CLXX. DYNAMICS OF THE WAXY GENE IN MAIZE.

II. THE NATURE OF WAXY STARCH¹.

By R. A. BRINK.

From the University of Wisconsin, Madison, Wisconsin, U.S.A.

(Received July 18th, 1928.)

INTRODUCTION.

THE waxy factor in maize occasions a conspicuous change in the character of the reserve starch. In analysing the physiological action of the gene we have sought to determine how this storage product differs from the starch laid down in the endosperm and pollen of non-waxy maize. Two questions of primary importance are presented. Are the waxy and non-waxy starches composed of the same ultimate sugar units? And, secondly, are the sugar residues united in the same fashion in the respective elementary starch molecules? Perhaps a third query might be added with reference to degree of association.

A mode of attack somewhat different from that formerly employed [Brink and Abegg, 1926] has been adopted in the present investigations. Malt amylase has been used as the exploring agent with a view of ascertaining the particular sugar or sugars formed on hydrolysis of the starch. It is by this means that an answer to the first question set forth above has been sought. The rate of conversion of waxy starch to sugar under the action of malt amylase has likewise been carefully investigated for the light it might throw upon the structure of the starch molecule. The phosphorus content of the starch has been determined and the relative amounts of the two main constituents of the starch granule, namely the α -amylose and β -amylose of Meyer's [1895] terminology, have been reinvestigated by the barley diastase method [Ling and Nanji, 1923].

The chemistry of the polysaccharides has not yet advanced to a stage where the structural formula of starch can be set down. This fact imposes close limitations upon an investigation of the present type. Perhaps the time is yet distant when we shall be able to describe the effects of such a gene in maize as waxy in terms of changes in molecular structure. Even though the chemical foundation upon which such investigations might be built is incomplete it seems worth while, in view of the importance of the subject, to study the dynamic properties of the hereditary units by such methods as are available.

¹ Papers from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, No. 88. Published with the approval of the Director of the Station.

Proportions of α - and β -amylose by the barley diastase method.

According to Ling and Nanji [1923] precipitated barley amylase added to a starch paste at 50° converts the β -amylose (amylose) to maltose while the α -amylose (amylpectin) is scarcely attacked. These workers adopted this procedure in separating the two principal constituents of the starch grain.

In preparing the enzyme 200 g. ground barley are suspended in 600 cc. water and a few drops of toluene added as an antiseptic. The suspension is allowed to stand overnight at room temperature. The liquid is then removed in a lever press, filtered, and treated with three volumes of 95 % alcohol. The rather copious precipitate formed, containing the barley amylase, is promptly washed with absolute alcohol and redissolved in water.

The content of β -amylose was determined on waxy maize starch prepared in the laboratory [Brink and Abegg, 1927], and on a high grade commercial sample of common maize starch. 5 g. samples were gelatinised in boiling water, transferred to flasks and made up to 250 cc. after cooling. The p_H was adjusted to approximately 5.0 through the addition of dilute acetic acid. For the conversion 7 cc. of the barley diastase solution (equivalent to about 2 g. barley) and two drops of toluene were added to each sample. The flasks were corked and placed in a water-bath at 50°. After 18.5 hours the conversion was stopped by boiling. The solutions were cooled and filtered and the "total solids in solution" in the filtrate determined by the specific gravity method [Brown, 1904]. The amounts of maltose formed were measured iodimetrically according to the procedure outlined by Baker and Hulton [1920]. The proportion of starch converted was then directly computed from the maltose values.

Duplicate determinations gave 48 and 47 % respectively of the starch converted in the case of the common sample. One lot of the waxy starch was accidentally lost, the other showed 49 % of the starch converted. The barley diastase method, therefore, reveals little difference in the content of β -amylose in common and waxy starch. In both there would appear to be approximately equal amounts of α - and β -amylose.

It will be recalled that the centrifugation method of Sherman and Baker [1916] gave 8 % of β -amylose in common maize starch and about 60 % in waxy starch [Brink and Abegg, 1926]. Evidently different things are being measured by the two methods. The biochemical test indicates no important difference in the relative amounts of α - and β -amylose; but the two substances may occur in quite different physical conditions in the two kinds of starch pastes giving quite different results on centrifugation.

Hemicellulose in waxy and non-waxy starch.

