V. THE DISTRIBUTION OF MALTASE IN PLANTS. III. THE PRESENCE OF MALTASE IN GERMINATED BARLEY.

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It is still uncertain whether maltase, the enzyme which hydrolyses maltose to glucose, exists in germinated barley. Until recently no really satisfactory method existed of estimating glucose in presence of maltose and other sugars. Brown and Morris [1895] in a paper on the "isomaltose" of Lintner pointed out the danger of attempting to identify sugars solely by the properties of their osazones and their conclusions were supported by the results of Ling and Baker [1895, 1] with the same substance. The use of maltase-free yeasts, however, as suggested by Davis and Daish [1913] makes it possible accurately to estimate maltose when occurring with other sugars, and on this method the present work is based.

O'Sullivan [1872, 1876] showed that the sugar produced by the action of ordinary diastase on starch was not, as had generally been supposed, glucose, but maltose. Considerable confusion had in the past existed because the reducing powers of maltose and glucose were assumed to be identical; O'Sullivan showed that the reducing power of maltose is only 62 % that of glucose.

Brown and Heron [1879] also concluded that glucose is not formed by the prolonged action of malt extract on starch or maltose. It must be noted that in all their experiments the malt extract had either been heated before use to a temperature of 50° or the actual conversions were carried out at 55° or 60°. Brasse [1885], however, found that the diastase precipitated from an extract of germinated barley, providing the extraction is made

rapidly in the cold and the alcohol used in precipitating the enzyme is not left too long in contact with it, digests raw starch grains, causing corrosion similar to that found during germination and producing at 32–42° not maltose, but glucose. At 50–57°, no glucose but only maltose was formed. It is clear from these facts that Brasse was working with a preparation containing maltase, but this enzyme was not then known; no experiments were made by Brasse to show whether his enzyme acted on maltose. To Cuisinier [1885, 1886] is due the credit of realising that a special enzyme, to which he gave the name *glucase*, is abundantly present in maize and that this leads to the formation of glucose; but he did not show that the glucase converts maltose into glucose nor that the glucose formed from starch is not split off directly from the latter. It was left to Geduld [1891], working in Cuisinier's distillery, to show that glucase hydrolysed maltose to glucose. Cuisinier specially states that "glucase" is present in the organs and particularly in the seeds of a great number of plants, whether they contain starch or not, a statement which has been repeatedly denied by later workers but is undoubtedly correct.

Lintner and Eckhardt [1889] concluded that extracts of raw, ungerminated barley gave with soluble starch the same products, namely maltose and dextrin, as were obtained with malt extract but the optimum temperature of action of barley diastase was considerably lower, viz. 45°, than that of malt diastase. Brown and Morris [1890] considered that maltose and dextrin only were formed by the action of the enzymes of either germinated or ungerminated barley on starch; they always used chloroform as antiseptic in their experiments. Lintner [1893] confirmed Cuisinier's statement that maize contained an enzyme capable of forming glucose from starch and stated that dried malt might sometimes give rise to not inconsiderable quantities of glucose. Ling and Baker [1895, 1, 2] also found a small amount of glucose to be formed by the action of the diastase of *kiln-dried* malt on starch at 70° and found that such diastase formed small quantities of glucose from maltose, the glucose being approximately estimated as osazone. When *low-dried* malt was used, no glucose was formed and they considered the kilning so to modify the diastase as to confer the property of converting starch into glucose. In a later paper [1897] they modify their views and state that kilning is not the origin of the glucose-forming enzyme. Their later experiments lead them to state that glucose is *never* formed by the action of normal malt on starch. Kröber [1895] on the contrary took the view that a "glucase" existed in germinated barley but was not present after kilning. Morris [1896],

like earlier workers, found maltase in maize but no evidence of its presence in malted or unmalted barley; he states that "normal malt contains no enzyme capable of hydrolysing maltose."

Beyerinck [1895] whilst confirming the presence of "glucase" in maize denied its general distribution in plants as affirmed by Cuisinier. In wheat, rye and barley prior to germination no "glucase" could be found whilst during germination only very small quantities of glucase, if any, were formed. Beyerinck, as pointed out elsewhere [Davis, 1916], takes the view that "glucase" acts directly on *starch*, as well as on maltose, splitting off glucose directly.

Baker [1902] studying the action of the *precipitated* diastase of ungerminated barley on soluble starch found that after 6 hours at 50° only maltose and dextrins were formed; after 24 hours small quantities of glucose could be recognised by the osazone test. As glucose could not be detected after allowing this diastase to act on maltose but was formed by its action on "α-amylodextrin" he concluded that the glucose was formed from the latter and *not* from maltose. The action was regarded as not being due to maltase. At the ordinary temperature after 144 hours no glucose was formed from soluble starch, but only maltose and "α-amylodextrin." Ling and Baker [1902] found only maltose to be formed by the action of a diastase prepared from low-dried malt on starch paste at 50–60°. When the diastase was previously "restricted" by heating it above 65° its action was greatly modified and glucose was among the products of its long continued action on starch. B. F. Davis and Ling [1904] considered that the amount of glucose formed increased on raising the temperature of "restriction" until a maximum was reached at 65–70°. Glucose, however, was formed even when the enzyme had been restricted at 78°; but in this case the activity was greatly impaired. The maximum amount of glucose, estimated as osazone, was 12 % of the products of hydrolysis.

Marino and Sericano [1905] described the preparation from germinated barley dried at 25–30° of a "maltase" which hydrolysed maltose to glucose very vigorously. According to Marino and Fiorentino [1906] this "maltase" differs from the maltase of yeast in readily decomposing the β-glucosides and not the α-glucosides. In this respect this "maltase" resembled emulsin, but by a number of special experiments they considered they had shown that the property of decomposing the β-glucosides was not due to the presence of emulsin but to the "maltase" which hydrolysed maltose. No later work has been published to explain these abnormal results which contradict

the generally accepted notions as to the action of maltase and the structure of maltose. Huerre [1909, 1, 2, 3, 4] studied the properties of the maltase prepared from different varieties of maize and buckwheat. From a consideration of the differences of optimum temperature and stability towards heat he considers that a whole family of "maltases" exists, the members of which have different solubilities and different resistances to heat.

