

LXV.—*Contributions to the History of Starch and its Transformations.*

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EARLY in the present century the attention of chemists was directed to the curious modifications which starch undergoes under the action of heat, acids, and certain vegetable infusions; and owing in part to the great industrial applications of these discoveries, and in part to a belief that a study of the transformations would throw light upon many obscure points in vegetable physiology, the subject has attracted the attention of a great number of observers. Scarcely a year has passed since 1811, unmarked by the production of several memoirs upon starch or its products of transformation. In a catalogue recently prepared by one of us, these original publications are shown to exceed 400 in number. Amongst these there are, it is needless to state, some most valuable memoirs which mark distinct epochs in this special line of research. We may, perhaps, here mention the most important discoveries which lie at the base of all our present knowledge of the subject.

In 1811, Vauquelin (*Bull. Pharm.*, **3**, 54) discovered that starch, on being heated somewhat strongly, is converted into a substance entirely

soluble in water, and possessing many of the physical properties of gum arabic.

Kirchof, in or about the same year (*Schweigg. Journ.*, **4**, 108) found that starch, when boiled with dilute sulphuric acid, yields a crystallisable sugar; and two years later, in 1814, the same chemist observed that a similar transformation is brought about by the action of the vegetable albumin of grain, and that this body has its action much intensified by subjecting the grain to the malting process (*Schweigg. Journ.*, **14**, 389, 1815).

In the year 1812, Vogel (*Schweigg. Journ.*, **5**, 80) found that, in addition to sugar, there is formed a body resembling gum when starch is acted upon by hot dilute acid. This gum was described and examined more fully in 1833, by Biot and Persoz (*Ann. Chim. Phys.* [2], **52**, 72), who gave it the name of *dextrin*, owing to the property it possesses of strongly rotating to the right a ray of plane-polarised light. Biot and Persoz, in this memoir, laid the foundation for the accurate optical methods of the present day, which have yielded such important results in the hands of Bondonneau, Musculus, O'Sullivan, and others. The iodine reaction for starch was discovered as far back as the year 1813, by Stromeyer (*Schweigg. Journ.*, **12**, 349), and the reducing action of the starch-derivatives upon an alkaline copper solution was first investigated by Trommer (*Ann. Chem. Pharm.*, **39**, 360, 1841).

Payen and Persoz, in 1833 (*Ann. Chim. Phys.*, **53**, 73), stated that the action of an infusion of malted grain upon starch is attributable to the presence of a particular transforming agent, which they named *diastase*, from its supposed property of separating the interior of the starch-granules from their outer envelope.

The true structure of the starch-granule was first demonstrated by Fritzche, in an important memoir, published in 1834 (*Pogg. Ann.*, **32**, 129), and this work served some years afterwards as the foundation of C. Naegeli's classical researches on the morphology of the starch-granule, embodied in his celebrated monograph *Die Stärkekörner* (Zurich, 1858).

In the year 1860, Musculus threw an entirely new light upon the action of sulphuric acid and diastase upon starch. It had previously been supposed that the sugar produced in the reaction was a product of the hydration of dextrin, a body intermediate between starch and sugar, and isomeric with the former. Musculus brought forward strong experimental evidence to show that this view of the matter was wrong, and that the sugar and dextrin are produced simultaneously by a splitting up of the starch molecule, accompanied by hydration (*Ann. Chim. Phys.* [3], **60**, 203; *Compt. rend.*, **54**, 194).

Although this theory found strong opponents at the time, and was

attacked by many chemists, notably by Payen and Schwarzer, yet it has stood its ground, and is now pretty generally admitted to be in the main correct.

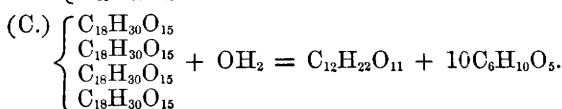
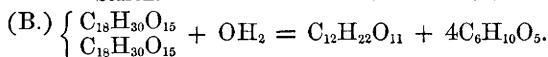
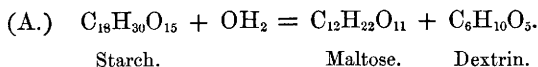
Since it would be out of place here to attempt even a sketch of the large amount of research based upon the above-mentioned discoveries, we must take up the subject from a point of comparatively recent date.

In a series of remarkable and most important papers, published by O'Sullivan, in the *Journal of this Society* (*Journ. Chem. Soc.* [2], **10**, 579; *ibid.*, [3], **2**, 125; *ibid.*, [3], **1**, 478), from 1872 to 1876, the author proved that the sugar produced by the action of diastase or acid on starch, is not, as had been almost universally supposed, *dextrose*, but is a sugar of the saccharose type, having a considerably higher optical activity than dextrose, and a much less reducing action upon Fehling's solution.

This body had been previously isolated by De Saussure, in 1819 (*Ann. Chim. Phys.*, **11**, 379), who, however, merely records its crystalline habit, which he describes with accuracy. It was prepared again in 1847, by Dubrunfaut (*Ann. Chim. Phys.* [3], **21**, 178), who recognised it from its optical properties as a distinct sugar, and named it *maltose*. Dubrunfaut's observations, however, obtained but little notice at the time of their publication, and appear to have been entirely forgotten until the re-discovery of maltose by O'Sullivan, many years afterwards.

Having established the principal properties of maltose and dextrin, which, with dextrose, are shown to be the only products obtained from starch, O'Sullivan determined the particular way in which starch is split up under the action of malt-extract, and varying conditions of temperature. His results led him to agree with Musculus, that the transformation of starch into dextrin and maltose is not a gradual act of hydration, the dextrin appearing first, and the maltose afterwards, but that the two bodies are formed simultaneously and in equivalent quantities, by the splitting up of the starch molecule.

The decomposition of starch is considered to take place according to one of the three following equations, the resulting transformation depending upon the temperature employed in the reaction.



The transformation, according to Equation A, is stated to take place when starch-paste is acted upon by malt-extract at any temperature below  $63^{\circ}\text{C}$ .; according to Equation B, at temperatures between  $64^{\circ}$  and  $68\text{--}70^{\circ}$ ; and according to Equation C, at temperatures from  $68\text{--}70^{\circ}$  to the point at which the activity of the transforming agent is destroyed. The specific rotatory power, and the cupric oxide reducing power of the mixed products of transformation corresponding to these equations are:—

	$[\alpha]_j$ .	Cupric oxide reducing power per cent.
A .....	170.6°	44.1
B .....	191.8	22.4
C .....	202.8	11.3

The dextrins produced in the above transformations are all considered identical, and capable of yielding maltose by a slow and gradual act of hydration on continuing to digest the solution with malt-extract. By continuing the digestion for many hours, the maltose was found apparently to yield a small quantity of dextrose, or a mixture of dextroses having a united optical activity of  $[\alpha]_j + 67^{\circ}$ . This production of dextrose was attributed to the small amount of acid always present in the malt-extract used.

O'Sullivan considers that the results represented by the three equations are due to some change brought about by heat in the character of the transforming agent, and are not due to any difference in the size of the starch molecule.

Märker states (*Landw. Versuchs-Stat.*, **22**, 69: and *Ber. der Naturfor. München*, 1877) that at a temperature of  $60^{\circ}\text{C}$ . four molecules of starch yield three of maltose and one of dextrin; at  $65^{\circ}$  the yield of maltose is lowered somewhat; and at a still higher temperature two molecules of starch yield one of maltose and one of dextrin.

Märker concludes from this that there are possibly two diastatic ferments, one producing much maltose and little dextrin, and destroyed at a comparatively low temperature; the other producing more dextrin and less maltose, and destroyed at a higher temperature. The latter transforming agent does not act upon dextrin, whilst fresh unheated diastase readily converts it into sugar.

In July of last year Musculus and Gruber (*Bull. Soc. Chim.*, **30**, 54) described the results of a new series of experiments upon starch. They look upon starch as a polysaccharide, containing five or six times the group  $\text{C}_{12}\text{H}_{20}\text{O}_{10}$ .

Under the action of diastase, or of dilute acids, this carbohydrate undergoes a series of hydrations and successive decompositions, resulting in the production of maltose, and of a new dextrin of less molecular weight. This action goes on by successive stages, until the

$\gamma$ -achroo-dextrin is reached, which is probably then converted into maltose by a simple act of hydration.

The authors recognise the existence of three distinct achroo-dextrins, (*i.e.*, dextrins which are not coloured by iodine), varying in optical activity and cupric oxide reducing power. These are—

	$[\alpha]_D^{20}$	Cupric oxide reducing power.
Achroo-dextrin $\alpha$ .....	210°	12
Achroo-dextrin $\beta$ .....	190	12
Achroo-dextrin $\gamma$ .....	150	28

It will be seen, when we come to describe the results of our own experiments, that we have obtained strong evidence of the non-identity of the achroo-dextrins. We have found them to differ in some of their properties in a way which can be accounted for only by the greater or less complexity of their molecule. These results were obtained some months before the appearance of the above paper of Musculus and Gruber, a fact which we mention, not with any wish to raise the question of priority of discovery, but as affording a greater probability of the correctness of this theory. The optical and other properties of our dextrins do not, however, correspond with those of Musculus and Gruber.

Musculus and Gruber used in their experiments precipitated diastase prepared from malt. We have ourselves made some experiments, using the so-called diastase prepared by Hufner's glycerin method (*Journ. f. prakt. Chem.* [2], 5, 372), but have found its action vary very much with its mode of preparation. By repeated solution in glycerin and precipitation with alcohol and ether, the activity of the body is constantly lessened, until a point is reached at which it merely produces limpidity in starch-paste, without any further transformation. During the whole of the following research we have consequently used the cold aqueous infusion of malt as the converting agent, which admits of any degree of accuracy if proper precautions are taken.

#### *Method of Experiment.*

All the experiments described in this paper were conducted with potato-starch, which, after being well washed with water, was treated successively with a very dilute solution of potassic hydrate and a 1 per cent. solution of hydrochloric acid. The starch, after being thoroughly washed with water until the last trace of acid had disappeared, was dried at a temperature not exceeding 25° C. Potato-starch thus prepared is, under favourable conditions, so completely transformed by malt-extract as to yield a solution of almost perfect

transparency, the residue from 8 or 10 grams of starch being inappreciable in amount. The ash from a starch treated by this method does not amount to more than 0.15 per cent.

The smaller-grained starches, *i.e.*, those from maize and rice, are purified with greater difficulty by the above treatment, and are consequently less suited for such work as we are about to describe.

In the preparation of the starch-paste the greatest possible care must be taken that the gelatinization is complete, and that the paste is perfectly homogeneous, otherwise it is impossible to obtain uniformity in the results. This end is best attained by mixing the starch thoroughly with a sufficient quantity of cold water to form a thin mud, and then pouring it, with constant and rapid stirring, into water at 100°.

The most suitable quantity of starch to be used per 100 c.c. is from 3 to 5 grams. It is not advisable to have the paste too thick, especially when small quantities of malt-extract are to be used.

The malt-extract was invariably prepared as follows:—100 grams of very finely ground pale malt were mixed with 250 c.c. of distilled water. The mixture, after being well stirred, was allowed to stand from six to twelve hours, and then filtered perfectly bright. The clear filtrate so obtained has a specific gravity of 1.036—1.040 (water = 1.000), varying somewhat with the kind of malt used; this we call *normal malt-extract*, and, except where specified to the contrary, it was with such an extract, after previous treatment in various ways, that all our experiments were conducted.

The specific gravity, optical activity, and cupric oxide reducing power of the malt-extract must be determined, either in the original liquid before treatment of any kind, or after heating and filtration, according to the conditions of the experiment.

A definite quantity of the malt-extract is dropped from a burette, or accurately graduated pipette, into the starch-paste, which has meanwhile been brought in a water-bath to the temperature at which the transformation is to be made. The water-bath is kept at a constant temperature by means of a thermostat. We have found Page's regulator answer the purpose admirably.

At the close of the experiment the volume of liquid, after cooling to 15.5° C., must be measured off, or, if very great accuracy is required, the solution is weighed, and its volume deduced from its specific gravity. If during the transformation it is necessary to take out samples in order to apply the iodine test, these must be carefully measured with a pipette, and allowance made for them in finally determining the total volume of the liquid.

In case the transformation has to be continued for more than 15 to 20 minutes, and the malt-extract employed exceeds 2 or 3 c.c.

per 100 c.c. of starch-paste, it is necessary to digest some of the malt-extract in a separate flask for the same length of time and under the same conditions of temperature as the transformation-liquid itself, the analysis of the malt-extract being made in such a case *after* the digestion. The reason for taking this precaution will be rendered evident when we come to consider the changes brought about in malt-extract by heat.

The solution of the starch-products has now to be analysed. Its specific gravity is first taken at  $15.5^{\circ}$ , referred to water at  $15.5^{\circ}$ , and the total solid matter per 100 c.c. is estimated by dividing the specific gravity minus 1000 by the constant 3.86 (water = 1000).\*

From the total solid matter thus obtained there are deducted the total solids per 100 c.c. introduced in the malt-extract. The difference gives the number of grams of starch-products per 100 c.c. of liquid.

The cupric oxide reducing power of the liquid must then be determined. This is estimated *gravimetrically* with Fehling's copper solution. Here the precaution of weighing the liquid to be analysed must be taken, for since the quantity required to be used seldom exceeds 5 or 6 c.c., a very small error of measurement would be fatal to accuracy. The volume of the liquid taken is found by dividing the weight in grams by the specific gravity. A sufficient quantity of the liquid is taken to afford from .2 to .3 gram of cupric oxide. The reduction with the Fehling's solution is carried on in a water-bath for 12 or 15 minutes. It is not advisable to continue the heating longer if any of the higher dextrans are present, since these are slightly converted by heating with the potash solution. The precipitate of cuprous

\* This constant is absolutely correct for solutions of cane-sugar of sp. gr. 1050. We are aware that the true divisor for solutions of dextrin and maltose differs somewhat from this number; but our percentage results will not be influenced by this fact, since the numbers used throughout this paper, representing the specific rotatory powers of maltose, dextrose, and dextrin, and the cupric oxide reducing power of maltose and dextrose, are all calculated for the same constant, 3.86. Although this divisor is fairly correct for solutions of cane-sugar of from 1 to 10 per cent., it is too high for solutions of high specific gravity, the volume occupied by a unit of weight of sugar in a solution of high specific gravity being greater than the volume occupied by a unit of weight in a solution of low specific gravity. In other words, if a given volume of a strong solution of cane-sugar is mixed with a given volume of water, or of a sugar solution of less specific gravity, then the resulting volume will be *less than the sum of the volumes*.

The results of our experiments in this direction with cane-sugar are given in Table I in the form of a curve. By means of this curve we are enabled to ascertain by inspection the divisor to be used for solutions of any specific gravity from 1000 to 1070, in order to determine the number of grams of sugar per 100 c.c. of liquid.

The curve is almost truly parabolic.

oxide, after filtration and drying, is thoroughly oxidised by ignition and then weighed.

The weight of cupric oxide that would be reduced by 100 c.c. of the liquid under examination must then be calculated. If this weight be represented by  $x$ , the volume of the liquid taken in c.c. by  $V$ , and the weight of the  $\text{CuO}$  by  $W$ , then

$$x = \frac{100 W}{V}.$$

From the value of  $x$  must then be deducted the  $\text{CuO}$  corresponding with the number of c.c. of malt-extract in 100 c.c. of the transformation-liquid.\*

There now only remains to determine the optical activity of the substance.

All the optical determinations were made with a Soleil-Ventzke-Scheibler instrument manufactured by Schmidt and Haensch, of Berlin. The analyser, polariser, and compensator are adjusted so as to give at the zero point a disc of light uniformly illuminated by the so-called "neutral tint," corresponding in refrangibility to the medium yellow ray of the solar spectrum. The solutions of the starch transformation products, which were almost always colourless, were examined almost invariably in a column of 200 mm. in length. In no case was animal charcoal used for decolorising the liquid. The slight difficulty presented by the colour of the malt-extract, in the examination of that liquid, was avoided by using a column 100 mm. in length, and slightly rotating the extra Nicol's prism placed at the anterior end of the instrument for the special purpose of neutralising any slight colour that a solution may possess. The presence of this third Nicol's prism renders the Soleil-Ventzke-Scheibler instrument especially adapted for such observations as we are describing, since in delicate work no reliance can be placed upon decolorisation with animal charcoal: for even when the first portions of the filtrate are rejected, it is seldom that the filtrate from a solution containing much lævorotatory albuminoids has exactly the same optical activity as the original liquid.

In the instrument used by us we find that 100 divisions of the scale are equal to 38.22 degrees of arc. The error of reading, when a good light and a transparent solution are obtained, does not amount, to

\* This method of determining the amount of substance reducing cupric oxide is capable, if all proper precautions are taken, of yielding extremely accurate and concordant results. The severe condemnation of the gravimetrical process recently uttered by Soxhlet (*Chem. Centr.*, 1879, 219) is perfectly unfounded. We state this with great positiveness after upwards of three years' experience of the process, and after carrying out numerous series of test analyses.



a practised eye, to more than one-tenth of a division of the scale, or 2·3 minutes of arc.\*

The reading in the 200 mm. tube is divided by 2, and corrected for the deflection due to the malt-extract used.

We have now obtained three factors for the starch transformation-products corrected for the malt-extract used.

(1.) The total solid matter expressed in grams per 100 c.c. (corrected).

(2.) The amount of cupric oxide reduced by 100 c.c. of the solution (corrected).

(3.) The optical activity (corrected) of the solution in the 100 mm. tube expressed in divisions of the Soleil instrument.

From these data we have to deduce the specific rotatory power of the solid substance and its percentage cupric oxide reducing power.

The *absolute* specific rotatory power of a substance is the angular deflection experienced by a ray of plane polarised-light of definite refrangibility on passing through a layer of its aqueous solution 1000 mm. in thickness, and containing 10 grams of the substance per 100 c.c. Where the neutral tint, corresponding with the medium yellow ray of the solar spectrum, is employed, the specific rotatory power is

\* We may here notice a curious phenomenon attending the working of the polariscope, to which we believe attention has not been before directed. We find that when the instrument is accurately adjusted to the zero point by one of us (*i.e.*, when the two halves of the disc are illuminated equally by the true neutral tint), the other observer, in order to obtain the same even neutrality, must move the compensator  $\frac{2}{10}$  of a division to the right. We imagined at first that this "personal equation" might be due to one of us having a finer appreciation than the other of very small differences of tint; but if this were the case, there ought to be two points equidistant on either side of the true zero point, which would be capable of satisfying the less sensitive eye, for at these two points the colours would be of equal intensity, but reversed in position. As a matter of fact, however, this is not the case, the differences of reading of two observers being *always in the same direction*, and constant in amount. We are at present unable to give a satisfactory explanation of this phenomenon, but believe that it will be found in the region of physiological optics. An examination of the humours of the eye (eye of ox) by one of us has revealed the fact that whilst the aqueous and vitreous humours exert no action whatever on plane-polarised light, the crystalline lens itself has a marked levorotatory power. We find that a section of the lens, 4·5 mm. thick, is capable of rotating the plane of polarisation for the neutral tint through  $1\cdot34^\circ$  to the left. This levorotation is as large in amount as is the dextro-rotation of a fourteen per cent. cane-sugar solution in a layer of equal thickness. It is possible that this property of the crystalline lens may have something to do with this question of the "personal equation" of the polariscope: it cannot, however, be the direct and only cause, since the interposition of any substance capable of circular polarisation between the last Nicol and the eye can have no effect in altering the relative tints of the two sides of the illuminated disc.

denoted by the symbol  $[\alpha]_j$ , whilst  $[\alpha]_D$  represents the specific rotatory power for the light emitted by incandescent sodium vapour.\*

In such experiments as we are describing, the amount of matter in solution must necessarily be determined from the specific gravity, and since the density of the various carbohydrates in solution differs somewhat, it is evident, when two or more such bodies are present, that the determination of the solid matter will be somewhat complicated. In consideration of this, and also of the still more important fact that, up to the present time, the solution-densities of the carbohydrates have been but very imperfectly determined, we resolved to employ a constant and unvarying divisor for the determination of the solid matter, to which all specific rotatory powers should be referred. This divisor is, as we have before stated, 3·86, and when this number has been used in the determination of the constant, we append it to the ordinary symbol denoting the specific rotatory power, thus  $[\alpha]_{j3\cdot86}$ . In order to convert  $[\alpha]_{j3\cdot86}$  into *absolute* specific rotatory power it is only necessary to increase or diminish the angular value in the proportion of 3·86 to the absolute divisor when this has been determined.

