LXXIV.—*Thermo-chemistry of Carbohydrate Hydrolysis.*

By HORACE T. BROWN, F.R.S., and SPENCER U. PICKERING, F.R.S.

- (1) *Hydrolysis of Starch by Vegetable and Animal Diastase.*
- (2) *Hydrolysis of Cane-sugar by Invertase.*

So far as we are aware, no serious attempt has hitherto been made to determine by *direct* means the amount of energy liberated or absorbed during the hydrolysis of any one of the carbohydrates under the action either of enzymes or of dilute acids. The few determinations which are recorded are altogether of an indirect nature, and, for the most part, are based on the observed differences between the *heats of combustion* of the hydrolysable substance and the products of its hydrolysis.

Such a method, however, cannot give results of any real value, since the thermal changes to be measured are considerably within the experimental error of the combustion values, and, even if these could be determined far more accurately than is possible, the results obtained would merely apply to the substances in the solid state, and would, of course, have to be corrected for the heats of dis-

solution. As an example of the erroneous conclusions which may be drawn from such indirect methods, we may cite the experiments of C. v. Rechenberg (*J. pr. Chem.*, 1880, **22**, 1 and 223), and of Stohmann and Langbein (*J. pr. Chem.*, 1893, [ii], **48**, 305), which are practically in accord with each other. The results of the last-mentioned observers give +9.1 cal. per gram for the inversion of cane-sugar as deduced from the heats of combustion of cane-sugar, dextrose, and levulose respectively. This value, however, represents only 0.1 per cent. of the sum of the quantities measured in order to determine it, and it is not surprising, therefore, that, when we compare it with the values which we have obtained by direct measurement, we find but little agreement with the latter, the 9.1 cal., when combined with the heat of dissolution of the substances concerned, leading to -1.0 cal. per gram for the heat of inversion in the dissolved condition, whereas the actual value would appear to be +11.2 cal.

Fleury, in 1875, recorded the fact (*Compt. rend.*, **81**, 196) that a distinct elevation of temperature occurs when a solution of cane-sugar is poured into dilute hydrochloric acid. Although he appears to have made no correction for the heat of dilution of the acid, nor of the cane-sugar, he considers that his experiment proves that the inversion is an *exothermic* reaction.

In the year 1882, F. Urech (*Ber.*, **15**, 2457), whilst studying the influence of mass and time on the acid inversion of cane-sugar, incidentally noticed the same fact, and, a short time before this, one of us had observed a distinct evolution of heat attending the inversion of a cane-sugar solution with the invertase of yeast.

As regards the heat phenomena of starch-hydrolysis, but very few references are to be found in chemical literature. Following the indirect method based on heat of combustion, C. v. Rechenberg (*loc. cit.*) estimated that the conversion of 1 gram of starch into *maltose* by diastase would be attended with the liberation of +28 heat units, a result which we shall presently see is very wide of the mark indeed, and that +17 heat units would be liberated by the complete conversion of 1 gram of starch into *dextrose* by dilute acid.

On the other hand, C. O'Sullivan states (*Trans.*, 1879, **35**, 785) that there is a considerable *absorption* of heat when the hydrolysis of starch by diastase takes place according to his equation C, that is, when the mixed products of transformation apparently contain 17.4 per cent. of maltose.

From the above remarks, it will be seen that the whole question of the heat phenomena of carbohydrate hydrolysis is practically untouched from the quantitative side. It is an important question, whether we regard it merely in its chemical or in its physiological aspects; for, on the one hand, any information must be welcome which will tend to throw

light on the still obscure processes of starch-hydrolysis, and, on the other, starch and cane-sugar play such an important part in plant life, that a knowledge of the amount of energy absorbed or liberated during their deposition or re-dissolution within the living cell may ultimately influence our views of the *modus operandi* of assimilation, translocation, and the temporary storing-up of these carbohydrates in the various organs.

It would be very difficult in any delicate calorimetric experiments, such as we are about to describe, to work at temperatures above that of the surrounding air, especially when the reactions do not progress very rapidly; we have, therefore, at the outset, confined ourselves to such hydrolytic actions as are capable of progressing with fair rapidity at ordinary temperatures, and the products of which admit of accurate determination.