Since the present work was undertaken Wierzchowski [1913, 1, 2] has published two papers in which he shows that not only is maltase present in maize but that it exists also in smaller quantities in all cereals, for example rye, barley, wheat, oats, buckwheat and millet. In his first paper he failed to find maltase in barley or barley diastase, but in his second paper succeeded in doing so. He clearly realised that the maltase is sparingly dissolved by water, and is easily destroyed by precipitation with ordinary alcohol. He therefore worked, as we have done, not with water extracts but with the ground meal from the grain, and at a low temperature (42–45°). Wierzchowski's view that the enzyme can form glucose directly from starch is discussed by Davis [1916].

The experiments described below confirm Wierzchowski's view that germinated barley contains maltase. As it was possible to raise the objection to Wierzchowski and earlier workers whose results pointed to the presence of maltase, that the latter arose from moulds or fungi present on the grain, special precautions were taken to work with seeds which had been sterilised beforehand. To Dr H. B. Hutchinson thanks are due for valuable help. The grain was sterilised by a short immersion in a solution of mercuric chloride; after washing thoroughly with sterilised water it was allowed to germinate in sterile Petri dishes in which each grain could be observed. In no case could the growth of any mould or foreign organism be detected. The action of the germinated grain after drying and grinding was tested directly on starch and maltose at 38° in presence of toluene as antiseptic.

The results which are given show that gelatinised starch is acted on by the ground meal, the cupric reducing power of the product increasing as time proceeds whilst the rotatory power falls. The fermentations with maltase-free yeasts show that maltose and glucose are both formed, the latter increasing with time, whilst the maltose correspondingly falls. Control experiments carried out by digesting the barley alone, in which case self-digestion of the starch of the grain occurred, show that after prolonged digestion practically all the starch is converted into glucose; but in the earlier stages, considerable proportions of maltose, as well as some dextrin,

are present. When an excess of starch is used, as the maltase is relatively deficient, considerable amounts of maltose are found, but the latter is gradually transformed into glucose as time proceeds.

The results obtained with maltose show clearly that the germinated grain contains a true maltase. The action of the grain on starch is strictly comparable with that of taka-diastase [Davis and Daish, 1914; Davis, 1914] and the degradation of the starch by the enzymes of the germinated barley occurs in successive stages:

Starch ⟶ soluble starch ⟶ dextrins ⟶ maltose ⟶ glucose

[see Davis, 1916].

Experimental.

Series I. *Preliminary experiments with Unsterilised Barley.*

(i) *Self-digestion of germinated barley.* A sample of good barley, free from damaged grains, was soaked for two days in several changes of water. The seeds were then allowed to germinate in a heap on a cement floor until the rootlets were two to three times as long as the grain, the temperature being kept at 15–20°. The germinated material was dried in the air at 25–30°. Portions were then ground up in a mortar and allowed to digest in presence of 200 cc. of water at 38° for 24 hours, toluene being added (1 to 2 cc.) and the mixture well stirred at intervals. After the action the solution was boiled to destroy the enzymes, 10 cc. of alumina cream were added and the volume made up to 500 cc. at 15° (solution *A*). The weights of germinated barley represent the weights of vacuum-dried material. Air-dried material was weighed out but the weights have been reduced by the amount of water found to be present when similar material was dried to constant weight *in vacuo* at 130°. The percentage of water present was 13·2 %.

The analyses were made by the methods described by Davis and Daish [1913], the cupric reducing powers being determined under Brown, Morris and Millar's standard conditions. The values given in each case are the mean of two closely concordant results. All weights are in grams. The rotations are in true angular degrees.

For comparison of the results obtained on digestion of the barley by its own enzymes with those given by taka-diastase digestion, a similar quantity of the ground barley was heated with boiling water (200 cc.) to gelatinise the starch and destroy the enzymes. After cooling to 38°, 0·1 g.

TABLE I.

Self-digestion of Germinated Barley at 38° (24 *hours*).

Expt.	Weight of vacuum-dried germinated barley	CuO from 50 cc. of *A*	$\alpha_D^{20°}$ of *A* in 400 mm. tube	$\alpha_D^{20°}$ calc. for reducing sugars as maltose	$\alpha_D^{20°}$ calc. for reducing sugars as glucose
1	4·0933	0·2605	1·076°	2·108°	0·434°
2	4·5561	0·4287	2·696°	3·475°	0·756°

of taka-diastase was added and the mixture digested 24 hours at 38°. The solution was then treated as above, 10 cc. of alumina cream added and the volume made up to 500 cc. (solution *B*). The results in Table II are corrected for the 0·1 g. of taka-diastase by a control experiment under similar conditions.

TABLE II.

Digestion of Germinated Barley with 0·1 *g. Taka-diastase.*

Expt.	Weight of vacuum-dried barley	CuO from 50 cc. of *B*	$\alpha_D^{20°}$ of solution *B* in 400 mm. tube	$\alpha_D^{20°}$ calc. for reducing sugars as maltose	$\alpha_D^{20°}$ calc. for reducing sugars as glucose
3	4·0937	0·4485	1·899°	3·633°	0·798°
4	4·4948	0·4842	2·093°	3·923°	0·873°

The results in Tables I and II show that the digestion of the barley by its own enzymes produces nearly the same result as that effected by taka-diastase, which contains maltase, after destroying the barley enzymes. In all cases a mixture of maltose and glucose is formed; the rotations observed are roughly the mean of those calculated for the two sugars, showing that nearly equal quantities of these were produced in 24 hours. In the case of the self-digestion of the barley it should be noted that the starch was not previously gelatinised so that in this case the action took place on the starch granules.

