . . .

"The effect of varying the one essential condition of the change still remaining, namely, the temperature of the solution . . . appears to be of this kind. If a be the rate of change in any mixture at 0°C ., the rate at any other temperature, say at h degrees, is ak^h . The value of k is about 1.07; whence it happens, conveniently for the memory, that for every 10°C . the rate of change is doubled."

The above are Harcourt's principal conclusions. They are supplemented by Esson (*Phil. Trans.*, 1867, pp. 123 *et seq.*), who shows that the equation applicable to a reaction proceeding according to the above laws is—

$$y' = ye^{-a(t'-t)},$$

when y and y' are the amounts of changing substance present in the solution at the beginning and end of an interval $t' - t$; a , the fraction disappearing in a unit of time; and e the base of the Napierian logarithms. This equation may be written in the form more convenient for calculation—

$$\text{Log log } \frac{y}{y'} - \text{log } (t' - t) = \text{log } a + \text{log log } e.$$

Each separate observation supplies a value for $\log a + \log \log e$, and these values should be identical.

In giving a quantitative expression to the function of invertase, we have not found it necessary to use this equation. Having once mapped out a theoretical curve on square paper, sufficient accuracy for our purpose was obtained by the quicker process of referring all future observations to it in the manner described under the heading "Estimation of the Strength of Invertase."

It may be convenient here to describe the methods and apparatus we employed. In performing experiments connected with the action of invertase on cane-sugar, the latter was first dissolved in boiling water and the solution allowed to cool. If the presence of sulphuric acid was required, the requisite amount was now added in the form of a decinormal solution (1 c.c. = 0.008 gram SO_3), and the solution was made up to the required bulk at 15.5° , space being left for the invertase-containing preparation. It was then placed in a water-bath automatically kept at the required temperature, and the vessel was covered over to prevent evaporation. The preparation under examination was now weighed or measured into a small beaker, and as soon as the temperature of the sugar solution was constant some of it was poured into the solution of invertase, and the whole poured back into the sugar solution. The small beaker was then rinsed with the solution and the time noted. The mixture was now left for inversion to proceed, and if at any time it was desirable to ascertain the progress of the reaction, it was accurately done as follows:—Into a small beaker was put half a drop of a very concentrated solution of potassium or sodium hydroxide. At a carefully noted time some of the solution was poured into this and well mixed with it. By this means the reaction was entirely stopped, and the sample could be examined at leisure; but we found it necessary, for reasons we shall presently give, to allow it to stand a quarter of an hour before doing so.*

When we desired to stop an inversion, it was done by adding sufficient alkali to give a reaction with litmus.

In order to ascertain the progress of inversion in the stopped solutions, they were examined at a temperature of 15.5° , by means of a Soleil-Ventzke-Scheibler polarimeter, and the result was recorded in the form:—100 mm. of the solution yields so many divisions, + or —, of the polarimeter scale. In order to save calculations, we always worked with these divisions, but they may be converted into degrees of $[\alpha]$; by multiplying by 0.384. It was generally necessary to calculate from the number of divisions observed the actual per-

* We may note here that we have only lately been acquainted with the necessity for this delay, and no doubt many of the minor irregularities in our results may be accounted for by not having regard to it.

centage of cane-sugar that had undergone inversion, and as the strength of the sucrose solution used was nearly always at the rate of 20 grams sucrose per 100 c.c. of solution, we used the following formula:—

$$(38.4 - d) \div 0.518 = p.$$

d = divisions of polarimeter scale given by 100 mm. of the solution.

p = per cent. of cane-sugar inverted.

38.4 is the number of divisions corresponding to the original sucrose solution, and 51.8 is the total number of divisions that the polarimeter reading would fall through if the sugar were all inverted.

These figures are equal to an optical activity for cane-sugar $[\alpha]_j = +73.8^\circ$, for dextrose $[\alpha]_j = +57^\circ$, and for levulose $[\alpha]_j = -106^\circ$.

In dealing with solid invertase preparations we have, for the purpose of drying them perfectly, always used a water-bath, so constructed as to admit a constant stream of well-dried air at a temperature of 100° to circulate over the powdered substance, thus causing rapid and perfect elimination of water even in very hygroscopic preparations.

The estimations of nitrogen were all done by combustion with copper oxide, and the carbon and hydrogen determinations by combustion in oxygen. The estimation of the optical activity of the purified substances was, whenever possible, performed by drying the powder in the water-bath until constant, then dissolving in a weighed quantity of water, and taking, first, the specific gravity, and then the optical activity of the solution. The divisor, D , was calculated from this by the formula—

$$\frac{G - 1000}{W} = D.$$

G = the specific gravity of the solution, taking water at 1000.

W = grams of the substance per 100 c.c. of the solution.

We shall now proceed to describe in detail the results we have obtained in examining the action of invertase on cane-sugar, and to compare them carefully with what we should expect if the reaction followed the laws we have quoted. We shall at the same time state any other facts of interest which we have come across, in the hope that we may be able to throw some light on the chemistry of the action.

PART II.—THE ACTION OF INVERTASE ON CANE-SUGAR.

Rapidity of the Reaction.

It is well known that the action of invertase is much more rapid at the beginning of the reaction than at the end; but the exact curve which the reaction follows has not hitherto been mapped out. We find that whatever the conditions under which an inversion takes place, as long as these conditions remain unaltered the reaction proceeds on a definite and regular curve, and, the speed of the reaction being once established by means of two separate observations, we can predict the course of the whole of the rest of the reaction. An alteration of the conditions under which the reaction takes place simply alters its speed, but, if allowance is made for this altered pace, the reaction follows the original curve.

In Plate I (p. 844) are shown the results of three carefully conducted experiments, undertaken to establish this curve. The vertical lines indicate time-units (that is to say, equal time intervals), whilst the horizontal ones show the percentage of the total sucrose that has been inverted. The three experiments were made at different temperatures and with different proportions of the same preparation of invertase. We have also made other experiments with very different degrees of concentration of the sugar solution and varying conditions of acidity, &c., but we have always found the inversion to follow a curve similar to the ones here plotted; we only publish these three as being those most carefully performed. The plain line in the plate shows the course a reaction would take if it followed the law laid down by Harcourt for a reaction in which the only varying condition is the diminution of the changing substance.

In plotting the theoretical curve, we have taken it that 10 per cent. of the total of the changing substance was changed in the first of the time-units (or "T"). Then, according to the above law, 10 per cent. of the remainder of the changing substance would be changed in the second time-unit (or T). As the changing substance would be only 90 per cent. of the whole original amount, this means that in the second T 9 per cent. would be changed. In the third T there would be only 81 per cent. to act upon, so that only 8.1 per cent. would be changed during this time-unit, leaving only 72.9 per cent. of changing substance for the reagent to act upon in the fourth T. By this mode of calculation, a theoretical completion of the reaction is never reached: at the conclusion of 44 time-units we still find 1 per cent. of the original changing substance left.

The following are the details of the three experiments on the inver-

sion of cane-sugar above alluded to; the results are plotted, side by side, with the theoretical curve on the Plate.

Experiment A.—Temperature 15·5°. 100 grams of cane-sugar, 4 c.c. decinormal sulphuric acid, and 2 grams of the solution of invertase preparation were mixed and made up to 500 c.c. In order to compare the course of the action with the theoretical curve plotted as above described, it was necessary to make the two curves correspond at two points of their course. For these we have chosen the starting point and the point of no optical activity. The theoretical line crosses the point of no optical activity at 12·7 time-units. In the experiment the inversion took 180 minutes to reach that point.

$$\frac{180}{12\cdot7} = 14\cdot17 = \text{the value in minutes of 1 T.}$$

In Plate I the points of observation are surrounded by small circles, marked "A," and they are connected by a line of dashes.

In Table I, the details of the experiment are given. The third column gives the number of time-units which have elapsed since the start. It is obtained by dividing the observed minutes by 14·17.

The percentage of sucrose inverted is calculated by the formula already given.

TABLE I.—*Rapidity of Inversion at 15·5°.*

Time.	Minutes from start.	T.	Reading in 200 mm. tube.	P. c. inverted.
*8.36 A.M.	start	0	—	0
8.41 "	5	0·35	+ 73·6	3·1
8.51 "	15	1·06	+ 66·6	9·8
*9.6 "	30	2·12	+ 56·9	19·2
*9.33 "	57	4·01	+ 42·0	33·6
10.6 "	90	6·36	+ 27·4	45·8
*10.36 "	120	8·47	+ 16·2	58·5
11.6 "	150	10·59	+ 7·0	67·4
11.38 "	182	12·84	— 0·4	74·5
*12.6 P.M.	210	14·83	— 5·9	79·8
12.36 "	240	16·95	— 10·7	84·4
1.6 "	270	19·05	— 13·7	87·3
*2.57 "	381	26·89	— 20·1	93·5
3.46 "	430	30·35	— 21·7	95·1
5.4 "	508	35·85	— 23·0	96·3
*9.6 A.M.	1470	103·7	— 26·0	99·2
48 hours	—	—	— 26·9	100·0

Experiment B.—Temperature 35·5°. 1 c.c. of decinormal sulphuric acid, and 1 gram of the invertase solution. The other conditions are the same as in Experiment A. The value of T was found to be

8.43 minutes. In the Plate the points of observation are marked "B," and are connected by a line of dots.

TABLE II.—*Rapidity of Inversion at 35.5°.*

Time.	Minutes from start.	T.	Reading in 200 mm. tube.	P. c. inverted.
*9.31 A.M.	start	0	—	0
9.35 "	4	0.47	+71.7	4.9
*9.50 "	19	2.25	+56.9	19.2
*10.10 "	39	4.63	+40.2	35.3
10.30 "	59	7.00	+25.5	49.5
*10.50 "	79	9.16	+12.8	61.8
11.10 "	99	11.74	+ 3.3	71.0
*11.43 "	132	15.66	— 8.0	81.9
12.15 P.M.	164	19.45	—15.1	88.7
*1.2 "	211	25.03	—20.4	93.8
3.50 "	379	44.96	—24.5	97.8
*5.31 "	480	56.94	—26.2	99.4
24 hours	—	—	—26.9	100.0

Experiment C.—Temperature 54°. 0.25 c.c. decinormal sulphuric acid, and 0.25 gram invertase solution. The other conditions are the same as in Experiments A and B. The value of T was found to be 16 minutes. In the Plate, the points of observation are marked "C," and are connected by a line of alternate dots and dashes.

TABLE III.—*Rapidity of Inversion at 54°.*

Time.	Minutes from start.	T.	Reading in 200 mm. tube.	P. c. inverted.
*9.4 A.M.	start	0	—	0
9.9 "	5	0.31	+73.6	3.1
*9.21 "	17	1.06	+67.7	8.8
*9.39 "	35	2.19	+57.8	18.3
9.59 "	55	3.44	+48.1	27.7
*10.22 "	78	4.88	+38.2	37.3
10.52 "	108	6.75	+26.0	49.0
*11.23 "	139	8.69	+15.5	59.2
11.54 "	170	10.63	+ 7.7	66.7
12.28 P.M.	204	12.75	— 0.2	74.3
*12.59 "	235	14.69	— 5.7	79.6
*3.3 "	359	22.44	—17.3	90.8
5.1 "	477	29.81	—23.1	96.4
*9.4 A.M.	1440	90.0	—24.0	97.3
48 hours	—	—	—26.8	100.0

From the Plate it will be seen that the curves founded on these three experiments follow the line of the above-quoted law with great

closeness. Considering the nature of the experiments, the deviations are far within the limits of errors of manipulation. It must be remembered that the slightest alteration in the temperature causes considerable variation in the speed of the reaction. There are also other sources of error connected with the taking and the killing of the samples, and, towards the end of the reactions, errors due to the want of absolute accuracy in the sugar solution and to the optical activity of the inverting preparation are sufficient to account for very considerable discrepancies. We maintain, therefore, that taking the individual experiments the deviations are considerably less than those which might reasonably be expected, and the evidence is so far conclusive that the reaction follows the law we have enunciated. But if we look more closely at the diagram, we see that the deviations from the theoretical line, though small in themselves, are constant in the three experiments; in fact they correspond in a very striking manner. This might possibly be accidental for three experiments only, but we find that the curves of many other reactions that we have followed deviate from the theory in a similar manner.

A careful analysis of Tables I, II, and III will give the different rates of the reaction at different points of its progress. We have taken the observations marked with an asterisk in these tables, and have calculated the speed of the reaction between every two of them. Then, taking it for granted that the rest of the reaction proceeds on the theoretical curve, we have, from this rate of progress, calculated the time required to reach the point of no optical activity. For purposes of comparison between the three experiments, we have assumed the average speed of each reaction to this point to be 100 minutes, and we have thus obtained a series of numbers which are strictly comparable among themselves. If the reactions followed the theory exactly, the figures would all be 100·0; and, as the figures represent minutes, the higher the figure, the slower the reaction—in simple proportion.

TABLE IV.—*Rapidity of the Reaction at Different Points compared with the Theoretical Reaction.*

Per cent. inverted.	A Expt.	B Expt.	C Expt.	Average.
	minutes.	minutes.	minutes.	minutes.
0—20	105·9	112·7	112·2	110·3
20—35	102·1	110·3	107·5	106·6
35—60	96·7	94·9	93·0	94·9
60—80	96·2	88·6	92·3	92·4
80—93	83·2	103·0	105·6	97·3
93—99	275·5	104·1	496·8	292·1

These figures all show a continuous increase in activity up to a certain point, about 80 per cent. of inversion, and after that a large and increasing decrease. The figures themselves are of course not very accurate; the intervals between the observations are too small to permit this, and in the case of those in the last line, 93—99 per cent. inverted, the difference between the optical activities is too small, and the accidental errors are too great to admit of high accuracy; still the numbers agree wonderfully among themselves, and, as we have said before, we have done many other inversions, and all of them point to similar results.

In conclusion, we repeat that the reaction may be considered to follow a curve similar to that obtained from a reaction following Harcourt's law, but that, owing to some slight modifying circumstance, which we cannot explain, the reaction, compared with the theoretical curve, keeps increasing in rapidity until about 80 per cent. of inversion is reached, when it begins to get slower in comparison with the curve, and ultimately, just before the completion of the reaction, it gets very much slower than it should do if it adhered to the law.

Influence of the Proportion of Invertase Present.

We experienced great difficulty in discovering any relationship between the proportion of invertase preparation employed and the work done by it. For a long time we got the most incoherent results. At last, after hundreds of experiments, we found that it is absolutely necessary, in order to get comparable results, that the acidity of the solution should be such that it is in the most *favourable condition* for the action of invertase. This degree of acidity varies with every variation of the conditions under which the reaction takes place, and details of our experiments on this point will be found under heading, "Influence of Foreign Substances." Now, granted that the acidity is in the most favourable proportion, we find a very simple rule holds good, namely:—The time necessary to reach any given percentage of inversion is in inverse proportion to the amount of the inverting preparation present, and, therefore, to the amount of the invertase; that is to say, the time is in inverse proportion to the inverting agent.

In Table V we give details of two series of experiments made with the object of establishing this law. 10 grams of sugar were dissolved in 50 c.c. of water for each experiment. The most favourable degree of acidity was in each case determined beforehand; but as this most important factor varies a little with every different sugar solution, it is probable it was a source of error.

TABLE V.—*Rapidity of Inversion with Varying Proportions of Invertase Preparation.*

Exp.	Temp.	Invertase preparation. Grams.	SO ₃ , parts per 100,000.	Times of		Reading in 200 mm. tube.	Minutes to point of no optical activity.	
				Start.	Stopping.		A.	B.
1	15·5	0·15	7·5	11.40	4.41	— 2·0	283·0	424·5
2	15·5	0·45	12·5	3.0	4.40	— 1·8	94·8	426·6
3	15·5	1·50	20·0	11.56	12.26	+ 1·0	30·7	460·5
4	56·5	0·0345	1·0	11.0	12.43	+ 16·5	157·6	54·4
5	56·5	0·0722	1·5	11.22	12.15	+ 13·5	74·8	54·0

In the column marked "A" we give the time necessary for the reactions to reach the point of no optical activity, and in column "B" the time that would have been necessary to reach this point if 0·1 gram of the invertase preparation had been employed—assuming that the time is in inverse proportion to the inverting agent.

The method of calculation is as follows :—

From the polarimeter reading we calculate by the formula already given the percentage of sucrose that was inverted. This factor we refer to the theoretical time curve in the Plate, and note the number of time-units (T) to which it corresponds. Now, when a solution of sucrose has no optical activity, it is shown by the formula to be inverted to the extent of 74·1 per cent., and this number will be seen to correspond on our theoretical time curve to 12·7 T. We can now easily calculate the time when the reaction would arrive at 12·7 T by simple proportion ; and this number we give in column A.

As an example of the method of calculation we will take Experiment 1. The percentage of inversion calculated by the formula is 76·1 ; and if we refer this to the Plate, we shall see that it corresponds to 13·5 T. The number of minutes required to reach this was 301.

$$13·5 : 12·7 :: 301 : 283.$$

This last is the number of minutes the reaction took to arrive at the point of no optical activity.

In order to find out how long the reaction would have taken to reach this point if only 0·1 gram of the invertase preparation had been used, we have, if our law is correct, only to multiply the number already obtained by the grams of invertase preparation employed and then divide by 0·1. We thus get the number 424·5, which we give in column B.

As the same invertase preparation and the same temperature were employed throughout each series, we should find, if our law is correct, that in each series the figures given in column B are identical. This is substantially the case with the exception of Exp. 3, which differs from Exps. 1 and 2 to the extent of about 7.5 per cent. In experiments of this kind this may be considered to be almost within the errors of manipulation; but in this case we believe it to be due to an insufficient amount of acidity.

Further confirmation of this law will be found in Tables X—XV. the results of which are plotted out in Plates III and IV (pp. 856, 858). In these diagrams it will be seen that at the most favourable degree of acidity the curves attain approximately the same height. In each case the curve produced from the smallest amount of invertase comes between the other two, and in one case the greatest amount of invertase is at the top, and in the other case at the bottom. Under these circumstances we can only suppose that these are errors of manipulation, and conclude that we have proved the law:—The time necessary to reach any given percentage of inversion in a solution containing the amount of acid most favourable for the action of invertase is in inverse proportion to the amount of invertase present.

This result is in exact accord with the results of Harcourt's investigations on the Laws Regulating Chemical Change.

Influence of the Concentration of the Solution.

The following table shows some results obtained by varying the amount of water present, all other conditions remaining the same.

The temperature was 54°, and the raw material (yeast) was used as the inverting agent.

TABLE VI.—*Rapidity of the Reaction with Varying Concentration of the Solution.*

Cane-sugar. Grams per 100 c.c.	Yeast. Grams per 100 c.c.	Times of		Reading in 100 mm. tube.	Minutes to point of no optical activity.
		Start.	Stopping.		
15	0.15	10.37	12.27	+ 12.5	274
20	0.20	10.38	12.28	+ 11.0	194
25	0.25	10.39	12.29	+ 14.0	200
30	0.30	10.40	12.30	+ 18.8	215
40	0.80	10.56	12.53	+ 6.4	278*
50	1.00	10.56	12.53	+ 23.8	386*

* In these numbers the increased proportion of yeast to sugar has been allowed for.

In calculating these results, we have proceeded generally in the same manner as in Table V. In the first four experiments the proportion of yeast was 1 per cent. on the weight of sucrose, so the results were directly comparable; but in the last two experiments the yeast was raised to 2 per cent. on the sugar, so, in obedience to the law proved under the last heading, it was necessary to divide the minutes by 2 before comparing with the former numbers.

From these results it will be seen that about 20 grams of cane-sugar per 100 c.c., or probably a little more, is the most favourable degree of concentration for inversion. More dilute solutions are very unfavourable to the reaction, whereas concentration greater than 20 grams is only slightly detrimental until 40 grams to the 100 c.c. is reached.

These remarks, of course, only apply to a temperature of 54°.

Harcourt gives no separate rule for the effect of varying the amount of the solvent, but it is no doubt included in the statement that the amount of change varies directly "with some function of each of the other conditions under which the change occurs."

It is evident from the results we have given that this is not the case with this reaction; and we believe the reason to be that the conditions under which the experiments were conducted were not in this respect comparable. Harcourt's experiments were conducted with comparatively dilute inorganic solutions, ours with very strong and, to some extent, viscous solutions.

In his experiments the increase of viscosity obtained by decreasing the amount of the solvent would be trifling, and would not, practically, affect his results. In our experiments, on the contrary, every decrease in the amount of the solvent affects very seriously the fluidity of the solution, and hence, no doubt, impedes chemical action. If the concentration is low, the increase in viscosity is trifling, and not nearly sufficient to counteract the stimulative effect of the increase of concentration. The effect of the viscosity increases rapidly as the concentration increases, and at about 20 per cent. the two are balanced. Beyond that point the effect of the viscosity becomes greater and greater until in the case of a saturated solution of cane-sugar the viscosity is so great that invertase only acts with extreme slowness. We think in this manner our results are satisfactorily accounted for without coming into conflict with Harcourt's observations.

Influence of Temperature.

We made some experiments with a view to determining the comparative rate of inversion at different temperatures. In Table VII we give the results obtained from the solutions which contained

approximately the most favourable amount of acid. Some experiments were done at 60°, but the influence of the minutest traces of acid was so excessive that we did not pursue these experiments. The number given for 60° must therefore be taken as very doubtful; this will be seen from the full results given under the heading "Influences of Foreign Substances." It is there shown that though the experiments were conducted at intervals of one-half part of acid per million of solution, this degree of accuracy was not nearly sufficiently great.

At each temperature we made three or more experiments with slightly different amounts of acidity. Several complete series are given in Tables IX and XVI. In Table VII we have, however, only given the one which yielded the most favourable results. Each experiment was performed with 10 grams sucrose dissolved to 50 c.c. The same sugar solution and the same invertase preparation were used throughout.

TABLE VII.—*The Influence of Temperature on the Rapidity of Inversion.*

Nos.	Temperature.	Invertase preparation. Grams.	SO ₃ parts per 100,000.	Times of		Reading in 200 mm. tube.	Minutes to point of no optical activity calculated for 0.1 gram invertase preparation.
				Start.	Stopping.		
1	0.0	0.6	15.0	10.56	2.33	+3.8	1440.0
2	15.5	0.18	4.0	11.16	2.29	+5.0	398.0
3	29.5	0.05	1.0	11.45	5.4	-1.0	155.5
4	45.0	0.051	0.6	10.37	12.39	+6.3	73.8
5	55.0	0.033	0.6	10.27	1.34	-6.0	51.8
6	60.0	0.0342	0.5	11.38	3.22	+1.8	80.4

The calculations were the same as in previous cases; the variants, other than temperature, which were necessitated by the requirements of the experiments, being allowed for in accordance with the facts already described. Hence the numbers given in the last column represent proportionately the activity of the inverting preparation at the various temperatures.

In order to compare these results with Harcourt's law, we refer to Plate II (p. 852), in which the horizontal lines represent temperature, and the vertical ones time. The places where the observations fall are marked with small circles, and they are joined by a dotted line.

We have denoted by a continuous line the course the reaction

should take if it followed Harcourt's law, that for every 10° increase of temperature the rate of change is doubled.