(1) *Hydrolysis of Starch by Vegetable and Animal Diastase.*

It has been shown (Brown and Heron, Trans., 1879, 35, 626, and Brown and Morris, Trans., 1895, 67, 309) that the action of malt-extract on gelatinised starch at ordinary temperatures can, under favourable circumstances, be rendered very energetic, and that the reaction becomes stationary when the products of hydrolysis contain about 81 per cent. of maltose. Owing to the extreme viscosity of the starch-paste produced by the gelatinisation of solid starch, it was not found practicable to use this in the calorimeter, as will be explained later; most of our experiments, therefore, have been made with Lintner's "soluble-starch," produced by the limited action of acid in the cold on ordinary, well purified potato-starch. We have in some cases, also, used the mixed transformation-products of starch with diastase, obtained by arresting the reaction at some intermediate point.

For the active agents of hydrolysis we have used (1) *malt-extract*, made by digesting a very actively diastatic, air-dried barley-malt with $2\frac{1}{2}$ times its weight of water for 12 hours; (2) a solution of *Taka-diastase*, containing an active diastatic enzyme extracted from a cultivation of *Eurotium Oryzæ* on bran (Japanese Koji ferment); (3) diluted "mixed" human *saliva*; (4) a solution of *pancreatin*, containing the active diastatic agent of animal pancreas. As regards the last-mentioned hydrolytic agent, the ordinary "Liquor Pancreaticus" (Benger), which had been successfully employed in previous researches, was not applicable to the present enquiry, owing to the heat disturbance introduced by a small amount of alcohol contained in it. We are indebted to the courtesy of Mr. Benger for the preparation of a solid pancreatin which is free from this defect. This was dissolved in water at the rate of 4 grams per 100 c.c., and the filtered liquid was

used as the hydrolytic agent. The solid could not be used directly in the calorimeter owing to its floating on the water without becoming wet.

It was shown in 1880 (Brown and Heron, *Trans. Roy. Soc.*, 1880, 395) that the starch-hydrolysing enzyme of the pancreas has an action on starch which, for the first 50 or 60 minutes, is in every way comparable with that of malt-diastrase as regards the chemical nature of the products. If, however, the action is continued for several hours at 40°, a certain amount of dextrose is formed at the expense of the maltose. In the calorimetric experiments with pancreatin, presently to be described, the time and temperature were so restricted that only maltose and dextrin were formed, so that the results compare with those obtained with malt-extract.

We have found, on the other hand, that the Taka-diastrase, when acting at ordinary temperatures even for a short time, gives rise to so much *dextrose* that the thermal results obtained with it are not strictly comparable with those obtained with malt-extract or pancreatin.

The action of the diluted "mixed" saliva was not found sufficiently energetic at ordinary temperatures to render the thermal results obtained with it of any value.

The analysis of the products of hydrolysis was carried out by the methods described in detail by Brown, Morris and Millar (this vol., p. 72). The amount of hydrolytic change can be expressed either by the amount of apparent maltose produced during the action, or by the amount of hydrolysed *amylin* corresponding to the maltose; we have preferred to adopt the latter mode of expression.

When adding an enzyme to a solution of starch in order to measure the thermal value of the conversion, it is scarcely possible to adopt the same method as that employed when adding an alkali to dextrose, &c., as described in the previous paper, for the enzyme solution to be used measures about 50 c.c., and any error in determining its temperature—such as may always be expected when the temperature has to be taken as that of the air-bath in which it is placed—would produce an appreciable error in the results, especially as the thermal changes to be measured are very small. To obviate this difficulty, the enzyme solution was placed in a pipette, the end of which was drawn out into a long fine tube, bent first upwards and then downwards, so that the liquid could be left in it without running out, and this pipette with its contents was immersed in about 500 c.c. of the starch or other solution in the calorimeter. The pipette was connected with a combined force and suction pump, so that when necessary the liquid could be forced out of it into the other liquid in the calorimeter, and then some of the mixture drawn in and forced out again, in order to wash the pipette out. By following the fall or rise of temperature in the