In the following experiments the maltose was estimated by the use of maltase-free yeasts so that the proportion of the two sugars could be calculated.

(ii) *Estimation of maltose and glucose formed by the self-digestion of germinated barley and by the action of the barley on starch.* Approximately 5 g. of the germinated barley was ground up and digested in presence of 200 cc. of water (*a*) with 2·1210 g. of vacuum-dried potato starch, after gelatinising the latter; (*b*) without adding any starch. The digestions were continued for 48 hours at 38°, sufficient toluene being added. The solution

was then boiled, 10 cc. of alumina cream being added, and the mixture diluted to 500 cc. at 15° (solution *C*). After determining the reducing and rotatory powers, 100 cc. portions of the solution were sterilised by heating in the autoclave, after adding 5 cc. of yeast water, and fermented in the usual way with pure cultures of *S. exiguus* and *S. marxianus* for 28 days. The solutions were then boiled and 5 cc. of alumina cream added. Owing to the evaporation which had taken place in the course of the month the total volume was now slightly below 100 cc. and could be adjusted exactly to 100 cc. by adding water (solution *D*). 50 cc. portions of *D* were used to measure the cupric reducing power.

TABLE III.

Sugars formed by the Self-digestion of 5 g. of Germinated Barley and by its action on Gelatinised Starch at 38° (48 hours).

Expt.	Conditions	CuO from 25 cc. of *C*	CuO after fermentation from 50 cc. *D* = 50 cc. solution *C*: *S. exiguus*	CuO after fermentation from 50 cc. *D* = 50 cc. solution *C*: *S. marxianus*	Total maltose in 500 cc. *C*	Total glucose in 500 cc. *C*
5	5 g. ground germinated barley + 2·1210 vacuum-dried starch	0·2938	0·2590	0·2574	1·892	1·300
6	5 g. germinated barley only	0·1194	0·0508	0·0526	0·3746	0·7256

Expt.	Conditions	Glucose / Maltose	$\alpha_D^{20°}$ observed in *C* (400 mm.)	$\alpha_D^{20°}$ calc. for maltose + glucose in *C*	Total starch from sugars[1]	Percentage of starch in barley (air-dried) digested to sugars
5	5 g. ground germinated barley + 2·1210 vacuum-dried starch	0·69	2·700°	2·631°	2·964	14·9
6	5 g. germinated barley only	1·93	0·637°	0·565°	1·008	20·2

In Exp. 5, where the germinated barley has acted on gelatinised starch, far more reducing sugars (3·192 g.) have been formed than from the 5 g. of barley alone (1·100 g.). On the other hand, in the first case the greater part of the sugar is in the form of maltose (glucose/maltose = 0·69) whilst in the second it is present as glucose (glucose/maltose = 1·93). Whilst the grain contains sufficient maltase to convert the greater part (two-thirds) of

[1] In calculating these results the sugars originally present in the germinated barley have been neglected. Strictly these are by no means negligible as the results in Section v show, but for the purposes of *comparison* and the argument of the present paper they can be ignored. The results for starch given are in reality higher than the true values by the amount of pre-existent sugars.

its own starch into glucose, when an excess of starch has been added more maltose is formed than can be dealt with by the limited amount of maltase present. The liquefying and maltose-forming enzymes are in relative excess as compared with the maltase, so that far more maltose is formed from the excess of starch than can be transformed into glucose.

In Exp. 5 the sugars formed correspond with considerably more starch (2·964 g.) than that taken (2·1210 g.), owing to the fact that the starch of the grain has been partly digested. It is probable that the whole of the gelatinised starch has been converted into sugars in the 48 hours and that the starch of the grain is undergoing a slower conversion, owing to its granular nature. This would explain the fact that the excess of the rotation observed over that calculated for the sugars, which in Exp. 5 is + 0·069° and in Exp. 6 + 0·072°, is practically identical in the two cases. This excess no doubt is due to the presence of a nearly constant quantity of dextrin in both cases: the amount of this dextrin is relatively very small, as compared with the sugars, the dextrin being transformed into sugars almost as fast as it is produced from the starch. If we calculate in Exp. 5 the quantity of starch *of the grain* which has been converted into sugars by assuming that the whole of the gelatinised starch has disappeared, it amounts to 14·9 % of the weight of the barley taken. In Exp. 6, the digested starch represents 20·2 % of the barley, showing that the addition of the gelatinised starch has arrested to some extent the self-digestion of the grain itself. The barley probably contains some 50 % of starch (see Table VII), so that in 48 hours only a small proportion of the total has been digested.

(iii) *Action of small quantities of germinated barley on soluble starch.* When small quantities of germinated barley (0·1 and 0·2 g.) are added to soluble starch considerably less conversion of the starch occurs than in Exp. 5 where 5 g. were used. After 36 hours, more than half of the soluble starch is left in the form of dextrin, whilst the greater part of the sugar remains in the form of maltose, insufficient maltase being present to effect anything like complete transformation of the latter.

In these experiments 2·17 g. of vacuum-dried soluble starch were digested with 0·1 and 0·2 g. of powdered germinated barley for 36 hours at 38°; 5 cc. of alumina cream were then added, and, after boiling, the volume was made up to 500 cc. (solution *E*). Portions of 100 cc. after adding 5 cc. of yeast water were fermented for 28 days, as in the earlier experiments, and finally made up exactly to 100 cc.

The values given show that after 36 hours' digestion 44·3 % of the soluble

starch has been converted into sugars by 0·1 g. of germinated barley, and 56·3 % by 0·2 g. of the latter. In both cases, nearly the whole of the sugar is in the form of *maltose*, the ratio of glucose to maltose in the two experiments being 0·037 and 0·077 respectively. The use of the doubled quantity of

TABLE IV.

Conversion of Soluble Starch by small quantities (0·1–0·2 *g.*) *of Germinated Barley* (36 *hours at* 38°).

