III. THE DISTRIBUTION OF MALTASE IN PLANTS. I. THE FUNCTION OF MALTASE IN STARCH DEGRADATION AND ITS INFLUENCE ON THE AMYLOCLASTIC ACTIVITY OF PLANT MATERIALS.

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In an investigation of the sugars present in the leaves and stems of various plants [Davis, Daish and Sawyer, 1916; Davis and Sawyer, 1916] it was found that maltose was invariably absent. Many different types of plants were examined, some of which, for example the potato, turnip, *Tropæolum* and sunflower, elaborate large quantities of starch in the leaf during the day-time to be used as a reserve during the night. If the degradation of the starch were effected merely by the ordinary diastatic enzymes, which convert starch into maltose and dextrin, maltose should be found in the leaves or circulating system of these plants, especially at night. In no single case, however, throughout the night or day could even traces of maltose be detected in starch-forming plants. Negative results were also obtained with other plants such as the mangold, dahlia and grape vine which store reserve carbohydrates other than starch (saccharose, inulin and glucose). For the first time in work of this kind a method of analysis has been used, based on the action of selective yeasts, such as *S. marxianus* and *S. exiguus* [Davis and Daish, 1913], which enables maltose to be estimated accurately in presence of other sugars such as saccharose, glucose, laevulose and pentoses. From the results of some 500 analyses, we can affirm with certainty that maltose was entirely absent from the leaf and stems of all the plants we examined. The writer is of opinion, on grounds which will shortly be stated, that this absence of maltose will be found to be a general if not universal phenomenon in plants.

From the work referred to above the writer has been led to extend the views put forward by Brown and Morris [1893] as to the mechanism of starch degradation in plants, by assuming that the enzyme *maltase* plays a part also in the transformation of starch into soluble sugars. This enzyme, instead of having a very limited distribution as assumed hitherto, probably takes part invariably in breaking down the starch in plant tissues. This degradation undoubtedly takes place in a series of successive stages, of which at least three may be distinguished:

(1) Starch → soluble starch and dextrins (*"Liquefying" enzymes*).
(2) Dextrins → maltose (*Dextrinase*).
(3) Maltose → glucose (*Maltase*).

The Presence of Maltase in Foliage Leaves and Seeds.

The fact that maltose was invariably absent from the leaves and circulating vessels of plants suggested that experiments should be made to ascertain whether maltase existed in foliage leaves, especially during the times when starch degradation is at its maximum. Such experiments carried out in this laboratory by A. J. Daish have shown that this is invariably the case; they are described in the following communication [Daish, 1916, 1].

The part played by maltase in the germination of seeds in which large stores of starch are gradually utilised has been hitherto almost entirely ignored. Since the pioneer work of Brown and Morris [1890] on the germination of some of the Gramineæ it has been always assumed that the starch is resolved by the enzymes of the seed simply into maltose and dextrin, and all calculations as to the development and activity of the enzymes in germinating seeds have been based on the assumption that a *single* reducing sugar, maltose, is formed. In the very careful work of Brown and Escombe [1898] and more recently of Stoward [1911] on the distribution of enzymes in the barley grain during germination, the fact that two saccharifying enzymes are present, capable of forming *two* sugars, has been left out of consideration. In the writer's opinion, if this fact had been recognised, certain results obtained by Stoward which are at present difficult to understand, such as the power possessed by the non-living inner endosperm of augmenting its amylolytic activity, would have found an explanation.

The results obtained by A. J. Daish [1916, 2] clearly show that in germinating barley considerable quantities of maltase are present and that the starch of the grain is resolved by this enzyme finally into glucose. We are conscious that these experiments could have been made more complete in several

directions, but as they were carried out more than two years ago, being interrupted by the war, and there is little prospect that further work can be done in the immediate future, they are published as they stand.

Maltase was first discovered in maize by Cuisinier [1885, 1886] who termed it "glucase" and patented a process of converting starch into glucose based on its use. The later work of Geduld [1891], Beyerinck [1895], Morris [1896], Huerre [1909] and Wierzchowski [1913, 1, 2] has confirmed that of Cuisinier and shown that maize is particularly rich in maltase. Regarding the presence of maltase in other cereals, especially barley, there has been a great divergence of opinion which it is now necessary to explain.