When common maize starch paste is treated at 50° with malt diastase there appears, in 10 to 30 hours, depending upon the enzyme concentration, a rather copious flocculent precipitate. Schryver and Thomas [1923] found that this material gives a blue coloration with iodine but resists digestion with

taka-diastase. They conclude that the substance is a hemicellulose. It is not known whether the hemicellulose is an integral part of the starch grain or whether it is present as an impurity. The former seems more probable. Owing to the rather small supply of laboratory-prepared waxy starch at hand a quantitative estimation of hemicellulose content was not made. It was evident, however, from the relatively clear appearance of the waxy starch conversion liquids that practically no hemicellulose was present. Common maize starch, on the other hand, contains significant amounts of it. Schryver and Thomas [1923] report 3.6 %.

The presence of hemicellulose in starch introduces difficulties in measuring rate of conversion by noting the time required for the solution to lose the power of giving a colour reaction with iodine. Our first experiments [Brink and Abegg, 1926], in which this method was employed, afforded results which led to the conclusion that waxy starch is more readily hydrolysed than the common sort. The difference was apparent when 1 % HCl was used as the hydrolysing agent but it seemed particularly large when salivary amylase or the amylases from waxy and non-waxy maize seedlings were employed. The procedure used, however, has been shown to be inadequate. If starch were the only substance present giving the colour reaction the method would be valid but, as noted above, Schryver and Thomas [1923] have shown that maize starch contains hemicellulose which reacts with iodine but is resistant to amylase. Owing to the hemicellulose present, colour may be given by the conversion liquid long after the starch has been hydrolysed.

In the present studies the behaviour of the waxy and non-waxy starches on treatment with malt amylase has been carefully investigated and our former conclusions on comparative rates of hydrolysis are revised.

The sugar formed on treating waxy and common maize starches with amylase.

The view is commonly held that under the action of amylase starch is split quantitatively into a single sugar, maltose. A number of investigators, however, dissent from this conclusion. Pringsheim [1923] contends that adequate proof of the formation of but one sugar has not been furnished and, according to Armstrong [1924, p. 164] "There is little doubt that the starch molecule contains glucose as well as maltose residues." Ling and Nanji [1923] hold that, on hydrolysis with malt amylase, β -amylase (amylose) forms maltose, and α -amylase (amylpectin) produces two sugars, maltose and isomaltose. The weight of evidence, nevertheless, seems to be in favour of the contention that starch is composed entirely of maltose residues [Maquenne and Roux, 1905].

We have sought to determine whether the reserve starches of the waxy and non-waxy races of maize differ with respect to the sugar formed on diastatic hydrolysis. The conversions were carried out in two stages. The starch paste was first treated with alcohol-precipitated barley diastase which hydrolyses the β -amylase and scarcely changes the α -amylase. The latter substance was

then separated from the other products of the first conversion and hydrolysed with an active preparation of malt amylase (D.P. = 170°)¹. Reducing power, specific rotation and the osazone test were employed in identifying the sugars in the respective conversion liquids.

β-Amylose fraction of common maize starch.

40 g. of a high grade commercial sample of common maize starch were gelatinised by boiling and made up to 800 cc. 0.4 g. of barley diastase was added and the conversion allowed to proceed on a water-bath at about 50°. After 24 hours hydrolysis was stopped by boiling. The liquid was evaporated to a small volume and poured into alcohol giving a final concentration of 60 %. The precipitated α-amylase was filtered off and set aside for further treatment.

The filtrate containing the conversion products of the β-amylase was concentrated to a small volume and poured into an amount of alcohol giving a final concentration of 90 %. From the resulting precipitate the sugars were taken up by boiling, leaving a light, sticky precipitate, which was discarded. The supernatant liquid was filtered and the alcohol removed by heating on a steam-bath. The alcohol-free syrup from the β-amylase portion of the starch was taken up with water, a little toluene added as a preservative, and allowed to stand for two days in a corked bottle.

During the period of standing a light, brownish precipitate formed. This was filtered off leaving a fairly clear but yellowish solution. The concentration or "total solids in solution" was found by the specific gravity method to be 7.25 %. The reducing power was measured iodimetrically and the specific rotatory power determined. These constants were as follows²: $R = 93$ and $[\alpha]_{D_{4.0}} = +144.6^\circ$. The corresponding values for maltose are $R = 100$ and $[\alpha]_{D_{4.0}} = +138^\circ$. It seems probable from these results that some material of a dextrinous nature was still present as an impurity in the solution after the second treatment with alcohol. The formation of a precipitate on standing also bears out this contention. Dextrins would tend to lower the reducing power and increase the specific rotation. When the osazone was prepared definite evidence for the presence of maltose was obtained. A trace of glucosazone was also observed but no crystals of the isomaltosazone type [Ling and Nanji, 1923] were present.