Expt.	Conditions	CuO from 50 cc. solution *E*	CuO from 50 cc. solution *E* after fermentation by *S. exiguus*	CuO from 50 cc. solution *E* after fermentation by *S. marxianus*	Total maltose in 500 cc. *E*	Total glucose in 500 cc. *E*	Glucose / Maltose
7	2·17 g. soluble starch + 0·1 g. germinated barley	0·1443	0·1348	0·1349	0·9785	0·0368	0·037
8	2·17 g. soluble starch + 0·2 g. barley	0·1882	—	0·1644	1·200	0·0923	0·077

Expt.	Conditions	Percentage of starch taken converted to sugars	$\alpha_D^{20°}$ of solution *E* (400 mm.)	$\alpha_D^{20°}$ calculated for sugars only	Δ	$\alpha_D^{20°}$ calc. for sugars + unaltered soluble starch[1]
7	2·17 g. soluble starch + 0·1 g. germinated barley	44·3	2·874°	1·092°	1·782°	2·967°
8	2·17 g. soluble starch + 0·2 g. barley	56·3	2·805°	1·360°	1·445°	2·831°

barley has just doubled the ratio. The large differences between the rotations actually observed and those calculated for the sugars are due to unconverted soluble starch, as shown by the values in the last column. The differences (Δ) are strictly proportional to the starch unconverted: thus

$$\frac{1·782}{1·445} = 1·233; \text{ and } \frac{100 - 44·3}{100 - 56·3} = \frac{55·7}{43·7} = 1·274.$$

(iv) *Attempt to separate the maltase from the germinated barley by extraction and precipitation.* Two attempts were made to separate an active preparation of maltase from the germinated barley. For this purpose a method similar to that stated to be successful by Marino and Fiorentino [1906] was used.

[1] The soluble starch used had $[\alpha]_D^{20°} = 193·7°$ and this value has been used in the calculation for the total rotation of the solution due to maltose, dextrose and unchanged soluble starch. The values actually observed are very slightly lower than those calculated. In presence of the excess of soluble starch it would appear therefore that practically none of the starch of the added grain has passed into solution. No correction has been made for the small amount of soluble sugars in the added grain.

The powdered *air-dried* grain was extracted with 1·5 times its weight of water saturated with thymol, the extract filtered and the solid matter pressed to remove as much liquid as possible. The extract was slowly dropped from a dropping funnel into eight to nine times its volume of 95 % alcohol, kept rapidly stirred. A large quantity of a slightly rose-coloured precipitate was obtained; this was immediately filtered off under pressure and dried in a vacuum desiccator. It was then redissolved in the smallest quantity of water saturated with thymol and again precipitated by dropping into alcohol. After filtering rapidly the precipitate was again dried *in vacuo*. The preparation so obtained acted very rapidly on soluble starch, but gave only maltose and dextrin as products, and no glucose. A second preparation gave a similar result. It is clear that either the method of extraction failed to dissolve the maltase or the latter was destroyed in the subsequent operations. The method described differs from that of Marino and Fiorentino in that the latter concentrated the extract *in vacuo* at 35–40° before precipitating with alcohol. They also finally dialysed their product. It is probable that differences in the nature of the alcohol may explain the differences between our results and those of Marino and Fiorentino; similar differences are to be found between the results of Beyerinck and Wierzchowski as regards the maltase of maize [see Davis, 1916, p. 34]. In any case, the negative results obtained show the instability of the maltase and the difficulty of isolating it.

SERIES II. *Experiments with Sterilised Barley.*

(v) *Analysis of the sugars present in germinated barley.* Two hundred barley grains (weight = 8·4223 g.) were sterilised in mercuric chloride solution and, after washing with sterilised water, were soaked for three days and allowed to germinate separately in sterile Petri dishes for a period of seven days. They were then dried in air at 25–30° and after grinding in a mortar were extracted in a Soxhlet extractor with 80 % alcohol for 18 hours. The extract was evaporated *in vacuo* at 35° and after the addition of 2 cc. of basic lead acetate solution made up to 100 cc. (solution *F*). 20 cc. portions were used to estimate the reducing sugars and saccharose (by inversion with 10 % citric acid); two further portions were fermented with *S. marxianus* and *S. exiguus* to give the maltose.

The values given, which must be regarded as only preliminary, show that maltose and glucose are present in nearly equal proportions; a slightly smaller amount of saccharose is present. The maltose was calculated as usual from the reducing power left after fermentation; the difference of

reducing power, 0·3149–0·1127 CuO, was assumed to be due to glucose. The sum of the rotations, 1·924°, due to the three sugars, assuming the whole of the reducing sugar other than maltose to be glucose, is slightly *less* than the value actually observed. This excludes the possibility that the reducing sugars consist largely of invert sugar. If the rotation be calculated for a

TABLE V.

Sugars present in Germinated Barley.

Expt.	Weight of air-dried barley	CuO from 20 cc. of solution *F*	CuO from 20 cc. *F* after inversion	CuO from 20 cc. *F* after fermentation with		Maltose percentage on barley
				S. marxianus	*S. exiguus*	
9	8·4223	0·3149	0·4456	0·1117	0·1137	4·91

Expt.	Glucose percentage on barley	Saccharose percentage on barley	$\alpha_D^{20°}$ of *F* in 200 mm. tube	$\alpha_D^{20°}$ calc. for mixture of sugars in *F*	Total sugars percentage in barley
9	4·80	3·21	2·265°	1·924°	12·92

mixture of maltose, invert sugar and saccharose it becomes + 1·325° which is much further removed from the value observed (2·265°) than on the assumption that glucose is present. It is probable therefore that the sugars are maltose, glucose and saccharose, with a trace of dextrin sufficient to cause the difference between the observed and calculated values.