A comparison of these two curves shows us that they do not absolutely agree. At lower temperatures, the differences are in fact, comparatively, so small for experiments of this sort that we think ourselves justified in saying that the reaction is, in this respect, in accord with Harcourt's law. At higher temperatures there is, however, a decided falling off from the rate indicated by the curve. According to this law invertase should be 16 times as strong at 55.5° as at 15.5° , whereas we found it to be only 7.5 times as active. We should like, however, to point out that we have not seen any record of experiments made at a temperature so high that it approaches the point where the reaction would cease. Possibly, under these circumstances, the results obtained would be in accord with those here noted.

We have made some experiments showing that invertase in solution, heated to a temperature of 60° in the presence of an excess of cane-sugar, is not permanently affected. Some results which clearly show this will be given when we come to Table XXXI. This is an important point, as it shows that the effect that temperatures under 60° have on a solution undergoing inversion is limited to the time the temperature lasts. To make this more clear, we will suppose that a certain inversion is proceeding at a rate which would be expressed by the formula $\pm 0 = 100'$, the temperature being 15.5° . If the temperature is now raised to 60° , the rate of inversion would be greatly increased, and for the time it remains at 60° the rapidity might perhaps be expressed by $\pm 0 = 15'$. If now the temperature is again lowered to 15.5° , the inversion will again proceed at that temperature at its former rate, $\pm 0 = 100$.

Influence of Foreign Substances.

The Action of Alkalis.—The caustic alkalis, even in very small proportions, are instantly and irretrievably fatal to the reaction, and the hydrates of the alkaline earths are extremely detrimental to it. It is a remarkable fact that if an inversion is stopped with a little alkali only—sufficient to render good test-paper distinctly blue, and the solution at once observed through a polarimeter, it will be seen that the angle continues to decrease for some considerable further time. This looks as if the amount of alkali was not sufficient to kill the invertase instantly, but required time to do it. That this is not so is proved by the fact that if a second sample is taken at the time of "stopping," and stopped with a large excess of alkali, it does not show any slow residual movement downwards, but at once assumes the angle that the slightly alkaline solution eventually attains to.

PLATE II. THE INFLUENCE OF TEMPERATURE ON RAPIDITY OF INVERSION.

We give an example of this in the following experiment. A solution of cane-sugar was mixed with a large amount of invertase solution. After allowing the reaction to proceed for a quarter of an hour, two small samples (about 20 c.c.) were taken and simultaneously stopped, A with one-fourth of a drop of very strong potash solution, and B with 4 drops. They were both quickly examined in the polarimeter with the result that B at once showed an angle = $+32.2$, which fell after 18 hours standing to 30.8 ; whilst A started at $+39$, and kept slowly descending for a long time, eventually reaching $+29.8$.

The following are the readings from A sample:—

Time.	Reading.	Time.	Reading.
4.0	stopped	4.23	$+33.7$
4.2	$+39.0$	4.35	$+33.2$
4.5	$+36.6$	4.55	$+32.6$
4.7	$+36.0$	5.25	$+32.5$
4.12	$+34.3$	after 18 hours	$+29.8$

We see from this that the small amount of alkali must have killed the invertase just as quickly as the larger one. It is also evident that in the case of the large amount of alkali there must have been a large jump down on rendering alkaline. We further proved this in the following experiments:—

A 20 per cent. solution of sugar was prepared and divided into two portions, A and B. To A, 5 per cent., and to B, 10 per cent. invertase solution was added. At intervals, polarimetric readings were made of each, and at the same instant, small samples of them were stopped by the addition of several drops of strong potash, and readings of these also made. The observations were made in a 200 mm. tube; the results are given in the following table:—

TABLE VIII.—*The Decrease of Optical Activity on stopping Inversion with Alkali.*

A Expt. 5 per cent. invertase solution.			B Expt. 10 per cent. invertase solution.		
Before stopping.	After stopping.	Decrease.	Before stopping.	After stopping.	Decrease.
$+74.7$	$+70.3$	4.4	—	—	—
$+67.8$	$+58.5$	9.3	—	—	—
—	—	—	$+64.8$	$+49.0$	15.8
$+38.3$	$+21.0$	17.3	—	—	—
—	—	—	$+21.6$	-2.5	24.1
$+8.7$	-7.0	15.7	—	—	—
—	—	—	-16.0	-24.2	8.2

The decrease of 24.1 divisions observed in B experiment is equivalent to the inversion of 23.3 per cent. of the total sucrose originally present.

We call this fall, after stopping, "the residual decrease."

Of course all the numbers given for the inverting process were obtained from observations taken after the residual decrease had taken place; we prove this method to be correct under the heading "Molecular Weight of a Solution of Cane-sugar undergoing Inversion." It is already clearly evident from the general agreement of our results, that the decrease is in no way due to any direct inverting action of the alkali. One law alone would prove that the whole of the inversion is due to the invertase; we refer to our numbers showing that the action of invertase is in proportion to its weight. We shall explain the cause of the residual decrease under the heading "Theory of Inversion by Invertase."

From these facts will be seen the necessity for allowing a stopped solution to stand a quarter of an hour before taking the final reading.

The action of acids is peculiar and complicated. Kjeldahl states, with perfect correctness, that in extremely minute quantities they greatly aid the reaction; but if these quantities are increased this action is reversed, and they are very detrimental. This is a most important matter, as without a knowledge of the action of dilute acids it is impossible to estimate the strength of invertase preparations.

In the following tables we give the effect of varying the amount of sulphuric acid present. In each experiment 10 grams of sugar was used, and the bulk of the total solution was 50 c.c. As most of these tables contain results from more than one series of experiments, they are not always absolutely regular, the proportion of acidity being so small that the most favourable point varies for each series. We have thought it best, however, to put in *all* the experiments, as they cover a wider range. In a single series we always find that the results are perfectly regular. In the column headed "Series" we have distinguished each series with a different letter. The sulphuric acid is expressed as SO_3 in parts per 100,000 on the total solution; a very dilute solution of it was made, and a measured quantity used. In the case of the invertase solution it was either measured, with or without dilution, or weighed, according to the amount wanted; its sp. gr. was 1.020; it was generally added to the sugar solution before subdividing into lots of 50 c.c.

The results are calculated in every way the same as those in Table VII.

TABLE IX.—*Influence of Sulphuric Acid on Rapidity of Inversion at 0°.*

Sugar, 10 grams. Invertase solution, 0.6 gram = 6 per cent. on the sugar. Total solution, 50 c.c. Temperature, 0°. In Plate III (p. 856) the curve is indicated by a line of crosses.

Experiment.	SO ₃ , parts per 100,000.	Time of start.	Time of stopping.	Reading in 200 mm. tube.	Minutes to point of no optical activity calculated for 0.1 gram invertase preparation.
1	15	10. 56	2. 33	+ 3.8	1440
2	25	"	"	+ 4.9	1476
3	35	"	"	+ 8.0	1608
4	56	"	"	+ 25.6	2586

TABLE X.—*Influence of Sulphuric Acid on Rapidity of Inversion at 15.5°.*

Sugar, 10 grams. Invertase solution, 0.15 gram = 1.5 per cent. on sugar. Total solution, 50 c.c. Temperature, 15.5°. In Plate III the curve is indicated by a dotted line.

Experiment.	Series.	SO ₃ , parts per 100,000.	Time of start.	Time of stopping.	Reading in 200 mm. tube.	Minutes to point of no optical activity calculated for 0.1 gram invertase preparation.
1	A	none	10. 43	3. 15	+ 8.8	513
2	A	2.5	"	"	+ 0.2	412
3	A	5.0	"	"	— 1.0	396
4	D	5.0	12. 27	5. 5	— 1.9	395 } 396
5	A	7.5	10. 43	3. 15	— 1.4	390
6	A	10.0	10. 43	3. 15	— 1.3	391
7	B	10.0	10. 33	3. 20	— 3.7	386 } 391
8	D	10.0	12. 27	5. 5	— 1.7	395 }
9	B	20.0	10. 33	3. 20	+ 0.5	437
10	B	30.0	"	"	+ 6.0	502
11	B	50.0	"	"	+ 20.4	738
12	B	150.0	"	"	+ 62.0	3640
13	E	250.0	23 hours	"	+ 58.4	14000

TABLE XI.—*Influence of Sulphuric Acid on Rapidity of Inversion at 15.5°.*

Sugar, 10 grams. Invertase solution, 0.45 gram = 4.5 per cent. on the sugar. Total solution, 50 c.c. Temperature, 15.5°. In Plate III

the curve is indicated by a line of dashes; the first portions of it (up to 10 parts SO_3) and some of the latter portions are obtained from a different source, and need only be regarded as probable.

Experiment.	Series.	SO_3 , parts per 100,000.	Time of start.	Time of stopping.	Reading in 200 mm. tube.	Minutes to point of no optical activity calculated for 0.1 gram invertase preparation.
1	F	10	2.28	5.2	-15.6	415
2	F	15	2.28	5.1	-15.4	415
3	F	20	"	"	-14.6	430
4	F	250	"	"	+60.4	5453
5	F	500	"	"	+72.0	19950

TABLE XII.—*Influence of Sulphuric Acid on Rapidity of Inversion at 15.5° .*

Sugar, 10 grams. Invertase solution, 1.5 grams = 15 per cent. on the sugar. Total solution, 50 c.c. Temperature, 15.5° . In Plate III the curve is indicated by a line of alternate dots and dashes.

Experiment.	Series	SO_3 , parts per 100,000.	Time of start.	Time of stopping.	Reading in 200 mm. tube.	Minutes to point of no optical activity calculated for 0.1 gram invertase preparation.
{ 1	H	none	3.24	3.49	+12.3	405
{ 2	J	none	4.8	4.38	+16.5	542
{ 3	K	none	11.25	12.0	+13.7	590
{ 4	H	10	3.24	3.49	+8.6	368
{ 5	J	10	4.8	4.38	+2.8	380
{ 6	H	20	3.24	3.49	+7.1	354
{ 7	J	20	4.8	4.38	+2.1	372
{ 8	H	30	3.24	3.49	+7.5	357
{ 9	J	30	4.8	4.38	+3.1	381
{ 10	K	30	11.26	12.0	-7.4	319
11	J	40	4.8	4.38	+3.6	38
12	K	45	11.27	12.1	-5.4	340
13	J	50	4.8	4.38	+4.1	391
{ 14	J	60	4.8	4.38	+6.0	413
{ 15	K	60	11.27	12.0	-3.4	351
16	J	120	4.8	4.38	+22.2	634
17	K	250	11.28	12.0	+54.6	2090

In Plate III we have mapped out the results given in Tables IX to XII.

PLATE III. THE INFLUENCE OF SULPHURIC ACID ON RAPIDITY OF INVERSION AT 0° AND AT 15.5° C

TABLE XIII.—*Influence of Sulphuric Acid on Rapidity of Inversion at 56°.*

Sugar, 10 grams. Invertase solution, about 0.04 gram = 0.4 per cent. on the sugar. Total solution, 50 c.c. Temperature, 56°. In Plate IV (p. 858) the curve is indicated by a dotted line.

Experi- ment.	Series.	Invertase solution in grams.	SO ₃ , parts per 100,000.	Time of start.	Time of stop- ping.	Reading in 200 mm. tube.	Minutes to point of no optical activity, calcu- lated for 0.1 gram invertase preparation.
1	Q	0.0355	none	10.51	12.37	+ 22.0	66.0
2	O	0.04	0.5	12.43	3.27	- 9.6	48.4
3	„	0.042	1.0	12.44	3.27½	- 12.4	45.9
4	„	0.04	1.5	12.45	3.27½	- 10.6	46.5
5	„	0.04	2.0	12.45	3.27	- 8.6	49.7
6	Q	0.0367	6.0	11.14	1.1	+ 37.0	108.4

TABLE XIV.—*Influence of Sulphuric Acid on Rapidity of Inversion at 56°.*

Sugar, 10 grams. Invertase solution, 0.07 gram = 0.7 per cent. on the sugar. Total solution, 50 c.c. Temperature, 56°. In Plate IV the curve is indicated by a line of dashes.

Experi- ment.	Series.	Invertase solution in grams.	SO ₃ , parts per 100,000.	Time of start.	Time of stop- ping.	Reading in 200 mm. tube.	Minutes to point of no optical activity, calcu- lated for 0.1 gram invertase preparation.
1	R	0.07	none	11.19	12.52	+ 10.0	84.3
2	N	„	0.5	3.49	4.58	+ 0.6	49.1
3	„	„	1.0	3.50½	4.58	- 2.6	44.0
4	„	„	1.5	3.52	4.58	- 1.6	44.3
5	„	„	2.0	3.53	4.58	0.0	45.5
6	M	„	2.0	3.57	5.24	- 10.4	44.0
7	„	„	3.0	„	„	- 8.0	47.7
8	R	„	3.0	11.19	12.52	- 8.6	49.7
9	M	„	4.0	3.57	5.24	- 4.0	54.1
10	„	„	5.0	„	„	+ 4.6	68.4
11	R	„	9.0	11.19	12.52	+ 54.0	344.0

TABLE XV.—*Influence of Sulphuric Acid on Rapidity of Inversion at 56°.*

Sugar, 10 grams. Invertase solution, about 0.15 gram = 1.5 per cent. on the sugar. Total solution, 50 c.c. Temperature, 56°. In Plate IV the curve is indicated by a line of alternate dots and dashes.

Experiment.	Series.	Invertase solution in grams.	SO ₃ parts per 100,000	Time of start.	Time of stop-ping.	Reading in 200 mm. tube.	Minutes to point of no optical activity, calculated for 0.1 gram invertase preparation.
1	P	0.156	0.5	3.32½	4.7½	+1.0	54.8
2	„	0.153	1.0	3.36½	4.11½	-3.4	47.3
3	„	0.153	1.5	3.40	4.15	-3.9	46.8
4	„	0.156	2.0	3.43½	4.18½	-3.8	47.9

TABLE XVI.—*Influence of Sulphuric Acid on Rapidity of Inversion at 60°.*

Sugar, 10 grams. Invertase solution, about 0.034 gram = 0.34 per cent. on sugar. Total solution, 50 c.c. Temperature, 60°. In Plate IV the curve is indicated by lines of crosses.

Experiment.	Series.	Invertase solution in grams.	SO ₃ parts per 100,000.	Time of start.	Time of stop-ping.	Reading in 200 mm. tube.	Minutes to point of no optical activity calculated from 0.1 gram invertase preparation.
1	S	0.0343	0.2	3.29	6.18	+68.0	920.0
2	„	0.0343	0.4	„	„	+14.3	85.4
3	„	0.0343	0.6	„	„	+42.8	196.0
4	T	0.0342	0.3	11.38	3.22	+55.0	442.0
5	„	0.0342	0.35	„	„	+50.0	336.0
6	„	0.0342	0.4	„	„	+34.4	195.0
7	„	0.0342	0.45	„	„	+20.6	131.0
8	„	0.0342	0.5	„	„	+1.8	80.4

In Plate IV we have mapped out the results given in Tables XIII to XVI.

A study of Plates III and IV enables us to see the remarkable influence exerted by minute quantities of acid on the rapidity of the action of invertase: and it shows the absolute necessity, if we wish to obtain the greatest possible amount of work from a preparation of in-

PLATE IV. THE INFLUENCE OF SULPHURIC ACID ON RAPIDITY OF INVERSION AT 56° AND 60° C.
PARTS OF SO_3 PER 100,000 OF THE SOLUTION.

vertase, of obtaining that degree of acidity which is most favourable for the action. The amounts of acidity involved are so extremely minute that it is absolutely necessary to ascertain this factor for each separate experiment. We have already referred to these facts under the heading "The Influence of the Proportion of Invertase Preparation Present."

From these Plates it will also be seen that the most favourable degree of acidity varies with the temperature and with the percentage of the active agent present. We may add that it is a different quantity for each different invertase preparation. We have not been able to find the law which governs these variations. In Table XVII we give approximately the most favourable amount of sulphuric anhydride for the invertase solution that we used. The numbers are inferred from the results recorded in Plates III and IV.

TABLE XVII.—*Comparison of the most Favourable Amounts of Sulphuric Acid.*

Invertase solution per cent. on the sugar.	Most favourable amount of SO_3 expressed in parts per 100,000 of solution.	
	Temperature 15.5° .	Temperature 56° .
0.4	—	1.25
0.7	—	1.25
1.5	7.5	1.5
4.5	15.0	—
15.0	25.0	—

These numbers do not seem to bear any relationship to one another, but it is evident that the more invertase is used the greater the amount of acid necessary; though the two are not in simple proportion. It is also noticeable that the higher the temperature, the less acid is required. We cannot see the reason of these variations. It is evident that they are not due to the inverting action of sulphuric acid *per se*, as these minute amounts are perfectly inactive at the temperatures employed. It is also remarkable how fatal to the reaction is a small variation in the acidity. In Table XVI it is shown that at 60° 2 parts per million of acid alters the rapidity of the reaction elevenfold.

The effect of alcohol is adverse to the reaction.—In Table XVIII the results of experiments are given in which we substituted alcohol for a portion of the water. These experiments were conducted in the manner already described.

Each beaker was made to contain 10 grams of sugar, 0.24 gram of invertase solution, and 0.4 c.c. of decinormal sulphuric acid. After the addition of the alcohol it was made up with water to 50 c.c. Temperature, 15.5°. Time, 9.39 to 12.18 = 159 min.

TABLE XVIII.—*The Influence of Alcohol on the Rapidity of Inversion.*

Beaker.	Alcohol, per cent. on the solution.	Reading in 100 mm. tube.	Per cent. inverted.	Minutes to point of no optical activity calculated for 0.1 gram invertase preparation.
1	0	0	74.1	382
2	0.25	+ 0.6	73.0	390
3	0.50	+ 2.0	70.3	412
4	1.00	+ 4.2	66.0	471
5	4.00	+ 12.0	51.0	713

We have now the means of comparing the influence of two foreign substances with the law laid down by Harcourt, namely, "It varies directly with some function of each of the other conditions under which the change occurs." Taking the alcohol first, it is evident from the following comparison that its influence varies in arithmetical progression with the amount present:—

Theory	382,	402,	422,	462,	702.
Found	382,	390,	412,	471,	713.

The agreement here is within the limit of the errors of experiment. We may note that the addition of 5 per cent. of alcohol about halves the rapidity of the reaction.

The case of sulphuric acid is different. It is at once evident that the influence of varying amounts of this substance is not at all in accord with the above-stated law of Harcourt. This fact is similar to that we mentioned under the heading "Influence of Temperature," namely, that Harcourt's experiments are not truly comparable with these, because they were not done with a substance which has a stimulating effect up to a certain percentage only, but afterwards has the opposite tendency. Possibly under these circumstances, too, the results obtained would accord with those here noted. It may be objected to this that the law, if it is accurate, precludes the possibility of such a reaction. This is true: but we must remember that the theory was based upon a few reactions only, and it by no means follows that some others may not have a point of maximum influence, and would therefore follow lines similar to this one. This is the more probable, because we can see from Plates III and IV that

if we only examined the reaction some distance on either side of the maximum point, we should obtain numbers very similar to those which would be yielded by a reaction following Harcourt's theory.

Molecular Weight of Cane-Sugar undergoing Inversion.

Under the last heading we showed that if an alkali is added to a solution of cane-sugar undergoing inversion, the optical activity suddenly falls many degrees. It became, therefore, a question whether, in calculating the percentage of inversion, it was right to take as a basis the optical activity before or after this sudden change. With this object we determined the freezing point of a solution of sucrose that was undergoing inversion (Raoult's method, see heading "Molecular Weight of ζ -Invertan"), and compared it with the optical activity of the solution both before and after stopping with alkali. Theoretically the optical activity should have been determined and the sample should have been killed at the same moment that the solution was frozen. The two last factors were practically possible, but for the first the solution had to be warmed up before it could be observed. We did this as quickly as possible; the inversion proceeding during the interval can only have been slight, and the error introduced thereby would be such as to make the residual decrease appear smaller than it really was. It will be seen later on that this point is not material to the results. The experiment was conducted as follows:—

50 grams of cane-sugar, 3 c.c. of a solution of invertase, and 0.8 c.c. of decinormal sulphuric acid were dissolved together to 250 c.c. of solution at 11.51 A.M. The freezing point of the mixture was at once determined. As soon as this was done, the half frozen mass was rapidly thawed, and 20 c.c. of it was killed by pouring into a beaker containing a drop of the strongest potassium hydroxide. The unkilld portion was rapidly warmed and its optical activity taken as quickly as possible in the 200 mm. tube. After a short time, the optical activity of the portion killed by alkali was observed.

After this first series of observations had been made, all the portions which had not been killed were added together and allowed to stand in the cold for half an hour: a second similar series of observations was then made. In all there were five such series, the last of which was made the following morning, so that the solution had the whole night to complete the inversion. The following table gives the numbers obtained:—

TABLE XIX.—*Comparison of the Freezing Point and Optical Activity of Sucrose Solution undergoing Inversion.*

Series.	Time of freezing.	Freezing point.		Optical activity of the solution, divisions in 200 mm.		Residual decrease of optical activity calculated in per cent. of sugar.
		Observed.	Corrected.	Unkilled.	Killed.	
1	11.53	-1.49	-1.57	+74.5	+69.4	4.9
2	12.30	-1.86	-1.95	+57.9	+37.6	19.6
3	2.25	-2.58	-2.65	+0.6	-20.4	20.3
4	4.15	-2.74	-2.82	-19.1	-24.8	5.5
5	22 hours	-2.76	-2.84	-27.8	-27.8	none

The column "Freezing point, Corrected," gives the freezing point after allowing for the inaccuracies of the thermometer. The last column gives the amount of cane-sugar which apparently became inverted on adding the alkali (see "Influence of Foreign Substances"). It is calculated in percentages on the total sugar present.

During the process of inversion, the molecular weight of the sugar decreases from 342 to 180. The decrease in the intermediate stages is of course in proportion to the amount inverted, and therefore should be in arithmetical progression with the decrease of optical activity. The calculation of the molecular weight from the optical activity is therefore a simple matter. To take the first number, +74.5 div., the original optical activity must have been 76.8 div.; so the decrease was 2.3 div. The total decrease when inversion is complete is 103.6 div., whilst the total decrease in molecular weight is 162.

$$103.6 : 162 :: 2.3 : 3.6.$$

3.6 represents the decrease in molecular weight corresponding to 2.3 div. decrease in optical activity. The molecular weight of the sugars would therefore be 338.4 (342 - 3.6).

In calculating the molecular weight from the freezing point, we must first make allowance for the effect of the alcoholic solution of invertase, &c., on the freezing point. The only practicable way of doing this is by taking the number obtained after standing 22 hours and (as we know that all the sugar is inverted) deducting the theoretical amount due to the invert-sugar present. The remainder we look upon as a constant error running through all the observations. The freezing point of a solution of cane-sugar containing 20 grams of sugar per 100 c.c. should be -1.27° . This figure would be increased to -2.71° after inversion, making allowance both for hydra-

tion and contraction. If we compare the latter number with the one obtained, 2.84° , we find a difference of 0.13 , and this we look upon as the constant error. The conversion of these results into molecular weights by the ordinary means is difficult, because of the necessity of making allowance for the varying concentration of the solution due to hydration and contraction, but if we adopt the same method of calculation that we gave for the optical activity, the affair is simple. We take the theoretical number for the cane-sugar, -1.27° , and the numbers obtained between this and -2.71° are in inverse arithmetical progression with the molecular weights between 342 and 180. The calculation is the same as in the case of the optical activity. It will be noticed that the numbers obtained from the freezing point and from the optical activity are calculated quite independently of each other.