The experiments of Butkewitsch [1908] on the enzymes present in the barks and woods of plants during the spring period of starch dissolution are interesting as showing the probable presence of maltase in these parts, although Butkewitsch does not specifically recognise this. Such bark, *or even an aqueous extract of it,* hydrolyses starch and maltose; but when the extract is precipitated by alcohol the property of acting on maltose is destroyed. It is clear that this is due to the presence of *maltase.* The influence of chloroform and toluene in promoting starch dissolution is shown; when twigs are cut and stored in water containing toluene or chloroform, the starch dissolving enzymes rapidly come into action and remove the starch stored in the rind. In water alone the starch is not affected. Toluene has a far greater effect than chloroform, no doubt owing to the fact that the latter largely destroys the maltase. Butkewitsch's experiments [1908, p. 339] showing the limitation of the starch dissolving action by the presence of glucose are also important.

Failure of earlier Workers to detect Maltase in Cereals.

The failure of earlier workers to recognise the universal distribution of maltase in cereals is largely due to the fact that it is an endo-cellular enzyme which does not readily diffuse out of the plant cells and is not easily extracted by water. Extracts of the cereals (with the exception of maize) as a rule have little action on maltose and produce only small quantities, if any, of glucose, from maltose or starch. Many workers have failed to detect maltase owing to its instability. They have worked under conditions which destroyed the enzyme. Thus they used enzyme preparations which had been precipitated by alcohol or worked in presence of chloroform as an antiseptic, both of these substances *under ordinary conditions* rapidly destroying maltase.

It seems probable that *pure* alcohol and *pure* chloroform would not destroy maltase and that their usually destructive action is due to the presence

of small quantities of acid or alkali. Ordinary chloroform, when kept in a white glass bottle, frequently contains large quantities of hydrogen chloride and carbonyl chloride; when its decomposition into these substances is prevented by adding lime, the chloroform may become distinctly alkaline. Ordinary alcohol is generally acid, but in some cases, when stored in soft glass bottles, it becomes alkaline. Differences in the nature of the chloroform would explain the fact that Tebb [1894] succeeded in extracting maltase from animal tissues with chloroform water whereas Brown and Heron [1880] failed; in the latter case it is probable that the chloroform used destroyed the enzyme. In the same way the contradiction between Fischer's [1894] positive results as to the action of yeast on maltose in presence of chloroform and his later negative results [1895] under the same conditions, which confirmed the equally negative results of Morris [1895], may be explained. Similarly Beyerinck [1895] succeeded in precipitating the maltase of maize in an active condition with alcohol and Marino and Sericano [1905] the maltase of germinated barley; whilst Huerre [1909] and Wierzchowski [1913, 1, 2] state that this method gives with maize a preparation which contains little active maltase but behaves like ordinary diastase, converting starch into maltose, a result clearly due to destruction of the maltase. Our own experience [Daish, 1916, 2, p. 65] with germinated barley has been similar to that of Wierzchowski with maize.

In other cases the action on starch has been studied at a temperature of 55–60°, at which the maltase is either destroyed or at least has its activity greatly restricted. Huerre [1909] has shown that the maltase of the "*blanc hâtif*" maize of the Landes is very rapidly destroyed at 65° and somewhat less rapidly at 50° (completely in 2 hours). In other varieties of maize the maltase was found to be more resistant, probably owing to the presence of protective substances. The writer [1914] has shown that the very active maltase of taka-diastase has its power of converting maltose enormously restricted at 55°. Beyerinck's [1895] maltase from maize resisted a temperature of 55°, whilst the maltase prepared from yeast was rapidly killed at this temperature.

As a rule, the optimum temperature of plant maltases lies in the neighbourhood of 38–40° as in the case of yeast maltase; above 50° rapid weakening occurs. In some cases, however, for reasons which have not been ascertained, certain plant maltases, for example the "*haute maltase*" of maize "*jaune hâtif*" [Huerre, 1909], show a higher temperature of optimum action.