Recent experiments of Tollens and of Schmitz upon dextrose and cane-sugar (*Deut. Chem. Ges. Ber.*, **9**, 1531; *ibid.*, **10**, 1403; *ibid.*, **10**, 1414; *ibid.*, **11**, 1807) indicate that the specific rotatory power of these bodies is not the same in concentrated as in dilute solutions. In the case of cane-sugar, both Tollens and Schmitz found that for highly concentrated solutions the value of  $[\alpha]_D$  was sensibly less than for weaker solutions, whilst in the case of dextrose the former observer noticed a marked increase in specific rotatory power in highly concentrated solutions.

It is probable that further research will indicate similar anomalies in the case of all the carbohydrates possessing optical activity, but since the phenomena described are only observed in highly concentrated solutions, they do not affect the accuracy of our experiments, which were all conducted with dilute solutions.

The value of  $[\alpha]_{j3\cdot86}$  is thus obtained :—

Let  $x$  = the total starch transformation-products in grams per 100 c.c. deduced from the specific gravity, and corrected for the malt-extract.

$y$  = the reading of the solution in the 100 mm. tube expressed in divisions of the scale, and corrected for the malt-extract.

Then

$$[\alpha]_{j3\cdot86} = \frac{3\cdot822 y}{x}.$$

\* The relation of  $[\alpha]_j$  to  $[\alpha]_D$  for the carbohydrates is 24 to 21·54.

where 3.822 is a constant for the instrument used, 100 scale-divisions being equal to 38.22°.

In determining the cupric oxide reducing power of a substance, O'Sullivan has adopted *dextrose* as his standard of comparison. He defines the cupric oxide reducing power to be "the amount of cupric oxide calculated as dextrose which 100 parts reduce" (*Chem. Soc. J.*, 1876, **2**, 130); hence if the amount of cupric oxide reduced by 1 gram of dextrose is known, the amount of cupric oxide reduced by 1 gram of any substance, calculated upon this number as a percentage, will represent the cupric oxide reducing power of the substance, which we denote by the symbol  $\kappa$ . Here, just as in the case of the specific rotatory power, when the constant 3.86 has been used in determining the value of  $\kappa$ , this number is appended thus:  $\kappa_{3.86}$ .

The amount of cupric oxide reduced by 1 gram of dextrose has been determined as 2.205 grams. We have satisfied ourselves of the accuracy of this number by repeated experiments with dextrose prepared both by the inversion of cane-sugar and by the action of acid upon starch. The value of  $\kappa_{3.86}$  is determined by the formula—

$$\kappa_{3.86} = \frac{100 y}{2.205 x}.$$

where

$y$  = grams of cupric oxide reduced by 100 c.c. of the solution after correction for the malt-extract.

$x$  = the total starch-products in grams per 100 c.c. deduced from the specific gravity and corrected for the malt-extract.

#### *Action of Heat upon Malt-extract.*

In previous experiments upon starch in which malt-extract has been employed, it has been assumed that the optical activity, cupric oxide reducing power, and percentage of solid matter in the extract are but little liable to change, and that it is necessary merely to determine these factors for a given volume of the cold infusion of malt, or for a definite weight of the malt exhausted with cold water, in order to obtain the correction to be applied to the mixture of the transformation-products. As a matter of fact, however, we find that freshly prepared and filtered malt-extract is constantly undergoing change, even at ordinary temperatures. The result of this change is a gradual increase in specific gravity and cupric oxide reducing power, and a decrease in optical activity. In the cold, and at temperatures below 45° C., the progress of these changes cannot be observed for any length of time, owing to the rapid fermentation to which cold infusions of malt are particularly liable. This fermentation is generally due to

the presence of *Bacillus subtilis*, with the occasional appearance of one or two small species of saccharomyces. If the malt-extract is digested at a temperature of 40—45° C. the saccharomyces do not often appear, the *Bacilli*,\* unmixed with any other organism, in 48 hours running through their cycle of growth and development, which terminates in sporulation, and an entire disintegration of the spore-bearing rods. At the same time torrents of carbonic anhydride and hydrogen are evolved in equal proportions, and butyric acid appears in the liquid. If cane-sugar and calcic carbonate, or zinc oxide, are added to a cold water malt-extract, the mixture, submitted to a temperature of 40—45° C., affords an excellent means of obtaining butyric acid, and of studying the organisms active in bringing about the change. Under these conditions little or no lactic acid is formed, or, if formed, is at once converted into butyric acid.

The optical and chemical changes in malt-extract previously referred to are not in any way dependent upon the presence of organised ferments, since the alterations still take place after the malt-extract has been submitted to temperatures sufficiently high to arrest the fermentative activity of such organisms. At 50° C. the changes take place very rapidly and fully. If malt-extract is heated to a temperature higher than 50° for a few minutes, and is, after filtration and analysis, digested for some time at 50°, the above-mentioned changes in specific gravity, optical activity, and cupric oxide reducing power are less rapid and complete the higher the temperature to which the liquid has been heated; showing that heat has a decided weakening action upon the special converting agent. Malt-extract which has been heated above 76° C. undergoes little or no change on subsequent digestion at 50° C. for many hours; a proof that the acids of the malt-extract exert no influence in bringing about the change.

The following examples, taken from a large series of experiments, illustrate what has been said above :—

\* Occasionally *Sarcina* will appear.

*Experiments illustrating the Action of Heat upon Filtered Normal  
Malt-extract.*

Number.	Description of malt-extract.	Solid matter in grams per 100 c.c.	CuO reduced in grams per 100 c.c.	Deflection in 100 mm. tube, scale-divisions.
1	Malt-extract heated to 58° C. for one hour . . .	9·888	9·261	8·5
2	The same after digestion at 50° C. for 16 hours	10·025	11·798	6·8
3	Malt-extract prepared quickly (one hour) . . . . .	9·129	4·857	8·8
4	The same heated for 20 minutes to 60° C. . . . .	9·295	6·200	8·8
5	The same heated for 20 minutes to 60° C., filtered, and afterwards digested at 55° C. for 15 hours . . . . .	9·453	9·208	6·7
6	Malt-extract freshly prepared . . . . .	7·759	4·657	7·7
7	The same digested at 58° C. for 15 hours . . . . .	7·979	7·454	6·3
8	Malt-extract prepared for 16 hours in the cold. .	9·956	8·653	8·5
9	The same digested for 16 hours at 55° C. . . . .	10·152	11·263	7·2
10	No. 8 heated to 62° for 20 minutes . . . . .	10·349	9·552	8·8
11	No. 10 digested at 55° for 16 hours . . . . .	—	11·002	7·5
12	Malt-extract heated to 76° for 20 minutes . . . . .	9·577	6·282	9·4
13	The same digested for 16 hours at 55° . . . . .	9·549	6·146	9·2
14	Malt-extract quickly prepared and boiled for 15 minutes . . . . .	7·562	5·157	8·0
15	The same digested after boiling at 55° for 16 hours . . . . .	7·523	5·020	8·3
16	Malt-extract heated to 66° for 20 minutes . . . . .	9·336	6·185	8·8
17	Digested for 16 hours at 55° after heating to 66°	9·401	7·633	8·5

From the increase in specific gravity observed when malt-extract is digested at a moderate temperature, it is evident that the infusion contains a carbohydrate or carbohydrates capable of hydration.

In the year 1875, Kühnemann announced (*Deut. Chem. Ges. Ber.*, 8, 202; *ibid.*, 387) the discovery of cane-sugar among the soluble constituents of barley malt, and, consequently it seemed to us possible that the changes induced in malt-extract by time and elevated temperature might be due to the inversion of this substance.

If the entire alteration in composition were due to this cause, it is evident that the amounts of cane-sugar, separately calculated from the increased density of the solution, and from the increased cupric oxide reducing power respectively, should correspond within the errors of experiment.

Such, however, is found not to be the case, as will be seen from the following results:—

No. of experiments in previous table.	Cane-sugar in grams per 100 c.c., calculated from hydration.	Cane-sugar in grams per 100 c.c., calculated from increase in CuO reduced.
I and II	2·441	1·155
IV and V	3·038	1·364
XVI and XVII	1·250	0·656

In the above experiments there was no deposition of solid matter on digestion.

The changes in optical activity were too small to afford trustworthy data for calculation, but in all cases the decrease in rotatory power was very much less than would correspond to the inversion of an amount of cane-sugar equal to that given in either of the above series of numbers.

It is evident, therefore, that the above-mentioned modifications in the properties of malt-extract are not at any rate entirely due to the presence of cane-sugar. We have convinced ourselves, however, of the accuracy of Kühnemann's observations. A cold aqueous infusion of malt certainly contains a very appreciable quantity of cane-sugar; and that this cane-sugar must, on digestion, be gradually hydrated as shown by the following experiments, which conclusively prove, we believe for the first time, that malt contains an unorganised ferment capable of inverting cane-sugar:—

*Action of Normal Malt-extract upon Cane-sugar.*

*Series I.*—Cane-sugar solution, sp. gr. 1015·26,  $[\alpha]_D$  72·8°; 75 c.c. of sugar solution with 25 c.c. malt-extract.

	$[\alpha]_D$ .	Percentage of invert- sugar.
(1.) Cane-sugar solution digested <i>without</i> addition of malt-extract at 55° for 16 hours . . . . .	72·8°	—
(2.) Digested with malt-extract in the cold for 16 hours. . . . .	69·3	3·5
(3.) Digested with malt-extract at 55° for 16 hours . . . . .	52·6	20·4

*Series II.*—Cane-sugar solution, sp. gr. 1015·78; 75 c.c. digested with 25 c.c. malt-extract. Action in each case continued for 16 hours.

(4.) Digested at 45° with malt-extract previously heated for 20 minutes to 60° . . . . .	66·2°	6·6
(5.) Digested at 45° with malt-extract previously heated for 20 minutes to 66° . . . . .	71·2	1·6
(6.) Digested at 45° with malt-extract previously <i>boiled</i> for 15 minutes. . . . .	72·6	0·2

In No. (2) there was a slight development of bacterial life which may have somewhat influenced the results. The other experiments are free from this objection, not a trace of organic life appearing during the whole time.

It is evident from the results given above, that malt-extract contains an unorganised ferment capable of inverting cane-sugar. This ferment appears to exert its maximum effect at about  $55^{\circ}$  C., its action being very considerably weakened at  $60^{\circ}$ , and almost destroyed at  $66^{\circ}$ . Boiling entirely destroys its invertive power. The organic acids present in malt-extract have no invertive action, the very slight apparent inversion of No. (6) being within the errors of experiment.

We believe that the unstable nature of ordinary cold water malt-extract is mainly attributable to the presence in solution of a small amount of the transformation-products of starch, resulting from the action of the converting agent upon those granules which have been partially disorganised (1) during germination, (2) during the drying of the malt, and (3) during the process of grinding. Under any one of these conditions, as will be shown hereafter, the starch-granules will be attacked by the transforming agent, resulting in the rapid production of maltose, and the lowest achroo-dextrin of the series; the latter body, by the continued action of the malt-extract, being afterwards hydrated. The results obtained on the continued digestion of malt-extract can be readily explained by the simultaneous hydration of a little dextrin and the inversion of cane-sugar.

It is very possible that a cold aqueous infusion of malt may contain other carbohydrates capable of hydration, and derived immediately from the malt, but this is a matter we must leave for further examination. Our object in drawing attention so fully to the changes brought about in malt-extract by heat is mainly to insist upon the great importance, in all experiments on the transformation of starch, of digesting a duplicate sample of the malt-extract under identically the same conditions of temperature, and for the same length of time, as the transformation liquid itself; and of applying to the latter the corrections obtained by an examination of this duplicate sample.

#### *Nature and Properties of Starch and Starch-cellulose.*

Starch-granules are built up of distinctly stratified layers, mainly consisting of a substance which has been called by C. Naegeli *granulose*. The outer layers are, as a rule, denser than those nearer the nucleus, and they increase from without inwards in the amount of water which they contain.

Every part of the granule contains, besides granulose, small quantities of another substance, very closely allied to granulose, which has

been called by recent observers *starch-cellulose*. We find that starch-cellulose preponderates in the outer layers of the granule, if indeed the entire outer coating does not consist of this substance. This outer layer is the oldest in point of growth, the starch-granule increasing by intussusception, *i.e.*, the formative material, absorbed from without during the growth of the starch-grains within the protoplasm of the plant cell, is deposited between the already existing particles in the direction of the surface of the layers (C. Naegeli, *Die Stärkekörner*).

To the presence of this protective coating of cellulose is to be attributed the fact that starch-granules when intact are absolutely unacted upon by cold water. Although water is absorbed to a very large extent by the granulose, none of this substance, which is to the highest degree colloidal, is able to diffuse into the surrounding medium.

When the outer layer of the granule is ruptured by attrition, the contents become much swollen from absorption of water, and a small quantity of matter having all the properties of soluble starch goes into solution. By repeated treatment of the ruptured starch grains with cold water, the greater part of the granulose may be removed, most of the cellulose being left behind in the form of extremely thin layers, which, unlike the granulose, are only coloured by iodine a dirty yellow colour. Granulose itself, and water which has been only for a short time in contact with it, are coloured an intense indigo-blue on the addition of a solution of iodine.

Fr. Schulze (Henneberg's *Journ. Landwirth.*, New Ser., 7, 214) has obtained starch-cellulose by digesting starch for several days at 60° C. with a saturated solution of common salt containing 1 per cent. of hydrochloric acid.

The same substance was also obtained by C. Naegeli, by acting upon starch at 45—55° C. with saliva, which contains an active agent (ptyalin) capable of attacking and dissolving only the granulose (*Die Stärkekörner*, p. 113).

Finally, Fitz has recently observed (*Deut. Chem. Ges. Ber.*, 1877, 282) that certain schizomycetes can easily ferment the granulose of starch-paste, but leave the starch-cellulose unattacked.

We have found that the action of malt-extract upon starch-paste in the cold affords a ready means of obtaining the cellulose of the starch-granule in large quantities. A starch-paste containing from 5 to 6 grams of starch per 100 c.c. is, when quite cold, treated with about one-tenth of its volume of freshly prepared normal malt-extract. The mixture becomes perfectly limpid in from four to eight minutes, and can then be easily filtered. The flocculent matter left on the filter, after being freed from malt-extract and the products of transformation of the granulose, by washing with cold water, consists of the so-called starch-cellulose. The filtered liquid, at first perfectly bright, becomes



in a few minutes turbid from the separation of a further quantity of flocculent matter which does not appear to differ, except in want of structure, from that previously separated by filtration. This further separation of cellulose is due to the fact that soluble starch and the higher dextrins, the first products of the action of malt-extract upon granulose, are capable of holding some of the cellulose in solution; as the soluble starch and the higher dextrins are degraded most of this separates in the insoluble form. The matter left upon the filter consists of fine laminae still retaining the shape of the swollen starch-granules, whilst the precipitated portion is of course amorphous.

Starch-cellulose after separation in this way is perfectly insoluble either in cold water or in water at 70—80° C., nor is it possible to dissolve it in solutions of soluble starch or of the higher dextrins. That it is however capable, under certain conditions, of entering into solution is shown by the facts mentioned above, and by the impossibility of preparing it with a very dilute starch-paste, *i.e.*, 1 to 1·5 grams per 100 c.c.\*

Insoluble starch-cellulose is not acted upon by malt-extract in the cold, nor yet at a temperature of 60°; we have good reason, however, to believe that the soluble modification is acted upon by malt-extract, somewhat slowly it is true in the cold, but rapidly at temperatures from 50° to 78° C. Proof of these statements will be afforded later on.

Boiling with water converts starch-cellulose to a great extent into soluble starch, the solution having all the optical properties of that body, and yielding the characteristic indigo-blue reaction with iodine. By repeated boiling with water and frequent washing, there is left behind a body upon which a continuation of this treatment has no further effect. This body, amounting to about one-fifth of the original substance acted upon, is readily soluble in a solution of potash, under the action of which it is slowly converted into soluble starch on digestion at an elevated temperature.

It is evident that insoluble starch-cellulose consists of at least two substances, one readily acted upon by boiling water, and the other not. Both these bodies must be very closely allied to granulose, since all these substances yield under favourable conditions soluble starch as the first product of their transformation.

The amount of cellulose which we have obtained from well-washed potato-starch by the above process is from 2·0 to 5·5 per cent., accord-

\* C. Naegeli's *granulose* is evidently identical with the *amidone* of Payen and Persoz (*Ann. Chim. Phys.*, **56**, 337, 1834), and with Guérin Varry's *amidine* (*Ann. Chim. Phys.*, **56**, 225); the *amidin tégmentaire* and *amidin soluble* of the latter author being identical with the soluble and insoluble modifications of starch-cellulose.

ing to the previous treatment of the starch during the process of purification. A sample of potato-starch which had been purified by long-continued washing with very dilute potash solution, afforded by the above process 2·46 of cellulose, whilst another sample of the same starch which had been subjected to only a slight treatment with alkali yielded 5·54 per cent.

When starch-cellulose is dissolved in dilute potash solution, the greater part of it can be reprecipitated by the addition of acetic acid, or by passing  $\text{CO}_2$  into the liquid. If the alkaline solution is kept at ordinary temperatures and reprecipitated immediately with acid, about 30 per cent. is found to have been converted into soluble starch. If the solution is digested at a moderately elevated temperature, precipitation with acid indicates the formation of a larger quantity of soluble starch. In one case digestion for half an hour at  $60^\circ$  resulted in the recovery of only 57·3 per cent. of the original quantity of cellulose.

The specific rotatory power of an alkaline solution of starch-cellulose appears to be very variable, but always increases on digestion at an elevated temperature. We have found the specific rotatory power of a freshly prepared solution, calculated on the quantity of carbohydrate present, as low as  $[\alpha]_D 146^\circ$ , increasing on digestion for a few minutes at  $60^\circ \text{C.}$  to  $[\alpha]_D 173\cdot6^\circ$ . The rise of angle under these conditions is due to the conversion of cellulose into soluble starch. The entire optical activity of the solution cannot, however, be due to the presence of this latter substance; the cellulose itself must, when held in solution by potash, exert some effect upon polarised light.

When potato-starch is transformed under the influence of malt-extract at temperatures above  $45^\circ \text{C.}$ , cellulose separates out only when the paste is of a high degree of consistency, and the malt-extract is deficient in quantity; hence it is evident that starch cellulose, at the moment of its separation from granulose, must under these circumstances be readily soluble.

In view of this fact, we have attempted to redissolve starch-cellulose, obtained by the cold process described above, by disseminating it in small quantities through thin starch-paste, and acting upon the mixture with malt-extract at  $65^\circ$ . We have, however, never been successful in obtaining a solution even of a small portion of the cellulose in this way. We believe that there are as well-marked differences between the soluble and the insoluble modifications of starch-cellulose as there are between coagulated and uncoagulated albumin.

*Starch-paste.*—When starch is treated with hot water the contents of the granules, owing to a large absorption of water, swell up enormously, and ultimately rupturing the outer layer, form a very viscous liquid which is known as starch-paste.

Marked differences in the viscosity of this paste are produced by slight variations in the treatment of the starch during the processes of purification and drying. When it has been treated with potash and acid, a paste of less viscosity will be produced, *cæteris paribus*, than when no such treatment has been adopted. We also find that immense variations in the consistency of the resulting paste are produced by slightly altering the manner of drying the starch. Starch which has been dried slowly and at a low temperature will always yield a more viscid solution than if dried quickly at a more elevated temperature. The following experiments made upon different portions of the same sample of potato-starch will illustrate our meaning.

The relative viscosity of the solutions was measured by the weight required to sink in them a disc of very thin glass,  $\frac{5}{8}$  of an inch in diameter.

The paste was made in each case with 3 grams of starch to 100 c.c. of water, but since the samples did not all contain quite the same amount of moisture, the results are corrected for equal weights of dry starch.

No. I. Dried at 50° C. whilst very moist, and afterwards at 100° for 24 hours.

No. II. Dried whilst very moist under the air-pump at ordinary temperatures, and afterwards in the air-bath at 100° for 24 hours.

No. III. Dried under the air-pump and finished at a temperature never exceeding 30° C.

*Relative Viscosity of the Paste made with the above Samples. No. I taken as unity.*

I	.....	1.000
II	.....	2.306
III	.....	3.288

The differences in viscosity are very remarkable and unexpected. If the statement made recently by Whewell (*Chem. News*, **39**, 97) is correct, that the technical value of a starch varies with its power of producing a more or less viscid paste, we have here a fact of considerable importance to the manufacturer of starch, and one to which, as far as we know, attention has not hitherto been directed.