calorimeter for a considerable time till the rate became quite constant, it could be ascertained when the temperature of the contents of the pipette was the same as that of the starch solution outside, and when, therefore, the mixing might be performed. Check experiments, in which previously heated, and consequently inoperative, enzyme solution was added to a starch solution (experiments 2 and 14 in Table I) showed that the method was capable of great accuracy, the thermal disturbance in these two cases being -0.0004° and $+0.0006^{\circ}$ (about 0.05 mm. of the mercurial column) respectively. In some blank experiments, where the unheated enzyme solution was added to water (experiments 7 and 15), the heat disturbance was rather greater, -0.002° and -0.0017° , but still little more than might be attributed to the errors in reading the thermometer.*

The error in the determination with the active enzyme on starch, must, however, be greater than in these check and blank experiments, for, in order to get a fair amount of conversion, the action has to be allowed from 10 to 30 minutes (generally 15 to 20 minutes), and the correction for cooling during this interval is considerable, generally much larger than the actual rise of temperature to be measured. Even after the lapse of 30 minutes, the conversion is by no means complete, and it is necessary to stop it, both that the rate of cooling at the "final" temperature may be determined, and for the purposes of the subsequent analysis of the liquid. In the earlier experiments, Nos. 1, 3, 4, 8, 9, 10 and 11 in the table, the action was stopped by adding a little alkali, and the rate of cooling then determined; as, however, the alkali somewhat interfered with a complete polarimetric examination of the solution, in the later experiments (5, 6, 12 and 13) 50 c.c. were removed for analysis before the alkali was added, and were transferred quickly to a flask of boiling water, the contents of which were afterwards evaporated to 50 c.c. before analysis.

When starch is hydrolysed with diastase, the maltose is, as we have mentioned in the previous paper (p. 770), produced in the α form, and this in the presence of water is gradually converted into the optically stable β modification; since, however, this change to optical stability involves, as we have seen, no thermal change, it will not matter, so far as the heat determinations are concerned, whether it has occurred or not. Nevertheless, in order to make certain that no error was introduced in this way, the heat evolved on adding the ammonia to stop the reaction (which would also convert the α - into the β -maltose) was measured, and similar measurements were made with a mixture of

* Any thermal effect on dilution of the malt extract would naturally be greater when the dilution was effected by water than when effected by starch solution, so that the slightly larger heat changes measured in these cases are not, necessarily, due to error.

starch-solution and previously heated malt-extract, adding several doses of ammonia in each case: but the heat evolved in the two instances was strictly identical, as it should be according to our previous work. It has not been thought necessary to give the details of the experiments in the table.

The results of the action of freshly prepared *malt-extract* on a 3 or 4 per cent. solution of Lintner's soluble-starch are given in the first part of Table I. (p. 794). A rise of temperature is observed immediately, evolution of heat gradually slackens, until, after about 10 minutes, it becomes inappreciable under the conditions of the present experiments.

There is a certain amount of chemical evidence in favour of the view that the molecule of soluble-starch is simplified prior to its hydrolysis, and it seemed a matter of interest to determine if this molecular simplification is attended with any absorption of heat. Two different series of experiments were undertaken with this object in view.

In the first, the malt-extract was reduced to one-eighth of the usual strength, and the temperature was read at half-minute intervals. If any absorption of heat had occurred in the early stages of the reaction from the above-mentioned cause, it seemed reasonable to expect that it would show itself in a short initial period of "incubation," but nothing of the kind was observed.

In the other series, recorded in experiments 3 and 4 of Table I, instead of commencing with soluble-starch, we employed solutions of the mixed starch-transformation products which had been previously hydrolysed down to an intermediate point, and then fixed by boiling. These solutions consequently still contained readily hydrolysable dextrins and maltodextrins, but the complex molecular grouping of the starch, should such a thing exist, must certainly have been broken down. In experiment 3, the specific rotatory power had been reduced to $[\alpha]_D 173.7^\circ$, the apparent maltose (R) being 55.8 per cent., whilst in experiment 4, the mixed solids had a specific rotatory power of $[\alpha]_D 171.9^\circ$, and the apparent maltose was 53.0 per cent. During subsequent hydrolysis of these solutions in the calorimeter, 7.28 grams and 4.79 grams respectively of the amylin constituents were converted into maltose, and the amount of heat evolved per gram of amylin hydrolysed is seen in both cases to be practically identical with the heat evolved under similar conditions from a similar amount of soluble-starch in solutions which had not been partially converted. In other words, the absorption of energy, if such there be, requisite to break down the larger molecule of soluble-starch, is so small as to be inappreciable by this method of experiment. We can, therefore, draw the important conclusion that in starch-hydrolysis by diastase the heat liberated is sensibly proportional to the amount of hydrolytic work done, or,