Bearing the above facts in mind it is possible to explain all the con-

tradictory statements as to the occurrence of maltase in leaves or in germinating or resting seeds. When maltase has *not* been found it has been due to the method of working either failing to extract the enzyme or destroying it. It is interesting historically to note that Brasse [1885] prior to the work of Cuisinier and Geduld on "glucase" clearly realised that the "diastase" isolated from germinated barley or from foliage leaves is capable of digesting *raw* starch to glucose, if the digestion *takes place at a low temperature* (34–42°). He points out that, providing the *extract is made rapidly in the cold* and "*subisse le moins longtemps possible l'action de l'alcool*" used to precipitate it, a product is obtained which effects the complete degradation of starch to glucose. Brasse, however, did not realise that a special enzyme (maltase) is concerned in the change as did Cuisinier shortly after, nor did he show that it acts by breaking down the maltose formed from starch by other enzymes. He realised, however, more clearly than later workers, the relative instability of the active enzyme and, in particular, points out that no glucose is formed at 50–57°. Had Brasse's paper been properly appreciated many of the contradictory statements of later workers would have been avoided.

It is not necessary here to analyse in detail the work of all those authors who failed to find maltase in plant material. Brasse, Cuisinier and Geduld considered that *all* cereals contain a glucose-forming enzyme (*glucase*). Lintner and Eckhardt [1889] contrasted the properties of the diastase of ungerminated barley with those of the diastase of malt, and although they concluded that the former gave rise only to maltose and dextrin, it would appear from the fact that they found the optimum temperature of activity (45°) to be considerably lower than that of malt diastase and a great falling off of saccharifying power to occur above 50°, that they worked with a preparation containing maltase. Later Lintner [1893] confirmed Cuisinier's statements [1885, 1886] as to the presence of "glucase" in maize and stated that glucose may also be formed in not inconsiderable quantities by the action of dried malt on starch. Brown and Morris [1893] in the case of foliage leaves observed only a transformation of the starch into maltose, and Morris [1896] stated that he had examined a considerable number of malted and unmalted grains but could find no evidence of a maltase. Beyerinck [1895] denied the general distribution of maltase in leaves and seeds (e.g. barley, wheat and rye) but found it to be present in the grain of maize, sorghum and millet. There is no doubt that in these last seeds it is especially abundant so that it could hardly escape detection even under adverse conditions of working. Later workers like Brown and Escombe [1898], Ling and Baker [1895, 1897 and

1902], B. F. Davis and Ling [1904], Ford [1904], Ford and Guthrie [1908] have all ignored the possible presence of maltase in barley and malt. On examining the conditions under which these authors worked it is clear that their negative results are to be attributed to their not realising the instability of the enzyme and the difficulty of extracting it.

Beyerinck [1895, p. 338] seems to have understood the difficulty of extracting "glucase" with water. Thus he says that rice meal contains much glucase, but on attempting to extract it he obtained, much to his surprise, an enzyme which acted strongly on starch but converted it only into maltose, without forming glucose. A similar result was obtained with oats. He is inclined to believe "though proof of this is difficult" that in the process of purification adopted, "glucase" is converted into "*granulase*" (that is, the enzyme which produces maltose and achroodextrin from starch). Obviously the maltase had been destroyed leaving behind the less sensitive ordinary diastatic enzymes.

The recent work of Wierzchowski [1913, 1, 2], published since our own was undertaken, clearly proves that all cereals contain considerable quantities of maltase, although as a rule it cannot be extracted with water owing to its endo-cellular nature. In buckwheat, millet and maize it is particularly abundant; in rye, barley, wheat and oats less so.

Wierzchowski realising the insolubility and instability of the enzyme made his experiments in the same way as we have done: the *finely-ground meal* was allowed to act upon the starch or maltose solution for 24 hours at 45° in presence of formaldehyde as antiseptic. The glucose formed was estimated as osazone; as the total reducing power was also determined, the course of action could be followed. The polarimeter was not used.