The following experiments were made in order to determine the specific gravity of starch (*i.e.*, granulose + starch-cellulose) when in the form of paste:—

*Experiment I.*—2.2500 grams of perfectly anhydrous starch were gelatinised with boiling water, and the volume approximately made up to 100 c.c. at 15.5°. The total weight of the paste was 100.78, and its specific gravity at 15.5° 1.008.95, consequently the volume was

99.88 c.c., and 100 c.c. of this liquid would contain 2.2527 grams of dry starch. The specific gravity of starch in the form of paste deduced from these numbers is 1.658 at 15.5°.

Another similar experiment gave 1.662. The mean of these two very closely concordant experiments gives 1.66 as the specific gravity of starch in weak solutions.

From this number can be calculated the divisor which has to be used for starch-paste, in order to convert specific gravities into grams of dry substance per 100 c.c. The general formula is—

$$x = 10 \left( 1 - \frac{1}{y} \right),$$

where  $x$  = the required divisor,  $y$  = the specific gravity of the body in solution at 15.5° C. referred to water at the same temperature.

In the case above  $x = 3.976$ .\*

There have been from time to time great differences of opinion as to whether granulose exists in starch-paste in a true state of solution, or merely disseminated through the liquid in a finely divided condition. The possibility of separating a great part of the granulose from a moderately thick paste by the ordinary methods of filtration appears at first sight to favour the latter view, which, however, is not necessarily correct, as will be seen from the following considerations. No one doubts that *soluble starch* is capable of forming a perfect solution with water, yet we have found that a solution of this substance on filtration under slight pressure through a thin septum of earthenware, is to a great extent deprived of its solid matter: a porous diaphragm of this kind allowing free passage to the less colloidal dextrins and all crystalloids. We believe that these and similar phenomena can be explained in the following way. We have reason to believe that the chemical molecule of an organic colloidal substance is, as a rule, much more complex than that of any crystalloid. The same relation probably holds good between the physical "solution-molecules" of the two classes of substances, the solution-molecule being larger the more perfectly colloidal the substance. When the pores of a filtering medium are small enough to bear a sensible relation to the size of the solution-molecule of the substance, this will be impeded in its passage through the filter, whilst other substances having a less solution-molecule may pass through with facility.

Starch-paste is one of the most perfect colloids known, and its solution-molecule being very large, its passage through the fine pores of ordinary filter-paper is rendered difficult. The less perfect colloid,

\* If  $x$  is known and the value of  $y$  is required, the formula becomes  $y = \frac{1}{1 - \frac{x}{10}}$ .

soluble starch, on the other hand, having a less solution-molecule than granulose, passes readily through ordinary filter-paper, but will not pass through the very fine pores of a septum of earthenware, which is, however, traversed by the less colloidal dextrans, and with the greatest ease by all crystalloids.

It appeared to us that the question of granulose existing in starch-paste in suspension or in solution might have some light thrown upon it in a different way.

We know that in most cases of solution the calculated mean of the volumes of the body and its solvent is almost always greater than the observed volume; or in other words, that a substance has a higher specific gravity in solution than when merely in a state of suspension. We have shown that the specific gravity of starch in the form of paste is 1.66. Starch gelatinised with water and precipitated with alcohol, has, after perfect drying, a specific gravity of 1.513. Hence it is extremely probable that granulose exists in starch-paste in a state of true solution.

*Optical Activity of Starch-paste.*—The determination of the optical activity of starch-paste is a matter of considerable difficulty, owing to the want of transparency in the liquid, and the difficulties attending the filtration of so viscous a solution.

It is not possible to obtain good observations with starch paste containing more than 1 gram of substance per 100 c.c., and even less has to be used if the starch has not undergone a lengthy treatment with dilute potash, in order to remove some of the cellulose.

The following results were obtained with gelatinised starch, the amount of matter in solution in No. I being 1.054 gram per 100 c.c., and in No. II, 1.00 gram.

	$[\alpha]_{j3.86}$	$[\alpha]_j$ absolute.
I .....	202.1°	208.8°
II .....	201.1	207.3

On boiling No. II for half an hour, the liquid became much more transparent, and increased decidedly in optical activity.

	$[\alpha]_{j3.86}$	$[\alpha]_j$ absolute.
II after boiling ....	213.3°	219.5°

This increase in angle is due to the greater part of the starch-cellulose being converted into soluble starch.

W. Naegeli found the specific rotatory power of a thin boiled starch-paste  $[\alpha] 198^\circ$ . Since, however, the solutions upon which this estimation was made contained only .16 gram of substance per 100 c.c., we cannot but look upon the approximation to correctness as accidental.

*Action of KHO on Starch-paste.*—When a little of a strong solution

of potash is added to starch-paste, the previously opalescent liquid is rendered perfectly transparent, without any alteration taking place in its viscosity. This increased transparency is brought about by the solution of the suspended integumentary cellulose. The optical activity of the solution is at the same time much reduced by the addition of the first portions of the potash solution, but rises again to its original height, or in some cases a little higher, when the alkali is neutralised by acetic acid or carbonic anhydride. These phenomena are dependent upon the production of a definite compound of starch with potash. Such a body has been described by Schmidt (*Ann. Pharm.*, **51**, 31), and by Ventzke (*Journ. prakt. Chem.*, **25**, 65), but the latter observer has erroneously stated that it has no action on polarised light.

We have obtained the following numbers expressing the specific rotatory power of the starch potash compound. The results are calculated on the quantity of starch present in the combination:—

	Starch with KHO.	After acidification with acetic acid.
	$[\alpha]_{j3.86}.$	$[\alpha]_{j3.86}.$
I .....	172.3°	198.8°
II .....	169.4	200.3
III .....	171.0	—
IV .....	174.2	206.8

In another experiment in which the potash compound was decomposed with CO<sub>2</sub>, the following results were obtained:—

	Starch with KHO.	After decomposing with CO <sub>2</sub> .
	$[\alpha]_{j3.86}.$	$[\alpha]_{j3.86}.$
V .....	166.9°	216.9°

Not a trace of sugar capable of reducing CuO was produced in these experiments, nor was there any considerable production of soluble starch unless the digestion with potash was continued for some time and at an elevated temperature. After acidification the solution remained transparent, and possessed all, or nearly all, the viscosity of the original starch-paste.

When soluble starch is acted upon with potash, the optical activity of the resulting compound is  $[\alpha]_{j3.86}$  182.6°, that of soluble starch itself being  $[\alpha]_{j3.86}$  216.0°.

A body possessing exactly these properties, and perfectly soluble in hot water with complete limpidity, may be separated in small quantities from a boiled starch-paste, by careful fractional precipitation with alcohol. Being somewhat more soluble than granulose, it is found in solution after that substance is no longer soluble in the dilute alcohol. It is no doubt due to the variable quantity of this substance,

soluble starch, and to the variable quantities of starch-cellulose, that is to be attributed the want of closer concordance in the numbers obtained for the optical activities of starch-paste given above. The value of  $[\alpha]_D$  for the pure potash compound of granulose is probably 165—168°.

### *Properties of Maltose.*

When estimating the maltose present in a solution of the transformation products of starch by means of the gravimetrical cupric oxide method, we always found that the calculated value of  $[\alpha]_D$  for the mixture was higher than the specific rotatory power observed. We were for a time unable satisfactorily to explain this want of coincidence. It was evident from its constancy in direction and amount that it could not be due to errors of experiment, neither did it seem probable that it was owing to the production of a little dextrose, otherwise the difference would have shown a constant tendency to increase, which was not the case. The only explanation possible seemed to be either that numbers too high have been taken for the specific rotatory powers of maltose and dextrin, or that the number 65 given by O'Sullivan as the cupric oxide reducing power of maltose is excessive. The latter seemed by far the more probable explanation, since we noticed that the difference between the observed and calculated numbers increased *pari passu* with the percentage of maltose, being greatest in the most complete transformation, and almost disappearing when the maltose only amounted to 10 or 15 per cent.

In order to investigate this important matter, we prepared maltose on several different occasions, using the method recommended by O'Sullivan, viz., exhaustion with alcohol of the syrupy mass obtained by evaporating a solution of dextrin and maltose prepared from starch. For this purpose as complete a transformation as possible should be made under the conditions mentioned later on.

The maltose on crystallising out was washed with alcohol and re-crystallised, until its properties were constant. By a little management this point may be reached on the second crystallisation.

*Solution Density.*—We find that anhydrous maltose has a somewhat higher specific gravity in solution than cane-sugar at an equal density. A solution containing 5.0655 grams of anhydrous maltose per 100 c.c. has a specific gravity at 15.5° of 1.019.915, hence the divisor to be applied to a 5-gram solution in order to calculate the number of grams per 100 c.c. is 3.9314.

*Specific Rotatory Power.*—The specific rotatory power was found in several different experiments to be as follows:—

*Specific Rotatory Power of Maltose.*

	$[\alpha]_{j3.85}$	$[\alpha]_j$ absolute.
(1.) . . . . .	150.7°	—
(2.) . . . . .	150.9	—
(3.) . . . . .	150.1	—
(4.) . . . . .	150.0	—
Mean . . . .	150.4	153.1°

The specific rotatory power according to O'Sullivan is  $[\alpha]_{j3.85}$  150.0°, the experiments he quotes giving as a mean 150.2°, a value almost exactly agreeing with ours.

We have estimated with extreme care the cupric oxide reducing power of several samples of pure maltose, and have invariably obtained a number lower than the usually accepted value 65.

The following are some of our results:—

*Cupric Oxide Reducing Power of Maltose.*

	$\kappa_{3.85}$	$\kappa$ absolute.
(1.) . . . . .	61.0	—
(2.) . . . . .	60.69	—
(3.) . . . . .	60.1	—
(4.) . . . . .	60.67	—
(5.) . . . . .	61.11	—
(6.) . . . . .	61.23	—
Mean . . . .	60.8	61.9

It is possible that these numbers may be a very little too low, but certainly not to the extent of more than .5.

We have adopted 61.0 as the true value of  $\kappa_{3.85}$  for maltose, and find when this number is used in our experiments instead of 65.0°, that the calculated and observed results agree with the greatest possible closeness.

*Action of Dilute  $H_2SO_4$  on Maltose.*—We have investigated with very great care the action of dilute sulphuric acid upon maltose.

The action of dilute acid is stated by O'Sullivan to result in the production of a dextrose, or a mixture of dextroses, having the united optical activity of  $[\alpha]_j + 67.0^\circ$  (*Chem. Soc. J.*, 1876, 2, 139).

Tollens (*Deut. Chem. Ges. Ber.*, 9, 487) has separated from the end products of the action of dilute acid upon starch a dextrose having all the properties of that obtained by the inversion of cane-sugar. The specific rotatory power was found by Tollens to be  $[\alpha]_D$  53.1° =  $[\alpha]_j$  59.1°.



We have prepared the starch-dextrose ourselves, and find its specific rotatory power in 5 to 10 per cent. solutions  $[\alpha]_{D^{86}} 58.65^\circ$ .

Our experiments point to this dextrose being the *only* end product of the hydration of maltose.

We have found that the best conditions for a favourable hydration of maltose by means of acid, are obtained by digesting the solution for a considerable time at a temperature not exceeding  $80-90^\circ \text{C}$ . By keeping the temperature within this maximum limit there is no risk of breaking up the dextrose into ill-defined bodies, giving more or less colour to the solution.

The first stages of hydration take place with considerable rapidity, the solution increasing in specific gravity as its optical activity diminishes. If further proof were required than that already given by O'Sullivan, of maltose being a sugar of the  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$  type, it is afforded by this marked increase in specific gravity, accompanied by a diminution in volume, a certain sign of hydration. In this respect maltose exhibits exactly the same phenomena as cane-sugar when undergoing inversion, the increase in total solid matter per cent. at the close of the two reactions being apparently the same.

The fall in optical activity, which was rapid at first, becomes gradually slower and less marked as the cupric oxide reducing power approaches 100. This value for  $\kappa$  is, however, never reached until the value of  $[\alpha]_D$  for the matter in solution has fallen to  $58-59^\circ$ .

The following experiment was made upon a solution of pure maltose containing, when acidified, 4.8510 grams of the sugar per 100 c.c.; 5 c.c. of dilute (one-fifth acid) sulphuric acid being used for the reaction:—

Time of digestion.	Temperature.	$[\alpha]_{D^{86}}$	$\kappa_{D^{86}}$
1 hour	$70^\circ$	147.2°	
3 hours	„	142.4	—
8 „	80	114.7	—
15 „	„	98.9	—
20 „	85	83.9	—
26 „	„	73.2	97.4
32 „	„	66.6	—
38 „	„	63.6	—
47 „	„	61.3	99.7
53 „	90	58.6	—
59 „	„	58.6	100.2

Although the latter part of the reaction is slow, it does not show any tendency to cease until the final angle of  $58.6^\circ$  and the cupric oxide reducing power of 100 are reached. These are the properties of ordinary dextro-glucose.

*Action of Malt-extract upon Maltose.*—In order to throw light upon the results obtained by the prolonged action of malt-extract upon the transformation-products of starch, it became necessary to investigate the effect of digesting a solution of maltose for a considerable time with an excess of malt-extract.

During this inquiry the same precautions were taken as in ordinary transformations of starch extending over a considerable time, *i.e.*, all the solutions used were carefully weighed and their volume deduced from the specific gravity, whilst duplicate samples of malt-extract were digested for the same length of time as the maltose solution itself, and the corrections applied as usual.

*Experiment I.* 75·47 c.c. maltose solution, sp. gr. 1020·94, digested with 24·53 c.c. of unheated normal malt-extract at 55° C.

	$[\alpha]_{j3.86.}$	$\kappa_{3.86.}$
Original solution . . . . .	151·4°	60·1
After 3 hours at 55° . . . .	148·9	—
„ 16 hours „ . . . .	148·0	57·9

*Experiment II.*—71 c.c. of maltose solution digested with 29 c.c. of normal malt-extract at 63°, the malt-extract having previously been heated to 65° for 20 minutes.

After 20 hours' digestion,  $[\alpha]_{j3.86}$  150·5°,  $\kappa_{3.86}$  60·07.

*Experiment III.*—75·00 c.c. maltose solution, sp. gr. 1027·00, mixed with 25·00 c.c. normal malt-extract previously heated to 55°.

Digestion at 50° C.

	$[\alpha]_{j3.86.}$	$\kappa_{3.86.}$
Original solution . . . . .	150·7°	66·67
After 16 hours at 50° . .	150·5	60·7

From the above experiments, it is evident that malt-extract does not contain any active agent capable of hydrating maltose. Even the acid, always present in small amount, is unable to effect any change.

Although maltose is a sugar of the saccharose group, it is a much more stable body than cane-sugar, which we have seen is decidedly inverted by malt-extract at the lower temperatures.

#### *Action of Malt-extract upon Starch.*

*Action upon Ungelatinised Starch.*—O'Sullivan has shown (*Chem. Soc. J.*, 1876, 2, 133) that malt-extract is incapable of acting upon unaltered starch. We can fully confirm this statement after repeated experiment. Even when the contact of malt-extract and starch-granules has been prolonged to a considerable time, we have never, at ordinary temperatures, obtained any evidence of action.

This fact, at first sight, appears to be at variance with the known

disappearance of starch in seeds during the process of germination, a disappearance which has almost invariably been attributed to the action of diastatic ferments. We therefore considered it necessary to investigate more fully than had been hitherto done the phenomena attending the *metastasis* of starch under these conditions.

Our experiments were conducted upon germinating wheat, barley, and oats. In order to render our results more clear, it will be necessary very briefly to describe the principal parts of the fruit of these cereals.

This consists of a cellular *endosperm* rich in starch and surrounded by a layer of rectangular cells containing the so-called *aleurone grains*, which consist for the most part of proteids.

Immediately around the layer of aleurone cells is the *testa* or envelope of the true seed, and exterior and closely adherent to this the *pericarp*. This combination of fruit and seed is known as a *caryopsis*, and is, in most species of barley, covered with a thick outer skin consisting of the adherent *paleæ*. The paleæ are not adherent to the caryopsis in the case of ordinary wheat, but separate from the grain in the form of chaff.

The *embryo* is enveloped in a cellular mantle which, on the posterior side nearest the endosperm, forms a thick pellate epidermis known as the *scutellum*.

When wheat commences to germinate, the plumula of the embryo on bursting through its envelope finds itself in immediate contact with the inner surface of the pericarp, which in this case, not being sufficiently strong, is ruptured, the plumula continuing its growth outside the grain.

In barley the pericarp is of sufficient strength (even in the case of the so-called naked barley, where the paleæ, as in wheat, are not adherent) to retain the plumula within it. In consequence of this the growth of the plumula takes place between the testa and pericarp, and in very close proximity to the reserve materials of the endosperm, until it finally issues at the end of the grain the farthest removed from the embryo.

The following are the principal physical modifications observed in the endosperm during the growth of the plumula.

The starch-bearing cells in the immediate neighbourhood of the scutellum of the embryo are deprived to a great extent of their protoplasmic lining, and become more or less disintegrated. This action gradually extends in the direction of the length of the grain, being in all cases most marked in the portions of the endosperm nearest an absorptive surface, *i.e.*, the scutellum or the portions of the testa immediately underlying the growing plumula.

The contents of the aleurone cells are considerably modified as the

growth proceeds, and where a grain has been taken in which the cellular structure of the endosperm is of a delicate nature, as in the case of fine barley grown upon light soil, distinct evidence can be obtained of the disappearance and absorption of the cellulose constituting the walls of the more delicate starch-containing\* cells.

It is these changes which mainly constitute the malting process.

The metastasis of the reserve materials other than starch is continued by the growing embryo until these begin to fail or are obtained with great difficulty, and then only are the starch-granules attacked.

We will first consider the case of a barley with an endosperm containing cells, the walls of which are delicate and not much thickened by adhering protoplasm, and where in consequence diffusion can take place with a tolerable amount of ease. In such a case as this little or no action upon the starch is apparent until the plumula has grown considerably beyond the apex of the grain. The first signs of action upon the granule are afforded, as might be expected, by those portions of the endosperm which have been the most thoroughly drained of all other formative material, *i.e.*, those in immediate proximity to the scutellum of the embryo. If the growth of the plumula is allowed to continue, the action upon the starch granules is seen to extend in the same direction as did the first disintegration of the cells of the endosperm, that is immediately subjacent to the plumula and in the direction of its growth.

If the cellular tissue of the barley is strong, and the cell walls have a thick albuminous lining, the metastasis of the reserve materials other than starch is much impeded, and the starch-granules at the base of the endosperm are in consequence disintegrated at a much earlier period than when there is no physical impediment to ready absorption.

In the case of wheat the action upon the starch-granules commences long before the endosperm has been modified to its fullest extent. This is due to two causes, first to the comparative tenacity and strength of the cellular tissue produced by thick cell-walls and the large quantity of protoplasm adherent to them, thus producing great difficulty of diffusion; and secondly to the fact that the plumula, not being retained as in barley between the testa and the pericarp, does not in any way contribute to the absorption of the reserve materials, which have consequently all to be taken up by the comparatively small surface of the scutellum. This results in a draining of the portions of

\* The disappearance of cellulose during the germination of certain seeds has been previously observed by Sachs. The greater part of the reserve-material constituting the endosperm of the date consists of cellulose which is absorbed during germination by the growing plant.

the endosperm nearest to the embryo of all readily assimilated material, and through this tract all the other formative material has to pass before reaching an absorptive surface. Hence it follows that the less easily assimilated starch may in such a case as this be attacked when abundance of other formative material may still exist in the endosperm.

In the case of barley, owing to the plumula being closely applied to the testa through the entire length of the corn, it is evident that the longest distance which has to be traversed by a particle of matter before reaching an absorptive surface is the shortest diameter of the grain, whilst in the case of wheat, it is the whole length of the grain.

In general terms we may state that in germinating cereals the point of time at which the starch-granules are attacked is dependent upon the relative ease with which the more readily absorbed reserve materials can be obtained.

The first evidence of any action upon the starch-granules is generally afforded by the appearance\* of small pits on the surface of the granule, which by-and-bye is traversed radially by fissures, the granulose being at the same time rapidly dissolved. In a comparatively short time there is nothing left of the original granule but a skeleton of starch-cellulose, which retains for a long time the form of the granule, but this, too, at a much later period of the growth of the young plant, is broken up and disappears.

When the outer coating of the granule has been ruptured there can be little doubt that the rapid action upon the granulose is brought about by the diastatic action of the cell-sap. We have made many attempts to obtain this "pitting" of the granule by treating starch in various ways with the aqueous extract of malt, but have, at ordinary temperatures, invariably failed to obtain any result. We are consequently obliged to look upon this phenomenon as one incapable of separation from the living functions of the vegetable cell. We have seen that there are strong indications of an absorption of cellulose by the embryo during the germination of cereals, and it is possible that there is the commencement of a similar action upon the cellulosic envelope of the starch-granule resulting in its partial disintegration and the exposure of the granulose to the solvent action of the cell protoplasm.