Table XX shows the results of these calculations.

TABLE XX.—*Molecular Weight of a Sucrose Solution undergoing Inversion.*

Series.	Freezing point, deducting constant error.	Molecular weight of sugar calculated from the		
		Freezing point.	Optical activity before killing.	Optical activity after killing.
1	-1.44	323	338	330
2	-1.82	280	312	281
3	-2.52	201	223	190
4	-2.69	182	195	183
5	-2.71	180*	180	180

The first column gives the freezing point after allowing for the constant error.

It will be seen that the molecular weights obtained by Raoult's method agree extremely closely with those calculated from the optical activity after stopping the reaction with alkali, whereas they are throughout too low for the numbers obtained without stopping.

We can, therefore, only assume that the molecular weight is the same before adding the alkali as after, although the optical activity would indicate a considerable decrease in it, and we may further say that the optical activity of a solution undergoing inversion does not give a true measure of the amount of cane-sugar that has been

* This number was assumed in the calculations, and is therefore of no value in the comparison.

split up into dextrose and levulose; but if the solution is stopped by an alkali, and time is allowed for the optical activity to become constant, it is a correct indicator of the amount of cane-sugar that has undergone inversion at the moment of stopping.

Extent of the Power of Invertase.

The strongest sample of invertase we ever prepared reduced the optical activity of 100 times its own weight of sucrose to the zero point in 25.1 minutes; that is to say, it inverted 74 times its own weight of cane-sugar in 25.1 minutes. If a temperature of 54° had been employed, it would have inverted 7.5 times as much, = 550 times its own weight in 25.1 minutes, or at the rate of about 22 times its own weight every minute; and, provided that the invert-sugar could be occasionally extracted and replaced by cane-sugar, we have every reason to think that the invertase would go on inverting at this rate for an infinite time. Many samples have been obtained nearly as strong as this.

To test whether there is any limit to the action of invertase, we made a considerable bulk of cane-sugar solution, and added to it the requisite minute amount of sulphuric acid. It was then boiled in a large flask, a small sample of it was taken in a small flask, and the two flasks were then placed side by side in a water-bath at 50—54°. A very small weighed quantity of invertase solution was added to the larger flask, and the two were kept at the above temperature for a fortnight. At the end of this time the blank experiment showed about 10 per cent. of the sugar inverted, while we found, after making allowance in the large bulk for this amount, that the invertase had acted upon over 100,000 times its own weight of the sucrose. Inversion was still proceeding slowly.

Comparison of the Laws governing Inversion with Harcourt's Laws.

Having now given in sufficient detail the results of our experiments on the action of invertase on sucrose under various conditions, and having shown in each case to what extent they agree with those laws which Harcourt found to regulate the chemical action of inorganic substances, we now proceed to review the comparisons as a whole.

We will, in the first place, sum up the results.

I. *The Rapidity of the Reaction.*—This agrees very closely with Harcourt's law, but we find that there is some slightly disturbing cause at work which varies in strength during the course of the reaction, and which we have not been able to discover.

II. *The influence of the proportion of invertase preparation present* agrees exactly with the theory.

III. *The influence of the concentration of the solution* does not agree with Harcourt's results; but we have pointed out that the experiments are not comparable.

IV. *The Influence of Temperature.*—At low temperatures, the reaction follows the theory very closely, but at temperatures approaching those at which the activity is destroyed the agreement ceases. We have put forward considerations to show that this result is such as might be expected.

V. *The Influence of Foreign Substances.*—The influence of varying proportions of alcohol is in exact accordance with Harcourt's law, but sulphuric acid gives results which by no means tally with it. In this case also we have accounted for the discrepancy by showing that the experiments are not comparable.

We can sum up these results by saying that Nos. I, II, and parts of IV and V agree with Harcourt's laws; but No. III and the remainder of IV and V do not agree, and that this disagreement may be accounted for by the fact that the conditions of experiment are not comparable.

From this we do not hesitate to draw the conclusion that the action of invertase on cane-sugar is governed by laws similar to those which govern simple inorganic reactions, and therefore we may safely conclude that it is a simple chemical action differing in no important way from the reactions of inorganic substances, and that there is no reason whatever for supposing that vital force enters in any way into the action.

Method of Estimating and Recording the Activity of Preparations of Invertase.

Before any attempt could be made to isolate invertase, it was, obviously, of the greatest importance to establish a trustworthy and practical standard method of measuring the inverting power of any given material. Indeed, as the inverting power is the only known attribute by which invertase can be recognised, so it is the only means we have of judging the amount of the substance present. A study of the laws which govern the reaction shows that the time factor is the most convenient one for expressing the intensity of this function, because the time that is necessary for a sucrose solution to reach a given percentage of inversion is in inverse proportion to the amount of invertase present. In the preceding work we have always, where possible, employed a solution containing 20 grams of sucrose per 100 c.c., and we have expressed the results in minutes necessary to reach the point of no optical activity. We have generally, also, calculated the results so as to allow for the different

proportions of invertase preparation employed, taking the standard amount of the preparation as 1 gram per 100 grams of sugar. It now only remains to fix the standard temperature; we take 15.5° as being the most convenient. It is needless to remark that the experiment is always supposed to be conducted under the most favourable possible conditions of acidity; otherwise the results are of no value.

For results thus obtained we use the expression $\pm 0 = x$ min., x being the number of minutes that the invertase preparation takes to do a certain standard amount of work. The amount of invertase present in a given sample is, of course, in inverse proportion to the value of x , and hence, if this factor is known, it is easy to calculate the relative amounts of invertase contained in two or more inverting preparations.

We repeat that, in the formula $\pm 0 = x$ min., ± 0 is a certain standard amount of work, and x is the time that is taken to do it.

The standard conditions of the work are as follow:—

1 gram of the invertase preparation and 100 grams of sucrose, together with the amount of sulphuric acid most favourable for inversion, are dissolved to 500 c.c. of solution, and maintained at a temperature of 15.5° . The standard amount of work is the inversion of 74.1 grams of sucrose under these conditions. This is equivalent to reducing the optical activity of the solution to ± 0 .

The practical method of ascertaining the value of x is very simple. 50 grams of sucrose is dissolved in water to nearly 250 c.c. solution, and put into a water-bath carefully maintained at 15.5° . When the temperature is constant, 0.5 gram of the material to be examined is added, and the time noted. The solution is then quickly made up to 250 c.c. and poured into five beakers placed in the water-bath. The five portions need not be accurately equal. To these beakers the following amounts of decinormal sulphuric acid (1 c.c. = 0.008 gram SO_3) are added, 0.1, 0.3, 0.6, 1.0, and 1.4 c.c. These numbers may be varied in any way that is thought best for the particular sample that is under examination. After standing an hour at 15.5° , the reaction is stopped in a small quantity taken from Beaker 3 by adding a few drops of strong potassium hydroxide, and the time of doing so is noted. A reading of this is taken in the polarimeter, and from this the percentage of sugar inverted is calculated by the formula already given ("Mode of Procedure"). The time which the contents of Beaker 3 will take to reach optical inactivity is calculated in the same manner as was given in the calculation of Table V (A column). It would be more accurate to use Esson's equation for this purpose: but it takes considerably longer than our way of calculating, and the results are practically identical. When the calculated time approximately arrives,

a few drops of potassium hydroxide solution are added to each beaker, and the time accurately noted. The beakers are then allowed to stand a quarter of an hour in order to allow the residual decrease to take place, and a reading of each is then taken in the polarimeter. If the numbers thus obtained are such that some amount of sulphuric acid outside the above series would give still more advanced numbers, which will be obvious on inspecting the results, the experiment must be repeated with more suitable quantities of sulphuric acid.

Of the five readings obtained for the above experiments, only the one which shows the greatest amount of inversion is calculated. This is done in exactly the same manner as described in arriving at the numbers in the A column of Table V, and the result gives the number of minutes equivalent to x .

The circumstances are sometimes such that it is necessary to perform the experiments under conditions different from the standard ones. The variation may be either in the amount of invertase used, or in the temperature at which the inversion takes place. It is convenient to vary the percentage of inverting material when it is either a very strong or a very weak preparation. It is generally best so to arrange the amount of material that the point of no optical activity is attained in from one to three hours. If any proportion of invertase different from the standard amount is employed, it will be necessary to correct the number calculated as above in accordance with the rule that the time is in inverse proportion to the amount of invertase preparation employed. In Table V, the figures corrected for this factor are given in column B. In the case of a determination of the inverting power of yeast it is not possible to get even approximate results in the cold; so the standard temperature has to be discarded in favour of 54°. At this temperature, yeast gives trustworthy and constant results, perfectly comparable among themselves, though whether the whole of the invertase contained in the yeast is brought into action is doubtful, as we shall show under the heading "Liquefaction of Yeast." Further particulars of the action of yeast at this temperature will be given in a future paper.

The estimation of the inverting power of yeast or any other material, for which a high temperature is desirable, is otherwise conducted in exactly the same way as we have described above. The cold yeast is mixed directly into the sugar solution at a temperature of 54°, and the mixture is kept at this temperature during the whole reaction—a degree more or less is not of paramount importance. Very much smaller amounts of sulphuric acid are required: about 0.0, 0.05, 0.1, 0.2, 0.3 c.c. of decinormal sulphuric acid are the best amounts for the five beakers. After calculating the results obtained by this method, it is necessary to correct them for the temperature. This is

done by multiplying the number of minutes by 7·5, which brings the results to the standard condition.

In ordinary approximate estimations of the strength of a sample of invertase, we have only, as a rule, used three beakers, instead of five. The method remains, of course, the same, but proportionate amounts of sugar, invertase, &c., must be taken. For the amounts of decinormal sulphuric acid we generally take 0·0, 0·4, and 0·8 c.c.

It should be noted that if the invertase preparation to be tested is in solution, it is advisable to have it as free as possible from alcohol, because this has, as we have shown, a deterrent action on the progress of the reaction. However, as the solution cannot contain more than about 20 per cent. absolute alcohol, the effect produced by it will scarcely be perceptible, if not more than 0·5 c.c. is employed to each beaker. We have generally partially freed from alcohol by allowing the solution to stand all night in a vacuum over sulphuric acid.

Many instances of the way in which this method of estimation is used in practice will be found later on.

In order to show the reliability of the method, we cannot do better than give some results obtained with a solution of invertase which was kept for a long time in a stoppered bottle. From the dates given it will be noticed that the experiments were made at irregular intervals during nearly two years. The reason for this is that they were not made with the object of testing the accuracy of the process, or, indeed, even with that of estimating the inverting power of the solution: but they are results which were obtained incidentally in the course of our investigations, and the majority of the figures have been given in the previous tables.

TABLE XXI.—*Estimations of the Inverting Power of a given Solution of Invertase Preparation under widely varying conditions.*

No.	Date.	Temperature.	Invertase solution per cent. on sugar.	SO ₃ , parts per 100,000.	Minutes.	Optical activity in 100 mm. tube	± 0 = min.
1	May, 1888..	54·0°	0·42	1·0	163·5	-6·2	344
2	"	54·0	0·7	1·0	67·5	-1·3	330
3	"	54·0	1·53	1·5	35·0	-1·95	351
4	Feb., 1889..	15·5	1·5	7·5	272·0	-0·7	390
5	"	15·5	4·5	10·0	154·0	-7·8	415
6	April, 1889.	15·5	15·0	30·0	25·0	+3·75	357
7	"	54·0	0·345	1·0	103·0	+8·25	414
8	May, 1889..	15·5	2·0	2·0	182·0	-0·2	358
9	"	54·0	0·25	trace	204·0	-0·1	383
10	Aug., 1889..	15·5	2·4	2·0	159·0	0	382
11	Jan., 1890	15·5	1·8	4·0	193·0	+5·0	398

The first thing that strikes us on examining this table is the remarkable agreement of the numbers for x in the column $\pm 0 = \text{min.}$; they differ, no doubt, somewhat, but when we consider the various conditions under which they were arrived at, we must admit that the agreement is remarkable. First, they were obtained for the same preparation of invertase at intervals during two years; secondly, the experiments of which they express the results were made at different temperatures; and, thirdly, the amount of preparation employed varied from 0.25 to 15.0 per cent. on the sucrose. When we add to these considerations the fact that the time taken by the experiments varied from 25 minutes to 272, and that a slight error in taking the time materially affects the results for the shorter times, while it has little effect on those for the longer ones, it will be conceded that the numbers are sufficiently concordant to establish the method of determination and record.

It will be noticed that between Nos. 3 and 4 there is an interval of nine months, and this corresponds with a considerable increase in the minutes necessary to reach ± 0 . This probably indicates an actual decrease in the inverting power of the solution, as a slight precipitation took place during this period.

It is with the above solution that practically all our investigations on the hydrolytic power of invertase were conducted. We called it the "A" sample of invertase, and the method of its preparation is given under the heading "Separation of Invertase from Yeast Liquor."

In concluding this description of our method of estimating the activity of a material containing invertase, we should like once more to state that when we use the expression $\pm 0 = x$ minutes, we mean that the given inverting material takes x number of minutes to do a certain standard amount of inversion under certain standard conditions. What these standards are, and why we chose them, we have already described. We may further state that the number, x , obtained by our method is a perfectly reliable number, and that it varies in inverse proportion to the amount of invertase contained in the material or materials under examination.

PART III.—THE PREPARATION OF INVERTASE.

Liquefaction of Yeast.

Having clearly shown the method by which a quantitative expression can be given to the function of invertase, and thus established a factor for describing the relative quantity of the substance

in different materials or preparations, we now proceed to describe our efforts to prepare it in a pure state. A sample of brewers' ordinary pressed top fermentation yeast was obtained from Messrs. Bass & Co. The inverting activity was estimated by the method before described, and it was found to be $\pm 0 = 2020$ minutes. The yeast was then allowed to stand at a temperature between 15° and 20° for seven days. At the end of this time it had become a thick, yellow liquid; the smell arising from it was not disagreeable, and it was evident that the change occurring was not ordinary putrefaction. It was thrown upon a filter, when a clear, yellow liquid filtered through. The strength of this was found to be $\pm 0 = 7100'$, so that it only possessed about 30 per cent. of the power of the original yeast. The soft, yeasty mass remaining on the filter was pressed between blotting-paper, and then its strength was found to be $\pm 0 = 1020'$, or twice that of the original yeast. This shows that although the yeast had given up most of the water it contained, yet the greater part of the active agent was still confined to the cells.

The partially dry residue was left for another 10 days at the same temperature as before, and was then made up with water to the same bulk as the original yeast occupied after it became liquid. It was well mixed and then filtered. This time the inverting power of the filtrate was $\pm 0 = 1470'$, which shows that the yeast cells now allowed the invertase to pass freely out of them; indeed, there is an apparent increase in the amount of invertase present, even after allowing for the separation of the insoluble portions of the yeast; this is always the case, as will be shown further on.

Another sample of yeast was obtained, this time from a stout brewing, also by Bass & Co. The activity was estimated by the method before described, as follows:—50 grams of cane-sugar was dissolved to nearly 250 c.c. of solution, and the temperature adjusted in the constant bath to 54° . At 9.35 A.M., 0.5 gram of the pressed yeast was added and thoroughly mixed. It was now without delay fairly equally divided among five beakers of equal size, placed in the bath, and varying amounts of sulphuric acid added.* At 11.31 A.M., 116 min. from the start, the reaction was stopped by adding a few drops of potash solution to each beaker, care being taken that each one was alkaline. The beakers were then placed on one side to cool. When cold, each one was made up to 50 c.c., a little of the solution passed through a filter, and the optical activity taken.

* In cases where the inverting power of the preparation is approximately known, it is not always necessary to make the preliminary trial of Beaker 3, which we described under the previous heading. We omitted it in this and most of the following estimations.

Beaker.	C.c. decinormal H_2SO_4 .	Reading in 100 mm. optical activity tube.
1	nil	+ 3.9
2	0.1	+ 5.4
3	0.15	+ 8.0
4	0.2	+ 9.6
5	0.3	+ 12.2

From this it will be seen that Beaker 1 was in approximately the most favourable condition as regards acidity. The difference between 2 and 5 is 6.8 div., or 3.4 div. per 0.1 c.c. decinormal sulphuric acid, whilst between 1 and 2 it is only 1.5 for the same amount of acid. A glance at Plate IV (page 858) will show that the curve is a very sharp one, and there is no doubt that Beaker 1 was about at the top, and it was only necessary to calculate the results obtained from it. The reading + 3.9 represents 66.6 per cent. inverted, $(38.4 - 3.9) \div 0.518 = 66.6$.

66.6 per cent. inverted, referred to the time curve (see Plate I), gives 10.4 T (T = time units). On the same curve $\pm 0 = 12.7$ T. Now the 10.4 T took 116'; how many will 12.7 T require?

$$10.4 : 12.7 :: 116 : 141.7.$$

We must still multiply by 7.5 to compensate for the temperature. $141.7 \times 7.5 = 1063$. The freshly pressed yeast had, therefore, an activity $\pm 0 = 1063'$.

The yeast was placed in a covered beaker and allowed to stand for a month at a temperature of 15—20°. At the end of this time it was quite liquid, but the smell was not at all disagreeable. Its activity was determined again in the same manner as before, but, as we had the former experiment to guide us, only two beakers instead of five were used. The time was 131', and the results were as follows:—

Beaker.	C.c. decinormal H_2SO_4 .	Reading in 100 mm. tube.
1	nil	—3.4 div.
2	0.1	—3.5 „

The acidity was evidently at the most favourable point. The observation made for Beaker 2 was calculated as before.

$$-3.5 \text{ div.} = 80.9 \text{ per cent. inverted} = 15.7 \text{ T.}$$

$$\text{Then } 15.7 : 12.7 :: 131' : 106'.$$

$$106 \times 7.5 = 795'.$$

So that the pressed yeast which when fresh had an activity $\pm 0 = 1063'$ had increased its power on standing a month to $\pm 0 = 795'$.

It will be noticed that in this case also there is an apparent increase in the invertase of yeast on standing. Evidently this increase must either really take place, or else our method of estimating the invertase of *fresh yeast* does not give sufficiently high results. We do not deny that the former explanation is very probable, but we are inclined to attach more importance to the latter, because we have noticed that when the yeast in the liquefied mass is quite dead, and, therefore, presumably incapable of forming invertase, a further apparent increase of this substance is caused by filtering. We give an example of this later on. Whichever be the true explanation of the increase, we can say with confidence that the results obtained by our method of estimating the inverting activity of yeast are constant numbers, and perfectly comparable among themselves.

It is evident from the above that sound pressed yeast does not putrefy on keeping, as has generally been supposed. Pressed yeast from winter brewings kept for a month at a temperature of 22° becomes very liquid, but is free from bacteria except on the surface, where there is a comparatively small growth of bacilli, which causes it to darken. Underneath this the yeast retains its original colour and smell. We have known summer-grown yeast under the same conditions to develop large quantities of *acetic acid* while liquefying, but apparently there was no other change. A microscopical examination at this stage shows that the yeast cells have shrivelled up into a comparatively small bulk, whilst their outline is irregular and ill-defined. The cell wall has almost entirely disappeared, but the large majority of the cells are unbroken. The vacuole occupies practically the whole of the interior of the cell, and the granulations are very marked, being, in fact, by far the most distinct feature of it. There are at times a few—a very few—bacteria present, resembling *Bacillus subtilis*. In great contrast to the shrivelled yeast cells, there are a few cells that seem to have changed very little; their walls are dark and thick, and they themselves are round. Nearly the whole of the interior of these cells is occupied by the vacuole, but there are absolutely no granulations. These cells—comparatively few in number—stand out very distinctly from the rest, and as no intermediate stages are to be seen, it seems probable that they are a distinct species.

Kept for a longer time, say a couple of months, a growth of blue mould slowly appears on the surface of the liquid yeast; but this does not seem to impair the activity of the invertase. The yeast liquor, as we call the fluid obtained from the liquefied yeast, soon assumes a somewhat darker tint, but shows no other sign of decomposition. This immunity from putrefaction is probably partly due to the concentration of the solution, as dilution with water

causes it to become black and putrid. We have seen a sample of yeast that was washed at the end of the first seven days, and then pressed, become black and smell badly, in a short time, whilst almost the whole of the inverting power disappeared. The sp. gr. of the liquid mixed with the dead yeast cells is about 1080; this concentration alone would not be sufficient to prevent putrefaction; there must, therefore, be some antiseptic present. No doubt the hop resins present—the yeast is always exceedingly bitter—act as a preservative. If at the end of seven days from the start, 30 per cent. of absolute alcohol is added, no further change takes place; the yeast cells do not give up their invertase, and filtrates obtained later are only slightly active.

The following experiment shows quantitatively the distribution of the invertase during the liquefaction and filtration of yeast:—

Some yeast from an ale of or. gr. 1068 was pressed and allowed to liquefy for a month. At the end of that time its activity was estimated, with the following results:—

Beaker.	C.c. decinormal H_2SO_4 .	Readings in 100 mm. tube.
1	nil	+11·3 div.
2	0·1	+ 9·7 „
3	0·2	+10·5 „

Time, 3.38 to 5.40 = 122 min. Temperature, 54° . Calculation for No. 2 beaker, + 9·7 divisions = 55·4 per cent. inverted = 7·7 time intervals.

$$7\cdot7 : 12\cdot7 :: 122 : 201.$$

$$201 \times 7\cdot5 = 1508.$$

$$\pm 0 = 1508'.$$

The water in the liquefied mass was also estimated. Weight taken, 1·466 grams. After drying it was 0·400 gram = 27·2 per cent. of solids and 72·8 per cent. of water.

The liquid was filtered. The filtrate or yeast liquor was slightly alkaline, and gave the following results:—

Beaker.	C.c. decinormal H_2SO_4 .	Readings in 100 mm. tube.
1	0·05	+4·8
2	0·15	+5·0

Time, 3.24 to 5.31 = 127'. Temperature, 54° .

Calculation for No. 1 beaker.

$$+4\cdot8 \text{ divisions} = 64\cdot9 \text{ per cent. inverted} = 9\cdot9 \text{ T.}$$

$$9\cdot9 : 12\cdot7 :: 127 : 163.$$

$$163 \times 7\cdot5 = 1223.$$

The activity of the yeast liquor was therefore $\pm 0 = 1223'$.

We estimated the total solids. Solution taken, 4.17 grams. Solids dried at 100° = 0.933 gram. Ash = 0.109.

This is equal to 22.4 per cent. total solids containing 2.6 per cent. of ash and 19.8 per cent. of organic matter.

We have then the following comparison:—

	Liquefied yeast.	Yeast liquor.
Inverting power.....	1508'	1223'
Water	72.8 per cent.	77.6 per cent.
Solids	27.2 „	22.4 „

Now if 77.6 grams of water hold 22.4 grams solids in solution, 72.8 would hold 21.0 grams.

$$77.6 : 72.8 :: 22.4 : 21.0.$$

This leaves 6.2 grams solids (27.2 — 21.0) in an undissolved state in the liquefied yeast = 22.8 per cent. on the total solid matter. From this we can calculate what we should expect the inverting activity of the yeast liquor to be by simply allowing for the 6.2 per cent. of undissolved solids in the liquefied yeast.

$$100 : 93.8 :: 1508 : 1414.$$

Calculated activity of yeast liquor	$\pm 0 = 1414'$
Actual „ „	$\pm 0 = 1223'$

So that there was a considerable increase in activity on filtration, as has been already pointed out.