As will be seen later, Wierzchowski incorrectly interprets his results, in so far as he considers that his "maltase" could act directly upon starch, as well as upon maltose; the whole of his results, as well as our own, are best explained by the view that the maltase simply breaks down maltose which has been formed from starch by the other diastatic enzymes (see p. 39).

Presence of Maltase in Malt and Malt Extracts.

Germinated barley as Daish's [1916, 2] results show, as well as those of Brasse [1885], Marino and Sericano [1905], Marino and Fiorentino [1906] and Wierzchowski [1913, 1, 2], undoubtedly contains maltase. Whether a malt or malt extract made from such barley contains maltase or not will depend upon circumstances. If the malt be dried at a low temperature, the maltase will largely survive, but if it be kilned at a high temperature, part, if not the

whole, of the maltase may be destroyed, so that such malt will contain principally the ordinary diastatic enzymes which break down starch only to the stage of maltose. On the other hand, kiln drying, if not too prolonged or carried out at too high a temperature may actually facilitate the extraction of maltase *by water,* by rupturing the cells or destroying the protoplasm in which the enzyme is imprisoned, just as drying facilitates the extraction of maltase from yeast, which fails to yield its enzyme when in a fresh condition. These views would explain the idea of Ling and Baker [1895] that kilning confers upon malt the power of converting maltose into glucose and that air-dried malt does not possess this property; Kröber [1895] asserted exactly the opposite. It is possible to reconcile these two statements by assuming maltase to be present which was rendered more easily soluble under Ling and Baker's conditions of malting and destroyed under those of Kröber. It is equally possible to understand Morris' statement [1896] that Ling had later been unable to confirm the observations of Ling and Baker that "the ability to convert maltose into dextrose which some malts possess is conferred by the kilning process and we may therefore take it that a normal malt contains no enzyme capable of hydrolysing maltose."

Most malt *extracts* probably contain little or no maltase, owing both to the difficulty of extracting this enzyme by water and the fact that it is readily destroyed during either kilning or extraction and concentration, especially if evaporation is carried out at too high a temperature (above 45°). Brasse [1885] stated that although germinated barley contained a glucose-forming enzyme, this was entirely absent from all the commercial malt extracts he had examined.

Whilst maltase is probably absent from most commercial malt extracts this should not be presumed in the case of any sample without proof. *Fine grinding* of malt, by rupturing the cells which contain the maltase, may increase the amount of the latter which is extracted by water. Thus Wierzchowski [1913, 2] by extracting maize which was ground to different degrees of fineness obtained the following comparative results for the glucose formed from maltose:

Coarse grinding (above 2 mm.)	...	...	6·0 % glucose
Less coarse (above 0·5 mm. sieve)		...	28·0 ,,
Fine (below 0·5 mm. sieve)	...	...	37·5 ,,

It is well known that the diastatic activity of a malt differs considerably with the fineness of grinding, a fact which is probably due in part to differences in the amount of maltase passing into solution.

Formation of Glucose by the action of Malt or Barley Diastases on Starch.

Ling and Baker's [1895] statement, that the diastase of high dried malt produces glucose whereas low dried malt does not, has been explained above. As a matter of fact low dried malt contains a considerable amount of maltase but it is not readily extracted. Baker [1902] working at 50° with the precipitated diastase of ungerminated barley found glucose to be formed from soluble starch after more than 6 hours; as the precipitated diastase failed to act upon maltose but converted "α-amylodextrin" into maltose and glucose, he considered that the glucose was formed from the dextrin and not from maltose. It is probable that Baker's enzyme, although it had been precipitated by alcohol and so weakened, contained some maltase, which in the long periods of digestion in his experiments gave rise to glucose. The action would in fact be similar to that of taka-diastase, which contains maltase, at 55° [Davis, 1914]; although the activity of maltase is greatly impaired at this temperature it is still capable of forming 20 % of glucose from starch after 50 hours (1 % solution of starch, 0·1 g. taka-diastase). The fact that maltose was not attacked by Baker's weakened enzyme is not surprising and may be attributed to impurities in the maltose used[1].