Having now ascertained that the integrity of the outer coating of the starch-granule is the cause of its being able to resist the action of the diastatic ferment, it became necessary to study the action of malt-extract upon *bruised* starch. For this purpose starch was triturated with sharp quartz-sand or powdered glass, with the addition of a little

\* The pitting of the starch-granules under the above circumstances was first noticed by C. Naegeli (vide *Die Stärkekörner*).

malt-extract. It was found that starch is very readily acted upon under these conditions.

The optical activity of the resulting transformation products is always very low, and in conjunction with the cupric oxide reducing power, does not always give results compatible with the presence merely of maltose and dextrin.

This cannot be due to the presence of dextrose, since, as a rule, on standing for some time, or on digestion at an elevated temperature, the solutions approach more nearly in optical and chemical properties a mixture of dextrin and maltose.

We attribute the disturbing cause to the presence in solution of a little starch-cellulose, which is gradually transformed into dextrin and maltose in the same way as ordinary starch. We shall see when we come to consider the action of malt-extract upon starch paste in the cold, we have in that case evidence of a similar body entering into solution, and that there is a strong probability of its possessing no optical activity.

The starch was in all cases triturated with sand or powdered glass, and normal malt-extract diluted to  $\frac{1}{10}$ . The malt-extract was treated in duplicate in a similar way to the transformation liquid.

	Grams of matter per 100 c.c. in solution.	Time.	$[\alpha]_{j3.86.}$	$\kappa_{3.86.}$
Experiment I . . . .	1.7303	20 hours	152.0°	40
Maltose . . . . .		65.5		
Dextrin . . . . .		24.9		
Cellulose . . . . .		9.6		
		<hr/>		
		100.0		
Experiment II . . . .	2.8497	6 hours	152.2°	52.7
Maltose . . . . .		86.3		
Dextrin . . . . .		10.5		
Cellulose . . . . .		3.2		
		<hr/>		
		100.0		
The same after 20 hours in the cold. . . . .			152.2°	55.8
Maltose . . . . .		91.4		
Dextrin . . . . .		7.0		
Cellulose . . . . .		1.6		
		<hr/>		
		100.0		
The same heated for 30 minutes to 57°. . . . .			152.4°	59.7

	Maltose .....	97.8		
	Dextrin .....	2.6		
		<hr/>		
		100.4		
	Grams of matter per 100 c.c. in solution.	Time.	$[\alpha]_{3.86}$	$\kappa_{3.86}$
Experiment III ..	3.155	4 hours	155.4°	51.0
	Maltose ....	83.6		
	Dextrin .....	13.9		
	Cellulose .....	2.5		
		<hr/>		
		100.0		
The same after 20 hours in the cold.....			152.0°	54.4
	Maltose .....	89.1		
	Dextrin .....	8.5		
	Cellulose .....	2.4		
		<hr/>		
		100.0		
The same after digestion for 30 minutes at 60°			152.0°	55.3
	Maltose .....	90.6		
	Dextrin .....	7.4		
	Cellulose .....	2.0		
		<hr/>		
		100.0		
Experiment IV ..	1.308	6 hours	131.0°	43.8
The same after lying 24 hours in the cold ..			130.6	51.4
	Maltose .....	84.2		
	Dextrin .....	1.9		
	Cellulose .....	13.9		
		<hr/>		
		100.0		

*Action of Malt-extract upon Starch-paste in the Cold.*

Contrary to what is generally believed, the action of malt-extract upon starch-paste in the cold is very energetic and rapid.

When *normal* malt-extract is added at the rate of from 5 to 10 c.c. to every 100 c.c. of a starch-paste containing from 3 to 4 per cent. of solid matter, complete limpidity ensues in from one to three minutes. The solution is, at this moment, at its maximum point of transparency, and iodine immediately afterwards ceases to give a blue coloration. The brown reaction for erythro-dextrin is, however, somewhat more persistent, seldom disappearing within five or six minutes of the com-

mencement of the experiment. If the solution is now filtered from the undissolved starch-cellulose, the filtrate is found to have a very low optical activity, and a cupric oxide reducing power which is incompatible with the assumption of there being nothing but dextrin and maltose produced from the starch. The filtrate, at first perfectly transparent, again becomes turbid on standing, and the turbidity increases slightly for about half an hour. This precipitate consists of a small quantity of starch-cellulose which has gradually become insoluble as the starch products are more degraded.

The optical activity of the solution is at a minimum two or three minutes after limpidity has taken place, and remains constant for 30 to 45 minutes. At the end of this time, always after the deposition of cellulose has ceased, there is a distinct rise both in specific rotatory power and in reducing power, which attains a maximum in about three hours. The respective values of  $[\alpha]_D$  and  $\kappa$  are now consistent with the presence of dextrin and maltose only.

We have no doubt that the abnormally low values for these factors obtained soon after limpidity is produced are due to a small quantity of starch-cellulose remaining in solution, the subsequent gradual rise of angle resulting from the breaking up of this dissolved cellulose.

The following results, which are also graphically represented in Table II, may be taken as typical of a starch-transformation conducted under the above-mentioned conditions.

3.7 grams of starch and 10 c.c. of normal malt-extract per 100 c.c. of liquid.

Time.	$[\alpha]_{D^{86}}$	$\kappa_{3^{86}}$	Iodine reaction.
5 minutes	154.6°	—	Slightly brown.
15 "	154.6	—	None.
30 "	154.6	—	"
60 "	157.5	44.1	"
3 hours	161.6	49.7	"

The value of  $\kappa$  at the end of 60 minutes indicates the presence of 72.2 per cent. of maltose; if the remainder were dextrin the angle ought to be 167.3° instead of 157.5° actually observed. At the end of three hours, the calculated angle is 162.2° against 161.6° observed.

The separation of a small quantity of starch-cellulose, after the reaction has continued for five or ten minutes, is never accompanied by any appreciable difference in the optical activity of the liquid, a fact which proves that the cellulose in solution has little or no action on polarised light. This is somewhat remarkable, when we consider the comparatively high optical activity possessed by an alkaline solution of starch-cellulose (see page 613).

The final composition of the starch transformation-products ob-



tained by the above process on several different occasions we give below :—

No.	Time occupied in obtaining a maximum angle.	$[\alpha]_{j3.86}$	$\kappa_{3.86}$	Composition.	Calculated optical activity.
I	3 hours	161.6°	49.7	Maltose.. 81.4 Dextrin.. 18.6	162.2°
				100.0	
II	3 hours	161.6	49.7	Maltose.. 81.4 Dextrin.. 18.6	162.2
				100.0	
III	1 hour	161.9	49.0	Maltose.. 80.3 Dextrin.. 19.7	163.0
				100.0	
IV	3½ hours	161.9	—	—	—
V	1 hour	162.9	—	—	—
VI	3½ hours	162.6	—	—	—

The solutions having once obtained the composition indicated above do not sensibly alter for several hours. In about 18 hours a small decrease in optical activity is generally appreciable owing to the dextrin being slightly attacked by the malt-extract.

No. II at the end of 30 hours was found to have the following composition :—

$[\alpha]_{j3.86}$ . . . .	159.5°	Maltose ..	84.4
$\kappa_{3.86}$ . . . . .	51.5	Dextrin ..	15.6
		100.0	

The calculated angle corresponding to this composition is  $[\alpha]_{j3.86}$ , 160.2°.

All our results obtained by the action of malt-extract upon starch paste in the cold have been so extremely constant, and the resulting mixture of the transformation products has proved so very stable in its composition, that we have no hesitation in asserting that there is produced under these conditions a definite molecular decomposition of starch.

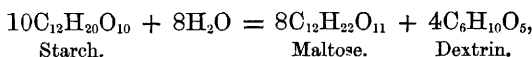
The mean of the above experiments gives the following numbers :—

$[\alpha]_{j3.86}$ . . . . .	162.1°
$\kappa_{3.86}$ . . . . .	49.1

This agrees with the composition—

Maltose .....	80·4
Dextrin* .....	19·6
	<hr/>
	100·0

The equation—



Starch.

Maltose.

Dextrin.

requires for the products of transformation—

Maltose ..	80·9	$[\alpha]_{j3.86}$ ..	162·6°
Dextrin ..	19·1	$\kappa_{3.86}$ ....	49·3
	<hr/>		
	100·0		

These numbers correspond very closely with those obtained in our experiments.

Evidence will be given later on that this is the decomposition towards which all the transformations tend, and that it is the most stable and well-defined of all the decompositions of starch brought about by malt-extract.

*Action of Previously Heated Malt-extract upon Starch-paste in the Cold.*—When to starch-paste in the cold is added malt-extract which has been previously heated and again cooled to ordinary temperatures, the converting action is, as might be expected, considerably weakened. Little difference, however, is observed in the time of producing complete limpidity under these varying conditions, but the higher the temperature to which the malt-extract has been heated, the longer can starch and erythro-dextrin be observed in the liquid, and *ceteris paribus*, the higher is the observed optical activity at any given time.

There is a most notable difference in the relative transparency of the solutions in the early stages of transformations made in the cold with heated and unheated malt-extract respectively. When the malt-extract has been previously heated to 66°, owing to the comparative slowness with which the soluble starch and higher dextrans are broken up, a great quantity of starch-cellulose is induced by these bodies to enter into solution. When unheated malt-extract is employed, the soluble starch and the higher dextrans are almost instantaneously converted, and consequently exert but little solvent action upon the cellulose. In consequence of this, in the earlier stages of the reaction, the transparency of the solution treated with previously heated malt-extract surpasses that of transformations conducted with unheated malt-extract; in the former case, however, there is a greater subsequent separation of the cellulose than in the latter.

\* Throughout this paper the optical activity of the dextrans has been taken as  $[\alpha]_{j3.86}$  216·0°.

*The Action of Malt-extract upon Starch-paste at Elevated Temperatures.*

In order to investigate the action of malt-extract upon starch-paste at elevated temperatures, it became necessary to devise some means of instantaneously arresting, in a portion of the liquid, all further transformation at any desired point of the reaction. The plan which naturally suggested itself was to raise the liquid to the boiling point. This, however, in rapid transformations, was found to yield by no means good results, for, no matter how quickly the solution might be heated, some further transformation of the starch products always ensued before the action of the diastatic ferment could be arrested by coagulation.

We then turned our attention to the action of *salicylic acid*, and found that we possessed in this body a most perfect agent for arresting the transformation, and one moreover which did not in the slightest degree influence the analytical results. The plan we adopted was to measure off samples of the transformation liquid at the desired moment into a small flask containing two or three centigrammes of salicylic acid. The sample was shaken with the acid whilst hot, and afterwards cooled and analysed at leisure. The specific gravity of the solution was always taken in another portion to which the acid had not been added, and which was taken out and merely cooled down rapidly; the increase in total solids, due to the small amount of hydration during this process being inappreciable.

The following experiments illustrate the marked effects of small quantities of salicylic acid in arresting the transformation of starch by malt-extract.

100 c.c. of starch-paste containing 5 grams starch used in each experiment, with 5 c.c. of normal malt-extract, and the specified quantity of salicylic acid.

*Conversion at 58° C.*

Grams of salicylic acid per 100 c.c.	Time of complete limpidity.	Time of disappearance of all soluble starch.	$[\alpha]_{j3.56}$ .
None	3 minutes	6 minutes	—
0·001	3 "	6 "	—
0·010	3 "	7 "	—
0·020	3 "	10 "	—
0·030	3 "	12 "	179·0°
0·035	3 "	1½ hours	191·5
0·040	4 "	2 hours	194·6
0·050	No action	No action	—
0·075	No action	No action	—
0·100	No action	No action	—

We see that under the conditions of the above experiments, quantities of salicylic acid up to 10 mgms. influence the reaction but little, whilst above this amount the retarding effect of the acid increases rapidly. 40 mgms. almost destroy the activity of the diastase contained in 5 c.c. of normal malt-extract, whilst 50 mgms. completely arrest all action.

It is a remarkable fact that as long as the transforming agent possesses any diastatic power, the times of production of complete limpidity in the starch-paste are sensibly equal.

Since the quantity of salicylic acid requisite to arrest all action varies somewhat, as might be expected, with the quality of malt used, we have here a means of estimating the relative converting power of malt, quite independently of another method which we shall point out further on.

Having now obtained a method of effectively arresting the action of malt-extract at any desired moment, it is only necessary to obtain a series of observations at short intervals of time in order to illustrate graphically the progress of a reaction by means of a curve, the points in this curve being determined by the length of rectangular co-ordinates. The line of the abscissæ is divided into equal parts corresponding to the duration of the action in minutes of time, whilst numbers corresponding to the angular specific rotatory powers between  $216^{\circ}$  and  $150^{\circ}$  are represented on the line of the ordinates.

*Modification of the Action of Malt-extract by Heat.*—It is evident that the varying results obtained by treating starch with malt-extract at different degrees of temperature may be due to one or both of two perfectly distinct causes.

These are (1) the direct influence of heat upon the splitting up of the starch molecule, and (2) the modifications brought about by heat in the transforming agent. O'Sullivan at the close of his last paper (*Chem. Soc. J.*, 1876, **2**, 143) touches upon this question, and inclines to the belief that the various observed decompositions are due entirely to the latter cause. Our experiments perfectly bear out this view of the case, for we find in every instance that the particular phase of the reaction is determined by the previous treatment of the malt-extract used, provided this treatment has been at the same or a higher temperature than that at which the transformation is made. For instance, when the results are shown graphically, the transformations conducted at  $60^{\circ}$  and  $66^{\circ}$ , with malt-extract heated respectively to these temperatures, are expressed by two totally distinct forms of curve, which are represented in Tables IV and V. When, however, the malt-extract has been previously heated for a few minutes at  $66^{\circ}$ , and the transformations are afterwards conducted with this malt-extract at temperatures of  $60^{\circ}$  and  $66^{\circ}$  respectively, both curves are found to be identical,

and correspond to that of the higher temperature, as will be seen by a comparison of Tables V and VI.

We may state the proposition in the following general terms for all temperatures above 50° C.:—

*If two different transformations of equal quantities of starch are brought about at different temperatures by equal quantities of a malt-extract, which has been heated for a few minutes to a point identical with or higher than the more elevated of the two temperatures of transformation, then the conversions will be similar in every respect.\**

*Action of Malt-extract at 40° C.*—The action of malt-extract upon starch at 40° differs in several important points from the transformations in the cold.

In the first place, unless the starch-paste is very thick, and the quantity of extract used very small, no separation of starch-cellulose takes place, neither can this body be detected in solution at any point of the reaction, as it is all converted at an early period. Owing to the conversion of the cellulose, at all stages of the transformation the values of  $[\alpha]_D$  and  $\kappa$  are perfectly compatible with the presence of dextrin and maltose only. The fall in optical activity speedily attains its lowest limit, and then remains very constant for a considerable time. The limit of the reaction appears to be exactly the same as that in the cold, viz.,  $[\alpha]_{D, 86}$  162·6°, and  $\kappa_{3, 86}$  49·3, corresponding to the composition—

Maltose .....	80·9
Dextrin .....	19·1
	<hr/>
	100·0

The following transformation may be taken as typical. It is represented graphically in Table III.

5 grams starch, and 10 c.c. of normal malt-extract per 100 c.c. of liquid.

Malt-extract previously heated to 40° for 20 minutes.

Temperature of conversion, 40°.

Time.	$[\alpha]_{D, 86}$ .	$\kappa_{3, 86}$ .	Iodine reaction.
2½ minutes	164·1°	—	Full brown
15     ,,	163·3	—	Brown
30     ,,	163·3	48·8	None

From these last numbers is deduced the following percentage composition of the starch products:—

\* The production of complete limpidity in starch paste, i.e., the conversion of granulose into soluble starch, appears to be favoured somewhat by higher temperatures, and this independently of the previous treatment of the malt-extract.

Maltose .....	80.0
Dextrin .....	20.0
	<hr/>
	100.0

This requires  $[\alpha]_{j3.86}$  163.2° and  $\kappa_{3.86}$  48.8.

Since the cessation of the action in the above experiment might be due to the malt-extract having to some extent lost its power, more malt-extract was now added, at the rate of 10 c.c. per 100, and the solution was digested for a further length of time, with the following result:—

After 60 minutes ....  $[\alpha]_{j3.86}$  161.6°  $\kappa_{3.86}$  49.7

The composition—

Maltose .....	81.3
Dextrin .....	18.7
	<hr/>
	100.0

requires  $[\alpha]_{j3.86}$  162.3° on  $\kappa_{3.86}$  49.7.

The solution could not be digested for any considerable length of time, owing to the extreme rapidity with which fermentation set in. We see, however, that the further production of maltose at the expense of the dextrin is very slow.

*Action of Malt-extract at 50° C.*—When malt-extract previously heated to 50° is made to act upon starch-paste at that temperature, the result of the action is almost exactly the same as when malt-extract heated to 40° acts upon starch-paste at 40°.

The following are the results of such an experiment. They are given graphically in Table III:—

5 grams of starch per 100 c.c.

10 c.c. malt-extract (previously heated for 20 minutes to 50°) per 100 c.c.

Temperature of conversion, 50°.

Time.	$[\alpha]_{j3.86}$	$\kappa_{3.86}$	Iodine reaction.
2½ minutes	168.1°	—	Full brown
5     "	166.5	—	Brown
15    "	166.5	—	"
30    "	162.7	—	No reaction
60    "	162.3	49.6	"

The percentage composition of the liquid at the end of 60 minutes is represented by—

Maltose .....	81.3
Dextrin .....	18.7
	<hr/>
	100.0

which requires  $[\alpha]_{j3.86}$  162.3°, and  $\kappa_{3.86}$  49.6.

On digesting the solution with a further quantity of malt-extract there was at first little or no action, but at the end of 16 hours the following numbers were obtained for the corrected starch-products:—

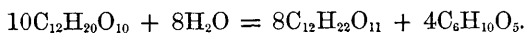
$[\alpha]_{j3.86}$ .....	147.7°
$\kappa_{3.86}$ .....	60.8

These numbers correspond closely with the properties of *maltose*. Hence the whole of the dextrin has been hydrated, but without the production of any *dextrose*.

*Action of Malt-extract at 60° C.*—When malt-extract is heated to 60—62°, its action is found to be somewhat weakened when compared with that of an equal quantity of the same extract heated only to 50°.

At 60°, according to generally received ideas, the transformation of starch ought to take place according to O'Sullivan's Equation A; *i.e.*, we ought to obtain indications of a stoppage of the reaction, when the value of  $[\alpha]_j$  for the mixed products of transformation has fallen to 170°, and the cupric oxide reducing power has reached 44.1°. This, however, is not the case, as will be seen by referring to Table IV, where two transformations at 60° with malt-extract heated to that temperature, are graphically delineated. It will be seen that when 10 c.c. and upwards of malt-extract are used for a normal conversion of 5 grams of starch per 100 c.c., there is no break in the continuity of the reaction, until the final angle of 162—163° is reached, which takes place within a period of time which is, roughly speaking, inversely proportional to the quantity of malt-extract used. It is true that when comparatively small quantities of malt-extract are used, it is possible, by stopping the reaction between about the fifth and twentieth minute, to obtain any desired angle from about 178 to 164°, with the corresponding cupric oxide reducing power; but there is no criterion by which we can judge of the exact moment when to stop the reaction, nor any indication between the points named of any slackening of the reaction which might correspond to a well defined molecular transformation.

The normal transformation of starch-paste at 60° is similar to that which takes place in the cold and at all intermediate temperatures, and is a close approximation to the reaction represented by the equation—



The following are a few of the final results obtained by acting upon starch under the above conditions:—

5 grams starch per 100 c.c.

The malt-extract was previously heated to 60° for 20 minutes.

Temperature of transformation, 60°.

No. of c.c. of malt extract used per 100 c.c.	Duration of the reaction.	$[\alpha]_{j3.86}$	$\kappa_{3.86}$
16.6	5 minutes	162.0°	49.2
20.0	15 "	162.4	49.3
10.0	20 "	162.8	49.0
15.0	20 "	163.8	49.3
10.0	60 "	162.9	49.6
15.0	60 "	162.5	50.5
10.0	15 "	162.3	49.3
10.0	60 "	163.6	48.9
Mean . . . .		162.8	49.3

The mean values of  $[\alpha]_j$  and  $\kappa$  in the above reactions, taken at hazard from a large number of experiments, correspond very closely with those demanded by theory:—

$$\begin{array}{ll}
 [\alpha]_{j3.86} & \dots\dots\dots 162.6^\circ \\
 \kappa_{3.86} & \dots\dots\dots 49.3
 \end{array}$$

We now give the details of some experiments which show the progress of the transformation as a function of the time; the action having been in each case arrested as described, with salicylic acid. These results are also represented graphically in Table IV.