what is the same thing, to the amount of water fixed, and that this is independent of whether we commence with starch bodies of a high, or of an intermediate degree of complexity.

A partially converted starch solution was used in some of the other determinations (5, 6, 12 and 13), as such a solution is quite clear, and cannot contain any undissolved starch.

The mean value of our five determinations is +2.60 cal. per gram of amylin transformed into maltose, when malt-extract is the hydrolysing agent. The difference between the extreme determinations is 0.45 cal., corresponding to 0.007°, which must be regarded as small when we consider the duration of the experiments.

The next experiments in the table are those in which *pancreatin* was used as the hydrolysing agent instead of malt-extract.

The blank determinations with water, and the check determinations with heated enzyme solutions, gave the same results as those with malt-extract: the actual determinations gave also similar results as to the amount of transformation, the instantaneity of the heat evolution, and the duration of the action; but as regards the *quantity* of heat liberated, the values, it will be seen, are sensibly *lower* than with the malt-extract. The highest value with the pancreatin, it is true, very nearly approaches the lowest with malt-extract, but they are all somewhat lower, and the mean of our six determinations is 1.79—a value 30 per cent. below that with malt-extract. Such a difference, we think, cannot possibly be attributed to mere experimental error, and the explanation must be sought in other directions.

That there should be such a difference in the heat evolved in the action of two different enzymes is very remarkable, for the amount of enzyme which is requisite for the hydrolysis of a substance like starch is so extremely small in comparison with the mass of the hydrolyte* decomposed, that it might reasonably be expected that the thermal results would be those due to the hydrolysis of the hydrolyte only, any heat disturbance due to the change in the enzyme concerned being quite inappreciable.

Indeed, it is most probable that the enzyme does not undergo any permanent change at all during the hydrolysis, but merely plays the part of a 'trigger' in the operation: that it forms in the first place an unstable combination with the hydrolyte,† and that this is rapidly broken up again by the excess of water present, the enzyme being once more liberated in its original state ready to

* The word *hydrolyte*, which is comparable with *electrolyte*, is used to signify the substance hydrolysed.

† That such a combination would be conditioned by the correspondence of the configuration of the enzyme with that of the hydrolyte, seems highly probable from some recent work of E. Fischer (*Ber.*, 1894, 2985 and 3479; *ibid.*, 1895, 1429).

enter into further combination with more of the hydrolyte. It is easy to see how, by such a cycle of operations, an extremely small amount of enzyme can do a very large amount of hydrolytic work. The heat disturbances caused by the union of the enzyme with the hydrolyte would, of course, on this assumption, be exactly balanced by disturbances of an opposite sign when the enzyme-hydrolyte compound was broken up again ; and, therefore, if we have two different enzymes acting on the same hydrolyte, *and producing the same final chemical products*, as we have every reason to believe is so in the cases under discussion, we must get the same final heat disturbance per unit of substance hydrolysed.

It is just this result which we do *not* get when starch is hydrolysed by malt-diastrase and by pancreatic diastrase respectively, and the only conclusion that can legitimately be drawn is, either that the operation of the enzyme is not such as is described above, or that there are some differences in the final products of change in the two cases which have escaped notice.

The attempts to investigate the thermal changes accompanying the hydrolysis of starch by *saliva*, and by the *Taka-diastrase* were not successful. In the case of *saliva*, as we have already said, we could not obtain a sufficiently rapid hydrolytic effect at ordinary temperatures to enable us to follow the reaction in the calorimeter.