B. F. Davis and Ling [1904], who found glucose to be formed from starch by the action of "restricted" malt diastase, worked with a precipitated diastase prepared from a malt dried below 33°, which therefore contained undestroyed maltase; they considered that when this diastase had its activity "restricted" by heating for a short time at 60–70°, the enzyme was so modified in its nature as to give glucose among the products of its action on starch. It is more probable that the glucose owed its origin, in the prolonged periods of digestion adopted, to traces of maltase which escaped destruction during the "restriction." The slow formation of glucose under these conditions is quite parallel to the results obtained by partly destroying the activity of taka-diastase by heating at 55°. That the heating operation, which was supposed to confer the power of forming glucose, actually destroys maltase appears from the gradual falling off of the power of forming glucose with the time of heating. "Restriction" at 70·5° for example gave an enzyme which after 67 hours' action on starch gave the following quantities of

[1] Maltose recrystallised several times from alcohol in *ordinary* glass may take up sufficient alkali to destroy the very sensitive maltase. The writer has had preparations of maltose which failed to yield glucose with maltase on this account; maltose to be used in such experiments should be recrystallised in Jena glass vessels.

glucose (measured as osazone): 5 minutes' "restriction," 10·8 %; 17 minutes', 10·0 %; 30 minutes', 7·3 %; 120 minutes', 3·9 % [B. F. Davis and Ling, 1904, Series vi, vii and viii].

Ling [1907, p. 182] considers that the action here discussed was not due to maltase, as the heated diastase solution did not convert maltose into glucose; but this was probably owing to the reason given above. In their recent work on the action of precipitated diastase from kiln-dried malt on starch granules Baker and Hulton [1914] generally observed the formation of glucose at low temperatures after digestions of 4 days or more. There is little doubt that this was due to the presence of traces of maltase which escaped destruction during the precipitation of the diastase by alcohol, although they regard the glucose as formed from dextrins of low molecular weight. They consider that "it is unlikely that maltose can undergo hydrolysis by an amylase separated from malt."

Ling and Rendle [1904] stated that commercial concentrated malt extracts invariably contain glucose. Part of this glucose is probably formed from the starch of the malt by the action of maltase, but as was pointed out by Beyerinck [1895] it is probable that part arises from the inversion of saccharose, which is invariably formed in barley during germination. After extraction, the saccharose would undergo inversion by the invertase present in the extract.

The Maltase of Plants does not act directly on Starch or Dextrin but only on Maltose.

Most of the investigators who have discussed the formation of glucose from starch have considered that this sugar may be split off from the starch or dextrin molecule by the direct action of an enzyme, without the prior formation of maltose. Thus Beyerinck [1895] held that the "glucase" of maize might act directly on starch forming dextrin and glucose; also that it is capable of resolving dextrin into maltose and glucose. Ling and Baker in their various papers expressed a similar view that glucose might be formed directly from amylodextrin [compare Baker and Hulton, 1914]. Kita [1913] held a similar view with regard to the action of taka-diastase or "koji" on starch. The writer [1914] has shown that in this case glucose is undoubtedly produced from pre-formed maltose; as the glucose appears an equivalent quantity of maltose disappears and the action is perfectly continuous.

In the case of the enzymes of germinated barley it is not so easy to give a definite proof that the glucose arises only from maltose, as the dextrin

stage persists longer than with taka-diastase; as shown by Daish [1916, 2; Table VI, p. 67], dextrin is present even in the later stages (263rd hour) of the action. The fact that the starch of the barley itself undergoes slight but progressive digestion is an additional complication, it being impossible to correct for the sugars formed from the starch in a rigidly exact way by a "control" experiment as the self-digestion of the barley is greatly modified by the presence of maltose or starch [Daish, 1916, 2, p. 70]. The following considerations founded on the data in Tables VI and IX of Daish's paper [1916, 2, pp. 67, 71] make it highly probable, however, that the glucose is derived entirely from maltose and not from starch or dextrin.

TABLE I.