*Experiment I.*—6 grams starch and 5 c.c. of malt-extract (previously heated to 60°) per 100 c.c. of liquid.

Temperature of conversion, 60°.

Time.	$[\alpha]_{j3.86}$	Iodine reaction.
1 minute	—	Blue
2½ minutes	189.6°	Violet
5 "	178.7	Full brown
15 "	166.3	Very light brown
30 "	164.1	None
60 "	163.4	"

At this point more malt-extract was added at rate of 5 c.c. per 100.

90 minutes      162.2°

More malt-extract added at rate of 5 c.c. per 100.

120 minutes      161.7°

More malt-extract at rate of 4 c.c. per 100.

135 minutes      161.2°

*Experiment II.*—Per 100 c.c. of liquid.

5 grams starch.

10 c.c. malt-extract, previously heated to 60°.

Temperature of conversion, 60°.



Time.	$[\alpha]_{53.86}$ .	$\kappa_{53.86}$ .	Iodine reaction.
1 minute	191.7°	—	Pure blue
2½ minutes	175.6	—	Brown
5 „	166.8	—	Very light brown
15 „	165.7	—	None
30 „	163.7	—	„
60 „	162.9	49.6	„

More malt-extract added at rate of 4.5 c.c per 100.

90 minutes	161.3°	—
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More malt-extract at rate of 4.2 c.c. per 100.

120 minutes	160.1°	52.0
-------------	--------	------

Continued digestion and the addition of further quantities of the malt-extract have in the above experiments only reduced the optical activity slightly below the point corresponding to the theoretical transformation, a proof that the cessation of the reaction is not due to a weakening of the diastatic power of the malt-extract. Neither is this cessation due to the converting agent being paralysed by the excess of maltose produced, for we find, at the end of such a transformation, that the liquid is fully able to reduce to its lowest terms of maltose and dextrin a further quantity of starch.

*Action of Malt-extract heated to 66° C.*—When malt-extract is heated rapidly to 66°, and added at once to starch-paste at the same temperature, the reaction varies very little in its first phases from one obtained at 60°, with malt-extract heated to 60°. If, however, the previous heating of the malt-extract is continued *for a few minutes*, its mode of action is considerably modified. A prolongation of the heating for from 10 to 15 minutes at 66°, is sufficient to induce the maximum amount of change in this respect; beyond this point further heating at the same temperature results in but little alteration.

On Table VII are shown graphically three different transformations of starch-paste at 66°, with malt-extract heated previously to 66° for 20, 40, and 60 minutes respectively. It will be seen that the extra time of heating the extract beyond 20 minutes has had but little effect upon the results.

The general character of starch transformations conducted with malt-extract heated to 66°, is much greater slowness of the reaction as compared with those conducted at 60°, the rapidity of the action being measured by the fall of angle in a given time.

The conversion of granulose into soluble starch, as indicated by the production of complete limpidity, is, however, produced fully as rapidly at 66° as at 60°, and with decidedly greater rapidity than at 40° to 50°.

When the iodine reactions obtained in transformations at  $66^{\circ}$  and  $60^{\circ}$  respectively are compared at points corresponding in the two cases to similar optical activities, there is found to be no correspondence between them.

In transformations at  $66^{\circ}$  the total disappearance of the iodine reaction takes place, when the specific rotatory power of the starch products has fallen to  $188-189^{\circ}$ ; whilst in transformations at  $60^{\circ}$  iodine very frequently gives a coloration indicative of erythro-dextrin, or even of soluble starch, when the angle has fallen as low as  $165^{\circ}$  to  $166^{\circ}$ . We believe that this want of correspondence is due to there being a difference in the action of the various transforming agents, contained in malt-extract, upon the dextrins. For instance, certain of the transforming agents contained in malt-extract heated only to  $60^{\circ}$ , are capable of splitting up and degrading the higher achroo-dextrins more rapidly than the erythro-dextrin can be produced or be converted into these higher achroo-dextrins; consequently we may obtain under these conditions a very low optical activity, and yet have a little starch or erythro-dextrin present. On the other hand, when malt-extract is heated to  $66^{\circ}$ , the transforming agent which degrades the higher achroo-dextrins is rendered much less active, whilst the agent bringing about the conversion of soluble starch into erythro-dextrin, and erythro-dextrin into the higher series of the achroo-dextrins, has its activity unimpaired, or even slightly increased. In this latter case it is easy to see that the disappearance of the erythro-dextrin and soluble starch will take place in conjunction with a much higher optical activity than in the former case.

In transformations at  $66^{\circ}$ , as is the case at the lower temperatures also, there is no break in the continuity of the reaction, consequently any desired angle can be obtained by stopping the transformation at the right point, the analytical results of course varying with the duration of the reaction. There is, however, always a very close approximation to an angle of  $188-189^{\circ}$ , when iodine ceases to give any brown coloration.

The numbers obtained for the cupric oxide reducing power and specific rotatory power of the transformation products are at all periods of the reaction closely compatible with the presence of dextrin and maltose only.

The following results bear out this statement :—

	$[\alpha]_{j3.86}$	$\kappa_{j3.86}$	Maltose per cent.	Dextrin per cent.	$[\alpha]_{j3.86}$ calculated.
I .....	188.3°	25.9	42.4	57.6	188.0°
II .....	193.4	19.2	31.4	68.6	195.2
III .....	182.2	33.5	55.7	44.3	179.3
IV .....	192.6	22.6	37.0	63.0	191.0
V .....	190.3	23.4	38.3	61.7	190.7

The following experiments illustrate the progress of the reaction at 66° with malt-extract previously heated to that temperature. They are represented graphically with others of a similar nature in Tables V, VI, and VII, and were conducted in the usual way, the reaction being arrested at the desired points with salicylic acid.

*Experiment I.*—Per 100 c.c. of liquid.

6 grams starch.

8.92 c.c. malt-extract, heated to 66°.

Temperature of conversion, 65°.

Time.	$[\alpha]_{j3.86}$	Iodine reaction.
2½ minutes	204.0°	Full brown
5     "	201.2	Brown
10    "	195.5	Light brown
20    "	191.8	Very light brown
30    "	188.2	No reaction
60    "	185.0	"

More malt-extract added at rate of 4.3 c.c. per 100.

90 minutes     178.0°

More malt-extract at rate of 4.3 c.c. per 100.

120 minutes    173.0°

*Experiment II.*—13.04 c.c. of malt-extract (heated to 66°) per 100 c.c.

Time.	$[\alpha]_{j3.86}$	Iodine reaction.
2½ minutes	205.4°	Full brown
5     "	199.5	Light brown
10    "	195.2	Very light brown
20    "	191.0	"     "
30    "	187.0	No reaction
60    "	181.2	"

6.4 c.c. malt-extract added per 100 c.c.

90 minutes     176.9°

6.0 c.c. malt-extract added per 100 c.c.

120 minutes    171.8°

*Experiment III.*—25 c.c. malt-extract per 100 c.c.

Time.	$[\alpha]_{j3.86.}$	Iodine reaction.
2½ minutes	199·8°	Brown
5 „	195·9	Light brown
10 „	188·3	No reaction
20 „	186·4	„
30 „	182·6	„
60 „	176·9	„

9·7 c.c. more malt-extract added per 100 c.c.

90 minutes	170·7°
------------	--------

8·2 c.c. more malt-extract added per 100 c.c.

120 minutes	165·5°
-------------	--------

7·2 c.c. more malt-extract added per 100 c.c.

150 minutes	163·9°
-------------	--------

*Action of Malt-extract at Temperatures higher than 66°.*—When malt-extract is heated to temperatures higher than 66° its action upon starch becomes still further modified. This modification arrives at a maximum at about 75° or 76°. When 20 to 30 c.c. of malt-extract previously heated to this temperature are allowed to act upon a starch-paste containing 5 to 6 grams of starch per 100 c.c., the fall in optical activity proceeds with great regularity, and with tolerable rapidity for 10 or 12 minutes, but at the end of this time the reaction either continues with extreme slowness, or altogether ceases.

The minimum angle attained is seldom much lower than 195°, and as a rule the addition of a further quantity of malt-extract similar to that at first employed reduces the optical activity, but very slightly, even on long digestion.

The following are the results of some experiments conducted in this manner.

*Experiment I.*—Per 100 c.c., 5 grams starch. Conversion at 75°. 30 c.c. of malt-extract heated to 76°.

Time.	$[\alpha]_{j3.86.}$	$\kappa_{9.86.}$	Iodine reaction.
2½ minutes	205·0°	—	Much erythro-dextrin
5 „	200·3	—	„ „
10 „	196·5	—	Slight coloration
20 „	195·5	—	No reaction
30 „	195·5	—	„
45 „	194·6	—	„
60 „	194·6	23·0	„

15 c.c. of same malt-extract added per 100 c.c.

70 minutes	194·4°
90 „	194·0

*Experiment II.*—Per 100 c.c., 5 grams starch. 25 c.c. malt-extract heated to 76°. Conversion at 75°.

Time.	$[\alpha]_{D^{25}}$	$K_{25}$	Iodine reaction.
2½ minutes	205.0°	—	Strong reaction for erythro-dextrin
5 „	204.0	—	„ „ „
10 „	200.3	—	Less erythro-dextrin
20 „	197.9	—	Small quantity of erythro-dextrin
30 „	197.9	—	„ „ „
60 „	195.8	21.0	No reaction
10 c.c. of same malt-extract added per 100 c.c.			
70 minutes	194.4°		„
90 „	191.4		„

These experiments, together with others, are given in a graphical form in Tables VIII and IX. Where the malt-extract was not sufficiently heated there was sometimes a sudden fall of angle on adding further quantities, as shown in No. 18, Table IX.

Under the above conditions limpidity of the starch-paste ensues very quickly, and the blue or violet reaction with iodine, indicative of the presence of starch, always entirely disappears in less than two minutes from the commencement of the experiment; these changes in fact taking place with as great, if not somewhat greater, rapidity than at lower temperatures. The iodine reaction for erythro-dextrin is, however, very persistent. The full brown colour, indicating the maximum production of this body, is obtained when the optical activity of the solution has fallen to 202—203°, the complete disappearance of the reaction only taking place at 194°, or a little lower.

According to O'Sullivan, at temperatures from 68° to the point at which the transforming agent is destroyed, starch under the influence of malt-extract affords a dextrin which is not coloured by iodine, and which corresponds to an angle, for the joint transformation products, of 202.8°. We have made very many attempts, varying the method of experiment in all possible ways, to obtain evidence of the existence of such a dextrin, without success. When an angle of 202° to 203° is obtained the solution always gives with iodine the strongest possible brownish-red action, the maximum coloration occurring between these points. We can therefore only conclude that the dextrin of O'Sullivan's equation C is an erythro-, and not an achroo-dextrin. Erythro-dextrin, or  $\alpha$ -dextrin, according to the nomenclature of Bondonneau and O'Sullivan, is consequently one of the ordinary transformation-dextrins proceeding from the degradation of soluble starch, and not merely an isomeric modification of that body, as has been generally assumed. We are strengthened in this belief

by the following considerations. It is evident if erythro-dextrin were only an isomeric modification of soluble starch, that it ought to be possible to obtain it from soluble starch without the simultaneous product of maltose. As a matter of fact, however, in every reaction which we have studied, a change in the iodine reaction from the pure blue of soluble starch, indicating the appearance of a trace of erythro-dextrin, is invariably accompanied by the appearance of cupric oxide reducing power in the liquid which previously was unacted upon by Fehling's solution.

*Iodine Reactions.*—We may here call attention rather more fully to the changes in the iodine reaction observed during the transformation of starch with malt-extract. These changes are best observed in reactions conducted at 66°.

At the moment of complete limpidity iodine gives a deep indigo-blue reaction, which gradually changes as the transformation proceeds, through violet to a deep reddish-brown. This last reaction, intense at first, gradually dies away, until iodine ceases to give any further coloration.

We have satisfied ourselves that these changes of colour from blue to brownish-red are produced by mixtures of the two bodies, *soluble starch* and *erythro-dextrin*. When these substances are isolated and their solutions mixed in varying proportions, on adding iodine all the differences of tint can be obtained which are observed in a starch transformation.

The iodine compounds of these two bodies do not yield, when examined in the spectroscope, any absorption bands, but cut off opposite ends of the spectrum.

Owing to the greater affinity of soluble starch for iodine a trace of that body can be detected in the presence of an excess of erythro-dextrin by adding only a very small quantity of iodine; the violet or blue reaction under these circumstances appearing before the brown of the erythro-dextrin. On the other hand, the affinity of erythro-dextrin for iodine is less than that of the achroo-dextrins.

During the entire progress of starch transformations at 76° we find no indications of the production of any other body besides dextrin and maltose, the angle calculated from the cupric oxide reducing power always agreeing fairly with the observed specific rotatory power. The coincidence is, however, not quite so close as at the lower temperatures, a fact which we are inclined to attribute to the higher dextrins being slightly degraded by the action of the Fehling's solution, a little too much maltose being consequently obtained.

The following are some examples of transformations at 76° arrested at different points:—

	$[\alpha]_{j3.86}$	$\kappa_{3.86}$	$[\alpha]_{j3.86}$ calculated from $\kappa$ .
(1.) . . . . .	204.1°	9.4	205.8°
(2.) . . . . .	198.7	17.9	196.6
(3.) . . . . .	195.9	20.5	193.8
(4.) . . . . .	194.1	22.3	191.8
(5.) . . . . .	194.6	23.0	191.1
(6.) . . . . .	195.4	18.7	195.8
(7.) . . . . .	192.1	23.8	190.2

*Influence of Neutralisation upon the Action of Malt-extract.*—When malt-extract, after being heated to 66° for a few minutes, is fully neutralised with *barium hydrate* and filtered from the resulting precipitate, its subsequent action upon starch-paste is found to be somewhat weakened, although the nature of the transformation curve does not materially differ in general form from that obtained with unneutralised malt-extract heated to 66°. This will be seen on comparing the Curve No. 19, Table X, with the results given on Table VI, where about an equal quantity of unneutralised extract was employed.

If instead of using barium hydrate the malt-extract after being heated to 66° is rendered very slightly alkaline with *sodium carbonate* and then momentarily heated to 66°, the transforming agent is still capable of rapidly reducing the starch products to a specific rotatory power of 194—196°, but beyond this point it is impossible to push the reaction even by repeated addition of the same malt-extract. Malt-extract treated in this way behaves in fact towards starch exactly in a similar manner to malt-extract heated to 75—76°.

The following experiments illustrate these statements. The results are given graphically in Table X:—

*Experiment I.*—Per 100 c.c.

Starch 5 grams.

16 c.c. malt-extract heated to 66°, and neutralised with  $\text{Na}_2\text{CO}_3$ .

Temperature of reaction 65°.

Time.	$[\alpha]_{j3.86}$	Iodine reaction.
2½ minutes	214.0°	Blue
5     "	201.0	Brown
10    "	200.0	"
20    "	193.9	None
30    "	193.9	"
60    "	193.9	"
8.4 c.c. of same malt-extract added per 100 c.c.		
90 minutes	194.0°	"
8 c.c. of same malt-extract added per 100 c.c.		
120 minutes	194.0°	"

*Experiment II.*—Per 100 c.c.

5 grams starch.

19.8 c.c. of malt-extract treated as before with  $\text{Na}_2\text{CO}_3$ .

Temperature of reaction  $65^\circ$ .

Time.	$[\alpha]_{j3.86}$ .	Iodine reaction.
$2\frac{1}{2}$ minutes	$205.3^\circ$	Full brown
5     ,,	201.0	”
10     ,,	197.8	Light brown
20     ,,	196.0	”
30     ,,	195.6	None
60     ,,	194.7	”
10 c.c. of same malt-extract added per 100 c.c.		
90 minutes	$194.2^\circ$	”
8.7 c.c. of same malt-extract added per 100 c.c. ,,		
120 minutes	$192.3^\circ$	

When malt-extract heated to  $66^\circ$  is rendered *very slightly* alkaline with *sodium hydrate* the final angle of the starch products is not reduced below  $202^\circ$ , a point corresponding with the maximum iodine reaction for erythro-dextrin in all transformations with malt-extract heated to  $66$ — $76^\circ$ . Subsequent frequent additions of the same malt-extract do not bring down the angle, as is shown in the following experiment, which is also given graphically in Table XI:—

Per 100 c.c.

5 grams starch.

25 c.c. malt-extract heated to  $66^\circ$ , and rendered *very slightly* alkaline with  $\text{NaHO}$ .

Time.	$[\alpha]_{j3.86}$ .	Iodine reaction.	
$2\frac{1}{2}$ minutes	$207.8^\circ$	Violet.	Much starch
5     ,,	204.8	Full brown.	A little starch
10     ,,	204.0	Full brown.	No starch
20     ,,	202.0	”	”
30     ,,	202.0	”	”
60     ,,	202.0	”	”

9.3 c.c. of same malt-extract added per 100 c.c.

90 minutes      $203.0^\circ$      Full brown.   No starch

8.2 c.c. of same malt-extract added per 100 c.c.

120 minutes      $203.0^\circ$      Full brown.   No starch

If the malt-extract is rendered more than slightly alkaline with soda its diastatic power is quite destroyed.



In all experiments with neutralised malt-extract the modifications of its action were always attended with a distinct precipitation of albuminoids.

*The Molecular Transformations of Starch.*—We believe that the experiments described substantiate the existence of at least four well-defined molecular transformations of starch; by far the best defined and the most stable being the ultimate reaction most easily obtained with malt-extract heated not higher than  $60^{\circ}$ , and having  $162.6^{\circ}$  for the specific rotatory power of its products, and  $49.3$  for the cupric oxide reducing power.

The next fixed point is that of the disappearance of the iodine reaction for erythro-dextrin when starch is transformed with malt-extract heated to  $66^{\circ}$ . This takes place when the value for  $[\alpha]_{j3.86}$  is  $188.5^{\circ}$ , and for  $\kappa$   $25.0$  or thereabouts, and is very constant with varying quantities of malt-extract.

The next strongly marked reaction is that obtained with malt-extract heated to  $75^{\circ}$ , and also with malt-extract heated to  $66^{\circ}$ , and rendered alkaline with carbonate of soda; the numbers obtained being  $[\alpha]_{j3.86}$   $195$ — $196^{\circ}$ , and  $\kappa_{3.86}$   $18.9$ .

The highest stable transformation established by our experiments is obtained by the action of malt-extract heated to  $66^{\circ}$ , and made very slightly alkaline with soda, the same reaction being also marked in all transformations above  $66^{\circ}$  by the appearance of the maximum iodine coloration for erythro-dextrin. The numbers for this transformation,  $[\alpha]_{j3.86}$   $202$ — $203^{\circ}$  and  $\kappa$   $12.7$ , are very nearly those of O'Sullivan's equation C, the dextrin being, however, an erythro-dextrin.

*Transformation of the Higher into the Lower Equations.*—When the products of a transformation of starch, having an optical activity higher than  $162.5^{\circ}$ , are treated with a little unheated malt-extract at  $50$ — $60^{\circ}$ , the specific rotatory power of the matter in solution falls very rapidly to  $162.5^{\circ}$ , the cupric oxide reducing power at the same time increasing to about  $49.3$ , at which points these factors remain for a time constant. This change, under favourable conditions, takes place *per saltum*, and not by any slow and gradual act of hydration, as will be seen in the following experiments:—

A conversion of starch with malt-extract previously heated to  $65^{\circ}$  was made in the usual way, the reaction being stopped by boiling in about ten minutes from the commencement. The starch products gave on analysis, after correction, the following numbers:—

$[\alpha]_{j3.86}$ .....	$187.8^{\circ}$
$\kappa_{3.86}$ .....	$28.9$

corresponding to a percentage composition of

TABLE I.

Curve for cane sugar, showing the divisor to be used for solutions of sp. gr. of 1,000 to 1,115 (water = 1,000) in order to obtain the number of grms. of dry sugar per 100 c.c. of liquid at 15.5° C.