It is concluded that the sugar formed from the β-amylase portion of common starch is maltose. Very small amounts of glucose may also occur.

¹ The diastatic power (D.P.) is taken as 100 when 3 cc. of a solution containing 0.1 g. in 250 cc. (1.2 mg.) of diastase produce sufficient maltose in 1 hour at 70° F. to reduce 5 cc. of Fehling's solution.

² R = reducing power. Measured in terms of an equivalent amount of maltose taken as 100.

The subscript to $[\alpha]_{D_{4.0}}$ in this case 4.0, denotes the solution factor used in computing the concentration from the observed specific gravity.

α -Amylose fraction of common maize starch.

The α -amylose portion precipitated in 60 % alcohol was taken up with sufficient water to give a fairly limpid solution and reprecipitated in 60 % alcohol. The filtrate was discarded. After kneading out the excess liquid the α -amylose was obtained as a firm, non-elastic, non-adhesive, white mass, sparingly soluble in water. A portion of the solution was filtered by suction. The constants on this were: $R = 13.2$ and $[\alpha]_{D_{40}} = + 255^\circ$.

The remainder of the α -amylose was made up to a volume of 300 cc. with water and 0.2 g. malt amylase (D.P. 170) was added to it. It was placed on a water-bath at 50° for 48 hours. The solution was then cooled, and the hemi-cellulose filtered off. The clear solution was returned to the bath and a further 0.2 g. malt amylase added. Digestion was continued for another 48 hours.

At 96 hours a 50 cc. sample was taken, heated to boiling to inactivate the amylase, cooled and filtered. The solution was made up to 100 cc. and found to contain 0.76 % solids. The constants were: $R = 104.6$ and $[\alpha]_D = + 138.1^\circ$. Several osazone preparations were made. In recrystallising these it was invariably found that an insoluble residue was present. No precipitate formed during heating. The recrystallised material showed maltosazone and a small amount of glucosazone. *iso*Maltosazone did not appear to be present.

The evidence indicates that the principal sugar present was maltose. Some glucose also occurred and a small amount of dextrin. The difficulties in recrystallisation were probably due to the latter substance.

 β -Amylose fraction of waxy maize starch.

An examination of the sugars formed on the diastatic hydrolysis of waxy starch was carried out in the same way as with the common maize starch. 50 g. of waxy starch were gelatinised in hot water and the suspension made up to 1000 cc. 0.6 g. of alcohol-precipitated and dried barley diastase was added. The mixture was placed on a water-bath, at about 50° , and frequently shaken.

The digestion was stopped after 19 hours by boiling. The liquid was cooled and filtered and, after evaporating to 300 cc., poured into a volume of alcohol giving a final concentration of 60 %. The α -amylose was precipitated as an elastic mass. Suction was used in filtering and the free liquid pressed out of the α -amylose mass by kneading.

The filtrate was milky in appearance and formed a precipitate on standing. This was filtered off and the clear filtrate evaporated to 140 cc. The concentrated liquid was poured into alcohol, giving a final concentration of about 80 %. A further light precipitate formed which did not dissolve on boiling. The solution was cooled and filtered. The precipitate gave a distinctly violet reaction with iodine. The filtrate was concentrated to a thick syrup on the water-bath and allowed to stand at room temperature overnight. It did not crystallise. The syrup was then diluted to 250 cc. and the constants determined on the solution. These proved to be as follows: $R = 96.3$ and $[\alpha]_D = + 132.5^\circ$.

A number of osazones which were prepared showed well-defined crystals of maltosazone and some glucosazone. A few crystals resembling those which Ling and Nanji considered to be isomaltosazone were also observed, but these did not occur consistently. These results indicate that the waxy conversion liquid contained principally maltose, together with small amounts of glucose and dextrans. The latter substances, not entirely removed by the treatment with alcohol, account for the fact that the observed reducing power is below 100. The rotatory power of the liquid is below that expected for a solution of pure maltose ($[\alpha]_D = +138^\circ$). Dextrans would tend to make the value higher. Glucose, on the other hand, would depress it, and the observed deviation is probably due to the combined effect of the glucose and dextrin impurities.