(vi) *Action of germinated barley on gelatinised starch.* The sterilised barley was allowed to germinate in Petri dishes until the plumules were about $\frac{1}{4}$″ long. The grains were then removed, dried *in vacuo* over sulphuric acid and ground to a fine meal. About 1 g. of potato starch (vacuum-dried at 130°) was gelatinised by heating for 15 minutes with 50 cc. of water in a 150 cc. Jena flask and, after cooling to 38°, 0·2 g. of the barley meal was added and the mixture left to digest for various lengths of time, in presence of sufficient toluene to prevent any growth of micro-organisms, the flask being closed with a plug of cotton wool. After the digestion in each case 10 cc. of alumina cream were added, the solution raised to the boil, filtered and washed to 100 cc. at 15° (solution *G*).

20 cc. portions were used to determine the reducing power. 20 cc. portions were also mixed with 5 cc. of yeast water and fermented with the special yeasts for 21 to 25 days; after the fermentation 5 cc. of alumina cream were added, the mixture being boiled and filtered. The precipitate was washed until the volume of the filtrate was 50 cc., the whole of the latter being used for the reduction.

These results clearly show that as time proceeds the maltose present decreases in amount whilst the glucose increases. The ratio of glucose to

TABLE VI.

Action of Germinated Barley (0·2 *g.*) *on Gelatinised Starch.*

Expt.	Time of digestion hours	Weight of vacuum-dried starch	CuO from 20 cc. of *G*	CuO from 20 cc. of *G* after fermentation with *S. marxianus*	CuO from 20 cc. of *G* after fermentation with *S. exiguus*	CuO from 20 cc. of *G* after fermentation with Mean	Total maltose in 100 cc. of *G*
10	119	1·0532	0·3973	0·1598	0·1608	0·1603	0·5885
11	191	1·0192	0·4250	0·1458	0·1398	0·1428	0·5240
12	263	1·0468	0·4551	0·1363	0·1342	0·1353	0·4967

Expt.	Total glucose in 100 cc. of *G*	Glucose / Maltose	Total starch from sugars	Starch found as percentage of starch taken	$\alpha_D^{20°}$ of *G* in 400 mm. tube	$\alpha_D^{20°}$ of sugars of *G* in 400 mm. tube	Δ
10	0·4866	0·827	0·9959	94·6	5·443°	4·266°	+1·177°
11	0·5885	1·125	1·0263	100·7	4·838°	4·125°	+0·713°
12	0·6770	1·363	1·0802	103·1	4·691°	4·162°	+0·529°

maltose increases from 0·827 after 119 hours to 1·125 after 191 hours and to 1·363 after 263 hours. During the whole period part of the starch is present in solution in the form of dextrin as is shown by the value of $\alpha_D^{20°}$ observed being greater than that calculated from the glucose and maltose present. As time goes on, this difference, Δ, diminishes, being 1·177°, 0·713° and 0·529° at the different times of digestion, but even after 263 hours some dextrin is still present. The amount of enzyme taken in the 0·2 g. of barley is so small that conversion of the total starch into sugars has not been complete even after 263 hours. The action is not, however, at a standstill even after this prolonged period, as shown by the steadily increasing values of the glucose and total starch and the falling values of the maltose. The fact that the ungelatinised starch of the barley is being slowly converted into sugars accounts for the values found at the 191st and 263rd hours slightly exceeding 100 % of the gelatinised starch taken. It is noteworthy that during the last stages the increase of the glucose is greater than the corresponding fall of maltose in the same period, probably owing [see Davis, 1916, p. 42] to the rate of formation of maltose from the dextrin in solution being greater than that of the formation of glucose from maltose.

It is possible to calculate approximately at each interval the amount of the starch of the grain which has been converted into a soluble form. If this be done it is found that after the 119th hour, 33·5 % of the total weight of the added barley has been digested, after the 191st hour 37·2 %, and after the 263rd hour 38·9 %. As the actual starch present in the grain is probably about 50 %, some starch still remains undigested even after so long a period of action. For the purpose of control, experiments were made to ascertain the degree of self-digestion of 0·2 g. of the germinated barley

under exactly the same conditions as those of the experiments given in Table VI. The results are given in Table VII (solutions obtained in these controls are designated G').

It is necessary to make allowance for the pre-existent sugars in the germinated barley. The method of calculation is as follows: the values for the original sugars given in Table V are used.

		In 400 mm. tube
Maltose originally present in 0·2 g. barley	=0·0098 producing	$\alpha_D^{20°}$ = +0·054°
Glucose „ „ „ „	=0·0096 „	= +0·020°
Saccharose converted into invert sugar in 0·2 g. barley	=0·0061 „	= −0·005°

Therefore maltose actually produced from starch
=0·5885 − 0·0098 =0·5787 g. with rotation = +3·185°

Therefore glucose actually produced from starch
=0·4866 − 0·0096 − 0·0061 =0·4709 g. with rotation = +0·993°

Starch corresponding to maltose =0·5787 ÷ 1·055 =0·5485 g.
„ „ glucose =0·4709 × 0·9 =0·4238 g.

Total starch =0·9723 g.

Gelatinised starch not converted to sugars =1·0532 − 0·9723 =0·0809 g.

If this is in solution as dextrin with $[\alpha]_D^{20°} = +202°$, it produces rotation = +0·654°

Total rotation from above constituents ... = +4·901°
Actually observed rotation ... ... = +5·443°

$\Delta = +0·542°$

If Δ is due to the starch of the grain, transformed to dextrin (or soluble starch), with $[\alpha]_D^{20°} = 202°$, the concentration of the latter in 100 cc. of solution = **0·0671** g., which on 0·2 g. of germinated barley = **33·5** %.

The following table gives a summary of the results calculated in this way for the three periods of digestion.

Time in hours	Δ° due to dextrin in solution G	Dextrin arising from grain in g.	Percentage of barley transformed
119	+0·542	0·0671	33·5
191	+0·601	0·0744	37·2
263	+0·548	0·0678	38·9

At the 263rd hour more than the whole of the gelatinised starch taken has been converted into sugars and 0·0099 g. of the starch of the barley has passed into the form of sugars. Hence the falling off in the value of Δ from the 191st to 263rd hour.