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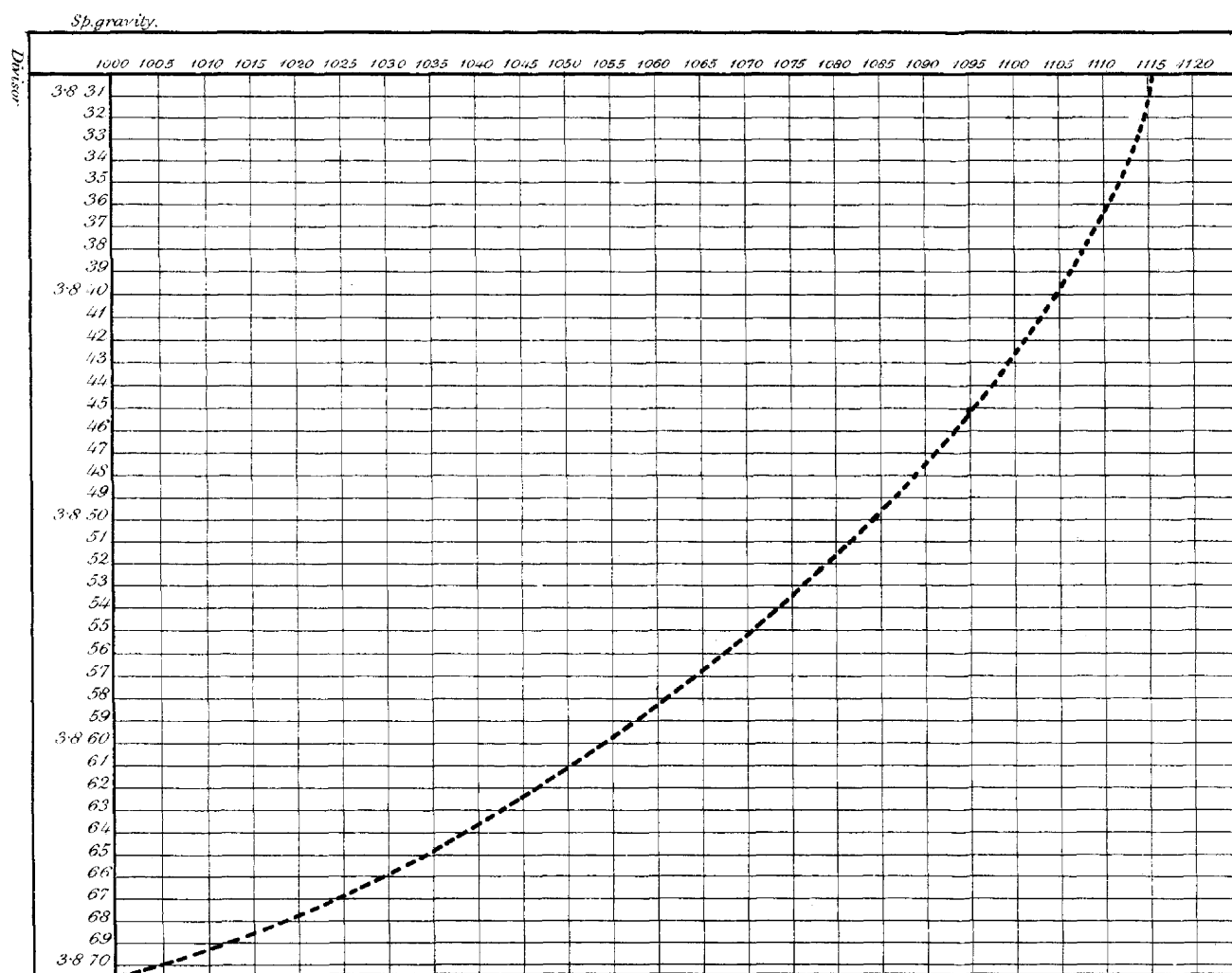


TABLE II.

Action of (unheated) malt extract on starch paste in the cold.

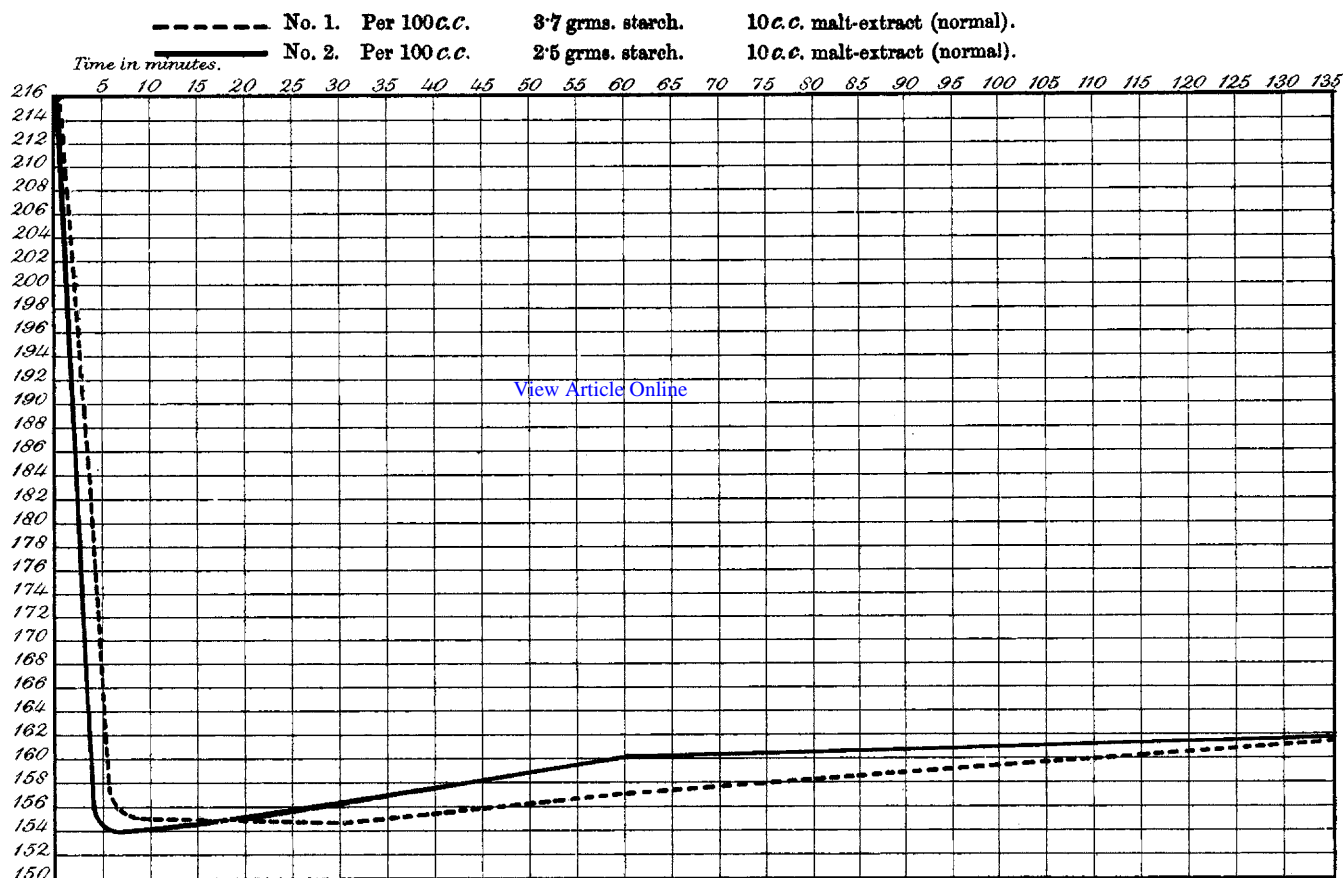
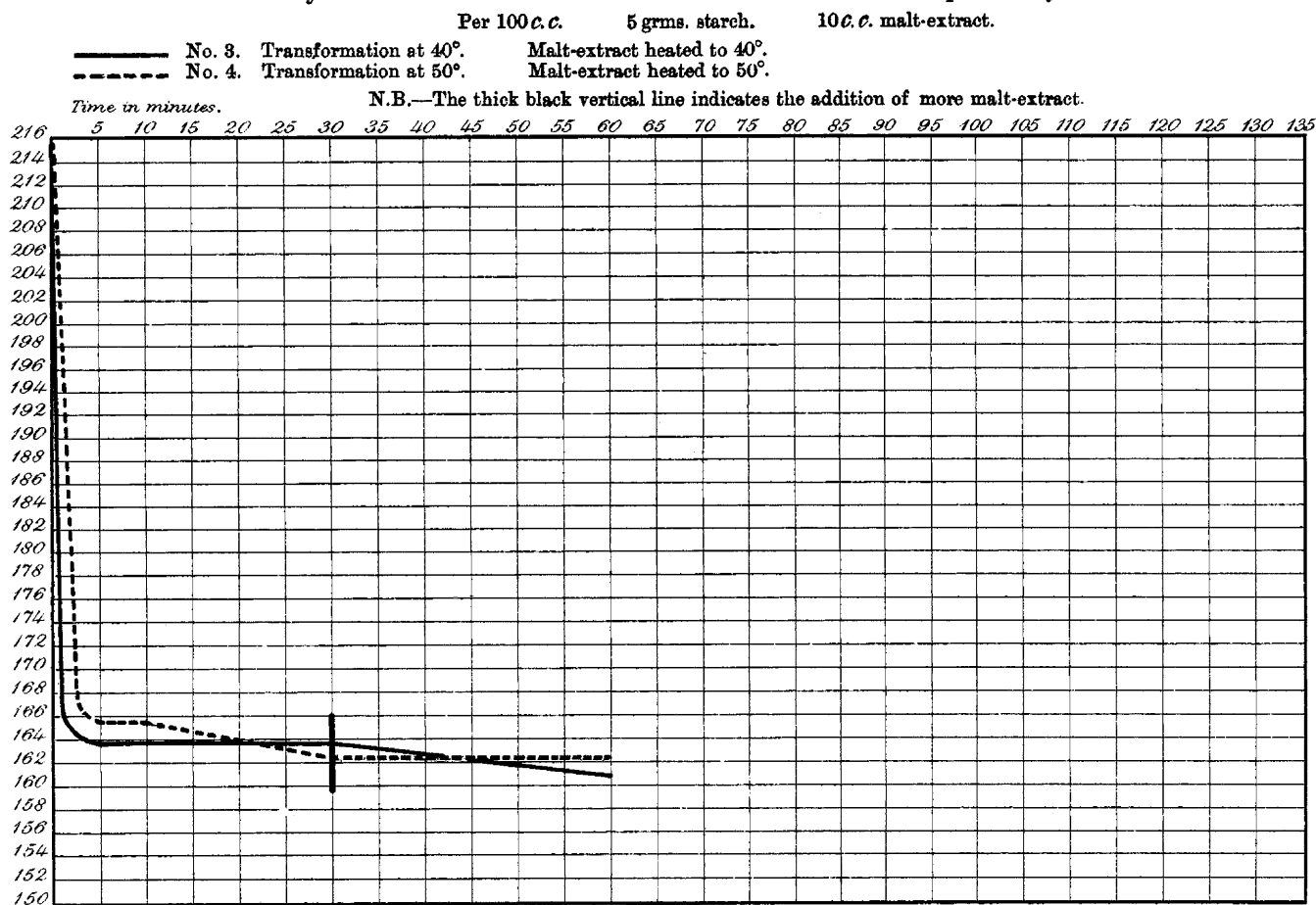


TABLE III.

Transformations at 40° and 50° with malt-extract heated to those temperatures for 20 minutes.



*Transformations at 60° with malt-extract previously heated to 60° for 20 minutes.*

————	No. 5.	Per 100 c.c.	5.5 grms. starch.	10 c.c. malt-extract.
-----	No. 6.	Per 100 c.c.	5.5 grms. starch.	5 c.c. malt-extract.

N.B.—The thick black vertical lines indicate the addition of more malt-extract.

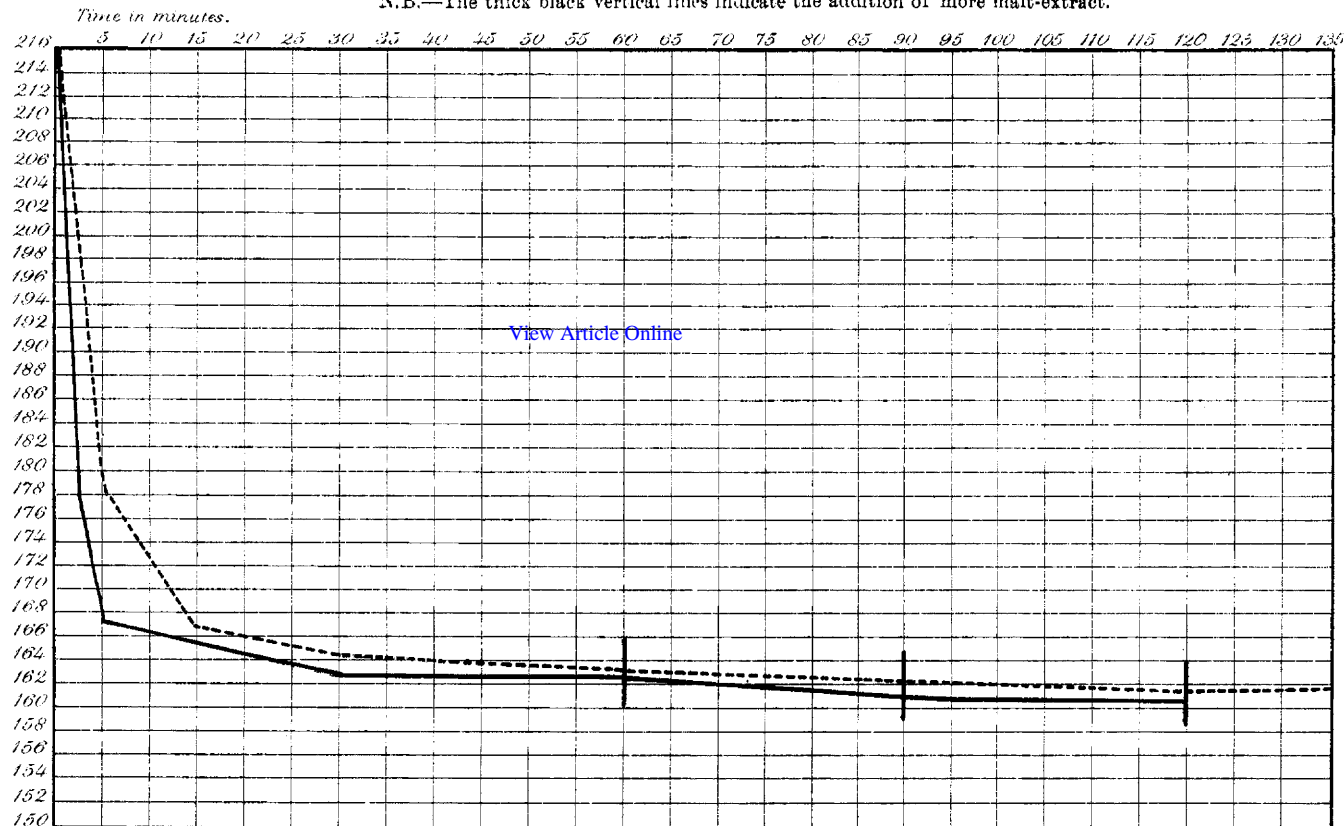


TABLE V.

*Transformations at 66° with malt-extract previously heated to 66° for 20 minutes.*

Per 100 c.c. 6 grms. starch. 8.92 c.c. malt-extract.

N.B.—The thick black vertical lines indicate the addition of more malt-extract.

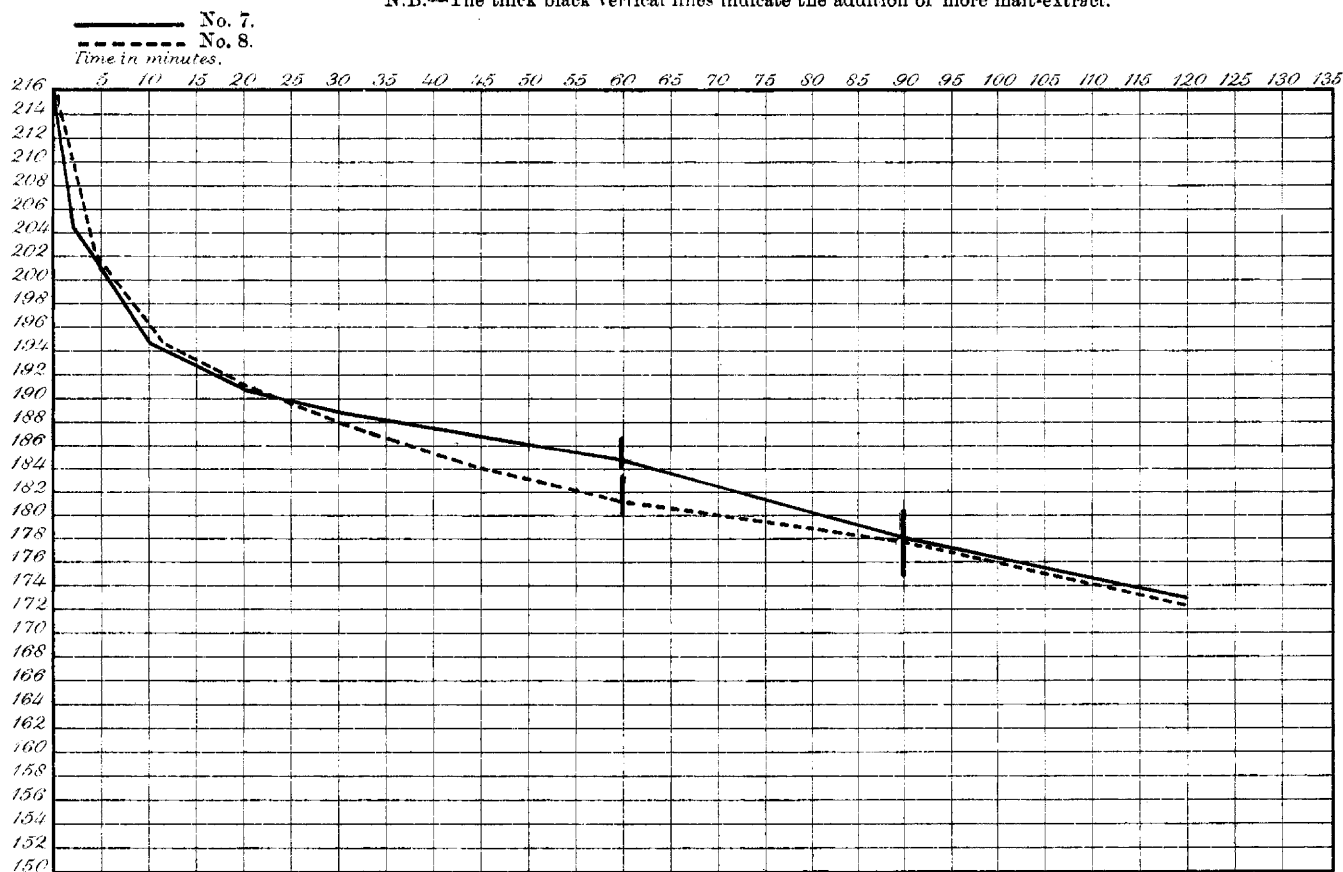


TABLE VI.

Transformations at 66° and 60°.

- No. 9. Malt-extract previously heated to 66° for 20 minutes, conversion at 66°.  
 - - - No. 10. Malt-extract previously heated to 66° for 20 minutes, conversion at 60°.

Per 100 c.c. 5 grms. starch. 15 c.c. malt-extract.

N.B.—The thick black vertical lines indicate the addition of more malt-extract.

Time in minutes.