We now have the following figures for the yeast:—

Water.....	72.8 per cent.
Dissolved solids.....	21.0 „
Undissolved solids.....	6.2 „
	<hr/>
	100.0 „

Inverting power of the liquefied yeast.....	$\pm 0 = 1508'$
„ „ calculated for the yeast liquor	$\pm 0 = 1414'$
„ „ found „ „	$\pm 0 = 1223'$

The solution thus derived from yeast has a somewhat high colour, which increases slightly on standing. Its odour is not unpleasant, by no means reminding one of putrefaction. If prepared from yeasts obtained from winter brewings, it is always slightly alkaline, but in summer it sometimes contains a considerable amount of acetic acid. It will keep for a long time unchanged and without any marked diminution of its inverting power, but in time, if left exposed, blue mould grows on it, especially in summer.* It throws down a precipi-

* Since writing the above we have examined a sample of yeast liquor prepared nearly a year ago, and kept since then in a stoppered bottle. There is a small growth of mould on the surface, otherwise it is still perfectly sound and active.

tate on boiling. It is partly precipitable by alcohol, and it yields the flocculent copper compound described further on. We have taken the specific gravities of three different samples of yeast liquor as follows:—A, 1082; B, 1076; C, 1080; but they naturally contain a little alcohol.

Separation of Invertase from Yeast Liquor.

Having thus far demonstrated that the whole of the inverting agent can be obtained in the yeast liquor by the method described, our next step was to determine whether the solid matter in solution in that liquid could be fractionated without loss of inverting power.

In September, 1887, some pressed yeast was kept for 11 days at a temperature of 13° to 15°. It was then well mixed up. After standing for a few hours, a little clear liquid again showed itself on the surface. A portion of this was sucked up in a pipette, and its inverting power determined by the side of that of a sample of the well-mixed mass of liquefied yeast. The results were—supernatant liquid $\pm 0 = 4000'$; the mixed liquid yeast $\pm 0 = 2020'$. It was evidently not yet nearly ready for filtration.

Three days later—14 days from the start—the activity was again estimated as before. The supernatant liquid gave $\pm 0 = 2410'$; the mixed liquefied yeast, 1660'. This also shows the increase in the inverting activity of the liquefied yeast already remarked upon. The liquefying mass was now allowed to stand for two months longer; at the end of that time it was well stirred up and filtered. The invertive power of the filtrate was found to be $\pm 0 = 1200'$, thus showing a higher activity than the whole mixture had at the end of 14 days. We, therefore, considered that we had the invertase, at least in greater part, in solution.

To the filtrate, sufficient alcohol was added to produce a precipitate; this was allowed to deposit. The alcoholic solution was decanted off the semi-liquid precipitate, and the activity of both was estimated. Alcoholic solution, $\pm 0 = 14100'$; thick semi-liquid precipitate, $\pm 0 = 370'$.* The alcohol was evidently not quite strong enough to precipitate the whole of the invertase, though the amount remaining in solution was very small, as was indicated by the inverting activity. The precipitate was dissolved in the least possible quantity of water, and the solution filtered and put by with a little alcohol in a stoppered bottle. The solution contains 13.6 per cent. of organic matter and 1.7 per cent. of ash.† It is with this solution

* Probably the real activity was greater than this. At the time this experiment was made, we were not aware that it was necessary to estimate the activity under the most favourable conditions of acidity.

† We shall refer to this as the "A" sample of invertase.

that nearly all our experiments on the hydrolytic action of invertase have been done. Its strength at different periods is given in Table XXI (p. 868).

The following is a more detailed account of the preparation of invertase from yeast liquor. The whole experiment was quantitative, and the inverting power was carefully followed to show that none was lost during the purification.

The yeast liquor employed was the last described under the heading "The Liquefaction of Yeast;" its inverting power was there shown to be $\pm 0 = 1223'$. It will be remembered that this estimation was not done at the standard temperature of 15.5° , but at 54° , and an allowance made for the difference. It was thought wise to do another estimation under standard conditions.

Beaker.	C.c. decinormal H_2SO_4 .	Reading in 100 mm. tube.
1	0.4	+1.0
2	0.6	+0.5
3	0.9	+0.1

Time, 3.30 to 5.31 = 121'. Temperature, 15.5° (standard). Sucrose, 30 grams in 150 c.c. solution. Amount of invertase solution used, 3 grams = 10 times the standard quantity. No. 3 beaker was evidently at about the most favourable acidity, and as the reading was practically ± 0 , it only remains to multiply the minutes by 10 to allow for the amount of the invertase solution used, and we get $\pm 0 = 1210'$. Comparing this with the number we got at 54° , $\pm 0 = 1223'$, we find an agreement so close that it shows in a striking manner the accuracy of the method of estimation.

The sp. gr. of the solution was 1.080. The total solids, we have already seen, amounted to 22.4 per cent., of which 2.6 per cent. was ash, and 19.8 per cent. organic matter. On 100 c.c., weighing 108 grams, this would amount to 24.2 (22.4×1.08) grams total solids, consisting of 2.8 grams ash and 21.4 grams organic solids. This is more than might be expected from the gravity, but there is, no doubt, a considerable amount of alcohol in the solution. Its inverting power was $\pm 0 = 1210'$, which, reckoned on the organic solids, is equal to $\pm 0 = 240'$ ($100 : 19.8 : : 1210 : 240$).

100 c.c. of this yeast liquor was now precipitated very slowly, and with frequent pauses, with alcohol of 70 per cent. (0.87). As soon as a precipitate appeared, the solution was allowed to stand for a couple of hours, and then some more alcohol of the same strength was added, and so on, until no further precipitate was produced. By this means the precipitate was obtained in a finely-divided state, and with a minimum of impurities. The solution was allowed to stand for two days, and then it was decanted and the precipitate washed several times

by decantation with 47 per cent. (0.925) alcohol. It was then thrown on to a filter, and again washed with the same alcohol and allowed to drain. The washed precipitate was carefully transferred to a beaker and made up with water to 100 c.c. It was by no means all soluble; a light, finely divided, and slightly coloured precipitate remained. It was well mixed with the solution, and the total solids estimated. 3.425 grams of solution gave 0.144 gram dried at 100°, of which 0.018 gram was ash. This gives for the whole of the turbid solution 3.68 grams organic solids and 0.53 gram ash. So the matter which had been discarded in the alcohol amounted to 17.72 grams organic solids (21.4 - 3.68) and 2.27 grams ash (2.8 - 0.53).

The turbid liquid was now filtered, and the insoluble portion discarded without washing. It consisted of *yeast albuminoid*, and is described under that heading.

The filtrate, after the separation of this insoluble albuminoid, was clear and almost colourless; it readily yielded the characteristic copper compound which we shall describe fully later on.

Estimation of total solids and ash in the clear solution:—3.300 grams solution gave 0.071 gram total solids dried at 100° and 0.011 ash, leaving 0.060 gram of organic solids. This gives 1.82 cent. of organic solids and 0.33 per cent. of ash.

So that the insoluble albuminoids which we had filtered out consisted of 1.86 grams (3.68 - 1.82) organic solids, and of 0.2 gram (0.53 - 0.33) ash.

The optical activity of the organic solids in solution was $[\alpha]_D = +74^\circ$. The inverting power was estimated under standard conditions, but with 10 times the standard weight of the solution.

Beaker.	C.c. decinormal H_2SO_4 .	Reading in 100 mm. tube.
1	0.6	-1.5
2	0.9	-0.8
3	1.2	+1.0

Time, 2.48 to 5.19 = 151'. The calculations for Beaker 1 are $-1.5 = 77$ per cent. inverted.

This is equal to 13.9 T. $13.9 : 12.7 :: 151 : 138$.

$$138 \times 10 = 1380.$$

$$\pm 0 = 1380'.$$

This, calculated on the organic solids in the solution, gives—

$$\pm 0 = 25.1' (100 : 1.82 :: 1380 : 25.1).$$

We can now calculate how much of the inverting power has been lost during these processes. As the volume of the purified solution was the same as that of the yeast liquor used, the inverting activity

of the two should agree if no inverting power was lost. The yeast liquor had an activity of $\pm 0 = 1210'$, the purified solution $\pm 0 = 1380'$, showing a loss of 12.3 per cent. of the total inverting power.

This is a most satisfactory result when we take into consideration the fact that precipitation by alcohol has a strong tendency to destroy invertase, as will presently be seen. We must also remember that nearly pure solutions of invertase will not long retain their activity, as will be shown later.

Under these circumstances the loss of 12.3 per cent. of the invertive power must be considered small and such as can be fairly attributed to the treatment; hence, we have satisfactorily proved that all the invertase originally present in the yeast is contained in the clear filtrate described.

In Table XXII the constitution of the 100 c.c. yeast liquor and of the yeast whence it was derived is given.

The ash, which is principally a mixture of potassium and magnesium phosphates, is all put together, as its presence in the various precipitates and solutions is probably purely accidental, and depends on the amount of washing, &c., they have been subjected to. This at least is the case with invertase, as will be shown later on.

TABLE XXII.—*Composition of Yeast and Yeast Liquor.*

The constituents of yeast.	Per cent. on the dry solid matter of the yeast.	Grams per 100 c.c. of the yeast liquor.	Inverting activity calculated on the yeast liquor.
<i>Matter Undissolved in Yeast Liquor.</i>			
Cellulose and other organic matter	22.80	—	—
<i>Matter Dissolved in Yeast Liquor.</i>			
Organic matter soluble in alcohol of 47 per cent.	56.53	17.72	none
Yeast albuminoid	5.93	1.86	none
Organic matter insoluble in alcohol, soluble in water (invertase)	5.81	1.82	1380'
Ash	8.93	2.80	—
Total.....	100.00	24.2	1210'

In the first column of figures we give the amounts of the constituents of the yeast calculated on the total dry solid matter. In this case we have taken it for granted that all the water in the thoroughly liquefied yeast holds in solution an equal amount of the

various solid constituents. That this is so we cannot be quite certain, as there may be a different solution in the interior of the dead cells. We, however, believe the mixture to be complete. It is difficult to see how the cell could allow a non-dialysable substance, like invertase, to escape so freely without the rest of the cell contents being equally diffused through the whole mass. The ash, too, which, calculated in this manner comes to 8.93 per cent., agrees very closely with the amount found in the original pressed yeast, viz., 8.75 per cent.

The 56.53 per cent. organic matter soluble in dilute alcohol and in water we neglected, having satisfied ourselves that it did not contain invertase.

This sample of invertase,* with an inverting activity of $\pm 0 = 25.1'$, is the purest we have ever been able to obtain. Many other preparations were made in the same way, but we have not again succeeded in obtaining one with an activity as high as this; the nearest approach to it being samples with an activity $\pm 0 = 27.9'$ and $28.5'$. These numbers, however, agree moderately well with those obtained for the strongest sample. When we take this into consideration, and other facts to which we shall refer later on, we believe we are justified in concluding that the solid matter in the solution with the activity $\pm 0 = 25.1'$ was invertase contaminated simply with the products of its own decomposition from the 12.3 per cent. of lost activity, and with ash.

It will be seen that by the above method we have a ready means of obtaining large quantities of invertase. At one time we prepared 1 kilo. of dry invertase for the purpose of this work: but as we did not know its properties so well then as we do now, the preparation was not nearly so active as the above, though we have reason to believe that the only impurities were the ash and the products of the decomposition of the invertase. In the preparation of this we proceeded as follows:—

A small unheaded cask was filled about two-thirds full of pressed yeast and allowed to stand, covered with a towel, in a warm place for a month. The liquid mass was then passed, in small portions at a time, through a screw filter-press. This is a matter of time, as the solution only comes out with great slowness—even under considerable pressure. As the clear solution was obtained, alcohol was added to raise the whole to a 47 per cent. (0.925) solution of absolute alcohol, and the mixture allowed to stand for two days. At the end of that time the supernatant liquid was quite inactive. Stronger alcohol only brings down more ash. This liquid was decanted off and discarded, and

* We shall refer to this as the "B" sample of invertase.

the precipitate allowed to settle in tall vessels. To this dense precipitate a little water was slowly added until it became pasty, showing that the invertase was redissolved. This was diluted with some 28 per cent. alcohol (0.960), and the whole well mixed to ensure there being no lumps. Alcohol of this strength is necessary—among other reasons—to get the large insoluble precipitate of albuminoid into such a state that it will not stop up the filter. The mass was then thrown on to a filter, allowed to drain, and washed with 28 per cent. alcohol. The filtrate was clear, and not much coloured; it was again made up to an alcoholic strength of 47 per cent.; the precipitate thus obtained was dehydrated with absolute alcohol, and dried in a vacuum over sulphuric acid, and powdered. The straw-coloured powder so obtained did not redissolve to a clear solution, and it contained 5.1 per cent. of ash. The analysis of the body free from ash was as follows:—N = 6.2 per cent., C = 46.5 per cent., H = 6.91 per cent. When first made it was very active, but in a few months its activity completely disappeared, and the powder was only partly soluble.

This large bulk was used in the preparation of most of the members of the invertan series.*

Since making the above preparation we have made many experiments with a view to obtain invertase in a greater state of purity. We have always found that every precipitation by alcohol means some loss of inverting power: and we shall presently show that when the invertase approaches a pure state, precipitation by alcohol destroys it entirely. We have, therefore, tried the effect of precipitating only once, and then thoroughly washing the precipitate with 0.920 alcohol. This we have found by far the most effective method. If alcohol weaker than 0.920 is used it generally destroys the invertase; and if the yeast liquor is precipitated very slowly in fractions, the result is often the same.

The mode of procedure we have found most effective in obtaining the highly active preparations of invertase is as follows:—Yeast is allowed to liquefy for at least a month in a fairly warm room. It is better not stirred, as the surface may become mouldy or putrid if the yeast has not been well pressed. At the end of this time the surface is removed, and any liquid on the top poured away; as far as our experience goes, the lower part will be found to be perfectly sound. This is thrown on to a quick filter, and after a couple of days the yeast liquor will have drained from it.

To the clear filtrate, alcohol, sp. gr. 0.870, to the extent of $1\frac{1}{2}$ times its bulk is gradually added, with vigorous and continued

* We shall refer to this as the "C" sample of invertase.

stirring; in this way a precipitate is obtained which can be easily washed. After this mixing, which should take about half an hour, the solution is allowed to stand for 24 hours. The light precipitate will then have settled, and should be washed on this and several successive days by decantation with 0.920 alcohol. When the washings become nearly colourless, the precipitate is thrown on to a filter and allowed to drain, and immediately taken off, and again mixed with a large bulk of 0.920 alcohol. The precipitate is again collected; the filtrate should be colourless. The residue is mixed with its own bulk of water and some 0.970 alcohol. It is thoroughly incorporated, allowed to stand for a few hours, and then thrown on a filter. The filtrate contains the invertase.* The residue on the filter may be taken off and washed again with 0.970 alcohol, but the resulting invertase is less pure.

We have tried very many modifications of the above process, but with less satisfactory results.

In this manner we have made the following preparations for analysis:—

Sample D, strength	$\pm 0 = 33.1'$
„ E, „	$\pm 0 = 28.5'$

Another sample was prepared by a slightly modified process as follows:—The first precipitation was effected with great rapidity; the heavy precipitate was dissolved in yeast liquor, and again rapidly precipitated. This precipitate was washed with 0.945 alcohol; but on account of the rapid precipitation we should expect the sample to contain yeast albuminoid. Its activity was found to be $\pm 0 = 32.9'$. This is sample F.

Yet another sample was prepared by precipitating the yeast liquor slowly, washing the precipitate by decantation, again dissolving in 1 part of yeast liquor, reprecipitating slowly, redissolving the precipitate, precipitating a third time, and washing as usual. The activity of this sample was $\pm 0 = 27.9'$; it is sample G. On account of the repeated precipitations, the sample probably contained some of the products of the decomposition of invertase.

A second sample, prepared in the same way, had an activity of $\pm 0 = 33.4'$, and it was called sample H.

The analyses of these samples will be found in Part IV, under the heading " *β -Invertan or Invertase.*"

If a preparation of active invertase is required to be kept for use when wanted, it is probably best to do so in the form of yeast liquor (see footnote, p. 874), and either use it direct or precipitate when required.

* We have not succeeded in precipitating invertase from this solution, as any attempt in this direction renders it inactive.

Separation of the Ash.

It will be noticed that in all the preparations of invertase hitherto made there was a large amount of ash. This consisted of potassium and magnesium phosphates, and the question arose whether it was an integral part of invertase or only an impurity. It was found to be impossible to separate it from the invertase by means of precipitation with alcohol, except by many precipitations in an acid solution, and in the meantime the inverting power had quite disappeared. Recourse was therefore had to dialysis, and, as it was found that dilute hydrochloric acid had no injurious effect on invertase in solution, the dialysing solution was kept constantly acid. Each day the dialyser and its contents was weighed, and the inverting power was calculated as if nothing had dialysed out. Under these conditions the inverting power should remain a constant figure, provided there was no change in the quantity of invertase. Unfortunately these experiments were done before the influence of the presence of very minute traces of acid was understood, and this influence was intensified by the estimations being made at 54°. They are, therefore, not very regular, and one estimation is a long way out, due no doubt to a slight excess of hydrochloric acid, but on the whole they are quite near enough for the purpose in hand. The invertase preparation used contained 4·6 per cent. of water and 8·05 per cent. ash on the dry substance. 30 grams was taken = 28·6 grams dry invertase, and containing 2·3 grams ash ($28·6 \times 0·0805$). The water outside the dialyser was always found to be inactive. The organic matter and ash were estimated in it each day. The following table gives the results of the experiment:—

TABLE XXIII.—*The Separation of the Ash by Dialysis.*

Time.	Passed through dialyser.		Inverting activity of the solution calculated on the whole original substance $\pm 0 =$
	Organic matter.	Ash.	
1 day	0·636	0·714	54'
2 days	0·558	0·402	50'
3 „	0·480	0·282	65'
4 „	0·306	0·168	48'
Total 4 days	1·980	1·566	—

The invertase originally contained 2·3 grams of ash, so that 0·73 gram is left; this is equal to 2·9 per cent. on the remaining

solid matter. The reduction of the ash to 2.9 per cent.* on the total solid matter has, in this case, had no appreciable influence on the inverting power, but it was advisable to try the effect of a still greater reduction.

With this view the experiment was repeated with the same amount of the same preparation of invertase, and the dialysis was continued for a longer time.

TABLE XXIV.—*The Separation of the Ash by Dialysis.*

Time.	Passed through dialyser.		Inverting activity of the contents of dialyser calculated on the whole of the original substance $\pm 0 =$.
	Organic matter.	Ash.	
1 day	1.77	1.71	57'
3 days			70'
4 „	0.52.	0.16	85'
5 „			78'
8 „	not weighed	—	70'
14 „			162'
15 „			104'
Total	2.29	1.87	—

The solution on the dialyser began to putrefy slightly on the 11th day, and, as it got slowly worse, it was thought useless to prolong the experiment beyond the 15th day.

An examination of the solution left on the dialyser gave the following result:—Sp. gr. = 1026.7. With a divisor of 3.85 this is equal to 6.93 grams per 100 c.c.

On estimating the ash, we found that 13 c.c. yielded 0.004 ash, so that 100 c.c. would contain 0.031 gram ash, equal to 0.45 per cent. on the solid matter. The strength was estimated, and it was found that 0.104 gram of the solution in 100 minutes gave a reading of + 5.2 div. = 9.6 T. The weight of the whole solution was 290 grams; so that, calculated in the same manner as on the previous days the strength was $\pm 0 = 104'$.

This figure, of course, is only useful for comparison with the results obtained on previous days, it does not take into account the amount that had dialysed or that was used for experimental purposes. It is, therefore, necessary to calculate the real activity of the organic solids in the solution. This we did as follows:—The 0 point would

* We shall refer to this preparation with 2.9 per cent. of ash as the “J” sample.

have been reached in 132'. Now 0.104 gram of the solution contained 0.007 gram solids. Allowing for this in the usual manner, we get 9.24 minutes ($0.1 : 0.007 :: 13.2 : 9.24$), and multiplying this result by 7.5 to correct for the temperature of the estimation, we obtain $\pm 0 = 69.3'$.

In passing we may state that we found the optical activity of the solid matter in the solution to be $[\alpha]_D = + 55^\circ$.*

Now in the above experiment we took a fairly active sample of invertase which had refused to part with its ash by precipitation with alcohol, and by means of dialysis with hydrochloric acid we reduced this ash from 8.05 per cent. to 0.45 per cent. on the solid matter, and that without any marked change in its inverting power. The first three days of its dialysis the activity was $\pm 0 = 57', 70'$, and $85'$. The last day it was $69'$.

We maintain that these figures show conclusively that ash does not enter into the composition of invertase.

Purification of Invertase.

It might be thought now that, having got rid of most of the ash, it would only be necessary to fractionally precipitate the invertase by means of alcohol, and then compare the several fractions to show its purity or the reverse. But here unexpected difficulties come in.

If the invertase is at all pure, it quickly loses its activity. As an instance of this we may refer to the B sample of invertase, which we stated to be the purest we succeeded in preparing. When we had examined the properties of this solution, we left it standing in a stoppered bottle for about a month, after first mixing with it sufficient weak alcohol to bring the total alcoholic strength to 0.975. At the end of the month the solution was quite clear and free from any organic precipitate, though some of the ash had crystallised. It was absolutely inactive. Weaker preparations of invertase are not so unstable, and they may sometimes be kept a long time with little injury, but anything like fractional precipitation completely destroys them, and even the inverting power of yeast-liquor itself may be destroyed by sufficiently slow precipitation.

We were not able to obtain more than slightly active precipitates from the solutions of the J and K samples of invertase after dialysis, although we tried everything we could think of to prevent the destruction of the invertase. We shall show later on that these samples must have been fairly pure mixtures of invertase with the products of its decomposition, this decomposition having been caused

* We shall refer to this preparation as the "K" sample of invertase.

by the number of preliminary precipitations which the substance had undergone.

If alcohol was added slowly to these solutions, they *milked*, that is to say, the solutions became white and opaque, exactly resembling milk. This term has been previously defined by one of us in treating of the amylans (Trans., 1882, 28). This milk was active, but if a trace of any acid was added a precipitate was thrown down which was perfectly inactive. We tried numerous experiments with the object of precipitating the invertase without destroying it; the following are typical ones:—

A. Strong alcohol was quickly added to the solution. The greater portion of it milked, but there was a small precipitate, which was tested and found to be active. Experience has shown us that a precipitate obtained thus suddenly cannot be called a fractional precipitate, and so it is useless for our purpose. It was, too, very minute. The milk was active. Nothing fell out of the solution on standing, and so it was necessary to add a drop of acetic acid to get a precipitate. The syrupy precipitate thus obtained was inactive.

B. Weaker alcohol added slowly produced no precipitate, but otherwise had the same effect.

C. We rendered the solution absolutely neutral with a little bicarbonate of soda and then added alcohol. Effect the same as in B.