TABLE VII.

Self-digestion of 0·2 g. of Germinated Barley.

Expt.	Time in hours	CuO from 20 cc. of G'	CuO from 20 cc. G' after fermentation with: *S. marxianus*	*S. eriguus*	Mean	Total maltose in 100 cc. G'	Total glucose in 100 cc. G'
10′	119	0·0506	0·0046	0·0034	0·0040	0·0145	0·0903
11′	191	0·0570	0·0034	0·0010	0·0022	0·00795	0·1062
12′	263	0·0607	—	0·0032	0·0032	0·0116	0·1114

Expt.	Glucose / Maltose	$\alpha_D^{20°}$ observed in 400 mm. tube	$\alpha_D^{20°}$ calculated for sugars	Δ	Starch corresponding with sugars	Percentage starch in barley
10′	6·2	0·345°	0·170°	+0·175°	0·0950	47·5
11′	13·4	0·241°	0·268°	−0·027°	0·1032	51·6
12′	9·6	0·255°	0·299°	−0·044°	0·1113	55·6

The above results show that the self-digestion of the barley by its own enzymes gives rise almost entirely to glucose when the digestion is allowed to proceed for a long period. On comparing these results with those of Table III and Table XII obtained with 5 g. of germinated barley it will be seen that whereas after 48 hours the ratio of glucose to maltose is 1·95, after 119 hours the maltose has practically disappeared, the ratio being 6·2; after 191 hours the ratio is 13·4 and after 263 hours 9·6. In Exp. 10′ some of the starch is still present as dextrin, $\alpha_D^{20°}$ observed being greater than $\alpha_D^{20°}$ calculated for the sugars ($\Delta = +0{\cdot}175°$). In Exps. 11′ and 12′ this dextrin has entirely disappeared and the values of Δ are slightly negative ($-0{\cdot}027°$ and $-0{\cdot}044°$). This is probably due to a small proportion of the reducing sugars consisting of laevulose, which has been formed by the inversion of the saccharose present in the original grain (see Table V).

The digestion of the grain is not complete after 119 hours and is still progressing slowly even after 263 hours. The sugars formed correspond with 47·5 % of starch after 119 hours, with 51·6 % after 191 hours and with 55·6 % after 263 hours. These values for starch are probably higher than the true starch in the grain as they include the original sugars present, which amount to 12·92 % (see Table V).

If we use the values given in Table VII as a "control" to correct the values for the digestion of gelatinised starch by 0·2 g. of germinated barley we obtain the following data.

TABLE VIII.

Action of 0·2 g. Germinated Barley on Gelatinised Starch corrected for the Barley used.

Time in hours	CuO from 20 cc.	Maltose CuO (corr.) from 20 cc.	Glucose CuO (corr.) from 20 cc.	Total maltose in 100 cc.	Total glucose in 100 cc.	Glucose / Maltose
119	0·3467	0·1563	0·1904	0·5745	0·3926	0·68
191	0·3680	0·1406	0·2274	0·5150	0·4641	0·90
263	0·3944	0·1321	0·2623	0·4849	0·5550	1·14

Time in hours	Total starch calc. from sugars	Starch as percentage of gelatinised starch taken	$\alpha_D^{20°}$ (corr.) observed in 400 mm. tube	$\alpha_D^{20°}$ calc. for sugars	Δ
119	0·8982	85·3	5·098°	3·990°	1·108°
191	0·9059	88·9	4·597°	3·814°	0·783°
263	0·9591	91·6	4·436°	3·838°	0·598°

The "corrected" values show again that as time proceeds the proportion of glucose increases whilst that of maltose falls; the ratio glucose : maltose

which after 119 hours is 0·68 rises to 0·90 after 191 hours and to 1·14 after 263 hours. The proportion of starch converted into sugars rises from 85·3 % after 119 hours to 88·9 % and 91·6 % after 191 and 263 hours respectively. The difference Δ between the values of $\alpha_D^{20°}$ observed and calculated for the sugars gradually falls from 1·108° at the 119th hour to 0·783° and 0·598° at the later times, as the dextrin in solution disappears owing to its transformation into sugars.

It is probable that actually the whole or the greater part of the gelatinised starch is converted into sugars, as suggested by the data of Table VI, before the more resistant starch granules of the barley are dissolved by its own enzymes, and that the latter change only makes marked progress when the gelatinised starch has largely disappeared. The self-digestion of the barley is in fact greatly modified in the earlier stages by the presence of the excess of starch so that it is incorrect to apply as a "correction" the values given in Table VII for the self-digestions carried out with the grain alone. As the gelatinised starch is transformed into sugars, the self-digestion of the added barley probably proceeds more rapidly but there is no means of ascertaining what proportion of the two kinds of starch present should figure in the calculations at the different intervals of time. As will be seen later, the self-digestion of the "controls" is greatly modified by the presence of an excess of maltose. In the latter case the effect can be more clearly traced (see p. 72).

(vii) *Action of 0·2 g. of germinated barley on maltose at* 38°. The values given above showing the transformation of starch suggest that the glucose is produced from pre-formed maltose by the action of a maltase. To test this point the action of the germinated grain on maltose was studied.