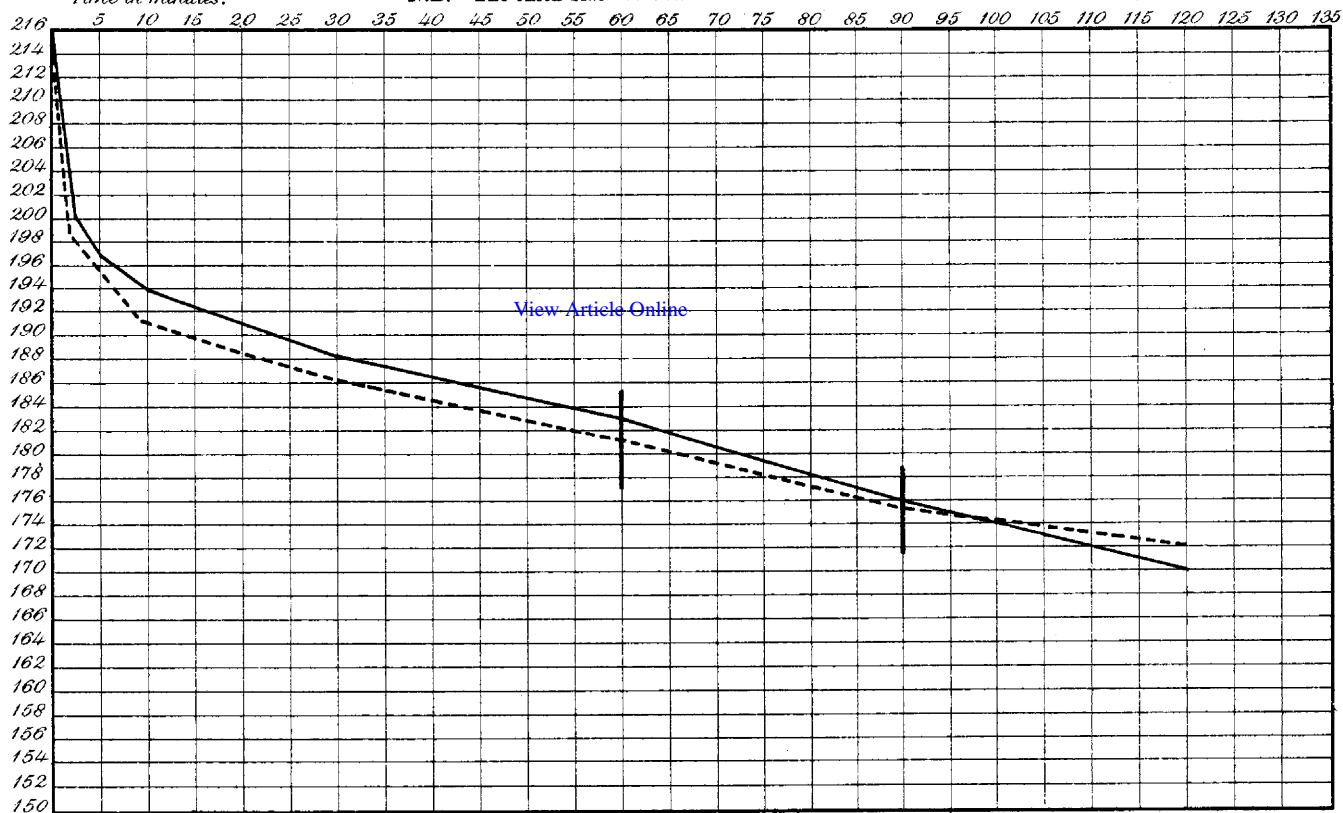


TABLE VII.

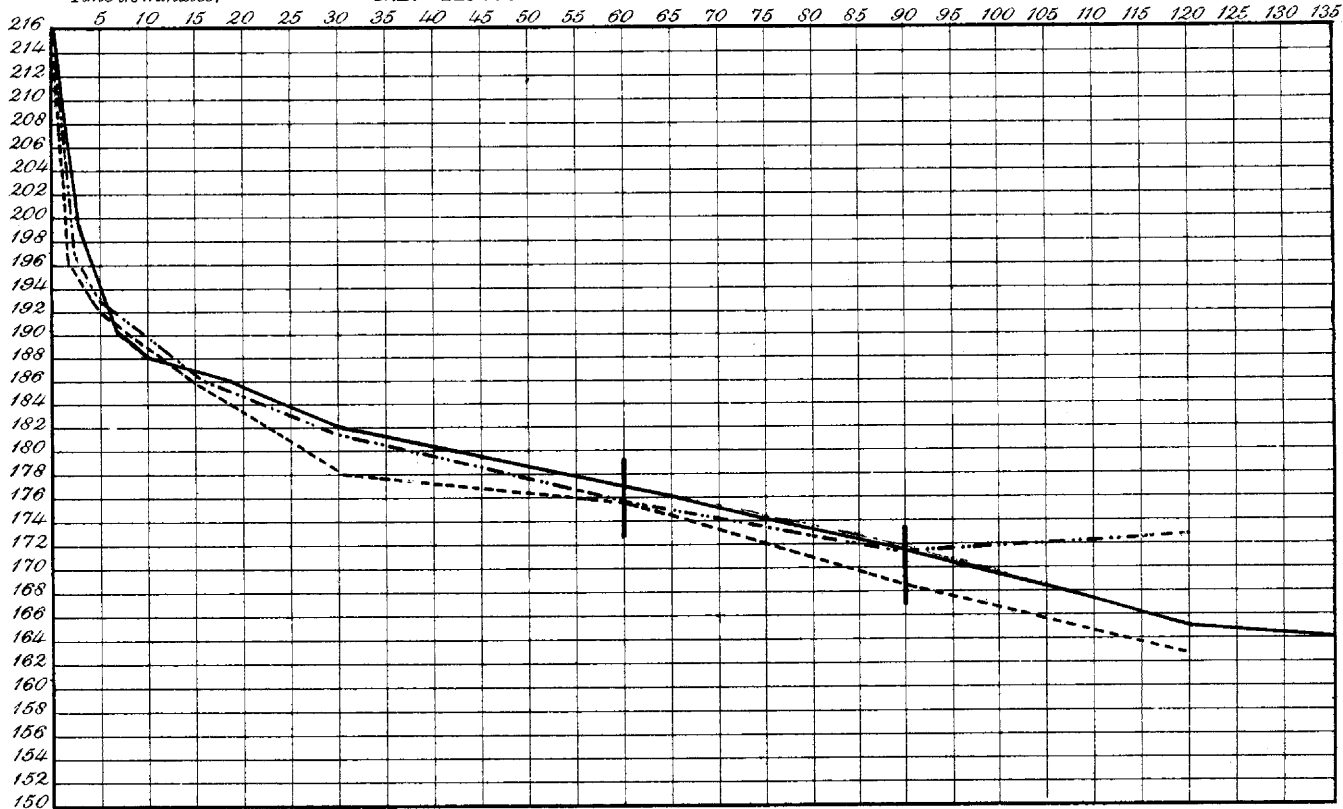
Transformations at 66° with malt-extract heated to 66° for different periods.

- No. 11. Malt-extract heated to 66° for 20 minutes.  
 - - - No. 12. Malt-extract heated to 66° for 40 minutes.  
 . . . . . No. 13. Malt-extract heated to 66° for 60 minutes.

Per 100 c.c. 5 grms. starch. 25 c.c. malt-extract.

N.B.—The thick black vertical lines indicate the addition of more malt-extract.

Time in minutes.



*Transformations at 75° with malt-extract previously heated to 76° for 10 minutes*

————— No. 14. }  
 - - - - - No. 15. } Per 100 c.c. 4 grms. starch. 20 c.c. malt-extract.  
 - · - · - No. 16. }

Time in minutes.

N.B.—The thick black vertical lines indicate the addition of more malt-extract.

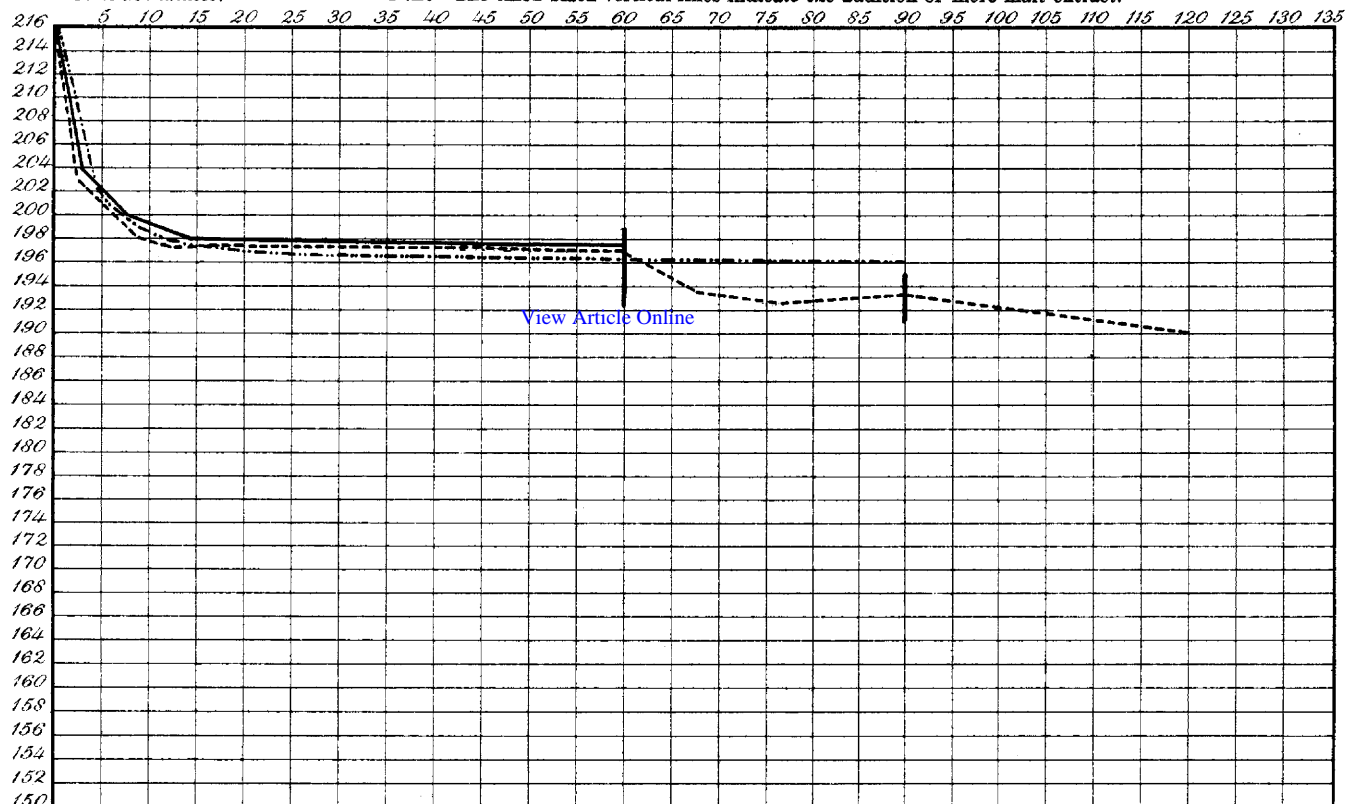


TABLE IX.

*Transformations at 75° with malt-extract previously heated to 76° for 20 minutes.*

————— No. 17. Per 100 c.c. 4 grms. starch. 20 c.c. malt-extract.  
 - - - - - No. 18. Per 100 c.c. 3·8 grms. starch. 23 c.c. malt-extract.

Time in minutes.

N.B.—The thick black vertical lines indicate the addition of more malt-extract.





TABLE X.

Transformations with neutralised and alkaline malt-extract.

- No. 19. Malt-extract heated to 66° for 20 minutes, and afterwards neutralised with  $BaH_2O_2$ .  
 Per 100 c.c. 5 grms. starch. 16 c. c. malt-extract.  
 - - - - - No. 20. Malt-extract heated to 66°, and rendered very slightly alkaline with  $Na_2CO_3$ .  
 Per 100 c. c. 5 grms. starch. 19.8 c.c. malt-extract.  
 . . . . . No. 21. Malt-extract heated to 66°, and rendered very slightly alkaline with  $Na_2CO_3$ .  
 Per 100 c.c. 5 grms. starch. 16 c.c. malt-extract.  
 + + + + + No. 22. Malt-extract heated to 66°, and rendered slightly alkaline with  $Na_2CO_3$ .  
 Per 100 c. c. 5 grms. starch. 25 c.c. malt-extract.

Time in minutes.

N.B.—The thick black vertical lines indicate the addition of more malt-extract.

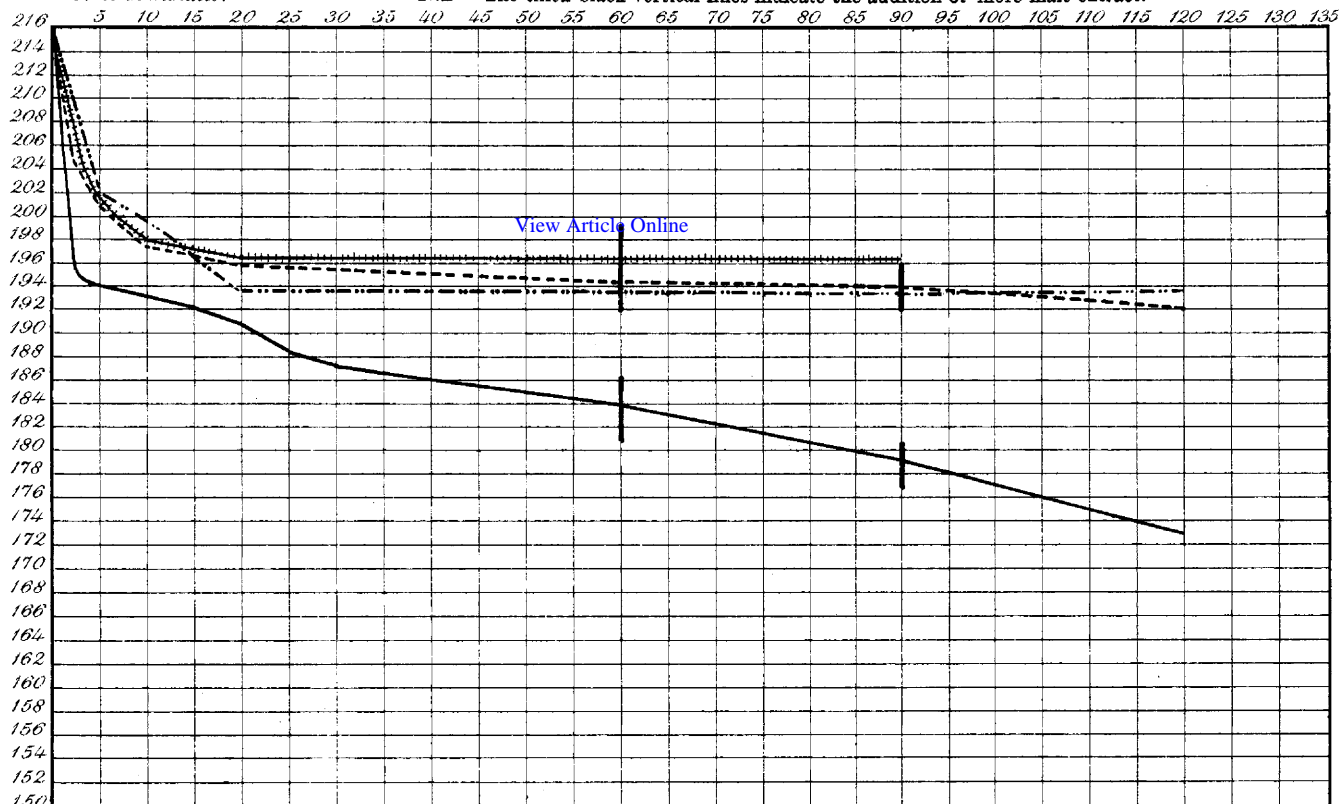


TABLE XI.

Transformation at 66° with malt-extract heated to 66°, and afterwards rendered very slightly alkaline with  $NaHo$ .

- No. 23. Per 100 c. c. 5 grms. starch. 25 c.c. malt-extract.

N.B.—The thick black vertical lines indicate the addition of more malt-extract.

Time in minutes.

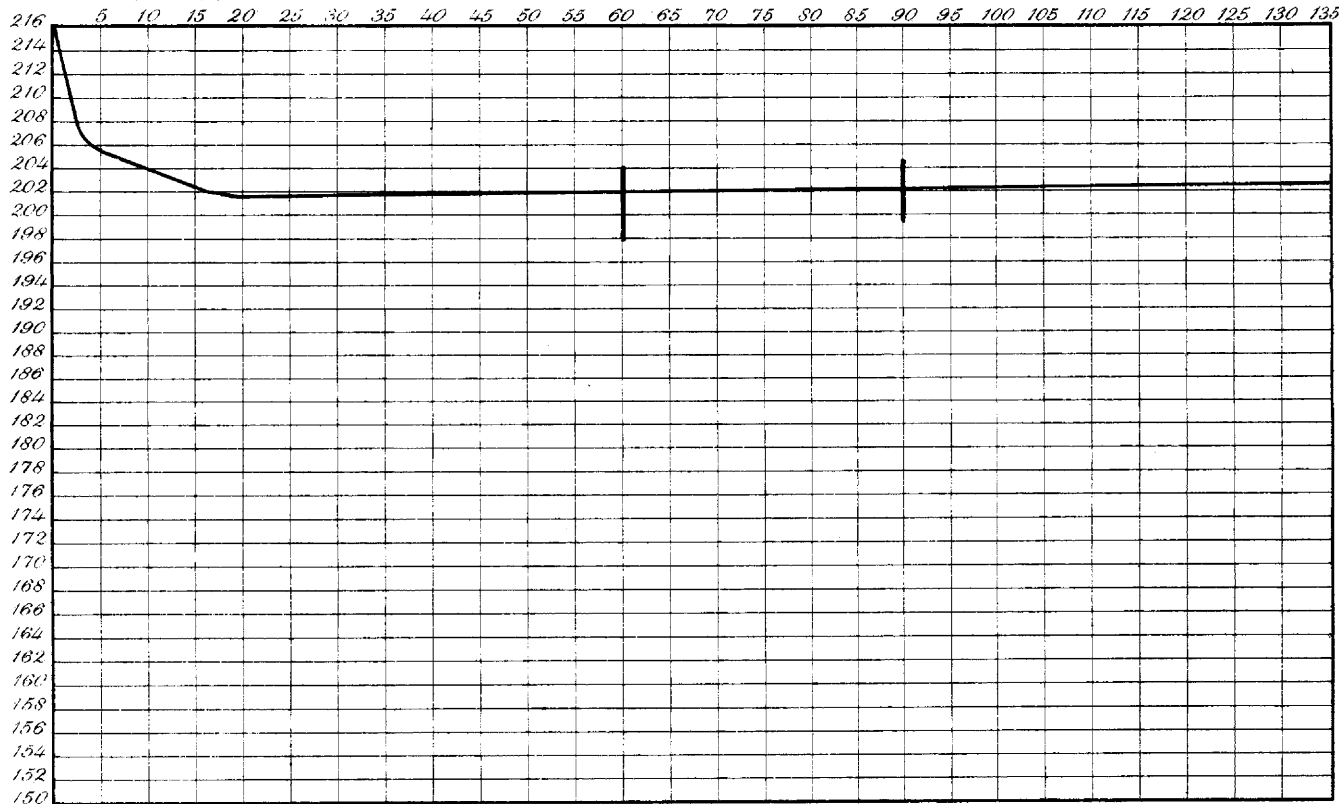


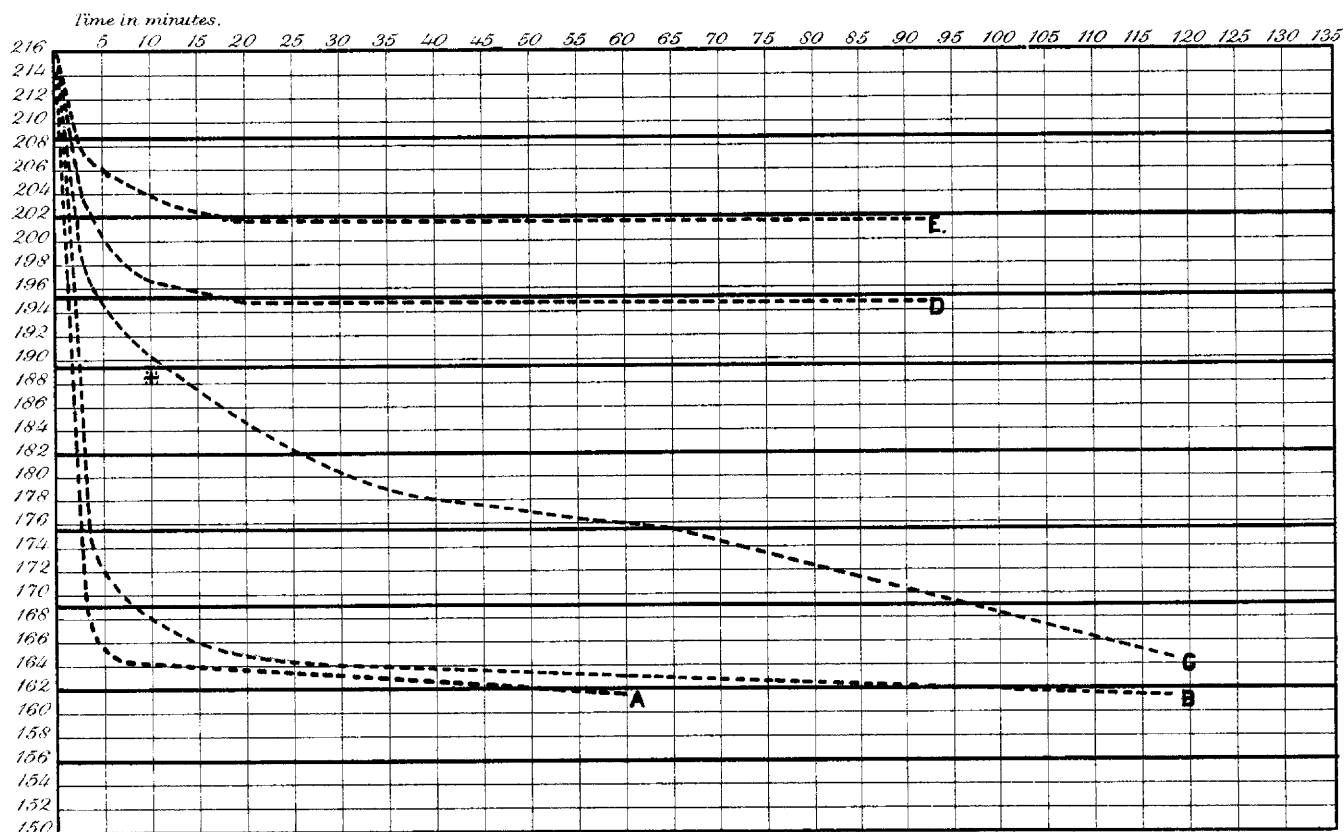
TABLE XII.