D. Rendered slightly alkaline with bicarbonate of soda. Result the same as in B, but the syrupy precipitate produced by acid was slightly active. This was dried, and the activity found to be $\pm 0 = 240'$ against $70'$ of the original substance. In this case no fractionation took place, because the precipitation was effected too rapidly.

E. Rendered slightly acid (HCl). Precipitate and supernatant liquid both inactive.

F. Acidified considerably. Results the same as E.

From these experiments and many similar ones it is evident that we had come to an end of the purification in this manner; we therefore tried the effect of dialysis on a very active solution, so as to avoid the necessity of fractional precipitation after dialysis.

Here again we were defeated by the old difficulty that these comparatively pure solutions rarely retain their activity for more than a few days, and even when they appear stable, any attempt to work with them ensures their rapid destruction. Particulars of an experiment of this nature, made with sample E, are given in Part IV under the heading "Splitting up of Invertase."

We had consequently to rely on an examination of the substances in the inactive solution for a knowledge of the composition of invertase. The analysis and properties of invertase are given in detail in Part IV under the heading " *β -Invertan or Invertase.*"

Yeast Albuminoïd.

Attention must now be directed to one of the substances eliminated in the preparation of invertase. We have shown that in this process a portion of the precipitate produced by alcohol in yeast liquor is not again soluble in water (see "Separation of Invertase from Yeast Liquor"). This is the albuminoïd of yeast. In the preparation of the B sample of invertase we have shown that this precipitate amounted to 5.93 per cent. on the dry solid matter of the yeast.

Preparation.—We prepared it in the following manner:—Alcohol was added to yeast liquor until a precipitate began to form. On standing, a large, flocculent precipitate fell to the bottom. This was collected on a filter; it was found to have only a very slight invertive action. It was repeatedly washed with water, through which it diffused readily, forming a thick semi-solution, which would not pass through a filter even under pressure. In order, therefore, to separate the water, it was necessary each time of filtering to add a little alcohol, though not sufficient to throw any invertase out of solution. The albuminoïd then fell to the bottom as a well-defined, broken precipitate. When this had been done several times, the precipitate was washed with strong alcohol, pressed between blotting-paper, and dried in a vacuum over sulphuric acid. Until this final desiccation, the precipitate was almost white, but in the vacuum it rapidly assumed a dark colour. Prepared in this manner, it is a hard, brown, dull-looking mass, which is very difficult to reduce to powder.

Analysis.—We made an analysis with the following results, and for the sake of comparison we give the highest and lowest numbers obtained from the analysis of albumins (Béchamp, *Mém. Académie Scien.*, 28, 459).

TABLE XXV.—*The Analysis of Yeast Albuminoïd compared with Albumin.*

Constituents.	Yeast albuminoïd.	Albumin.	
		Highest.	Lowest.
Carbon	54.33	55.4	49.4
Hydrogen	7.50	7.40	6.71
Nitrogen	14.88	18.83	14.60
Sulphur	none	—	none
Phosphorus	?	—	—

The numbers are calculated on the substance free from ash, which amounted to 3.9 per cent., consisting of potassium and magnesium phosphates. Further analyses are given under the heading, "Yeast Albuminoid derived from Invertase."

Properties.—Precipitated yeast albuminoid is insoluble in water, but dissolves readily in a small quantity of caustic alkali. It is generally precipitated on acidifying the alkaline solution, and it falls out completely if the acidified solution is placed on a dialyser till the acid is removed. In yeast liquor it is in an alkaline solution, but it is not thrown out by acid even on boiling.

If alcohol is added to an alkaline solution of yeast albuminoid, no precipitate is formed, but the solution is converted into a milk. Exposed to heat, it exhales the usual smell of burning albumin, and leaves the usual black residue difficult of combustion.

Solutions of yeast albuminoid are slightly levorotatory, but they are too opalescent to admit of reliable readings. It is impossible, therefore, to use this property as a criterion of purity; we may state, however, that we found the optical activity of our preparations, in alkaline solution, to be somewhere about $[\alpha]_D = -13^\circ$.

Yeast albuminoid is not precipitated by alkaline copper oxide solutions. With Millon's reagent, it gives no reaction in the cold, but on heating, the precipitate caused by the acid turns pink.

Béchamp (*Mém. Académie Scien.*, 28, 269) mentions an albumin which he separated from beer yeast; its properties were very similar to those given above. He does not seem to have analysed it, but he found its optical activity to be $[\alpha]_D = -43.9^\circ$.

PART IV.—THE INVERTAN SERIES.

Splitting up of Invertase.

Up to this point we have shown that practically the whole of the invertase of yeast can be separated (*a*) from the insoluble portions of the yeast cell; (*b*) from the yeast constituents soluble in 47 per cent. alcohol; (*c*) from yeast albumin; and (*d*) from ash. But when we tried to further purify the invertase, we found that on attempting to precipitate with alcohol the inverting power was destroyed, so that the product or products ceased to come within our definition of invertase.

Under these circumstances, we found it necessary to abandon the attempt at further purification of invertase, and to undertake in its stead an examination of the substances resulting from its alteration.

With this end in view, we turned to the K sample, which, it will

be remembered, was dialysed for 15 days. We took 200 c.c. of the solution off the dialyser; this, as we have already shown, would contain 13·87 grams of solid matter. To it alcohol was slowly added; the solution became a milk, but a small, crumbly precipitate was formed. This was put on one side, and, as it was too small for any practical purpose, some other small bits obtained during the fractionation were added to it, and the whole was marked "oddments."

By adding minute quantities of dilute hydrochloric acid, the milk was precipitated in three fractions. They all fell out as syrups, and the solution was eventually left perfectly clear with a slight red tinge. The 13·87 grams of solid matter was accounted for as follows. We also give the optical activity of the chief fractions:—

1st precipitate.....	5·6	grams	} = 85 p. c. {	[α] _j = +50·7° +55·5° +50·7°
2nd " 	2·3	"		
3rd " 	3·85	"		
Oddments	1·39	"		
Soluble in alcohol.....	0·73	"	= 10 "	
Total	13·87			

These fractions were all obtained by actual weighings, and the moisture in each was estimated and allowed for. The exact agreement of the numbers is a satisfactory coincidence.

We may note that in dealing with the bodies of the invertan series, a mixture of any two of them is easily separated by partial precipitation with alcohol and acid, as shall be described, and we have considered a sample homogeneous when we could separate it by this means into two or more fractions, all of which had the same or nearly the same optical activity as the original substance.

It will be seen from these results that 85 per cent. of the solid matter in the dialysed solution of active invertase consisted of a syrupy substance having [α]_j between +50° and 56°. The 10 per cent. of oddments was a crumbly substance, mainly insoluble in water. None of the fractions had any inverting power.

These three syrupy precipitates were mixed and dissolved in water to a clear solution. It was acidified, and then very carefully fractionated with alcohol. The first precipitate was very small and crumbly; the second was a small, syrupy precipitate; the third and fourth contained the large bulk of the solids.

TABLE XXVI.—*The Re-fractionation of the Syrupy Precipitates.*

Precipitate.			[α] _j .	D (divisor).*	N, per cent.
No.	Condition.	Bulk.			
1	crumby syrupy " "	very small	—	—	—
2		small	—	—	4·7
3		large	52·4	3·85	2·21
4		large	54·1	3·75	2·31

There was little doubt that we were dealing with a homogeneous substance.

These experiments were repeated with the J sample of purified invertase solution described above. The results obtained were exactly similar.

It may be remarked here that the readings in the polarimeter are not very accurate for these substances, as, even when they are clear and nearly colourless, they absorb a great deal of light. Hence absolute regularity in the results is not to be expected.

All the syrupy precipitates from these two series of fractionations were mixed, and again very carefully fractionated with alcohol and hydrochloric acid.

The fractions mixed had [α]_j varying from +52·4° to 56·8°. The total weight was 22 grams. The mixture was divided into six fractions, which yielded the following results:—

TABLE XXVII.—*The Re-fractionation of Precipitates of Optical Activity*
[α]_j = + 52·4° to 56·8°.

Fraction.	Weight.	[α] _j .	Divisor.*	N.	C.	H.
V.....	2·5	—	—	—	—	—
W.....	3·2	56·5	3·75	2·26	—	—
X.....	5·5	53·5	3·75	—	—	—
Y.....	5·7	53·5	3·71	—	—	—
Z ₁	1·3	53·4	3·74	—	46·5	6·82
Z ₂	1·45	—	—	2·59	—	—
Total.....	19·65	—	—	—	—	—

It will be seen that practically no differentiation has taken place.

Fractions X, Y, and Z₁ were again mixed and fractionated with the utmost care, as follows:—

* Determined.

Fraction.	$[\alpha]_j$.	D.*
A. A very small precipitate.....	—	—
B. A large precipitate	57.4	3.69
C. " " 	53.2	3.71
D. A very small precipitate.....	—	—

From these results it would seem as if there were a slow breaking down of the substance with $[\alpha]_j + 54^\circ$; one having a somewhat higher optical activity being amongst the products. We shall see later on that such is the case. Despite this fact, we see that we did not find it possible to differentiate the bulk of the syrupy precipitate by means of the most careful fractional precipitation with alcohol and acid, and we therefore came to the conclusion, justified by further experience, that the substance having $[\alpha]_j + 54^\circ$ (*circa*), and which we have named δ -*invertan*, was a homogeneous compound, and that it constituted at least 85 per cent. of the products of the breaking down of invertase on precipitation with alcohol.

The only other substance found in any quantity in the dialysed solution was the crumby precipitate mentioned above, and which we have called α -*invertan*. This substance is not soluble in water or in solutions of the syrupy substance. As, however, it exists in the perfectly clear dialysed invertase solution, in company with the syrupy substance it cannot be doubted that a combination of these two formed the soluble compound, *invertase*. This is absolutely shown to be the case by the following facts:—

First. It has not been found possible to separate the two substances without destroying all inverting power. Whenever a solution of invertase is precipitated by alcohol, the total inverting power is somewhat decreased, and at the same time it is found that the precipitate is not again entirely soluble in water, thus showing that a decomposition of some kind has taken place.

Secondly. Each of these two substances may itself be decomposed by suitable means into a crumby and a syrupy substance, and *these ultimate products are identical*.

Thirdly. Between the two ultimate products we shall show that a whole series of compounds exists, of which series all the substances we have mentioned are members.

There can, then, be no doubt that invertase is a compound of the two substances which we have called α - and δ -*invertan*. We shall, however, give further experimental proof of this fact; and, for this purpose, we employed the very purest samples of invertase solution we found it possible to obtain.

* Determined.

A solution of the E sample of invertase (see "Separation of Invertase from Yeast Liquor") was placed on a dialyser with a large excess of hydrochloric acid. The water outside was frequently renewed; at the end of four days the solution was nearly neutral, and a very small, flocculent precipitate had fallen out. Its inverting power and optical activity were then determined and compared with the same numbers before dialysis. In taking the optical activity, the flocculent precipitate was first filtered out.

	$[\alpha]_D$	$\pm 0 =$
Before dialysis	+ 69.7°	28.5'
After ,, 	+ 54.3°	nearly inactive

We see here, plainly, how difficult these nearly pure solutions of invertase are to work with. A treatment, which we have shown to have no effect whatever on the impure samples, is in this case fatal. Repeated experiments have shown us that the precipitate consists entirely of α -invertan, which has a slight minus optical activity. No mixture of this with the solution having $[\alpha]_D + 54.3^\circ$ could give an optical activity of +69.7°; hence the two must have been in combination, and the act of separating them destroyed the invertase. There is, however, just the possibility that some substance with a higher rotating power may have passed through the dialyser; in order to test this, we have made another experiment, as, in consequence of the high proportion of acid and the very small quantity of matter that passed through, it was impossible to make any satisfactory observations with the diffuse.

60 c.c. of the solution of the G sample of invertase were taken. It was acidified, and alcohol (0.870) added very slowly until a cloud appeared. It was then allowed to stand for two days. At the end of this time there was a large, flocculent precipitate at the bottom. This was collected and purified by mixing with water, and then filtering in the presence of very dilute alcohol. The washings were added to the original filtrate. To the latter a large excess of strong alcohol was added; this formed at first a milky solution, but after standing two or three days it became perfectly clear, and there was a colourless, transparent syrup at the bottom of the beaker. We had now divided the invertase solution into three portions: A, the flocculent precipitate; B, the syrup; and C, the alcoholic solution. Nothing had been discarded. The alcoholic solution, as we have repeatedly proved under similar circumstances, contained practically nothing but the ash, and this was thrown away. The A fraction was insoluble in water but soluble in alkali. It was dissolved to about 10 c.c., and the solution had a very faint dextrorotatory power, something below +1.0 division in 100 mm. It was impure α -invertan.

The B fraction was soluble in water to a colourless solution. It was dissolved in 25 c.c. of water, and in 100 mm. gave an angle of $+8.3$ divisions. The solution contained 6.000 grams per 100 c.c., and the $[\alpha]_D$ was therefore $+53.1$. It was evidently δ -invertan.

From these figures it is obvious that a mixture of A and B fractions would have had an optical activity of $[\alpha]_D =$ less than $+53^\circ$. Of course neither of them had any inverting power. Comparing the factors before and after the loss of inverting power, we have

	$[\alpha]_D$.	± 0 .
Before fractionation.....	$+72.4^\circ$	$27.9'$
After ,, less than	$+53.0^\circ$	none

The solid matter in the active solution had an optical activity $[\alpha]_D = +72.4^\circ$: that of the divided, inactive fractions, taken together, less than $[\alpha]_D = +53^\circ$; there can, consequently, be no further proof necessary that the higher optically active invertase breaks down into bodies of less optical activity, which we call α -invertan and δ -invertan.

We have already shown δ -invertan to be a homogeneous substance, and we shall, later on, show the same with regard to α -invertan; and, as 95 per cent. of the solid matter in the invertase solution was obtained in the form of these bodies (see "Splitting up of Invertase"), and as the remaining 5 per cent. was soluble in alcohol, the proof is complete that invertase yields α - and δ -invertan when an attempt is made to precipitate it.

Invertase does not always break down in the manner we have mentioned here: for sometimes other members of the series are found. α -Invertan is invariably present, but sometimes, instead of δ -invertan, either γ - or ϵ -invertan (to be described later on) takes its place. γ -Invertan we have only obtained on one occasion, and we do not know what was the cause of its appearance: but ϵ -invertan we have frequently found; and we believe that in this case the δ -invertan was first formed, and was then converted by the alcohol and acid into ϵ -invertan, as we shall, later on, show to be probable. We generally found ϵ -invertan when the process of purification was somewhat prolonged.

We will now take the yeast albuminoid prepared from invertase, and then each member of the invertan series separately, giving in each case the preparation, analysis, and properties.

Yeast Albuminoid derived from Invertase.

Preparation.—Some α -invertan, prepared from invertase in the manner already described, was dissolved in weak potassium hydroxide,

and an alkaline solution of a copper salt added. At first no precipitation took place, but on adding a very large excess of strong potassium hydroxide, a large blue precipitate slowly fell out.

The solution was allowed to stand for two days, and the clear, supernatant liquid was then decanted off. We shall treat of the precipitate thus obtained under the heading "*ζ-Invertan*."

The solution, containing everything not thrown out by copper, was acidified and precipitated with alcohol. The flocculent precipitate thus obtained was redissolved in very weak alkali and again thrown out with alcohol and acid. Finally the precipitate was redissolved in weak potassium hydroxide and an excess of acid quickly added. Nothing was thrown out of solution in this manner, so the solution was placed on a dialyser and kept there until it was perfectly neutral. This treatment caused a large, gelatinous precipitate to fall out. This was collected on a filter, dehydrated with alcohol, and dried in a vacuum. It took on much colour whilst drying.

We shall show that the substance thus obtained is very similar to the yeast albuminoid we have already described.

Analysis.—

TABLE XXVIII.—*Analysis of Yeast Albuminoid derived from Invertase.*

Sample.	Constituents of substance free from ash.			Ash.
	C.	H.	N.	
A	—	—	14·61	—
B	52·66	7·38	14·07	0·47
C	53·79	7·19	14·17	1·00

The samples marked A and B were obtained in the manner above described. In order to test the purity of the B sample, it was redissolved in a large excess of potassium hydroxide and some Fehling's solution added. As this did not produce any precipitate, a large excess of alcohol was added. After standing two days there was a small precipitate. The clear, supernatant liquid was decanted off, and the albumin was obtained from it in the manner before described. The analysis is given above as that of the "C" sample. It will be seen that, considering that we were working with an albuminoid, the figures agree as well as could be expected.

Properties.—The properties of this substance are, as far as we have been able to ascertain, the same as those of yeast albuminoid.

Conclusion.—In the following table we give a comparison of the results of the analysis of the albuminoid obtained directly from yeast and of the C sample of the albuminoid obtained from invertase :—

TABLE XXIX.—*Comparison of the Analyses of the Albuminoids obtained from Yeast and from Invertase.*

Source of the albuminoid.	Constituents of substance free from ash.		
	C.	H.	N.
Invertase	53·79	7·19	14·17
Yeast.....	54·33	7·50	14·88

These numbers agree pretty well, and leave no doubt that the substances are very similar, and although we cannot say that they are identical, we may take the mean of the two analyses as the composition of yeast albuminoid.

$$C = 54.06 \text{ p. c.}, H = 7.35 \text{ p. c.}, N = 14.53 \text{ p. c.}$$

We may note here that as this albuminoid contains no sulphur, and, probably, no phosphorus, we have no reason for attributing to it such a large molecule as the albumins are usually considered to possess. The formula $C_3H_{15}N_2O_3$ would give

$$C = 54.27 \text{ p. c.}, H = 7.54 \text{ p. c.}, \text{ and } N = 14.07 \text{ p. c.}$$

Although we do not for one moment believe that the molecular weight of yeast albuminoid is as low as this, yet we have thought it necessary to point out the possibility, because on this factor depends the molecular weight of the entire invertan series (see Part V, "Constitution of the Invertan Series").

α -Invertan.

Preparation.—We have already shown under the heading "Splitting up of Invertase," that invertase in an acid solution slowly breaks up in the presence of alcohol, with the separation of a flocculent precipitate. If this precipitate is purified by washing a few times with water and weak alcohol, and then by dialysis in the manner described in the case of yeast albuminoid derived from invertase, α -invertan is obtained. In this way it is easy to prepare considerable quantities of it.

That this substance is a true compound, will be apparent from the following considerations :—We shall show that α -invertan may be split up into yeast albuminoid and ζ -invertan. Now a mixture of these two substances containing 8·3 per cent. of nitrogen would have an optical activity of about $+40^\circ$, whilst α -invertan, on the contrary, has about the same levorotation as yeast albuminoid.

We consider that the approximate purity of the preparation is established by the general agreement of the percentage of nitrogen contained in the three samples analysed below.

TABLE XXX.—*Analysis of α -Invertan.*

Sample.	Constituents of substance free from ash.			Ash.
	C.	H.	N.	
A	48·03	6·65	8·35	0·70
B	—	—	8·53	—
C	—	—	8·15	—

The A sample analysed above was obtained by repeatedly precipitating 50 grams of the C sample of invertase with alcohol and an acid. The flocculent precipitate thus obtained was purified several times by precipitation with weak alcohol. The substance in solution we refer to under " ϵ -Invertan." Finally, the precipitate was dissolved in weak alkali, and a little Fehling's solution was added. A small precipitate fell out and was discarded. The α -invertan was separated from the copper solution in exactly the same manner as was described under the heading "Yeast Albuminoid derived from Invertase." It only took on very little colour on drying. The dry substance weighed 11·5 grams.

The B and C samples were obtained in a similar manner, but in the preparation of the C sample the treatment with Fehling's solution was omitted.

It will be noticed that we have only estimated the C and H in one sample of α -invertan. The reason of this is, that in the invertan series the C and H vary with the nitrogen, only in a very much smaller degree. For practical purposes, therefore, the nitrogen alone is required. In the case of α -invertan, the nitrogen of the A sample agrees with the mean of the B and C samples, so that we take for the composition of α -invertan :—

$$C = 48\cdot03 \text{ p. c.}, H = 6\cdot65 \text{ p. c.}, N = 8\cdot35 \text{ p. c.}$$

Properties.—The properties of α -invertan are exactly similar to those of yeast albuminoid, with the exception that in the presence of a very large excess of an alkali, it yields a copper precipitate as has been already described. The α -invertan is in this case split up into yeast albuminoid and ζ -invertan; the reaction is treated of under both these headings.

For its optical activity we have obtained numbers varying between $[\alpha]_D = -11^\circ$ and -20° .

β -Invertan or Invertase.

This is the substance which was primarily the subject of this investigation. We have already described our attempts to obtain it in a pure state, and we have given details of the preparation, among others, of five separate samples of which the inverting activity was found to be :—

D sample, ± 0	=	33.1'
E ,,	=	28.5'
F ,,	=	32.9'
G ,,	=	27.9'
H ,,	=	33.4'

Although these numbers do not differ very much amongst themselves, there is sufficient difference to indicate that we have not an absolutely pure body to deal with, but considering the different methods by which these samples were prepared, and the nature of the body itself, splitting up, when pure, as we have described, on the slightest handling, we are justified in concluding that we have pure invertase in our hands contaminated simply with the products of its own decomposition, a little ash, and possibly some yeast albuminoid which has not been rendered insoluble by the single precipitation with alcohol to which the preparation has been subjected. This statement is borne out by the fact that a careful examination of several of these solutions has failed to show the presence of any substances, except members of the invertan series and ash (see "Splitting up of Invertase"). We have not used the optical activity as a test of purity, because it is not nearly such a good criterion as the inverting power. We have already given reasons for our statement that this test is the only reliable one, and that it accurately indicates the relative proportion of invertase present. We shall show later on that $\pm 0 = 22.5'$ approximately represents the inverting activity of pure invertase, and from this factor it is easy to calculate the absolute amount of invertase present in any preparation. This would tend to show that the above samples are not sufficiently

pure for analysis, were it not for the fact mentioned above that the impurity consists almost entirely of the products of the decomposition of invertase, and therefore their presence would not have a very great effect on the analysis.

Analysis.—

TABLE XXXI.—*Analysis of β -Invertan or Invertase free from Ash.*

Sample.	C.	H.	N.
D.....	47.08	6.74	4.98
E.....	46.64	6.70	3.92
F.....	45.90	6.69	4.90
G.....	46.17	6.56	3.45
H.....	46.94	6.77	3.94

These members are calculated on the organic matter in the active solutions, the ash in each case being estimated and allowed for.

The strongest invertase we ever obtained was the B sample with $\pm 0 = 25.1'$, but this was not analysed. Under these circumstances we adopt the mean of the E and G samples as the nearest approximation to the composition of pure invertase that we have been able to attain. We do this with the more confidence as these two samples were prepared by different methods, one of which we should expect to give more and the other less nitrogen than the true quantity, and yet they agree well with each other. The composition of invertase calculated in this way would be—

$$C = 46.41 \text{ p. c.}, H = 6.63 \text{ p. c.}, N = 3.69 \text{ p. c.},$$

and this cannot be far removed from the truth.

Properties.—The action of invertase on cane-sugar is discussed in Part II of this paper.