α -Amylose fraction of waxy maize starch.

The α -amylose separated from the barley diastase conversion liquid by precipitation with alcohol is obtained as an amorphous mass quite different in texture from the corresponding fraction of common maize starch. The latter is firm and crumbly and difficultly soluble in water, whereas the waxy α -amylose is elastic, sticky and easily dissolved in water at ordinary temperatures.

The crude waxy α -amylose obtained in the initial precipitation with 60 % alcohol was dissolved in water and reprecipitated with 60 % alcohol. After filtering, the α -amylose was freed from alcohol by dissolving in water and concentrating on a steam-bath. A small portion of the material was taken for determination of the reducing power and the specific rotation. The constants were found to be as follows: $R = 12.6$ and $[\alpha]_D = +167^\circ$. It will be recalled that the corresponding values for the α -amylose from common maize starch were $R = 13.2$ and $[\alpha]_D = +225$. It may be remarked here that while the specific rotations in these two cases may be widely different, great emphasis is not to be put upon the magnitude of the values actually observed. In the case of the common α -amylose in particular the substance is imperfectly dissolved and the methods of clearing and filtering appreciably influence the rotatory power of the solution. Moreover the optical behaviour changes on standing. The sugars do not present these difficulties, but our experience with the more complex carbohydrates agrees with that of others in that comparable readings are very difficult to obtain.

A small portion of the waxy α -amylose was treated with an active culture of beer yeast and the accessory conditions were made favourable for fermentation. No fermentation ensued, however, showing that neither maltose nor glucose was present. The reducing power of the α -amylose preparation is probably due to the liberation of reducing groups in the substance itself by the alkaline iodine solution.

The remaining portion of the α -amylose was dissolved in water and treated with 0.2 g. of malt amylase (D.P. 170) at 50° . After 24 hours the liquid was cooled and a very light precipitate filtered off. A further 0.2 g. of malt amylase (D.P. 170) was then added and the material again digested at 50° .

At 90 hours the reaction was stopped by boiling. A light precipitate was filtered off after cooling. The constants were: $R = 100.2$ and $[\alpha]_D = +133^\circ$. After treatment with alcohol giving a final concentration of 90% the conversion liquid showed $R = 100.9$ and $[\alpha]_D = +136^\circ$.

The osazone test gave crystals rather ill-defined in appearance. Some of the crystals directly obtained simulated those of the *isomaltosazone* of Ling and Nanji [1923]. After recrystallisation, however, well-defined maltosazone crystals were formed. In these preparations no other types of crystals were present. The difficulties in obtaining a satisfactory test without recrystallisation were probably due to small amounts of dextrans present as impurities.

A sample of the conversion liquid from the α -amylose portion of waxy starch was inoculated with beer yeast and a little peptone added. After a few hours' incubation a vigorous fermentation developed. According to Ling and Nanji [1923] *isomaltose* is not fermentable with yeast, whereas maltose, as is well known, is readily attacked.

The experiments on amylase hydrolysis may be summarised as follows. The gelatinised starches of both waxy and common maize form maltose when treated with the amylase of ungerminated barley. Presumably only the β -amylose portion of the starch is attacked by this enzyme. Small amounts of glucose are also formed in both cases. The α -amylose residues of both kinds of starch when treated with malt amylase form maltose. Again traces of glucose may be found in the conversion liquids and small amounts of dextrin-like substances are present. These experiments afford no evidence for the existence of *isomaltose* among the diastatic conversion products of starch. They rather indicate that starch is split into maltose, the yield of this sugar closely approaching the theoretical.

From the genetic point of view the conclusion of particular interest to which these results point is that the two kinds of starches, waxy and non-waxy, are alike with respect to the ultimate sugar unit of which they are composed.

Rate of hydrolysis with malt amylase.

The foregoing studies indicate that waxy and common maize starches are composed of the same sugar residue, for both produce maltose on diastatic hydrolysis. Perhaps the essential difference between the carbohydrates lies in the manner in which the sugar molecules are united, that is, in the structure of the starch constituent molecules. If this is the case, one might expect that on treatment with a given amylase preparation the two starches would be hydrolysed at different rates.