*Shewing the average curves for transformations made under different conditions.*

- A. Transformations with unheated malt-extract at 40°-50°.  
 B. Transformations with malt-extract heated to 60°.  
 C. Transformations with malt-extract heated to 66°.  
 D. Transformations with malt-extract heated to 75°, and also with malt-extract heated to 66°, and rendered slightly alkaline with  $\text{Na}_2\text{CO}_3$ . [View Article Online](#)  
 E. Transformations with malt-extract heated to 66°, and rendered very slightly alkaline with  $\text{NaHO}$ .

The horizontal thick black lines mark the position of the different theoretical transformations possible under the 10-molecule theory of the constitution of starch.

\* Disappearance of iodine reaction for erythrodextrin.





Maltose.....	47·3
Dextrin.....	52·7
	<hr/>
	100·0

To 100 c.c. of this solution, containing about 5 grams of solid matter, were added 5 c.c. of unheated malt-extract, the temperature being at 60°.

At the end of two minutes the numbers for the starch products were found to be—

$[\alpha]_{j3.86}$ .....	162·6°
$\kappa$ .....	49·3

corresponding to a percentage composition of

Maltose.....	80·8
Dextrin.....	19·2
	<hr/>
	100·0

This composition was not further altered on digestion for 15 minutes longer.

In another similar experiment in which the numbers for the boiled solution were—

$[\alpha]_{j3.86}$ .....	184·2°
$\kappa_{3.86}$ .....	31·8

they were reduced by the action for two minutes of 5 c.c. of unheated malt-extract at 50° to

$[\alpha]_{j3.86}$ .....	164·4°
$\kappa_{3.86}$ .....	48·9

at which point they remained constant for some time.

If we assume that the achroo-dextrins of the higher and lower transformations are identical, there appears to be no sufficient reason why their hydration should proceed with such extreme rapidity to a certain point, and there be arrested. The phenomenon cannot be due to a weakening of the diastatic power of the transforming agent brought about by the presence of a large excess of maltose, since such a liquid is capable of readily reducing a further quantity of starch to the same point.

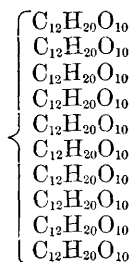
The explanation which most naturally suggests itself is that the dextrins are not *metameric* but *polymeric* bodies, those corresponding to transformations of high optical activity being of greater molecular complexity than those yielded by transformations of lower optical activity, the latter being produced from the former by a partial act of hydration with consequent elimination of maltose.

The separation and examination of the dextrans has confirmed this opinion.

Since arriving at these conclusions, Musculus and Gruber (*Bull. Soc. Chim.*, **30**, 69) have brought forward an identical theory to account for certain facts they have observed in connection with the action of precipitated "diastase" upon starch. They look upon starch as a polysaccharide having for its formula  $n(C_{12}H_{20}O_{10})$ , and undergoing under the influence of diastase and acids successive hydrations and decompositions.\*

This view of the constitution of starch, or more correctly speaking, of soluble starch, we believe to be essentially correct, and quite in accordance with our experiments.

When our results are attentively considered, it will be seen that the simplest formula we can adopt for soluble starch is  $10C_{12}H_{20}O_{10}$ , which we may write in the following way:—



The first action of the transforming agent of malt-extract upon this complex and unstable molecule results in the removal by hydration of one of the groups  $C_{12}H_{20}O_{10}$ , thus producing maltose, whilst the remaining nine groups  $C_{12}H_{20}O_{10}$  constitute the first dextrin of the series, erythro-dextrin  $\alpha$ . This dextrin on further hydration splits up into a molecule of maltose, and erythro-dextrin  $\beta$ , consisting of eight  $C_{12}H_{20}O_{10}$  groups. This dextrin in its turn splits up into a dextrin formed of seven such groups, which is the first achroo-dextrin.

In this way the degradation of the original molecule proceeds, until, by successive stages of hydration, maltose is reached.

The number of distinct dextrans which according to this hypothesis are possible, depend upon the molecular weight of the lowest possible dextrin. If a dextrin is capable of existing containing the same

\* Dr. Armstrong has shown to one of us a proof-sheet of the forthcoming new edition of Miller's *Organic Chemistry*, printed prior to the appearance of Musculus and Gruber's paper, in which it is pointed out that the results obtained by O'Sullivan indicate that starch has a very high molecular weight, and that according as we regard dextrin as  $C_{12}H_{20}O_{10}$  or  $C_{24}H_{40}O_{20}$ , it contains either 72 or 144 carbon atoms in its molecule.

number of carbon atoms as maltose in its molecule, nine distinct stages in the transformation may be expected; whereas if the lowest dextrin contain double as many carbon atoms as maltose, there can only be eight.

The following table represents the value of  $[\alpha]_{j3.86}$  and  $\kappa_{3.86}$  for the joint products of each of the theoretical transformations, the resulting dextrins being given in the second column:—

No. of transformation.	$[\alpha]_{j3.86}$ .	$\kappa_{3.86}$ .	Resulting dextrin.
Soluble starch . . . .	216.0°	0	—
(1) . . . . .	209.0	6.4	Erythro-dextrin $\alpha$
(2) . . . . .	202.2	12.7	„ $\beta$
(3) . . . . .	195.4	18.9	Achroo-dextrin $\alpha$
(4) . . . . .	188.7	25.2	„ $\beta$
(5) . . . . .	182.1	31.3	„ $\gamma$
(6) . . . . .	175.6	37.3	„ $\delta$
(7) . . . . .	169.0	43.3	„ $\epsilon$
(8) . . . . .	162.6	49.3	„ $\zeta$
(9) . . . . .	156.3	55.1	„ $\eta$
Maltose . . . . .	150.0	61.0	—

Of these possible transformations our experiments have unmistakeably established the existence of Nos. (2), (3), (4), and (8), whilst indications of (5) and (6) have been frequently obtained in various ways, but not with the same amount of certainty as in the other cases.

The dextrins corresponding to Nos. (1) and (2) are erythro-dextrins, all the others being achroo-dextrins.

By far the most stable dextrin is that of transformation (8). Between this point and the complete hydration to maltose we have been unable to determine any indication of a slackening of the reaction, which fact appears to favour the second of the views above expressed. It is true that transformation (9) can be obtained by continuing the digestion of (8) for some time with unheated malt-extract, but the fall in angle is continuous and regular until maltose is reached.

Throughout the whole range of the transformations of starch, conducted with malt-extract under the most varying conditions, the results obtained have always been compatible with the hypothesis that the hydration\* and splitting up of the molecule of soluble starch are

\* Dr. H. E. Armstrong has suggested the appropriate term *hydrolysis* to denote the assimilation of the elements of water with a simultaneous splitting up of the molecule; *hydration* being used to denote merely the fixation of the elements of water unattended by any splitting up of the resulting molecule.

The conversion of starch into maltose and dextrin, and the inversion of cane-

attended with the production of (1) a crystallisable body, *maltose*, having a specific rotatory power of  $[\alpha]_D^{150}$ , and a cupric oxide reducing power of 61, and (2) of a series of non-crystallisable polymeric bodies, the dextrins, having all the same specific rotatory power of  $[\alpha]_D^{216}$ , and no reducing power.

Musculus has stated that the different dextrins have a varying action on polarised light, and are all capable in a different degree of reducing cupric oxide. If this statement is assumed to be correct, it is evident that the values of  $[\alpha]_D$  and  $\kappa$  for the dextrins must throughout the series vary inversely, and in such a manner that these bodies may be looked upon as mixtures or combinations of maltose with a dextrin of  $[\alpha]_D^{216}$ , and no reducing power. Such a view has been taken throughout the course of this paper.

We are at present engaged with the isolation and study of the different dextrins, a work promising results of great interest, which we hope to communicate to the Society at a comparatively early date.

### *Is Dextrose a Product of the Action of Malt-extract upon Starch?*

In order to arrive at an answer to this important question, we have investigated the result of prolonged action of considerable quantities of malt-extract upon a solution of dextrin-maltose of known composition.

In these experiments the greatest possible care was taken to ensure that the duplicate samples of malt-extract were treated in an exactly similar way to the transformation liquids themselves.

*Experiment I.*—A solution of the transformation products was obtained in the ordinary way, having the following properties:—

$[\alpha]_{D^{86}} \dots$	163.6°	Maltose ....	80.1
$\kappa_{D^{86}} \dots \dots$	48.9	Dextrin ....	19.9
			<hr/>
			100.0

To 75 c.c. of this solution were added 25 c.c. of malt-extract previously heated to 62°. The mixture was digested for 16 hours at 55°, when the corrected starch-products yielded the following results:—

$[\alpha]_{D^{86}} \dots$	152.1°	Maltose ....	97.8
$\kappa_{D^{86}} \dots \dots$	59.6	Dextrin ....	2.2
			<hr/>
			100.0

sugar, are examples of *hydrolytic action*; whilst the conversion of ethylene oxide into glycol is an instance of *hydration*.

*Experiment II.*—A starch conversion at 60° C. with malt-extract heated to 62°; time of conversion 1 hour—

$[\alpha]_{j3.86}$ .....	164.0°
$\kappa_{3.86}$ .....	49.57

This solution after boiling was divided into three portions, A, B, and C.

A. 74.5 c.c. digested with 25.5 c.c. of unheated malt-extract for 16 hours at 53° C.

*Starch-products corrected.*

$[\alpha]_{j3.86}$ .....	147.0°
$\kappa_{3.86}$ .....	61.5

B. 74.6 c.c. digested with 25.4 c.c. of malt-extract (previously heated to 62°) for 16 hours at 53° C.

*Starch-products corrected.*

$[\alpha]_{j3.86}$ .....	150.5°
$\kappa_{3.86}$ .....	58.3

C. 74.5 c.c. digested with 25.5 c.c. of *boiled* malt-extract for 16 hours at 53° C.

*Starch-products corrected.*

$[\alpha]_{j3.86}$ .....	166.0°
$\kappa_{3.86}$ .....	49.3

In this last experiment, No. II, in order to obtain all the corrections for the malt-extract, it was necessary to make a separate analysis—

(1.) Of the malt-extract heated to 62° and digested for one hour at 60°.

(2.) Of No. (1) after raising to the boiling point.

(3.) Of unheated malt-extract after digestion for 16 hours at 53°.

(4.) Of malt-extract heated to 62° and digested for 16 hours at 53°.

(5.) Of malt-extract boiled and digested for 16 hours at 53°.

It is only by taking these precautions when dealing with such a changeable body as malt-extract, that results of any accuracy can be obtained.

In the following experiments the malt-extract was added in successive portions at different periods of the digestion, instead of only at the commencement.

*Experiment III.*—A solution of transformation products prepared, giving, when corrected—

$[\alpha]_{j3.86}$ .....	162.3°
$\kappa_{3.86}$ .....	49.6

75 c.c. of this solution on digestion at 50° for 16 hours with 24.7 c.c. of malt-extract, added at three different periods, gave for the corrected starch products—

$[\alpha]_{j^{3.86}}$ .....	147.7°
$\kappa_{3.86}$ .....	60.8

*Experiment IV.—Original Starch-products.*

$[\alpha]_{j^{3.86}}$ .....	164.3°
$\kappa_{3.86}$ .....	48.5

66 c.c. of this solution were digested with 34 c.c. of malt-extract for 28 hours, the malt-extract being added in four different portions.

*Starch-products corrected.*

$[\alpha]_j$ .....	148.2°
$\kappa$ .....	58.6

All the foregoing experiments prove most conclusively that *no dextrose is formed* by the prolonged action of malt-extract upon starch. The final product of the reaction is *maltose*, which, as we have previously shown, is not further hydrated either by the transforming agent itself, or by the small amount of acid contained in the malt-extract. The apparent production of dextrose in the experiments of other observers has resulted partly from the non-observance of the precautions we have shown to be so necessary, and partly from the fact that the cupric oxide reducing power of maltose has been hitherto taken at too high a value.

*The Nature of Diastase.*

When malt-extract is gradually heated coagulation is first apparent at about 46° C.

When this temperature is maintained, the maximum amount of precipitation possible under these conditions takes place in from 15 to 20 minutes, a continuance of the heating having no further influence. If, however, the temperature is raised a few degrees, a further coagulation takes place, which in turn soon attains a maximum. This increase of coagulation with the temperature continues up to about 95° C.

The amount of coagulation produced by heating equal quantities of normal malt-extract to a given temperature, is shown in the following table. The third column gives the quantity of precipitated albumin calculated as a percentage upon the total amount thrown down at 100°.

## 100 c.c. of Normal Malt-extract.

Temperature.	Grams of albumin coagulated.	Percentage of total amount.
50°	0·044	19·1
60	0·123	53·5
66	0·155	67·4
76	0·186	80·8
100	0·230	100·0

Every stage in the coagulation of malt-extract by heat is attended with a distinct modification of its starch-transforming power; and, conversely, we have never been able to discover any modification in starch-transforming power which is not attended with distinct coagulation.

In addition to this, at 80—81°, the point at which the diastatic power of malt-extract is destroyed, nearly the whole of the coagulable albuminoids have been precipitated. We are consequently led to conclude that the diastatic power is a function of the coagulable albuminoids themselves, and is not due, as has been generally supposed, to the presence of a distinctive transforming agent.

This supposition is rendered still more probable from the following considerations.

When malt-extract is filtered once or twice under slight pressure through a thin porcelain diaphragm, such for instance as that afforded by a porous battery cell, we find that the filtrate, on being heated to the boiling point, throws down no albuminoids, but only a trace of calcic phosphate. This phenomenon is doubtless due to the same cause as that described when calling attention to the properties of soluble starch treated in a similar manner. The albuminoids being highly colloidal, and having a large "solution molecule," refuse to pass through the diaphragm.\*

\* These results have an important bearing upon the experiment first described by Dr. W. Roberts, which has been frequently quoted as affording a most powerful argument against the doctrine of abiogenesis. This observer found that on filtration of a vegetable infusion, swarming with bacteria, through porous earthenware, the filtrate, when placed under conditions of temperature favourable to the production of organisms, remained barren and unchanged; a result which was attributed entirely to the separation of the bacteria and their germs.

We now see, however, that the liquid may have been modified very considerably by such a treatment, the albuminoids or plastic material upon which the heterogenists rely for the spontaneous production of life, being partially or entirely removed.

It must not be considered that we are in any way advocates of the doctrine of abiogenesis, which we consider opposed to a great mass of trustworthy evidence. We wish merely to point out that this so-called *experimentum crucis* of Dr. Roberts by no means possesses the value which is commonly attributed to it.

We have been unable to discover any alteration in the composition of malt-extract so treated, except this loss of albuminoids ; it is true that the dextrorotatory power of the liquid increases somewhat, but this is entirely due to the separation of the lævorotatory albumin.

When the action of the filtrate is tried upon starch-paste it is found to possess *absolutely no transforming power*. It has lost all diastatic action as fully and completely as if it had been heated to the boiling point.

In this case, without the application of heat, removal of the coagulable albuminoids has again been attended with loss of transforming power. Diastatic action appears to be as inseparably connected with the coagulable albuminoids as the production of alcohol is with the yeast-cell, and the evidence in favour of a causal connection of these phenomena appears to us to be as strong in the one case as in the other.

Barley before germination contains more soluble coagulable albuminoids than after the malting process is completed. The following table gives the results obtained by coagulating at different temperatures equal quantities of *normal* aqueous barley-extract. The barley was similar to that used after germination in the last experiment of the kind, 100 parts of barley being used for the preparation of the extract instead of 80 parts of malt.

100 c.c. of Normal Barley-extract.

Temperature.	Grams of albumin coagulated.	Percentage on total quantity pre- cipitated at 100° C.
50°	0·063	18·3
60	0·194	56·5
66	0·233	67·9
76	0·311	90·6
100	0·343	100·0

Although the actual amount of coagulable albumin is considerably greater in barley than in malt, the percentage of the total amount precipitated at the same temperature in each case is very similar.

The coagulable albuminoids of fresh barley-extract, although to all appearances differing but little from those of malt-extract, possess but very feeble diastatic power. Those portions which are coagulated only at temperatures above 66° are without action upon starch-paste, whilst those precipitated at temperatures below 66° have a feeble action, which, however, is probably only from  $\frac{1}{50}$  to  $\frac{1}{100}$  of the intensity of those existing in solution in malt-extract at the same temperature.

During the process of germination the coagulable albuminoids must evidently undergo some change, which, without materially altering



their physical properties, confers upon them a large amount of potential energy.

It is possible that these bodies consist of portions of the cell-protoplasm (still retaining some of the properties of living protoplasm) which are thrown into a state of varying molecular tension whilst undergoing the modifications necessary for the requirements of the growing embryo. The direction of these modifications is probably from the extreme colloidal form to a state admitting of freer passage through the cell-walls.

The molecular tension of the albuminoids results in great instability of composition, which is capable, under favourable conditions, of inducing the hydrolysis of starch.

We have up to the present time been unable to discover any marked change of state in the transforming agent attendant upon the liberation of so large an amount of energy. It is evident that some such change must take place, but it has hitherto escaped our notice.

It is possible to augment the transforming power of the comparatively inactive albuminoids of barley after their separation from the grain, and, consequently, without the aid of germination. An aqueous extract of barley, which is submitted for a few hours at a temperature of 30° C. to the action of ordinary yeast, has its power of transforming starch much increased by this treatment. A mixture of yeast and pure cane-sugar placed under identically the same conditions, affords a liquid which does not possess the slightest action upon starch. It is evident that the growing yeast-cell is capable of inducing certain modifications in the albuminoids which, during the ordinary process of germination, are brought about by the action of the living vegetable cell itself.

This curious action appears to be confined to the saccharomyces. Whenever schizomycetes only are present in the barley-extract, the infusion, far from gaining in diastatic power, rapidly loses that which it originally possessed.

We believe that a combination of the broad principles of Liebig's theory of fermentation with a portion of Frémy's hypothesis of "hemi-organisation" (*vide* "Sur la Génération des Ferments") will prove capable of connecting coherently all the known facts of the so-called *diastatic fermentations*.

On the above hypothesis, the influence of heat upon the subsequent action of malt-extract upon starch becomes more comprehensible than heretofore.

The first action of heat is to destroy by "heat-stiffening" those modifications of the cell-protoplasm which, during their action, liberate a large amount of energy, sufficient to effect the hydrolysis of the smaller and more stable molecules of the dextrins low in the

series. As these more active bodies are gradually reduced in quantity by an increase of temperature, the albuminoids still remaining uncoagulated are those which are capable of liberating a comparatively small amount of energy, which, although still sufficient to hydrolyse the more complex and less stable molecules of the higher dextrans, is insufficient to effect any change in the more stable molecules of the lower dextrans.

Unlike the organised ferments, the diastatic ferments do not possess any power of reproduction, consequently their transforming action is limited, and strictly proportional to the amount of transforming agent present. This being the case, the relative transforming powers of any two infusions of malt may be readily ascertained by conducting two transformations of starch under identically similar conditions, and constructing in each case a time-curve similar to those described. The relative energy of transformation is measured by the amount of degradation of the starch in a given time.

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