Invertase is a white substance readily soluble in water to a clear, but somewhat viscous solution, which is not rendered turbid by boiling. The solution slowly acquires colour in contact with the air. It is insoluble in alcohol of 0.94 sp. gr., and it is, like all the series, more soluble in warm than in cold alcohol. We have already shown that it may be precipitated out of solution by alcohol with only a small percentage of loss, provided the solution is not pure. The precipitate is semi-syrupy or semi-crumby, and partakes of the colour of the mother liquor. It also takes down large quantities of all the salts in solution, from which, however, it may be partially freed by washing with 0.920 alcohol. Solutions of impure invertase submitted to careful fractionation by means of alcohol are nearly always

destroyed, and so are solutions of invertase allowed to stand long in the presence of large quantities of alcohol. The purer solutions of invertase cannot be precipitated by alcohol without being destroyed, unless they are very concentrated, and a large excess of very strong alcohol is quickly added.

When invertase is precipitated by alcohol, the precipitate is never all soluble in water, showing that there is always some decomposition. Invertase will not pass through a dialyser, and, if impure, it may be dialysed for a long time without injury. It possesses no copper oxide reducing power, but readily forms with copper in alkaline solutions the characteristic copper compound described at length under a separate heading. With Millon's reagent, invertase gives no reaction in the cold, but, on heating, the solution turns a fine pink.

We have shown that it is very difficult to keep invertase, whether in solution or in the dry state, for an indefinite time, without a gradual loss of strength. We have found it best retains its power in solution (see Table I, and footnote, p. 874).

We are not able to give any definite number for the optical activity of invertase. The E sample had $[\alpha]_D = +69.7^\circ$ and the G sample $[\alpha]_D = +72.4^\circ$, whilst our strongest sample, the B sample, had $[\alpha]_D = +74^\circ$. We think that even the highest of these figures is below the truth, as the impurities have a lower optical activity than this, the highest being δ -invertan, with $[\alpha]_D = +54^\circ$. These figures, as well as the nitrogen estimations, bear out what we have already said of these two samples; viz., that the E sample is probably contaminated with some unprecipitated albumin, and the G sample with δ -invertan. We, therefore, take $[\alpha]_D = +80^\circ$ as approximately representing the optical activity of invertase.

Invertase in solution is not altered by dilute mineral acids; but dilute alkalis at once destroy its activity beyond the power of recall. Lime-water does not destroy the activity if quickly neutralised.

Powdered invertase slowly and thoroughly dried in a vacuum over sulphuric acid withstands with little alteration a temperature of 100° . Two samples thus dried were placed for some hours in a current of dry air at 100° with the following results:—

	± 0 before heating.	± 0 after heating.
J sample.....	131'	174'
K „	164'	222'

The effect of heat on the inverting power of invertase solutions is peculiar, and requires a more detailed description.

Experiment A.—A solution of invertase was placed in a test-tube in a bath of cold water. The temperature was then rapidly raised. In the test-tube were a pipette and a thermometer. The solution

was kept well stirred, and as the temperature rose samples was taken at intervals by means of the pipette.

Each sample, which consisted of 20 drops, about 1 gram, was placed in a cold empty beaker, and when all the samples had been collected, 25 c.c. of a 20 per cent. solution of cane-sugar was added to each. The samples all stood together for $3\frac{1}{2}$ hours, and were then stopped with alkali and their optical activity observed. 4 per cent. is added to the observed angle to allow for the invertase solution.

TABLE XXXII.—*The Effect of Heat on Solutions of Invertase.*

Temperature when sample was taken.	Readings in 200 mm. tube.	Corrected readings.	Time units.
15° C.	-14·0	-14·6	20·4
35 "	-12·0	-12·5	18·7
40 "	- 6·8	- 7·1	15·6
45 "	+25·6	+26·6	6·2
50 "	+70·4	+73·2	0·4
55 "	+74·0	+77·0	0·0

Under the heading "Time Units" we give the number that corresponds in Plate I (p. 844) with the percentage of inversion calculated from the polarimeter readings. As we only require the relative numbers, and all the other conditions of experiment are the same, it is not necessary to carry the calculations any further. Bearing in mind the rule, that the amount of invertase present is in direct proportion to its inverting power, we may consider that the original amount of invertase present is represented by the number 20·4, and the other figures are in proportion to this.

We shall again refer to these results after the next experiment.

Experiment B.—In order to find out what temperature invertase can endure when heated with cane-sugar, we rapidly heated a solution of cane-sugar containing invertase, and, as the temperature rose, we took samples, and rapidly cooled them. They were all placed together in a shallow vessel of cold water, and, when they had all cooled down, the optical activity of each sample was taken. They were then allowed to stand for twenty-four hours, and at the end of that time the optical activity was again observed.

From these figures the percentage of inversion was calculated in each case, and by comparing the results with the theoretical curve of inversion (see Plate I), it was easy to find the number of time units that each reaction had passed through in the twenty-four hours. These are given in the last column of Table XXXIII. The amount

of undestroyed invertase left in the samples is in proportion to the number of these time units; we have given the reasons for this in Experiment A.

TABLE XXXIII.—*The Effect of Heat on Solutions of Invertase in the Presence of Sucrose.*

Temperature reached before cooling down.	1st observation.	2nd observation.	Time units passed.
35° C.	+ 21·4	—11·1	26·0
55 „	+ 19·5	—11·3	26·2
60 „	+ 18·7	—11·5	26·9
65 „	+ 19·1	—10·6	23·4
70 „	+ 25·0	+ 1·6	8·9
75 „	+ 28·6	+ 28·6	0·0

These two experiments may be considered identical, except that in one case a large amount of cane-sugar was present, and in the other case none. The time of exposure to the high temperatures was no doubt not identically the same in the two experiments, but in each case as soon as the temperature reached the point named, the samples were rapidly cooled. Probably the one containing sugar, being the larger bulk, would be the longer time at the critical temperature, so this would not account for the difference in the results.

For comparison we have tabulated the results as follow :—

TABLE XXXIV.—*Showing how the Heat Resisting Power of Invertase is Affected by the Presence of Sucrose.*

Highest temperature attained.	Percentage of invertase destroyed.	
	A. Experiment (without sugar).	B. Experiment (with sugar).
15° C.	none	—
35 „	8·3	none
40 „	23·5	—
45 „	70·0	—
50 „	98·0	—
55 „	100·0	none
60 „	—	none
65 „	—	12
70 „	—	66
75 „	—	100

From this we see that invertase in solution requires a temperature fully 25° higher to destroy it when cane-sugar is present than when it is absent. In Part V of this paper we shall attempt to give an explanation of this.

For the manner in which invertase breaks down under various circumstances see heading "Splitting up of Invertase." We need only add that dry preparations seem to behave in the same way as solutions: after keeping for some time, a large portion is found to be insoluble, and the whole has lost its activity.

The inverting power of the most active invertase preparation we have ever succeeded in making was $\pm 0 = 25.1'$, calculated on the dry solid matter free from ash. We know that this was contaminated by the soluble products of the destruction of 12.3 per cent. of invertase. These soluble products would, we believe, amount to about 10.5 per cent. (see "Theoretical Constitution of the Invertan Series"). Making allowance for this, we have $\pm 0 = 22.5'$ for the activity of the remainder of the solid matter. We believe that this represents fairly well the activity of pure invertase. Our reason for this is that we have never found in the purer preparations any impurities but the products of the decomposition of invertase, ash, and sometimes a little albumin. This last, we believe, was not present in the sample we obtained these numbers from; and, as we have allowed for the other two impurities, we think ourselves justified in saying that $\pm 0 = 22.5'$ represents, at least approximately, the activity of pure invertase. We have shown that our purest preparations have $\pm 0 = 25.1'$, $27.9'$, and $28.5'$; the second of these was prepared from yeast liquor, quite differently from the other two. We think that these give probability to our estimations of the activity of the pure substance.

Taking this as the basis, it is easy to calculate the actual amount of invertase present in any material. In the case of yeast, for instance, from 2 to 6 per cent. of the dry solid matter would consist of invertase.

η -Invertan.

Preparation.—We have only obtained this substance once, but fortunately we had enough of it to be able to prove satisfactorily its homogeneity. From a sample of invertase we obtained by careful precipitation with alcohol and hydrochloric acid two syrupy precipitates. Their optical activities were respectively $[\alpha]_j = +64^{\circ}$ and $+45^{\circ}$. Of the former fraction we shall treat under the heading "*e*-Invertan." The 45 fraction was again carefully divided into three parts, and these were dried and powdered. Their optical activities were as follows: R, $[\alpha]_j = +67.5^{\circ}$, S, $[\alpha]_j = +47.8^{\circ}$, and T, $[\alpha]_j = +46.0^{\circ}$.

The S and T fractions were redissolved, and alcohol slowly added. The invertan nearly all fell out as a granular, syrupy precipitate, but a little was left in the solution as a milk. The precipitate was redissolved, and again treated in the same way. The two milks were added together, precipitated with acid, and the precipitate labelled Z. The main precipitate was redissolved, and weak alcohol added with great slowness. A large, flocculent precipitate gradually fell out; this was filtered off and called A. The remaining milk was fractionally precipitated in two parts, B and C. The A fraction was not soluble in water: it had the appearance of α -invertan. The remaining fractions were soluble, yielding clear solutions; their optical activities were B, $[\alpha]_D^{20} = +43.2^\circ$, C, $= +50.0^\circ$, and Z, $= +50.4^\circ$.

Taking all these facts into consideration, and knowing that β -, δ -, and ϵ -invertan break down in a similar way when precipitated by acid and alcohol, we have little hesitation in coming to the conclusion that the original precipitate obtained from the invertase, having $[\alpha]_D^{20} = +45^\circ$, was a fairly pure substance. On attempting to purify with acid and alcohol, it slowly broke down, as do nearly all the members of this series, into two invertans, one soluble and the other insoluble in water. We have failed in attempting to prepare a further quantity of γ -invertan, and the original amount was so much reduced by the continual breaking down that we had not enough for further experiments.

The above precipitations show how completely, by careful fractionation, the members of this series may be separated from one another; and we are perfectly convinced that if the original 45 fraction had consisted, as it did eventually, of a mixture of α -invertan with δ -invertan, the first fractionation would have separated nearly all the former from the latter. We lay some stress on this point, because it applies equally to δ - and ϵ -invertan, both of which, as well as invertase itself, split up during purification.

Analysis.—The fraction obtained above having $[\alpha]_D^{20} = +43.2^\circ$ contained 0.4 per cent. of ash. The substance free from ash yielded on combustion the following numbers: C = 45.62 per cent.; H = 6.55 per cent.; N = 3.15 per cent.

Properties.—Solutions of γ -invertan are liable to split up in the manner already shown.

We have also already given all the determinations of the optical activity of this substance that we made. From these figures it is evident that it has approximately $[\alpha]_D^{20} = +45^\circ$.

Its remaining properties it possesses in common with δ -, ϵ -, and ζ -invertan, and we will state them now in full, so that they need not be repeated when we describe these substances.

γ -Invertan is a non-crystallisable substance, easily freed from water

in a current of dry air at 100°. It is soluble in all proportions in water, forming clear, colourless solutions. There is, however, a peculiar blackness about the more concentrated solutions which renders them difficult to observe in the polarimeter. The solutions are viscous, and their divisor is about 3·85; they do not cloud on boiling, and the optical activity is not altered thereby. Alcohol transforms fairly dilute neutral or alkaline solutions into a milk, which remains unaltered a long time. Acids precipitate from this a colourless syrup. By using small drops of dilute acid the substance may be fractionated in this manner. Strong solutions are immediately precipitated by strong alcohol, thus affording a means of ridding precipitates of superfluous acid, which might be injurious during desiccation. Left for any lengthened period in alcoholic solution, it assumes colour, and is liable to split up as has been described.

Even dilute solutions of γ -invertan yield the characteristic copper compound common to all the series. This is probably the copper compound of γ -invertan, as we shall show in the case of several other members of the series, that we are able to convert them into the copper compound, and then recover them without alteration. This compound is extremely difficult to purify, and it seems to have the same tendency to split up as the uncombined invertan. We have, therefore, only been able to obtain the copper compound of ζ -invertan in a pure state. We shall treat of this compound under a separate heading.

We believe we have observed that a solution of γ -invertan has the power of dissolving traces of finely-divided α -invertan, but the amounts dissolved were too small for experiment. γ -Invertan dried in thin plates becomes a glassy mass. The dried preparation keeps unaltered an indefinite time.

If a concentrated solution of the substance is allowed to stand for a long time, the invertan seems to assume a modified form. It becomes extremely viscous, and, with difficulty, miscible in water. The optical activity is not altered, and its other properties seem to be unaffected. This state appears to correspond with the meta-state of the gum acids and similar substances. It is not easy, however, to reconvert these changed bodies into the ordinary state; raising to the boiling point seems to be the most effectual plan.

With Millon's reagent, γ -invertan gives no reaction in the cold; but on heating, the solution becomes a fine pink colour.

δ -Invertan.

Preparation.—This has already been described under the heading "Splitting up of Invertase," where we also showed that it was a

homogeneous substance. It is also formed by the breaking down of γ -invertan.

Analysis.—

TABLE XXXV.—*Analysis of δ -Invertan.*

Sample.	Constituents of substance free from ash.			Ash.
	C.	H.	N.	
A.....	—	—	2·31	—
B.....	—	—	2·21	—
C.....	46·5	6·82	2·59	1·4
D.....	—	—	2·26	—

The preparation of all these four samples has been described under the heading "Splitting up of Invertase." The average of the nitrogen estimations of C and D samples, the two most purified, is $N = 2·43$ per cent.

Properties.—Solutions of δ -invertan are liable, especially in the presence of acid alcohol, to split up into an insoluble invertan and ϵ -invertan. We shall give an example of this under the latter heading. The optical activity we have already shown under the heading "Splitting up of Invertase," to be about $[\alpha]_j = +54^\circ$.

The remaining properties are given under the heading " γ -Invertan."

ϵ -Invertan.

Preparation.—If we refer back to the heading α -Invertan, we shall find that in the preparation of the A sample we broke up the invertase into α -invertan and a substance soluble in water. This latter was fractionated in three parts, N, O, and P. Their optical activity was as follows:—N, $[\alpha]_j = +71·7^\circ$, O = $+64·5^\circ$, and P = $+65·0^\circ$. Fractions O and P were mixed, and constitute the B sample of ϵ -invertan analysed below.

In the above case ϵ -invertan was obtained directly from invertase. We will now give an instance of its preparation from δ -invertan. This experiment was done for the purpose of obtaining some carefully purified δ -invertan, but the latter broke down during manipulation.

Several fractions of fairly pure δ -invertan were dissolved together in water, and weak alcohol was slowly added until the solution became a milk. A small precipitate fell out, which was redissolved in water and again carefully milked. The minute precipitate which

fell out this time was not soluble in water, and it was discarded. The milks were all added together.

We now proceeded to fractionate this milk with the utmost care by means of minute drops of very dilute hydrochloric acid added with vigorous stirring. By this means we obtained a colourless syrup on the sides and bottom of the vessel. After standing a few hours, the milk was decanted off, and a further amount of acid added to it with the same precautions. After four fractions had been thrown out, the solution was no longer a milk, but was quite clear, and contained nothing in solution that could be thrown out by alcohol or acid. The optical activity of the four fractions was A, $[\alpha]_j = 65.4^\circ$, B = 65.8° , C = 65.1° , D = 60.0° . It is evident from this that, except about one-half of the D fraction, all the δ -invertan had been converted into ϵ -invertan. The A, B, and C fractions were added together. Their united weight was 15 grams, and the mixture constitutes the C sample of ϵ -invertan.

We undertook many experiments with a view if possible to divide ϵ -invertan into fractions of different optical activity. The following is one of them:—

In the preparation of γ -invertan, the two original fractions had $[\alpha]_j = +64^\circ$ and 45° . The former of these was dialysed with hydrochloric acid for several days; after that it was very carefully precipitated with alcohol and acid into five fractions, A, B, C, D, and E. A and E, being only very small fractions, were discarded; C also was small, and the bulk of the substance was contained in B and D. The optical activity of these two fractions was found to be B, $[\alpha]_j = +65.4^\circ$, and D = $+65.9^\circ$. Remembering that the original substance had $[\alpha]_j = +64^\circ$, this evidence of its homogeneity may be considered complete.

It was, however, our wish still further to test the homogeneity of ϵ -invertan. With this object we undertook a long series of fractional precipitations conducted on a principle similar to that adopted by Crookes in dealing with the rare earths ("Fractionation of Yttria," *Brit. Assoc. Rep.*, 1886). To a somewhat dilute acid solution of a fairly pure ϵ -invertan, alcohol was added with great care, until a slight haze appeared in the solution; it was then allowed to stand until the next day. The fall in temperature during the night caused a considerable syrupy precipitate to separate. The mother liquor was poured off and again treated in the same manner. In this way the substance was first divided into three fractions, labelled A, B, and C. Then these were again subdivided into A₁, A₂; B₁, B₂; C₁, and C₂. The fraction A₁ was kept by itself. A₂ and B₁ were mixed; B₂ and C₁ were mixed, and C₂ was kept by itself, so that the three fractions had become four. These four were then repeatedly treated in a similar

manner, until at last the number of fractions had increased to eight. The two end fractions, being very small, were discarded. The six middle ones had $[\alpha]_D = +70.5^\circ, +72.0^\circ, +68.6^\circ, +70.0^\circ, +70.0^\circ$, and $+70.9^\circ$. There was also considerable coloration. It is, therefore, evident that the substance is being converted into ζ -invertan, and this method is valueless as regards ϵ -invertan. We applied it with better results to ζ -invertan.

Analysis.—

TABLE XXXVI.—*The Analysis of ϵ -Invertan.*

Sample.	Constituents per cent. on substance free from ash.			Ash.
	C.	H.	N.	
A	44.80	6.43	2.09	1.00
B	44.56	6.31	2.07	0.62
C	44.00	6.34	2.06	0.50

We have already shown how samples B and C were prepared; the A sample was obtained in exactly the same manner as B. We have no reason to suppose that one of these is purer than the other, so we take the mean of the three as the nearest approach to the composition of ϵ -invertan.

C = 44.45 per cent., H = 6.36 per cent., N = 2.07 per cent.

Properties.—We have shown how the members of this series break down, especially in the presence of acid and alcohol, into two substances, one soluble and the other not. The soluble substance is generally that member of the series next below the one originally present. The insoluble one, on the other hand, contains much more nitrogen than the original substance. In the case of the splitting up of invertase, we showed this insoluble substance to be α -invertan. The insoluble substance, produced by the breaking down of δ - and ϵ -invertan, is exactly similar in appearance and properties to α -invertan, but from previous observations we had made we were not satisfied that it was this substance, so we made the following experiment:—

A well-purified mixture of δ - and ϵ -invertan with an optical activity $[\alpha]_D = +60^\circ$ was dissolved in water. It yielded a clear solution. A large excess of dilute hydrochloric acid was added, and then sufficient alcohol to cause cloudiness. It was now allowed to stand several days, a little weak alcohol being added occasionally. A

large, flocculent precipitate slowly fell out. This was filtered off and purified by repeatedly mixing with water, then throwing down with the weakest possible alcohol, and filtering, the washings being added to the original filtrate. The mixture was by this means divided into two parts, nothing having been discarded. The portion in the alcoholic solution was totally precipitated by strong alcohol. It fell out as a colourless syrup which on examination showed an optical activity of $[\alpha]_D = +69.8^\circ$. The portion insoluble in water was dehydrated and dried in a vacuum; it showed no tendency to take on colour. On combustion with copper oxide, it yielded 5.0 per cent. N.

The analysis of α -invertan showed 8.35 per cent. of nitrogen. Either, therefore, this was a different substance, or it was a mixture of *equal parts* of α -invertan with ϵ - or ζ -invertan. From our experience of the comparatively small amount of difficulty of separating α -invertan from the soluble members of the series, we should say that this latter supposition was not tenable.

In order to obtain further evidence on this point, we mixed the powdered substance with water. It swelled up into a thick jelly. A few drops of weak alkali caused immediate solution, and we were able to take a reading in the polarimeter. The result showed a slight dextro-rotation, probably about $+20^\circ$. This is about what might be expected from the mixture above described.

The solution was then diluted, acidified, and alcohol slowly added. As soon as the precipitate showed signs of falling out, the solution was put by for a day and then filtered. The filtrate yielded with more alcohol a well-marked, syrupy precipitate completely soluble in water. The insoluble portion was dried and found to contain 6.02 per cent. N.

From these facts it may be argued that the insoluble substance was simply a mixture of α - and ζ -invertan. We are, however, of opinion that there are some members of the series between α - and β -invertan, because we think that if the soluble substance separated in the last precipitation had been there earlier we could not have failed to have discovered it. These compounds must be very unstable, and we do not at present see any means, other than that we have employed, of proving their existence. We believe that δ - and ϵ -invertan in breaking down respectively into ϵ - and ζ -invertan do not yield α -invertan, but some compound or compounds situated between α - and β -invertan. These, however, being unstable substances, are soon converted into α -invertan and another member of the series containing less nitrogen.

In order to test whether an invertan is split up by being converted into the copper compound, we took some of the same mixture of δ - and ϵ -invertan ($[\alpha]_D = +60^\circ$) which was used in the last experi-

ment, and carefully added copper sulphate dissolved in excess of ammonia and a little potassium hydroxide. The precipitate does not fall out all at once, so it was allowed to stand all night. The solution was then decanted off, acidified, and a large excess of alcohol added. There was no precipitate. The copper compound was dissolved in a little weak acid. A large excess of alcohol was added, which produced a colourless precipitate. After deducting the weight of the ash, this was found to have $[\alpha]_j = +63.0^\circ$, showing a gain of 3° . This is probably not more than can be justly attributed to the ordinary breaking down of the invertan, and it conclusively shows that the copper oxide combines with the δ - and ϵ -invertan, and may be again separated from them without altering them. We may add that the precipitate showed decided signs of containing some of the meta-variety of the invertans.

The optical activity of the three samples of ϵ -invertan, of which we have given the analysis, was A, $[\alpha]_j = +65.1^\circ$, B = $+64.8^\circ$, and C = $+65.4^\circ$. Another sample that we fractionated with great care had $[\alpha]_j = +65.6^\circ$. The mean of these four is $[\alpha]_j = +65.2^\circ$.

The remaining properties are given under the heading " γ -Invertan."

ζ -Invertan.

Preparation.— ζ -Invertan may be obtained in two ways; either by the slow breaking down of ϵ -invertan, or by the action of an alkaline solution of copper oxide on α -invertan.

As to the former method, we have already shown in the preparation of ϵ -invertan, that on attempting to purify that substance by frequent precipitations with acid alcohol, we obtained a series of fractions having an optical activity $[\alpha]_j = +70^\circ$, or more. The ϵ -invertan being a more stable substance than either γ - or δ -invertan, this transformation is slower than in their case. We have, however, made several preparations of ζ -invertan by this means. We find the breaking down to be most rapid when the invertan is in very dilute solution, and a large excess of acid is present. When sufficient alcohol is added to form a cloud in the solution, a day's rest should be given, and then a little more alcohol added, and so on for several days.

In this manner there is no difficulty in preparing ζ -invertan from ϵ - or even δ -invertan.