Nine tests have been made on the rate of maltose formation from waxy and non-waxy starch pastes treated with a common enzyme under closely comparable conditions. The results are presented in Tables I and II. In tests 1 and 2 waxy starch prepared in the laboratory was compared with a high grade commercial sample. In the remaining experiments both the waxy and common maize starches used were separated from the two respective classes

of seed on segregating ears and purified by ourselves. The method employed in preparing these starches [Brink and Abegg, 1927] yields a product of very high purity.

Except in test 1, where an enzyme preparation of relatively low diastatic power was employed, one lot of malt amylase was used throughout. This was prepared from malt extract by diluting the latter with water to one-fifth and then treating it with three volumes of 95 % alcohol. The precipitated amylase was dried with absolute alcohol and stored in powder form. This preparation, which was used in several of the experiments discussed earlier, showed a diastatic power (D.P.) of 170 on the Lintner scale. This is a relatively high activity.

The starches were gelatinised in hot water and brought to volume after cooling. The acidity was adjusted with dilute acetic acid to approximately p_H 4.5. The amylase was weighed out and dissolved in water and the required amounts were measured with a pipette. In each trial the digestions of the waxy and non-waxy starches were carried out side by side in the same water-bath.

At the intervals shown in the tables, 50 cc. samples of the conversion liquids were taken for maltose determinations. The amylase was inactivated by boiling. The reducing power was determined by the iodimetric method of Baker and Hulton [1920].

In computing the results the amount of maltose found is expressed as a percentage of the amount of moisture-free starch initially present. In test 5 the maltose values are reported in terms of the "total solids in solution" computed from the specific gravity of the liquid by means of a solution factor. The two methods give values which are closely alike after about 1.5 hours' digestion but prior to this the gravity basis yields higher percentages.

The conditions under which several conversion tests were carried out differ particularly with respect to concentration of amylase and the temperature as follows.

Test 1. 5.0 % starch pastes; 0.3 g. malt diastase of D.P. 50 in 1000 cc.; temp. *ca.* 50°.

Test 2. 4.0 % starch pastes; 0.03 g. malt diastase of D.P. 170 in 800 cc.; temp. 35°.

Test 3. 4.0 % starch pastes; 0.01 g. malt diastase of D.P. 170 in 600 cc.; temp. 35°.

Test 4. Same as test 3.

Test 5. 4.0 % common starch paste, 4.2 % waxy starch paste; 0.2 g. malt diastase of D.P. 170 in 700 cc.; temp. 50°.

Test 6. 4.0 % starch pastes; 0.005 g. malt diastase of D.P. 170 in 350 cc.; temp. 35°.

Test 7. 4.0 % starch pastes; 0.01 g. malt diastase of D.P. 170 in 350 cc.; temp. 35°.

Test 8. Same as test 7.

Test 9. Same as test 7.

Table I. *Maltose (%) reckoned on moisture-free starch.*

Time (hours) ...	$\frac{1}{4}$	$\frac{1}{2}$	1	$1\frac{1}{2}$	2	3	5	8	25
Test 1 Common starch	33.8	43.6	47.9	51.4	51.1	53.9	56.5	56.2	62.0
Waxy starch	37.7	44.1	45.7	47.6	47.8	47.8	49.9	51.0	54.4
Time (minutes) ...	10	20	30	50	70	90	120	180	
Test 2 Common starch	13.0	19.7	27.5	39.3	48.1	50.2	51.1	67.2	
Waxy starch	12.5	14.6	19.0	28.7	30.8	36.3	40.5	54.5	
Time (minutes) ...	15	30	90	180	270				
Test 3 Common starch	22.5	33.5	47.5	63.5	71.0				
Waxy starch	21.0	33.5	47.5	66.0	71.5				
Test 4 Common starch	19.0	29.0	47.5	64.5	73.0				
Waxy starch	18.5	26.5	48.0	64.5	69.5				
Time (minutes) ...	15	45	120	210					
Test 6 Common starch	5.7	16.2	32.9	42.6					
Waxy starch	7.4	15.0	25.3	26.7					
Time (minutes) ...	15	45	90	180					
Test 7 Common starch	13.2	28.4	45.6	59.2					
Waxy starch	13.2	22.8	32.7	42.8					
Test 8 Common starch	20.9	36.9	52.8	63.3					
Waxy starch	18.4	34.5	48.3	58.1					
Test 9 Common starch	14.9	29.9	46.4	61.2					
Waxy starch	11.9	28.5	42.4	54.2					
Aver. Common starch	16.3	31.7	48.3	61.2					
Waxy starch	14.5	28.6	41.1	51.7					