With a 4 per cent. solution of Taka-diastrase, the first difficulty met with was a precipitation of phosphates on adding the ammonia to stop the reaction, and a gradual absorption of heat on this account, which interfered with the measurement of the final rate of cooling. This difficulty was obviated either by adding hydrochloric acid instead of ammonia, and then neutralising with ammonia after the rate of cooling had been determined, or by removing a portion of the liquid into boiling water before the acid was added.

So far as the evolution of heat is concerned, the action of Taka-diastrase appears to be much more feeble for an equal amount of amylin converted than that of either malt-extract or pancreatin. The results do not, however, admit of anything like strict comparison, as the products of hydrolysis are not the same. With the Taka-diastrase, a portion of the starch-products, unlike those obtained with malt-extract and pancreatin, always contain more or less *dextrose*, a secondary product derived from the maltose, and since the formation of this dextrose is, as in the analogous case of starch-hydrolysis, most probably an endothermic change, the action of Taka-diastrase, if confined to the maltose stage, would evolve even less heat than that which was measured, and considerably less than that evolved by either malt-diastrase or pancreatic diastrase.

Repeated endeavours were made to determine the heat of hydrolysis

of *starch-paste* by malt-diastrase, as it seemed probable that the change from the extremely viscid to the limpid state might be attended by heat disturbance.

When a 3 per cent. starch-paste was placed in the calorimeter it was found that the stirrer, however quickly it was rotated, cut through the viscid liquid at the bottom without producing the slightest motion at the surface, so that no uniform temperature could be obtained in this way. The procedure was then modified by placing the malt-extract in the calorimeter and the starch-paste in the pipette. A larger calorimeter was used, holding 1300 c.c. of water mixed with 100 c.c. of malt-extract, and the pipette held 180 c.c. of the starch-paste. There was thus only one-third as much starch present as in the former experiments, and the total bulk of liquid was nearly three times as great, so that the same percentage of conversion would produce a rise of temperature only about one-ninth of that in the former cases. The comparative magnitude of the thermometric error is, therefore, largely increased, and when small quantities are to be measured, the results can have but little value: in addition to this, when dealing with 180 c.c. of such a viscid substance as starch-paste, it is impossible to feel certain that it has really attained the temperature of the surrounding liquid before it is mixed with it. The two determinations which appeared to be most satisfactory are given at the end of Table I. * The mean of them leads to a value which is higher than that obtained with the soluble-starch, though one of them gives exactly the same value as the mean of the soluble-starch results. A blank determination in which starch-paste was added to water (experiment 19) gave perfectly satisfactory results, and fairly good results were obtained with a further check determination (experiment 18) in which the soluble starch solution was used instead of the starch-paste: the value obtained with it was 1.8 cal. instead of 2.6 cal., as it should have been according to the previous determination, but as the difference represents only 0.0027° , it cannot be regarded as otherwise than satisfactory. So far as the evidence goes at present, we cannot consider that we have any positive proof that the thermal disturbance attending the hydrolysis of starch-paste differs materially from that of soluble-starch, but the nature of such difference as has been observed is of an opposite character to that which might have been expected, the hydrolysis of the paste liberating the greater amount of heat, and thus indicating

* The 'satisfactoriness' of a determination may be inferred if the rates of cooling before the beginning and after the end of the experiment are nearly identical (the difference between the beginning and end temperatures being very small), and also if the expulsion of the contents of the pipette causes no sudden alteration of temperatures, as would be the case if its contents were not at the same temperature as the liquid in the calorimeter.

that the transformation of starch-paste into soluble-starch is probably an exothermic reaction.

The apparatus for this particularly difficult determination might be somewhat improved by substituting a coil of glass tubing for the pipette to hold the starch-paste, but the chief method of reducing the experimental error would have to be the multiplication of the number of determinations, interspersing them with similar determinations with Lintner's soluble-starch. In this way, a fairly trustworthy value might be obtained.

(2) *The Hydrolysis of Cane-sugar by Invertase.*

The heat of inversion of cane-sugar by invertase was investigated in the same manner as the heat of transformation of soluble-starch by diastase. The inverting agent was obtained from yeast by completely drying it at comparatively low temperatures on porous tiles, rubbing up the dry powder with water, and filtering until perfectly bright. The dark yellow solution thus obtained had an extremely energetic inverting power.