The preparation of ζ -invertan from α -invertan by means of the copper compound precipitated in the presence of a very large excess of alkali has already been partly described under the heading "Yeast Albuminoid derived from Invertase." The copper compound thus obtained was dissolved in weak acid, and purified in the usual

manner, and divided into two fractions, x and y , which gave the following results:—

Fraction x	$[\alpha]_j = +75.5^\circ$	$D = 3.95$.
„ y	$[\alpha]_j = +76.7^\circ$	$D = 4.00$.

The mixture of these two fractions constitutes the sample marked G in the table of analyses.

The peculiarity of ζ -invertan prepared in this manner is that it more resembles the meta-modification than the ordinary ζ -invertan, and we have not been able to convert it into the ordinary form.

This may be due to the excessive amount of alkali which it is necessary to use. The precipitate caused by alcohol falls out rather differently, and it is not a colourless syrup, but forms a tough, white skin at the bottom of the beaker. This skin is only very slowly soluble in water. From these indications it might be thought that this is not ζ -invertan, but we are strongly opposed to this view for the following reasons. With the exception of the differences noticed above, it has the same characteristics as ordinary ζ -invertan. It has the same optical activity, and gives approximately the same numbers on combustion. It yields a copper compound under similar conditions, though this substance seems to partake slightly of its peculiarities. Lastly, we have often noticed that the conversion of any invertan into the copper compound sometimes causes it to assume the meta-form after recovery, though generally speaking the invertan is not so affected. We have, however, never seen, under other circumstances, so complete a modification as occurs in the breaking down of α -invertan. On the whole we have little doubt that this substance is ζ -invertan in the meta-form.

We have also prepared ζ -invertan from the other members of the series by converting them into the copper compound, and then freeing them from copper by dissolving in hydrochloric acid and precipitating with alcohol. As we have, however, already shown that, at least, some members of the series are not altered by this process, we think that the conversion is not due to the formation of the copper compound, but to the manipulation and fractionation which the substance undergoes. As an instance of ζ -invertan prepared in this manner directly from invertase, it will be recollected that in the preparation of the A sample of α -invertan, a small precipitate was separated by means of Fehling's solution. The invertan was recovered from this precipitate, and divided into two fractions, having R, $[\alpha]_j = +75.0^\circ$ and S, $= +72.0^\circ$.

The former of these constitutes the D sample, the analysis of which is given below.

We have also prepared ζ -invertan from ϵ -invertan by the same

means, and samples E and F are from this source. These gave an optical activity of E, $[\alpha]_D = +78.5^\circ$ and F, $= 76.8^\circ$.

ζ -Invertan prepared in this manner does not, as far as we can see, differ in any way from that obtained by the slow breaking down of ϵ -invertan by means of alcohol and acid, though, judging by other experience, we believe it would, under some circumstances, partially assume the meta-form.

In order to thoroughly test the purity of ζ -invertan, we submitted it to the exhaustive process of fractionation we have already referred to in the preparation of ϵ -invertan as Crookes' method. A considerable number of fractions having $[\alpha]_D = +72^\circ - 78^\circ$ were mixed, and submitted to very careful fractionation in the manner then described. We obtained in this way, by means of between 40 and 50 precipitations, 10 fractions. These were reduced to eight by discarding the very small one at each end. The others gave the following results:—A, $[\alpha]_D = +76.6^\circ$; B, $= +75.0^\circ$; C, D, E, and F were not tested: G, $= +75.5^\circ$; and H, $= 73.0^\circ$. A and H were only very small fractions, and were discarded. The remaining six fractions were mixed, and again carefully fractionated into three parts. These form the samples, A, B, C, the analysis of which is given below. It was almost impossible to obtain a trustworthy reading of these fractions in the polarimeter, as they had taken on some colour, and a kind of black opalescence. However, we made out that the G sample had $[\alpha]_D = +73.7^\circ$, and the divisor was 3.85.

These figures leave not the slightest doubt that ζ -invertan is a homogeneous substance. They also show it to be very stable; indeed, we have only been able to alter it by boiling with sulphuric acid, and then only slowly.

Analysis.—

TABLE XXXVII.—*The Analysis of ζ -Invertan.*

Sample.	Constituents per cent. on substance free from ash.			Ash.
	C.	H.	N.	
A	44.74	6.38	1.55	0.50
B	44.45	6.13	1.50	0.40
C	44.76	6.26	1.84	0.38
D	44.98	6.49	—	0.67
E	44.73	6.75	1.50	1.45
F			1.68	
G	45.10	6.73	1.35	1.6

We have already detailed the origin of these samples. We may classify them as follows:—A, B, and C were obtained by the slow breaking down of invertase, and the intermediate products under the influence of alcohol and acid. D, E, and F were obtained from the same source by means of the copper compound. G was obtained by the same means from α -invertan, and it was in the *meta*-state. If we omit the G sample, we obtain as the mean of the others—

$$C = 44.73 \text{ p. c.}, \quad H = 6.40 \text{ p. c.}, \quad N = 1.61 \text{ p. c.},$$

and we take this as probably near the truth.

Properties.—Solutions of ζ -invertan are not liable to break down under any ordinary circumstances, though they slowly take on colour. Its optical activity is about $[\alpha]_j = +75^\circ$. We have obtained numbers as low as $[\alpha]_j = +73.6^\circ$, and, one occasion, as high as $[\alpha]_j = +78.7^\circ$, but we have little doubt that the number we have mentioned is approximately correct.

The properties given under the heading γ -invertan as common to most of the soluble members of the series also apply to ζ -invertan.

Some further properties of this compound are given under separate headings, namely:—The molecular weight, the absorption spectrum, and the action of boiling sulphuric acid.

Molecular Weight of ζ -Invertan.

The three preparations of dry ζ -invertan, which were analysed under the designation A, B, and C samples, were mixed, and an attempt was made to determine the molecular weight of the mixture by means of Raoult's freezing method. We followed the details of the process as described by Auwers (*Ber.*, **21**, 712). The powdered invertan was left for 16 hours in a current of dry air at 100° . It was then dissolved in a small quantity of water, and boiled vigorously for half-an-hour. This was done to be certain of getting rid of any alcohol that the dried substance might have retained. The solution was found to contain 6.19 grams ζ -invertan per 100 c.c., equal to sp. gr. = 1023.8 ($D = 3.85$). The zero point of the thermometer was determined by observing the freezing point of pure water. The freezing point of the invertan solution was next twice determined. In the first experiment the depression was 0.07° , and in the second 0.06° , the mean being 0.065° .

This number calculated in the manner given by Auwers is equal to a molecular weight of 1881 ($19 \div \frac{96.19 \times 0.065}{6.19} = 1881$).

If we take into consideration the fact that there was present in the solution about 0.4 per cent. of ash, consisting principally of the

acid phosphate of potash with a molecular weight of 136, we do not affect any material alteration in the number, merely increasing it to 1888. This is very much smaller than we consider probable for the compound, as we shall show under the heading "Constitution of the Invertan Series."

Absorption Spectrum of ζ -Invertan.

We have stated on several occasions that there was great difficulty with invertan solutions in obtaining readings in the polarimeter, even when the solutions were, comparatively, only slightly coloured, and clear. There is, too, a certain black look about such solutions which caused us to think that probably some well-marked absorption bands existed. We, therefore, examined the solution used in the last experiment but we did not observe any traces of a band, although a considerable portion of the violet end of the spectrum was cut off.

In view of Professor Hartley's researches, a more extended investigation of the absorption spectrum would probably yield valuable results.

Action of Boiling Sulphuric Acid on Invertan.

This reaction requires more investigation than we have been able to give it so far.

We boiled some mixed solutions of ϵ - and ζ -invertans of sp. gr. 1068 with 5 per cent. of strong sulphuric acid. The copper reaction slowly decreased, and on those occasions when the boiling was continued sufficiently long it disappeared altogether.

On one occasion, when about 60 grams of invertan was used, we stopped the boiling before this reaction had disappeared— $1\frac{1}{2}$ hours from the start. The solution was very carefully and slowly neutralised with baryta-water and filtered. Alcohol was added to the filtrate, and a precipitate was obtained having all the characteristics of the invertan series, including the copper reaction; it was purified in the usual manner. It weighed 7 grams, and was found to have $[\alpha]_D^{20} = +75.7^\circ$, $D = 3.95$, and $N = 1.05$ per cent. We shall describe this compound under the heading η -Invertan. The mother liquor left after the separation of this substance was evaporated in a vacuum to a syrup. The addition of 0.82 alcohol produced a further precipitate which, however, was considerably coloured. It was purified as before. It was much more soluble in alcohol than the last precipitate, and was too coloured to be read in the polarimeter. The dry substance contained 3.34 per cent. N.

The remaining solution was several times evaporated to a syrup,

and taken up with dry methyl alcohol; each time there was a flocculent precipitate, but the greatest care has not enabled us to obtain a crystallisation from the methyl alcohol solution. The substance in solution constitutes a large percentage of the original invertan, and it is probably a new carbohydrate. It has a high cupric oxide reducing power. On this occasion it gave $K = 84.9$ and $[\alpha]_D = +10.7^\circ$. A syrup, prepared at another time in a similar manner, gave numbers, $K = 108$ and $[\alpha]_D = +21^\circ$ and $+16^\circ$. Some ζ -invertan which we boiled with sulphuric acid until it ceased to give the copper compound yielded two substances, one soluble and the other insoluble in alcohol. The insoluble portion had an optical activity of $[\alpha]_D = +67^\circ$, and no action whatever on Fehling's solution. The portion soluble in alcohol had $[\alpha]_D = +22.2^\circ$ and $K = 120.6$; the reduction of the cupric oxide was very slow. In this case, as in every other, we completely failed to obtain any crystallisation from the reducing solution, though we took considerable pains to do so.

We are proceeding with a further study of the products of this reaction.

η -Invertan.

Preparation.—We believe this to be the first product of the action of sulphuric acid on ζ -invertan (see special heading). We have only made preliminary experiments in this direction, and have not had enough of this substance to submit it to the necessary exhaustive fractionation.

Analysis.—We have not thought it worth while to estimate more than the nitrogen, which we found in the sample under examination to be 1.05 per cent.

Properties.—Much the same as those of the rest of the series. It is readily soluble in water, and is thrown out of solution by alcohol, even when the solution is neutral. Our preparation yielded with Millon's reagent a coloration so slight that we took it to be due to impurities. It yields a copper compound, like the rest of the series.

Optical Activity.—Our sample gave $[\alpha]_D = +75.7^\circ$, $D, 3.95$.

It would seem, at first sight, as if we had hardly sufficient grounds for asserting the existence of η -invertan; it might be looked upon as a mixture of ζ -invertan with some substance containing less nitrogen. Opposed to this is the fact that the substance from which we separated it contained much more nitrogen than ζ -invertan, and, more important still, such a mixture would give a vivid coloration with Millon's reagent, which was not the case. We shall show later that the existence of η -invertan might have been, and, indeed, was predicted from the constitution of the rest of the series, and that probably it is the lowest member.

We are proceeding with a further study of this substance.

Invertan Copper Compounds.

Preparation.—If an alkaline solution of copper oxide is added to a solution of any member of the invertan series, together with a sufficient excess of potassium hydroxide, a very voluminous, blue precipitate is formed. The precipitate adheres strongly together, forming a mucilaginous mass filling the whole liquid. This forms a very delicate reaction for the members of this series; but if α -invertan only is present, a very large excess of potassium hydroxide is required to produce the precipitate.

We have found it practically impossible to obtain this copper compound in a pure state, and we believe that several different ones exist. The voluminous precipitate carries down with it almost everything that is in the solution: and it is hopeless to try to wash it.

The freshly-precipitated invertan copper compound is, with some difficulty, soluble in water. The presence of large quantities of ammonia does not seem to decrease its solubility: but it is much less soluble in water containing potash or soda, and quite insoluble in alcohol. Hydrochloric acid immediately decomposes it, yielding copper chloride and invertan. Taking these facts into consideration, we have attempted to prepare the pure compound by the following method:—

To a weak solution of ζ -invertan a little weak Fehling's solution was added slowly, the precipitate allowed to settle, and the supernatant liquid decanted off. A little alcohol was added to the precipitate, which was then collected. It was digested in lukewarm water, in which it slowly dissolved, and was then thrown out by means of alcohol. This purification was repeated three times. A portion of the precipitate was then dried, weighed, and ignited; the ash was weighed, then boiled with water, and reweighed. The difference in weight showed 3.1 per cent. of soluble ash. For reasons given below this was looked upon as an impurity, and was deducted from the original weight of the copper compound. The remaining insoluble ash was dissolved, and the copper oxide estimated. It was thus proved that the whole of the insoluble ash was CuO ; it amounted to 14 per cent. on the original copper compound after deducting the soluble ash. The percentages of CuO given below were all obtained and calculated in this manner.

The invertan copper compound was again dissolved in water and precipitated by alcohol from a very weak solution. It contained 15.0 per cent. CuO and 0.6 per cent. soluble ash. This is the smallest amount of soluble ash we have ever obtained: but we think it is small enough to render it most probable that the pure

compound would contain none. The copper compound obtained in this manner contains 14—15 per cent. CuO. If the precipitate is filtered out and repeatedly redissolved in water, and reprecipitated with potassium hydroxide, it always contains a proportion of soluble ash varying from 3 to 12·4 per cent., and the CuO is also higher, 19 to 23 per cent. A precipitate obtained in this manner was dried and powdered. It contained 12·4 per cent. of soluble ash. The fine powder was digested for a long time in water, in which it is very slowly soluble. The decanted solution was always alkaline, but it was not found possible to get rid of all the soluble ash. In the end, the residue contained CuO 18·6 per cent., soluble ash 4·4 per cent.

On another occasion, an excess of sulphate of copper was added to a solution of ζ -invertan, and the compound was precipitated from the mixture by potassium hydroxide in the presence of ammonia. Ammonia has the effect of consolidating the precipitate, so that it contains less of the mother liquor. The washed precipitate was redissolved by nearly neutralising the potassium hydroxide, and then reprecipitated by fresh potassium hydroxide and ammonia. Each time the discarded solution contained less of the excess of copper, until, on the fifth purification, it was quite colourless. The final precipitate contained 21·0 per cent. CuO and 2·3 per cent. of soluble ash.

In these two last experiments the precipitates were obtained in the presence of a large excess of potassium hydroxide, and the results were, CuO = 18·6 and 21·0 per cent. We performed several other similar experiments with approximately similar results.

Some freshly-prepared copper compound containing 23 per cent. CuO and 3 per cent. soluble ash, was boiled for some time in water. The greater portion of it dissolved; the insoluble portion was found to contain CuO 32·0 per cent., and soluble ash 1·7 per cent.

It will be noticed that we have only attempted to prepare the copper compound of ζ invertan. We have already shown that at least some other members of the series yield copper compounds. On the other hand, we have proved that α -invertan is broken down on entering into the combination.

We have, however, not attempted to purify the compound of any other member of the series than ζ -invertan, because we judge that this is probably the most stable, and that the others would be even more difficult to work with satisfactorily than this one.

Constitution.—It seems probable from the above that we have obtained three distinct compounds of ζ -invertan with copper oxide, containing approximately the following amounts of CuO:—A = 14—15 per cent., B = 18·6—21·0 per cent., and C = 32·0 per cent.

Now, if the molecule of ζ -invertan contains 1 atom of nitrogen, and this is equal to about 1·6 per cent., then ζ -invertan has a mole-

cular weight of about 875 ($1400 \div 1.6$). This, combined with CuO, would give the following percentages:—

Summary of the Invertan Series.

Before proceeding further with a consideration of the results just described, we may here conveniently summarise them.

From the purified invertase solution we have isolated a number of substances, some of them very much alike in general characteristics, but differing considerably in composition and optical activity. They may be tabulated as follows:—

TABLE XXXVIII.—*Composition of Yeast Albuminoid and of the Invertan Series.*

Substance.	Constituents per cent. on substance free from ash.			[α] _D approximately.
	C.	H.	N.	
Yeast albuminoid	54.06	7.35	14.53	-13° (?)
α -Invertan	48.03*	6.65*	8.35	-15° (?)
β - ,,	46.41	6.63	3.69	+80° (?)
γ - ,,	45.62*	6.55*	3.15*	+45
δ - ,,	46.50*	6.82*	2.43	+54
ϵ - ,,	44.45	6.36	2.07	+65
ζ - ,,	44.73	6.40	1.61	+75
η - ,,	—	—	1.05*	+75* (?)

The figures marked with an asterisk were obtained from the analysis of one sample only. On examining these numbers, it is to be observed to begin with that there is a great difference between the percentage of carbon in the albuminoid and in the α -invertan; between this latter and the next member of the series the drop is smaller, and between the remaining members of the series it is irregular, due, no doubt, to the fact that we were not analysing absolutely pure substances, but still, on the whole, the decrease of carbon is apparent even here.

The same observations hold good for the percentage of hydrogen.

In ζ -invertan the carbon and hydrogen percentages closely approach a carbohydrate.

On referring to the nitrogen, we find again this diminishes from 14.53 per cent. in yeast albuminoid to 1.05 per cent. in η -invertan. Between the albuminoid and α -invertan there is a large drop in nitrogen; between α - and β -invertan the diminution is proportionately large; but from this downwards the decrease is gradual and steady. The decrease in each of the three constituents evidently runs in parallel lines.

From these facts the idea suggests itself that the members of the series are mixtures, in various proportions, of the albuminoid with η -invertan, or indeed with a carbohydrate, but no other fact relating to these substances gives any support to this idea. For instance, yeast albuminoid after being dehydrated with alcohol is not again soluble in water, whilst all the lower members of the invertan series, including η -invertan, dissolve readily to perfectly clear solutions. Then, again, α -invertan is a substance of considerable stability. It is only by treatment with strong potash and an alkaline copper solution that it can be broken up into the albuminoid and ζ -invertan; after this treatment it is perfectly easy to separate the two products. If these substances were mixtures, the very first precipitation with alcohol of properly graduated strength would separate the albuminoid completely; we need hardly say that such is not the case.

Let us now turn to the evidence of the optical activity. The substance with which we started, invertase or β -invertan, has a specific rotatory power of about $[\alpha]_j = +80^\circ$. This substance, when its inverting activity is destroyed on attempting to fractionate it by treatment with alcohol, yields two bodies, the optical activity of one of which is about $[\alpha]_j = -15^\circ$, and of the other $[\alpha]_j = +45^\circ$, or more frequently $[\alpha]_j = +54^\circ$. No calculation is necessary to show that no mixture of these two substances could have an activity $[\alpha]_j = +80^\circ$. In the same way, if we use the percentage of nitrogen as a factor, it is clear that the other members of the series are not mixtures; for instance, if γ -invertan (3.15 per cent. N) was a mixture of α - and ζ -invertan, its optical activity would be $[\alpha]_j = +55^\circ$ instead of the observed number $[\alpha]_j = +45^\circ$. The difference between the optical activity calculated for mixtures and that observed for the lower members of the series is not so great as for those mentioned, but it is sufficiently great to be a link in the chain of evidence against their being mixtures; that they are of high stability we have shown, that their optical activities do not agree with those calculated for mixtures completes the evidence.

If we now refer back to Table XXXVIII we shall see that, with the exception of δ -invertan, we have a series of great regularity, and we

feel ourselves justified in saying that between β - and ζ -invertan no member has been overlooked.

When we compare the remaining properties of the members of the series, we find that the same regularity pervades them. The one exception to this rule is the power of inverting cane-sugar; this is the property of β -invertan only.

The two stable members of the series are those at the extreme ends. The intermediate members are, as we have repeatedly shown, very liable to break down, and whenever this happens, the immediate products of the dissociation are either α -invertan or a substance nearly allied to it, and the member next below the one so dissociated. If the breaking down force acts sufficiently long, the ultimate products of the reaction are α - and ζ -invertan. Omitting α -invertan, we find that each succeeding member possesses more stability than the one above it.

All the members of the series are non-crystallisable, undialysable, and colourless. α -Invertan is insoluble in water; the others dissolve readily, forming clear but viscous solutions which do not cloud on boiling. Their divisor is about 3.85. Alkaline or neutral solutions are transformed by the gradual addition of alcohol into a milk-like fluid which passes unaltered through a filter and will not clear. This applies also to alkaline solutions of α -invertan. Acids added to the milk cause rapid precipitation. The α -invertan falls out as a large, flocculent precipitate; β - and γ -invertan are more inclined to be syrupy, whilst δ -, ϵ -, ζ -, and η -invertan form heavy, transparent syrups. Any of these precipitates dried in thin plates becomes a glassy mass, and, with the exception of β -invertan, the dried preparations may be kept unaltered for an indefinite period. All the members of the series yield well-defined copper compounds, but α -invertan requires to be broken up with a very large excess of potassium hydroxide.

For the other properties of the members of the series, we refer to the separate headings.

We may note here that although these compounds possess many properties in common with some of the gum acids, they, however, are in no way related to those substances, inasmuch as the ash of their *neutral solutions* is never alkaline.

PART V.—THEORETICAL.

Theory of Inversion by Invertase.

When we look back over the whole of the facts we have adduced with regard to the action of invertase on cane-sugar, we are forced to

admit that there is not much which throws light on the actual chemistry of the reaction. We have, however, shown that hydrolysis by invertase is a simple chemical reaction, differing in no important particular from the ordinary chemical reactions.

From the time curve of the reaction it is easy to make the deduction that the invertase itself is not injured or destroyed by the act of inversion.

We have shown that the time curve is the one that expresses a chemical change "of which no condition varies excepting the diminution of the changing substance." It therefore follows that the activity of the invertase does not vary during the reaction.

The same argument shows that the products of inversion have no influence on the pace of the reaction.

A necessary corollary of the fact that invertase is not injured by inversion is that there is no limit to its inverting power. This we have practically shown to be the case.

In these respects the action of invertase on cane-sugar agrees very closely with that of boiling acids. We have little doubt that this reaction would follow the same curve as the one under consideration, though we have seen no figures bearing on the point.

In Table XXXIV we have shown that invertase when in the presence of cane-sugar will stand without injury a temperature fully 25° higher than in its absence. This is a very striking fact, and, as far as we can see, there is only one explanation of it, namely, the invertase enters into combination with the sugar. It is very difficult to see how it can otherwise be explained. Further, this combination between the invertase and the sugar must last until the compound molecule comes in contact with another molecule of cane-sugar, otherwise towards the end of the reaction, when cane-sugar is getting scarce, the invertase would be destroyed by the heat in the intervals between the combinations. It might be thought that our experiment on the molecular weight of cane-sugar undergoing inversion would throw some light on this point, but it does not throw much, because we were obliged by the conditions of experiment, to make allowance for a constant error in the observations, and this constant error would include the error due to the combination between the invertase and sugar if it existed. It may be held, however, to show that invertase does not combine with many molecules of sugar at the same time. We are not able to give further proof of the combination, which we believe to exist, between invertase and sugar, but it is obvious if our idea is right that the sugar must be invert-sugar, not sucrose, otherwise if much invertase was used the optical activity of the solution at the end of the reaction would not show complete inversion.

We next pass to the consideration of the phenomena which we have called the residual decrease, particulars of which are given in Table VIII (p. 853).