Table II. *Maltose (%) reckoned on moisture-free starch.*

Time (hours) ...	$\frac{1}{4}$	1	3	6	10
Test 5 Common starch	76.4	80.6	86.4	89.0	92.5
Waxy starch	70.6	76.0	79.3	84.1	88.1

In seven of the nine comparative tests maltose is formed more rapidly from the common starch than from the waxy starch. In the two remaining trials, tests 3 and 4, there is no certain difference in either direction. In test 1 the initial waxy samples gave higher percentages of maltose but the subsequent tests do not bear out this finding. Analysis of conversions attenuated through using small amounts of enzyme and a temperature of 35° shows that from the beginning the non-waxy starch is hydrolysed more rapidly.

In test 5 where conditions were favourable for rapid conversion the difference in rate in favour of the common starch is maintained to the stage where the digestion is about 90 % complete.

Tests 7, 8 and 9 were carried out with the greatest degree of refinement and the respective average percentages of maltose are plotted in Fig. 1.

The weight of evidence clearly favours the conclusion that malt amylase converts the common maize starch to maltose more rapidly than it does waxy starch. This is an important result for it gives some basis for thinking that there is a fundamental structural difference in the molecules of waxy and non-waxy starch.

Intermediate products in amylase hydrolysis.

It is a matter of interest whether the same intermediate compounds between starch and maltose are formed when the waxy and non-waxy reserve carbohydrates undergo diastatic hydrolysis. Some light is thrown on this question by observations on the optical activity of the conversion liquids during the digestion. In test 5 (Table II) such observations were made and were computed. In Fig. 2 the specific rotations are plotted against the percentages of maltose.

Fig. 1. Rate of maltose formation from the waxy and non-waxy starches when treated with a common enzyme, malt amylase. Average of results in tests 7, 8 and 9.

Fig. 2. Specific rotations of the conversion liquids during hydrolysis of the waxy and non-waxy starches with malt amylase. Test 5.

The specific rotation of maltose is $+138^\circ$. The values for the dextrins, intermediate products between starch and maltose, are higher. The conversion liquid contains maltose and probably a complex mixture of dextrinous materials. If the optically active substances in the conversion liquids are identical in nature, waxy and non-waxy samples containing the same percentages of maltose will have the same specific rotations. Fig. 2 shows, however, that the values for the common starch stand about 14° higher than those for waxy starch when comparison is made between solutions containing corresponding amounts of maltose. This means that the intermediate compounds between the initial polysaccharide and the sugar are different in the waxy and non-waxy conversion liquids.

These results afford further evidence in support of the conclusion that the waxy and non-waxy starches differ in molecular structure or in the degree of association of their elementary molecules.

Phosphorus content of waxy starch.

A number of workers have shown that starch contains phosphorus which is not removed by strong acids and bases at ordinary temperatures or by dialysis. Samec [1914] has concluded that the substance is present in organic combination. Karrer [1925] suggests that the phosphorus compound represents a small impurity in the α -amylose portion. Weight is lent to this view by the fact that Northrop and Nelson [1916] have isolated from starch an ester containing 5.3 % of phosphorus.

The amount of phosphorus in starch may be accurately and conveniently measured by a modification of the method of Briggs [1922]. The procedure is as follows. 5 g. of starch are incinerated at low heat. The ash is heated with about 80 cc. water on a steam-bath at 100°. 1 cc. of 5 % ammonium molybdate in 5N H_2SO_4 and 1 cc. of 1 % quinol slightly acidified with H_2SO_4 are added. Identical treatment is given to a blank containing a known amount of a solution of KH_2PO_4 containing 1 mg. P per cc. The concentration of phosphorus in the blank is adjusted by preliminary trial to approximate closely that in the unknown. After treating the solutions for 30 minutes, they are cooled and made up to 100 cc. 50 cc. of the blank and the unknown are taken and 1 cc. of a 20 % solution of sodium sulphite is added to each. After standing 10 minutes the solutions are made up to 100 cc. and the tints compared.