When the reaction had proceeded sufficiently far in the calorimeter, it was stopped by the addition of a very small quantity of ammonia, and the invert-sugar formed was estimated by means of the polarimeter and the cupric-reducing power of the solution, corrections, of course, being applied for the optical and reducing properties of the "yeast liquor."

The results of two concordant experiments are given in Table II (experiments 1 and 2), and they lead to a value of 11.21 cal. per gram of cane-sugar inverted. This is five times larger than the heat of hydrolysis of starch.

In the first experiment, the action was allowed to continue for about 40 minutes, and in the second for half this time. It will be seen that the amount of sugar inverted is very nearly proportional to the time allowed. The evolution of heat commenced at once on the addition of the invertase, and proceeded at the same rate for 30 minutes, after which there was a very slight slowing down.

When the action is stopped by the addition of ammonia, those portions of the dextrose and levulose which are still in the α form are, of course, converted into the optically stable β form (see preceding paper), the dextrose with an evolution of heat, the levulose with an absorption. Owing to the long duration of the experiment, however, a large proportion of both will have become converted into the optically stable modifications before the alkali is added, especially in the case of the levulose, where, as we have seen, the transformation is very rapid. In the determinations made with a special view to investigate

this point, the addition of ammonia, when all corrections were made, appeared to cause no temperature change, the small amount of heat evolved by the conversion of the residual α -dextrose to the stable form being probably counterbalanced by the heat absorbed in the conversion of the residual α -levulose, the greater value of the latter change being discounted by the smallness of the amount of unconverted α -levulose present.

The determinations in question are those in the two series 1—4 and 5—8, Table II (p. 795), in which the heat evolved on adding the ammonia after inversion had occurred (experiments 1*a*, 2*a*, and 5) was compared with that evolved by adding the ammonia to the same quantity of cane-sugar mixed with previously heated, and, therefore, inoperative invertase (experiments 3, 4, 6, 7, and 8). The heat evolved in the two cases is practically the same in both series— 0.0823° against 0.0883° in the first series, and 0.0611° against 0.0513° in the second—although the determinations with the heated invertase are not very concordant, a fact which is probably due to the yeast extract which was used containing a little of some volatile acid which was expelled to a greater or less degree during the heating. No alteration, therefore, will be made in the value of $+11.21$ cal. if the ammonia is added to reduce the products to the optically stable form, in other words, the value expresses the heat change accompanying the inversion of cane-sugar into the β forms of dextrose and levulose. From this value, with the help of our previous determinations of the heat of conversion of the α to the β forms, as described in our last paper, we can deduce the thermal value of the inversion into the α form of these substances. It must be borne in mind that 1 gram of cane-sugar yields on hydrolysis 0.526 gram of dextrose and the same amount of levulose, and that, whilst the heat change of α - into β -dextrose is $+0.588$ cal. per gram, that of levulose is -4.64 cal. The heat of inversion of cane-sugar to the “birotatory” α -forms of dextrose and levulose will consequently be

$$11.21 - (0.588 - 4.64) \times 0.526 = +13.34 \text{ cal.}$$

as against $+11.21$ cal. for the inversion to the stable β forms. The larger of these two values represents the true heat of inversion of cane-sugar.

We must express our thanks to Mr. J. H. Millar for assistance in carrying out the chemical details of the experiments described in this and the preceding paper.

TABLE I.—Conversion of Starch. Water equivalent of the Calorimeter and Apparatus = 14 grams.