We will briefly restate the facts. If a solution undergoing inversion is stopped with a moderate excess of a caustic alkali, the optical activity immediately drops several degrees, and then remains constant. If only a small amount of alkali is added, the drop is not sudden but gradual, taking an hour or so to accomplish, finally stopping at the same point that it would have done if much alkali had been used, and the fall in optical activity instantaneous. In one of the experiments given, this sudden drop was equal to the inversion of 23.3 per cent. of the total sugar present. We have there also shown, as is indeed obvious, that this residual decrease is not due to the action of the alkali on the sucrose. At first sight it would seem as if we had here good evidence of a gigantic combination between invertase and cane-sugar, and for some time we thought that this was the case. However, our experiment on "The Molecular Weight of Cane-Sugar undergoing Inversion" effectually disposes of this idea, because the figures obtained from observations of the freezing-point could not fail to yield well-marked indications of a combination on such a large scale, and to vary in proportion to the amount of the residual decrease. It was evident that an explanation of this very striking fact must be sought elsewhere, and it was suggested to us by Mr. A. L. Stern that the phenomenon might be due to bi-rotation of the dextrose. We have no doubt this is the case. We made several experiments on the effect of adding potassium hydroxide to solutions of bi-rotatory dextrose, and found the action similar to that which it has on solutions undergoing inversion. On one occasion, for instance, the optical activity of the dextrose dropped suddenly from $[\alpha]_D = +130^\circ$ to $[\alpha]_D = +57$. It was shown by Brown and Morris (Trans., 1888, 53, 614) that the freezing-point of solutions of dextrose is not affected by bi-rotation. This agrees with the fact observed by us, that in a solution of sucrose undergoing inversion, the molecular weight, before stopping, calculated from the freezing-point, is identical with the molecular weight after stopping, calculated from the optical activity. We thus see that the assumption that the nascent dextrose is bi-rotatory, explains in every particular the facts which we have observed, and which are so peculiar that they do not seem capable of any other explanation. We, therefore, think ourselves amply justified in stating that the nascent dextrose is in the bi-rotatory state; we have not been able to find any previous record of this fact, nor of the one that the bi-rotation of dextrose is instantly destroyed by potash.

About the extraordinary influence of minute traces of acidity on

the reaction we have nothing definite to say. The most favourable amount of acidity is not in proportion either to the amount of a given invertase preparation present, or to the differences of temperature, yet it varies with both. Under these circumstances, it is evident that it cannot depend for its action on a chemical combination with some constituent of the invertase preparation. It would, therefore, seem as if the influence depended upon its action upon the physical state of the solution, and yet it is quite inconceivable that a difference of two parts of SO_3 per million of the solution should so alter the physical state of the solution as to change the rate of the reaction from $\pm 0 = 920'$ to $\pm 0 = 85'$ (Table XVI, p. 858). We must, therefore, leave this problem unsolved.

In conclusion we are able to state, from a consideration of the above results, that the invertase simply acts the part of a carrier of water to the sucrose, and that in doing so a loose combination is formed between the invertase and the inverted sucrose, and that the invertase is again freed from this combination in its former active state.

Constitution of the Invertan Series.

If we examine Table XXXVIII (p. 916) we shall see that, roughly speaking, the numbers obtained from analyses of the members of the invertan series are such as would be yielded by mixtures of α - and ζ -invertan. We have shown that α -invertan itself breaks down into yeast albumin and ζ -invertan.

ζ -Invertan probably splits up into η -invertan and a substance containing more nitrogen, but as we have not fully examined either of these bodies, we will for the present take ζ -invertan as the base of our calculations.

The figures we have obtained on analysis of ζ -invertan are such as may be stated in terms of yeast albumin and a carbohydrate, for if, in the figures given for ζ -invertan, we make allowance for the amount of yeast albuminoid indicated by the nitrogen present, we obtain for the remainder the following composition:—

$$\text{C} = 43.56 \text{ per cent.}, \quad \text{H} = 6.28 \text{ per cent.}$$

The theoretical hydrogen for a carbohydrate having this percentage of carbon is, $\text{H} = 6.27$ per cent. It follows from this that the composition of the ζ -invertan may be expressed in terms of yeast albuminoid and a carbohydrate. In order to test whether this applies to the other members of the series, and, at the same time, to find out the composition of the carbohydrate, we have allowed for the yeast albuminoid in each member of the series, and calculated the per-

centage composition of the remainder. We took the nitrogen as the indication of the amount of albuminoid present, and for its composition we used the numbers already quoted, viz.:—C = 54·06 per cent., H = 7·35 per cent., N = 14·53 per cent.

In Table XXXIX we give the results of these calculations. The last column contains the relative amounts of the carbohydrate and albuminoid present.

TABLE XXXIX.—*Composition of the Carbohydrate after allowing for the Albuminoid.*

Member of series.	Constituents of the carbohydrate.		Parts of the carbohydrate to 1 of yeast albuminoid.
	C.	H.	
α -Invertan	40·00*	5·70*	0·74
β - "	43·81	6·38	2·94
γ - "	42·95*	6·33*	3·61
δ - "	44·98*	6·71*	5·00
ϵ - "	42·85	6·20	6·02
ζ - "	43·56	6·28	8·08
η - "	—	—	12·84

In comparing these figures it must be remembered that all the errors both of preparation and analysis are thrown on to these results, and very close agreement cannot therefore be expected.

We calculate the mean of these figures by first averaging those marked with an asterisk, and which were obtained from only one sample, we thus get C = 42·64 and H = 6·25 per cent. We again average these with the other members, and obtain C = 43·22 and H = 6·28 per cent. The theoretical hydrogen for a carbohydrate having this amount of carbon is H = 6·31 per cent., a number practically identical with that obtained.

These numbers would seem to prove that the composition of this hypothetical carbohydrate comes between an "*in*" and an "*on*" body.

In carbohydrate C = 44·44 per cent., H = 6·17 per cent.
 Invertan carbohydrate C = 43·22 " H = 6·28 "
On carbohydrate C = 42·11 " H = 6·43 "

Although we have thus shown that the figures we have obtained for members of this series are such as might approximately be obtained from certain mixtures of yeast albuminoid with a carbo-

hydrate of the above composition, yet at present we do not believe that the substances in question do directly combine to form the series. Our reasons are as follows:—If we turn back to our description of the individual members of the series, we shall find that η -invertan alone of the series gives little or no indication of albumin when tested by Millon's reagent. Of course we cannot say for certain that our sample was η -invertan, but we shall show directly that such a substance probably exists, and such being the case, we think it must be admitted, on referring to our description, that the substance we obtained was most probably η -invertan. Now from this behaviour with Millon's reagent we are led to believe that if albumin is present in η -invertan, it is in much closer combination than in ζ -invertan and the other members, and therefore we prefer to take η -invertan and not the hypothetical carbohydrate as the base of this series of compounds.

We regard the members of the invertan series as compounds of yeast albuminoid with η -invertan. Taking this hypothesis as a standpoint, it will be possible to form some idea of the manner in which the series is built up, but it will first be necessary to attribute an approximate composition to η -invertan. This is not difficult to do if we place it immediately below ζ -invertan in the regular series. In this way we attribute to it approximately the composition AS_{18} , where A represents 1 part by weight of yeast albuminoid, and S, 1 part by weight of the carbohydrate.

We have found some difficulty in attempting to convey our ideas of the proximate constitution of the invertan series. This is mainly due to the fact that we have no data to enable us to attribute a molecular weight to the albuminoid; hence, we have been obliged to employ parts by weight to represent the constitution instead of the usual molecular formulæ.

We next turn to α -invertan. The composition of this substance is approximately A_4S_3 , that is to say, 7 parts by weight of α -invertan contains 4 parts of yeast albuminoid and 3 of the hypothetical carbohydrate.

The other members are formed by the union of these two substances according to the general formula $\eta + \alpha_n$, where η represents three times η -invertan ($3AS_{18}$), and α , α -invertan (A_4S_3). It will be noticed that the symbol η represents 57 parts by weight, whilst α represents 7.

A homologous series is thus formed, each member differing from the one below it by α .

According to this idea, β -invertan would be $\eta\alpha$, that is to say, its composition is 57 parts by weight of η -invertan + 35 (7×5) parts by weight of α -invertan, making a total of 92 parts of β -invertan,

of which 23 are albuminoid and 69 the hypothetical carbohydrate. γ -Invertan, in like manner, is $\eta\alpha_1$, so that 85 parts by weight would contain 57 parts of η -invertan and 28 (7×4) of α -invertan, which is equivalent to 19 parts of albuminoid and 66 of carbohydrate.

According to this theory, the constitution and composition of the members of the invertan series are as follows:—

TABLE XL.—*Hypothetical Composition and Constitution of the Invertan Series.*

Name of substance.	Constitution.	Composition, parts by weight.	Ultimate composition.		
			C.	H.	N.
α -Invertan	α	A_4S_3	49·41	6·90	8·30
β - "	$\eta\alpha_5$	$A_{23}S_{69}$	45·93	6·57	3·63
γ - "	$\eta\alpha_4$	$A_{19}S_{66}$	45·64	6·54	3·25
δ - "	$\eta\alpha_3$	$A_{15}S_{63}$	45·30	6·51	2·79
ϵ - "	$\eta\alpha_2$	$A_{11}S_{60}$	44·90	6·47	2·25
ζ - "	$\eta\alpha_1$	A_7S_{57}	44·40	6·42	1·59
η - "	η	A_3S_{54}	43·78	6·36	0·76

A represents 1 part by weight of albumin and S, 1 part by weight of the carbohydrate. We have made the calculations on the following percentage composition:—

$$\begin{array}{ll} A \dots\dots & C = 54\cdot06 \text{ p. c., } H = 7\cdot35 \text{ p. c., } N = 14\cdot53 \text{ p. c.} \\ S \dots\dots & C = 43\cdot22 \text{ ,, } H = 6\cdot28 \text{ ,,} \end{array}$$

The carbon and hydrogen determinations (*v.* Table XXXVIII) agree as well with these theoretical numbers as could be expected when dealing with this class of bodies, and when we look at the numbers obtained for the nitrogen and compare them with the theoretical ones, we find the agreement very close indeed.

Now let us see what happens when active invertase breaks down during purification. The first reaction is expressed by the formula

$$\eta\alpha_5 = \eta\alpha_4 + \alpha$$

or more usually

$$\eta\alpha_5 = \eta\alpha_3 + 2\alpha.$$

The latter formula represents the splitting up of invertase into α - and δ -invertan, and bearing in mind the relative parts by weight represented by η and α , we see that 92 parts of β -invertan have split up into 78 parts of δ -invertan and 14 parts of α -invertan.

The δ -invertan, again, is destroyed by the action of acid and alcohol, breaking off of 7 parts by weight of α -invertan at a time, and being degraded successively to ϵ -, ζ -, and, perhaps, if the conditions are favourable, to η -invertan.

The α -invertan which is formed may be divided by means of the copper compound into ζ -invertan and albumin, $6A_4S = \eta + 23A$. This reaction involves a more complete splitting up into component parts than any of the others, and we have shown that it is only effected in the presence of very strong alkali.

It will be seen from Table XL that the existence of η -invertan, the last of the series, is rendered probable by the numbers obtained on analysis of the remainder of the series, and this, combined with the single impure sample we have obtained of this substance, leaves little doubt in our minds that we shall be able to prepare it in larger quantities.

We will now compare the numbers obtained for the molecular weight of ζ -invertan with the probable molecular weight if our hypothesis is correct.

It will be remembered that when speaking of the copper compound of ζ -invertan we calculated roughly that the molecular weight of the latter substance would be 875 or some multiple of that number. If we refer to Table XL, we shall see that according to our hypothesis ζ -invertan must contain at the very least 2 molecules of the albuminoid, whilst it probably contains more. We have already shown (heading "Yeast Albuminoid derived from Invertase") that yeast albuminoid may possibly have a molecular weight as low as 199, so that in ζ -invertan the molecular weight of the albuminoid portion must, at the very least, be 398. In Table XXXIX we have further shown that ζ -invertan contains 8.08 times as much carbohydrate as it does albuminoid. This gives us a total molecular weight of 3614 ($(398 \times 8.08) + 398$). This number, then, 3614, is the lowest possible molecular weight of ζ -invertan if our theory of the constitution of the invertan series is even approximately correct, unless, indeed, we take ζ -invertan instead of η - as the base of the series, under which circumstances the above figures, 3614, might be halved, but in all probability the molecular weight of ζ -invertan is very much higher than this figure. It, however, agrees fairly well with the quadruple of the number given above ($875 \times 4 = 3500$). With regard to the number obtained from the estimation of the molecular weight by Raoult's method, which it will be remembered was 1888, this again is too low, though it approximates closely to one half of the least possible number ($3614 \div 2 = 1807$). Under these circumstances we must say that although the evidence obtained from Raoult's freezing method is opposed to our hypothesis of the constitution of

the invertan series, we do not attribute much importance to the fact.

In conclusion, we wish to state that although we have been at some pains to point out in what simple manner a hypothetical series of combinations could be formed so as to satisfy all the requirements of the ascertained facts, we do not for one moment think that the series really is built up in exactly the way we have indicated. At the same time we fully believe that the invertan series will eventually be found to be formed in a manner in many points closely resembling our hypothesis, and we hold that for the present it will be convenient to look upon this as a good working theory. We consider we have advanced the knowledge of the subject a stage, and arrived at a position at which a record should be made; this we have done. We hope that a further study of the bodies we have only imperfectly described, either by ourselves or other workers, will lead to results as interesting and important as are foreshadowed in this paper.

PART VI.—SUMMARY.

The following is a short summary of the principal results of our work, arranged as much as possible in the same order as has been observed in the body of the paper:—

1. The rate of inversion of cane-sugar by means of invertase may always be expressed by a definite time-curve; this curve is practically that given by Harcourt as being the one expressing a chemical change "of which no condition varies, excepting the diminution of the changing substance." Whatever the conditions may be under which inversion is taking place, as long as these conditions remain unchanged, this curve is adhered to. There are, however, some slight, but apparently constant, deviations from the theoretical curve.

2. When the degree of acidity is that most favourable for the action of invertase, the rapidity of the action is in proportion to the amount of invertase present.

3. The most favourable concentration of the sugar solution at a temperature of 54° is about 20 per cent. Below that, there is a rapid decline in the speed of inversion. Greater concentrations are only slightly less favourable until about 40 grams per 100 c.c. is reached. In saturated solutions inversion only proceeds with extreme slowness.

4. The speed of inversion increases rapidly with the temperature until 55 — 60° is reached. At 65° , the invertase is slowly destroyed,

and at 75°, it is immediately destroyed. At the lower temperatures, the speed of the action increases with rise of temperature in accordance with Harcourt's law, the rate being about doubled for 10° rise, but above 30° the increase is not nearly as rapid. Elevated temperatures have no permanent effect on the activity of invertase, so long as they are not sufficiently high to destroy it.

5. The caustic alkalis, even in very small proportions, are instantly and irretrievably destructive of invertase.

6. Minute quantities of sulphuric acid are exceedingly favourable to the action, but a slight increase of acidity beyond the most favourable point is very detrimental. The most favourable amount of acid increases to some extent with the proportion of invertase, and decreases with rise of temperature, but we have not been able to discover on what it depends. We find that in studying the action of invertase, it is of the utmost importance that the most favourable amount of acid should be employed, otherwise correct results cannot be obtained. At a temperature of 60°, the action is almost stopped, unless exactly the right amount of acid is used, whilst if this factor is properly adjusted, inversion proceeds at (probably) the maximum speed.

7. The influence of alcohol varies in direct proportion with the amount present. 5 per cent. of alcohol decreases the speed of the action by about one half.

8. The dextrose formed by the action of invertase is initially in the birotary state, and, therefore, the optical activity of a solution undergoing inversion is no guide to the amount of inversion that has taken place.

9. If a caustic alkali be added to a solution undergoing inversion, and the optical activity be allowed sufficient time to become constant, it is a true indicator of the amount of inversion that had taken place at the moment of adding the alkali.

10. A sample of invertase which had induced inversion of 100,000 times its own weight of cane-sugar was still active; and we have shown that invertase itself is not injured or destroyed by its action on cane-sugar. Under these circumstances there is evidently no limit to the amount of sugar which can be hydrolysed by a given amount of invertase.

11. The inversion of cane-sugar by means of invertase is a simple chemical change, differing in no important way from those which inorganic substances undergo.

12. The products of inversion have no influence on the rate of the action.

13. A solution of invertase will withstand a temperature 25° higher in the presence of cane-sugar than in its absence. From this fact we

are of opinion that when invertase hydrolyses cane-sugar, combination takes place between the two substances, and the invertase remains in combination with the invert-sugar. The combination breaks up in the presence of molecules of cane-sugar.

14. A means of estimating the activity of a material containing invertase has been devised; the result is recorded by means of the time factor, $\pm 0 = x$ min. In this equation, $\pm 0 =$ a certain definite amount of work, and $x =$ the time necessary to perform it. The expression means that the given inverting material takes x minutes to invert a standard amount of cane-sugar under standard conditions. The number x varies in inverse proportion to the actual amount of invertase contained in the material or materials under examination.

15. If sound brewer's yeast be pressed, and then kept at the ordinary temperature for a month or two, it does not undergo putrefaction, but changes into a heavy, yellow liquid. The product possesses no power of fermentation, but an apparent increase takes place in the inverting power.

16. From such liquefied yeast, it is easy to filter off a clear solution of high hydrolytic power; and we have shown that all the invertase of the yeast is in this solution. We have named it "yeast liquor," and it has a specific gravity of about 1080. It will remain for a long time unaltered, excepting that the colour darkens. If exposed to the air it may slowly become covered with mould.

17. If spirit be added to yeast liquor until the mixture contains 47 per cent. of alcohol, the whole of the invertase separates with only a slight loss of power. This precipitated invertase may be washed with spirit of the same strength and then the residue either dehydrated with strong alcohol and dried *in vacuo*, or else it may be extracted by means of 10 to 20 per cent. alcohol and then filtered. The filtrate contains the invertase. On one occasion the extent of the loss involved by this process was determined and it was found that all the invertase of the yeast liquor was present in the filtrate except 12.3 per cent.

18. We have not been successful in further purifying invertase preparations carefully made in this manner, as the slightest attempt at purification destroys the invertase. We have succeeded in preparing invertase almost free from ash.

19. The inverting power of pressed English yeast varies from $\pm 0 = 1000'$ to $\pm 0 = \pm 3000'$; this is equivalent to about $\frac{1}{3}$ of these numbers on the dry solid matter of the yeast.

20. The inverting power of the most active invertase preparation made was $\pm 0 = 25.1$ on the dry solid matter. We believe that pure invertase would approximately have $\pm 0 = 22.5'$.

21. The dry solid matter of yeast contains from 2 to 6 per cent. of invertase. 5.8 per cent. of invertase was separated from one sample of yeast.

22. During the preparation of invertase from yeast liquor an albuminoid is obtained, which is not redissolved by water. This is termed yeast albuminoid, and it possesses all the characteristics of an albuminoid.

23. Invertase when it approaches a pure state is a very unstable substance. The products of its decomposition have been carefully examined and are found to constitute a new series of substances belonging to the *invertan series*.

24. The invertan series is a homologous series of substances which on analysis yield numbers which may be expressed in terms of an albuminoid and a carbohydrate. Seven members of the series are described.

25. We consider that invertase itself is a member of the invertan series, and call it β -invertan. If the products of its decomposition are examined, it is usually found that they consist of α - and δ -invertan. The former contains more, and the latter less, nitrogen than invertase.

26. α -Invertan is insoluble in water, and in all its other properties seems to resemble yeast albuminoid. It contains 8.35 per cent. N. It is a very stable substance.

27. β -Invertan or invertase is soluble in water, and is the only member of the series which has the power of inverting cane-sugar. It contains about 3.69 per cent. N, and its optical activity is $[\alpha]_j = +80^\circ$ (?).

28. γ - and δ -Invertan are products of the splitting up of invertase. One or other of these two substances seems invariably to be formed. They both contain less nitrogen than invertase, and are always accompanied by α -invertan. Both are readily soluble in water. They contain respectively 3.15 and 2.43 per cent. N, and their optical activity is $[\alpha]_j = +45^\circ$ and $+54^\circ$.

29. ϵ -Invertan is formed from the slow breaking down of δ -invertan. At the same time an insoluble substance, resembling α -invertan, is formed. This probably consists of an unstable member of the series coming between α - and β -invertan. ϵ -Invertan is soluble in water, and has $[\alpha]_j = +65^\circ$. It contains 2.07 per cent. N.

30. ζ -Invertan results from the splitting up of ϵ -invertan in the same way that the latter is formed from δ -invertan. Its optical activity is $[\alpha]_j = +75^\circ$, and it yields 1.61 per cent. N.

31. η -Invertan is formed by the action of boiling sulphuric acid on ζ -invertan. It contains less nitrogen than the latter substance, but the substance has not yet been sufficiently investigated to give reliable figures.

32. The further products of the action of sulphuric acid on ζ -invertan are two soluble substances, one containing a considerable amount of nitrogen, and the other with little or no nitrogen, a high cupric reducing power and a low (dextrorotatory) optical activity.

33. The properties of all the members of the series, except α -invertan, are very similar. They are all soluble in water, forming clear solutions, which do not cloud on boiling. They are all readily thrown out of solution by alcohol, provided a little acid is present; the precipitates so formed are transparent, colourless syrups, soluble in water in all proportions. The solutions are all dextrorotatory.

34. All the members of the invertan series, except η -invertan, yield a pink coloration on boiling with Millon's reagent.

35. All the members of the invertan series, except α -invertan, when submitted to the action of an alkaline copper solution, readily yield a very characteristic copper compound, from which the invertan (except β -invertan) may be separated unaltered. Invertan can probably form several such copper compounds, all having a similar appearance, but affording different percentages of copper oxide.

36. In the presence of a very large excess of alkali, a copper compound is also formed from α -invertan, but on examination it is found that the α -invertan has been split up into an albuminoid and ζ -invertan, the copper compound being that of ζ -invertan.

37. We believe that the members of the invertan series are combinations of yeast albuminoid with η -invertan, and that η -invertan is probably a combination of yeast albuminoid with a carbohydrate. According to this theory, the composition of the carbohydrate present in the invertan series, calculated from the average of all the analyses, would be C = 43.22 per cent. and H = 6.28 per cent. These numbers agree very closely with those required by a hypothetical carbohydrate, coming midway between the *in* and the *on* groups.

38. We find that η -invertan contains 18 parts by weight of this carbohydrate to 1 of albuminoid, and that α -invertan contains 3 parts of the carbohydrate to 4 of albuminoid. The other members of the series are formed by the union of these two substances according to the general formula $\eta + \alpha_n$, where η represents η -invertan, and α represents α -invertan. In this way, we look upon invertase (β -invertan) as $\eta\alpha_5$. This splits up into α - and γ -invertan, according to the formula $\eta\alpha_5 = \eta\alpha_4 + \alpha$. $\eta\alpha_4$ represents γ -invertan, and this may be further transformed, by the elimination of α , into $\eta\alpha_3$, δ -invertan.

39. The numbers calculated for the constitution of the above theoretical homologous series agree with considerable closeness with those obtained from the analysis of members of the invertan series.

40. The number obtained from a single determination by Raoult's freezing process for the molecular weight of ζ -invertan is considerably less than the possible molecular weight of ζ -invertan according to our theory of its constitution.