The same barley was used as in the experiments on starch. An approximately 5 % solution of maltose (recrystallised several times from 80 % alcohol) was used. 20 cc. portions of this were digested with 30 cc. of water and 0·2 g. of the finely ground barley as in the experiments with starch in presence of sufficient toluene to prevent the growth of micro-organisms. At the end of the digestion 10 cc. of alumina cream were added to each flask and the contents were boiled to destroy the enzymes. After filtering, the precipitate was washed until the volume of the filtrate was nearly 100 cc., and the solution was finally made up exactly to 100 cc. at 15° (solution *H*). 20 cc. portions of the filtrate were used to determine the reducing power and 20 cc. portions were fermented with *S. marxianus* and *S. exiguus* as in

the starch experiments. 20 cc. of the original maltose solution were diluted to 100 cc. and the reducing power and rotatory power determined with 20 cc. portions (+ 30 cc. water) so as to obtain values comparable with those of the digestions. That prolonged digestion with water alone has no action on maltose was shown by the following experiment. 1·006 g. of the maltose was added to 50 cc. of water and after adding 5 cc. of toluene the mixture was left in a flask closed with cotton wool for 165 hours at 38°. 5 cc. of alumina cream were added, the solution was boiled and made up to 100 cc. at 15°. The rotation of the original solution was 5·290° in a 400 mm. tube at 20°; after the prolonged digestion and treatment as above, the rotation was 5·291°.

TABLE IX.

Action of Germinated Barley (0·2 *g.*) *on approximately* 1 *g. Maltose in* 50 *cc. of water at* 38°.

Expt.	Time in hours	CuO from 20 cc. of solution *H*	CuO from 20 cc. of solution *H* after fermentation with			Maltose in 100 cc. *H*	Glucose in 100 cc. *H*
			S. marxianus	*S. exiguus*	Mean		
13	Nil	0·2722	—	—	—	0·9980	—
14	119	0·3672	0·1580	0·1585	0·1583	0·5810	0·4268
15	191	0·4006	0·1184	0·1247	0·1216	0·4463	0·5765
16	263	0·4262	—	0·1048	0·1048	0·3848	0·6710

Expt.	Glucose / Maltose	Total sugars in solution calc. as maltose	Percentage of maltose left unchanged	$\alpha_D^{20°}$ of solution *H* in 400 mm. tube		
				Observed	Calc. from sugars	Δ
13	—	0·9980	100·0	5·300°	—	—
14	0·735	0·9860	58·2	4·403°	4·098°	+0·305°
15	1·292	0·9939	44·8	4·035°	3·671°	+0·364°
16	1·744	1·0223	38·6	3·763°	3·533°	+0·230°

If we "correct" these values by the control experiments carried out with 0·2 g. of germinated barley (Table VII) we obtain the following data (Table X).

The results given in Table IX are very striking. The values for maltose and glucose are calculated from the reductions before and after fermentation in the usual way. The maltose is seen to fall steadily whilst the glucose increases. The ratio glucose : maltose rises steadily from 0·735 after 119 hours to 1·744 after 263 hours. From the values given in the 10th column for the total maltose corresponding with the sugars in solution[1] it is seen that during

[1] Calculated as follows, for example for the 119th hour:
Total sugars as maltose = 0·5810 g. + (0·95 × 0·4268) = 0·9860 g.

nearly the whole of the action these sugars correspond only with the maltose taken; practically no sugar has been formed from the starch of the barley. *Thus in presence of the excess of maltose the self-digestion of the barley, which gives rise to the values in Table VII, has been almost entirely suppressed.* It is only in the last stage of the action (263rd hour) that the total sugars expressed as maltose (1·0223 g.) exceed the maltose originally taken (0·9980 g.); even here the excess is small, but there is no doubt that in these latter stages additional sugar is being formed from the barley.

TABLE X.

Hydrolysis of Maltose by 0·2 g. Germinated Barley, "corrected" for the Barley used.

Expt.	Time in hours	CuO from 20 cc. H' (corr.)	Glucose CuO from 20 cc. of H'	Maltose CuO from 20 cc. of H'	Glucose in 100 cc. of H'	Maltose in 100 cc. of H'
13′	Nil	0·2722	—	0·1996	—	0·9980
14′	119	0·3166	0·1623	0·1543	0·3315	0·5650
15′	191	0·3436	0·2242	0·1194	0·4630	0·4383
16′	263	0·3655	0·2639	0·1016	0·5510	0·3730

Expt.	Glucose / Maltose	Percentage of maltose left unchanged	$\alpha_D^{20°}$ (corr.) in 400 mm. tube: Observed	Calc. for sugars	Δ	Total sugars calc. as maltose
13′	—	100·0	5·300°	—	—	0·9980
14′	0·586	56·7	4·058°	3·809°	+0·249°	0·8799
15′	1·057	43·9	3·794°	3·389°	+0·405°	0·8781
16′	1·478	37·4	3·508°	3·215°	+0·293°	0·8955

A comparison of the values of $\alpha_D^{20°}$ calculated for the sugars with those actually observed shows that up to the 191st hour the observed rotation is in excess of that calculated by a *nearly constant* but slightly increasing amount (+ 0·305° to + 0·364°); this is no doubt due to the formation of dextrin from the starch of the 0·2 g. of barley, the dextrin increasing only slightly in amount and undergoing very little if any transformation into maltose in the earlier stages. During the last stage of the action (191st to 263rd hour), when the concentration of the original maltose has greatly fallen, the dextrin from the barley starch apparently begins to be transformed into maltose, as shown by the value of Δ diminishing from 0·364° to 0·230°, whilst the total sugars expressed as maltose slightly exceed the original maltose taken.

The above results show that when working with enzymic material, such as germinated barley, a control experiment carried out with this material and water alone does not always give values which can be applied as a true correction, owing to the activity of the enzyme being greatly modified by the

presence of the substrate the change of which is being studied. From Table X it is seen that whilst the maltose values are only slightly changed by introducing a "correction" for the 0·2 g. of barley, since the maltose values obtained with the latter are in themselves very small, the results for glucose are very different from those in Table IX, and far lower than they should be. The sum of the sugars calculated as maltose is always far less than the original maltose taken (viz. 0·8781 to 0·8955 instead of 0·9980), the difference being greatest in the early stages of the action when the effect of the maltose in limiting the self-digestion of the starch is most pronounced. It is clear that the "correction" introduced in this way is far greater than that corresponding with the actual self-digestion of the starch under the modified conditions of experiment.