Phosphorus determinations carried out in duplicate according to the above procedure gave 0.0192 % for common maize starch and 0.0016 % for the waxy starch. A repetition of the test gave practically the same results, namely, 0.0194 % (common) and 0.0015 % (waxy). Waxy starch, therefore, contains only about one-twelfth as much organic phosphorus as does common maize starch.

The low content of phosphorus in the waxy starch is not due to a deficiency of this element in the endosperm tissue. Using the same methods of analysis as with starch, endosperm meal from common maize was found to have 0.0519 % P while comparable waxy endosperm meal contained 0.0507 %. Duplicate determinations again gave closely similar results.

Practically equal amounts of phosphorus are present in the endosperms of waxy and non-waxy maize but in the former case only a minute amount is combined with the starch.

SUMMARY.

1. The waxy and non-waxy maize starches contain nearly equal proportions of α - and β -amylose. It is considered that treatment with barley amylase is a better means of measuring the β -amylose content of a starch than the centrifugation method of Sherman and Baker.

2. When treated with malt amylase the waxy and non-waxy starches both produce maltose. Traces of glucose also occur in the conversion liquids, and the latter are never entirely free from dextrinous materials. The fact that maltose is the main hydrolytic product is taken to mean that the sugar unit entering

into the formation of the two kinds of starches is the same. It is concluded that the waxy gene exerts its differential effect subsequent to the synthesis of the sugar molecules.

3. Under the action of malt amylase common maize starch is hydrolysed to maltose more rapidly than waxy starch. It is suggested that the waxy gene may modify the starch molecule in some way which puts it slightly out of adjustment with malt amylase.

4. It is recognised, however, that the possibility is not definitely excluded that the essential difference between the two starches may be one of degree of association of a common fundamental molecule.

5. During the conversion to maltose with malt amylase waxy starch forms intermediate products of a lower specific rotatory power than does common maize starch. If dissociation and depolymerisation of the starch are completed in the initial stages of the conversion this means that the difference between the waxy and non-waxy carbohydrates lies in the character of their constituent molecules. It may be, however, that some undissociated or polymerised starch persists even until the later stages of digestion. If this is the situation the difference in the optical properties of the respective solutions may be due either to a difference in the constituent units or in the degree of polymerisation or association.

6. Common maize starch of high purity contains over twelve times as much organic phosphorus as does waxy starch prepared in the same way. The low content of phosphorus in waxy starch is not due, however, to a deficiency of this element in the endosperm tissue. Analyses show that the total phosphorus content of waxy and common endosperm meal is nearly the same. The significance of the differences in amount of combined phosphorus is not clear.

A considerable part of the work reported here was done in the Department of the Biochemistry of Fermentation at the University of Birmingham. Grateful acknowledgment is made to Prof. A. R. Ling and Dr D. R. Nanji for instruction in methods and for a critical interest in the problem. The investigation was carried on under the auspices of the National Research Council (U.S.A.), to which the author is indebted for a Fellowship in biology. The author is also indebted to his colleague, Dr K. P. Link, for helpful criticisms in the preparation of the manuscript.

REFERENCES.

- Armstrong (1924). The carbohydrates and the glucosides. (Longmans, Green and Co., London.)
- Baker and Hulton (1920). *Biochem. J.* **14**, 754.
- Briggs (1922). *J. Biol. Chem.* **53**, 13.
- Brink and Abegg (1926). *J. Genetics*, **11**, 163.
- (1927). *Plant Physiol.* **2**, 101.
- Brown (1904). Laboratory studies for brewing students. (Longmans, Green and Co., London.)
- Karrer (1925). Polymere Kohlenhydrate. (Akademische Verlagsgesellschaft, Leipzig.)
- Ling and Nanji (1923). *J. Chem. Soc.* **123**, 2666.
- Maquenne and Roux (1905). *Compt. Rend. Acad. Sci.* **140**, 1303.
- Meyer (1895). Untersuchungen über die Stärkekörner. (E. Fischer, Jena.)
- Northrop and Nelson (1916). *J. Amer. Chem. Soc.* **38**, 472.
- Pringsheim (1923). Die Polysaccharide. (Julius Springer, Berlin.)
- Samec (1914). *Koll. Chem. Beihefte*, **61**, 23.
- Schryver and Thomas (1923). *Biochem. J.* **17**, 497.
- Sherman and Baker (1916). *J. Amer. Chem. Soc.* **38**, 1885.