Experiment.	Initial contents of calorimeter.	Substance added.	Initial temp.	Rise observed.	Grams of starch hydrolysed.	Cal. evolved per gram. hydrolysed.
<i>Soluble-starch by Malt-extract.</i>						
1. Hydrolysis of starch-solution	556.3 c.c. 3 % starch-solution.	39.2 c.c. malt-extract.	18°	0.0461	10.30	2.72
2. <i>Check determination.</i> Heated malt-extract.....	556.3 c.c. 3 % starch-solution [α] _D = 173.7°	38.9 " "	"	-0.0004	—	—
3. Hydrolysis of partially converted starch-solution.	480 c.c. 3 % starch-solution [α] _D = 173.7°	39.1 " "	16°	0.0314	7.48	2.37
4. " "	556.3 c.c. 4 % " "	39.1 " "	"	0.0294	4.79	2.80
5. " "	500 c.c. 4 % " "	49.0 " "	"	0.0545	11.59	2.74
6. <i>Blank determination.</i> Malt-extract to water	500 c.c. water.	49.0 " "	"	0.0472	11.70	2.35
7. " "	500 c.c. water.	49.0 " "	"	-0.0020	—	—
				Mean.....		2.60
<i>Soluble-starch by Pancreatin.</i>						
8. Hydrolysis of starch-solution	500 c.c. 4 % starch-solution.	51 c.c. pancreatin-solution.	16°	0.0397	10.52	2.15
9. " "	" "	50 " "	"	0.0312	10.36	1.73
10. " "	" "	50 " "	"	0.0283	10.06	1.60
11. " "	" "	50 " "	"	0.0294	10.11	1.66
12. " "	partially converted	50 " "	"	0.0307	13.46	1.44
13. <i>Check determination.</i> Heated malt-extract to water	" "	50 " "	"	0.0445	12.01	2.17
14. " "	starch-solution	50 " "	"	0.0006	—	—
15. <i>Blank determination.</i> Malt-extract to water	500 c.c. water.	50 " "	"	-0.0017	—	—
				Mean.....		1.79
<i>Starch-paste by Malt-extract. Water equivalent of the Calorimeter and Apparatus = 53 grams.</i>						
16. Hydrolysis of starch-paste	1300 c.c. water and 100 c.c. malt-extract.	183 c.c. 3 % starch-paste.	16°	0.0140	5.35	4.3
17. " "	" "	182 " "	"	0.0092	5.77	2.6
				Mean.....		3.45
18. <i>Check determination.</i> Malt-extract to soluble-starch	" "	184 c.c. 5 % soluble-starch.	"	0.0061	5.69	1.8
19. <i>Blank determination.</i> Starch-paste to water	1400 c.c. water.	180 c.c. starch-paste.	"	-0.0006	—	—

TABLE II.—*Inversion of Cane-sugar.*
Water equivalent of the Calorimeter and Apparatus = 14 grams.

Experiment.	Initial contents of calorimeter.	Substance added.	Initial temp.	Rise observed.	Grams of sugar inverted.	Cal. evolved per gram inverted.
1. Inversion.....	568.9 grams sugar-solution.*	58.7 c.c. invertase.	20°	0.2892	15.74	11.28
{1a. Ammonia added to product	Ditto + 58.7 c.c. invertase.	11.4 c.c. of 7.9% ammonia.	"	0.0825]	—	—
2. Inversion.....	570.5 grams sugar-solution.*	58.4 c.c. invertase.	"	0.1467	8.11	11.13
{2a. Ammonia added to product	Ditto + 58.4 c.c. invertase.	11.4 c.c. of 7.9% ammonia.	"	0.0821]	—	—
3. Check determination. Ammonia to sugar {	568.7 grams sugar-solution * +	" "	"	0.0678}		
with previously heated invertase	58.9 grams heated invertase-solution.	" "	"	0.0583}		
4. Ditto ditto {	533.7 grams sugar-solution * +	" "	"	0.1057]		
{	54.8 grams heated invertase-solution.	" "	"	0.0611}		
5. Ammonia to products of inversion.....	545 c.c. of 10% sugar-solution +	10 c.c. of 1.77% ammonia.	16			
{	57 c.c. invertase-solution.	" "	"	0.0523}		
6. Check determination. Ammonia to sugar {	545 c.c. of 10% sugar-solution +	" "	"	0.0430}		
{	57 c.c. heated invertase-solution.	" "	"	0.0586}		
7. Ditto ditto- ditto	" "	" "	"			
8. Ditto ditto	" "	" "	"			

* Solution containing 9.525 grams per 100 c.c.; sp. gr. 1.03704.