The formation of glucose from maltose takes place even in short times of digestion. The following series of experiments was carried out to ascertain whether glucose can be detected in the earliest stages of the action. The details of the experiments were the same as in those described above but the times of digestion were shorter.

TABLE XI.

Action of 0·2 *g. Germinated Barley on Maltose Solution at* 38°.

Expt.	Time of digestion hours	Mean CuO from 20 cc. of solution	$\alpha_D^{20°}$ observed in 400 mm. tube
17	Nil	0·2746	5·36°
18	24	0·2857	4·98°
19	48	0·2927	4·92°
20	145	0·3395	4·04°

In these experiments no fermentations were made to ascertain the proportion of maltose and glucose present. It is clear, however, from the increase of reducing power and the fall of rotatory power that the glucose is formed from the start. Fig. 1 shows the result obtained in plotting the values of CuO and $\alpha_D^{20°}$; both the reducing power and the polarisation appear as linear functions of the time.

(viii) *Self-digestion of* 5 *g. of germinated barley.* 5 g. of the barley were ground up and digested at 38° with 100 cc. of water in presence of toluene during 48 hours. 10 cc. of alumina cream were then added, the solution was raised to the boil, filtered and made up to 500 cc. (solution *K*); 50 cc. portions were used for the direct measurements of cupric reducing power and similar portions were fermented as usual with the special yeasts.

After 48 hours' self-digestion of the barley at 38° roughly two-thirds of the sugar in solution are in the form of glucose (glucose : maltose = 1·95). This experiment has given almost identical results to those with 5 g. of the unsterilised barley in Exp. 6, Table III (glucose : maltose = 1·93). In both

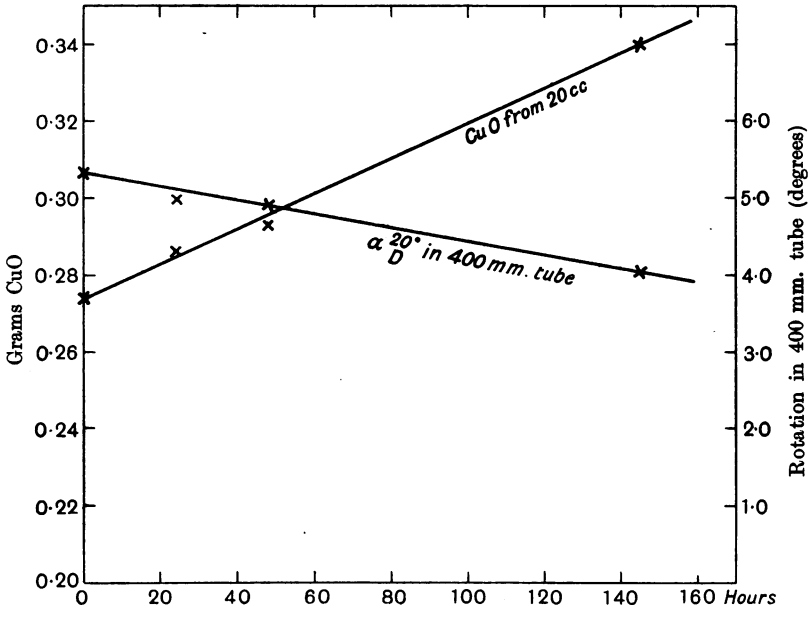

Fig. 1. Action of 0·2 g. of Germinated Barley on Maltose at 38°. (Table XI.)

TABLE XII.

Self-digestion of 5 g. of Germinated Barley.

Expt.	Mean CuO from 50 cc. of solution *K*	CuO from 50 cc. *K* after fermentation with			Glucose in 500 cc. *K*
		S. marxianus	*S. exiguus*	Mean	
21	0·2388	0·0526	0·0508	0·0517	0·7395

Expt.	Maltose in 500 cc. *K*	Glucose / Maltose	Starch in the form of sugars	Percentage of starch calc. on barley
21	0·3794	1·95	1·0252	20·5

cases practically the same proportion of the starch of the grain has been digested: in Exp. 6 the sugars calculated as starch represent 20·16 % and in Exp. 21, 20·5 % of the barley taken. This does not represent the total starch of the grain as shown by the results in Table VII, but only the amount digested in 48 hours. In the above calculation no allowance has been made for the pre-existent sugars in the germinated barley.

SUMMARY.

1. It is shown that air-dried germinated barley contains a *maltase* which hydrolyses maltose to glucose. Attempts to separate this enzyme by extraction with water followed by precipitation with alcohol caused its destruction; the preparation obtained only acted on starch in the same way as ordinary malt extract, forming maltose and dextrin.

2. The presence of maltase in germinated barley is shown, however, by allowing the finely powdered grain to act upon starch or maltose at 38°. Under these conditions a large amount of glucose is formed in both cases; as the proportion of glucose increases, that of the maltose falls. There is no doubt that in the case of the starch the glucose arises wholly from preformed maltose and not directly from starch. The action on starch is very similar to that of taka-diastase, which contains maltase in addition to the ordinary diastatic enzymes.

3. When the germinated barley is allowed to digest itself for a prolonged period, practically the whole of the starch disappears and is converted into glucose. In the earlier stages of the action dextrin and maltose are found but they gradually disappear until only glucose remains. The action probably takes place in the series of stages:

starch → soluble starch → dextrins → maltose → glucose.

4. When a small quantity of germinated barley (0·2 g.) is used to digest gelatinised starch or maltose, the self-digestion of the barley starch is largely inhibited until the greater part of the added starch or maltose has been converted into glucose. This makes it impossible to apply a "correction" for the enzymic material by carrying out a control with the latter in presence of water only.

5. When the germinated barley (0·2 g.) digests gelatinised starch, dextrin, maltose and glucose are found even after very prolonged periods. The dextrin and maltose gradually and continuously fall, but even after 263 hours both are still present; the glucose steadily increases in amount during the whole period of digestion.

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