Action of 0·2 g. Germinated Barley on Maltose—Increments of Glucose compared with Maltose Disappeared.

Interval	Time, hours	Glucose formed, g.	Maltose calc. as equivalent to glucose formed, g.	Decrease of maltose, g.	Remarks
1	0–119	0·4268	0·4054	0·4170	Maltose corr. with glucose nearly = maltose transformed
2	119–191	0·1497	0·1422	0·1347	Maltose corr. with glucose > maltose converted
3	191–263	0·0945	0·0898	0·0615	Maltose corr. with glucose much > maltose converted

TABLE II.

Action of 0·2 g. Germinated Barley on Starch—Increments of Glucose.

Interval	Time, hours	Glucose formed, g.	Maltose corr. with glucose, g.	Maltose apparently transformed, g.	Remarks
2	119–191	0·1019	0·0968	0·0645	Maltose corr. with glucose > maltose apparently converted
3	191–263	0·0885	0·0840	0·0273	Maltose corr. with glucose much > maltose apparently converted

The tables given above show the increases of glucose (calculated as their equivalent in maltose) compared with the maltose which has disappeared in the successive intervals during the transformation of maltose and starch by 0·2 g. of germinated barley [see Daish, 1916, 2, Sections vi and vii].

As stated on p. 32 we regard the degradation of starch as taking place in a series of successive stages, each characterised by an appropriate enzyme:

Starch ⟶ soluble starch ⟶ dextrins ⟶ maltose ⟶ glucose.

All these changes run concurrently during the whole time of action, but the relative velocities of the different changes are considerably modified by alterations in the concentration of the products of action at different periods. The fact that when germinated barley is used the starch of the latter is itself hydrolysed, especially during the later stages of action, is an additional complication. In the relatively simple case of the hydrolysis of pure maltose, this factor has no great disturbing effect in the early stages of the change, as the excess of maltose largely inhibits the self-digestion of the starch [see Daish, 1916, 2, Section vii]; consequently the glucose formed corresponds with the maltose that has disappeared (actually in the first 119 hours, see Table I, the glucose formed, calculated as maltose, 0·4054 g. is slightly less than the maltose which has disappeared, 0·4170 g.). In the later stages, however, with both maltose and starch, the glucose formed, calculated as maltose, is considerably greater than the maltose apparently transformed. This is no doubt due to the fact that in these periods, as the original maltose concentration falls, more and more of the starch of the barley undergoes transformation into maltose, so that there is a new formation of maltose to replace that transformed into glucose.

That the glucose is formed entirely from pre-formed maltose even in the case of the starch hydrolysis appears clearly on plotting the data in Tables I and II, so as to show the rate of formation of glucose from starch as compared with that obtained in the case of pure maltose (Fig. 1, p. 42). With starch this curve is practically a straight line from the 119th to the 263rd hour. With maltose, the curve can be plotted from the beginning of the change and it is seen that up to about the 180th hour the rate of formation of glucose gradually falls, but subsequently the glucose is formed along a straight line which almost coincides in position and direction with that showing the formation of glucose from starch.

In no period of the starch transformation is the rate of increase of the glucose greater than that found in the maltose transformation, so that there is no evidence pointing to a direct formation of glucose from starch. In fact during the first stage, up to the 180th hour, the rate of increase of the glucose is greater in the case of the maltose hydrolysis than in that of starch as shown by the greater steepness of the curve. This is well marked from the 119th to 180th hour when the *concentration* of the glucose present is less in the case of the maltose curve than in that of starch; it is this concentration which determines the rate of formation of fresh glucose. During the period from the 180th to 263rd hour, *when the concentration of the glucose is the same in both cases,*

the curves for starch and maltose are practically coincident and form strictly parallel straight lines.

Fig. 2 shows the rate of *apparent* disappearance of maltose in the two hydrolyses. With starch the curve is less steep than with pure maltose. This is no doubt due to the fact that, in the case of starch, maltose is being formed from dextrin at the same time as it is being transformed into glucose by the maltase of the barley. Maltose therefore *apparently* disappears less rapidly in this case than in that of the maltose alone.

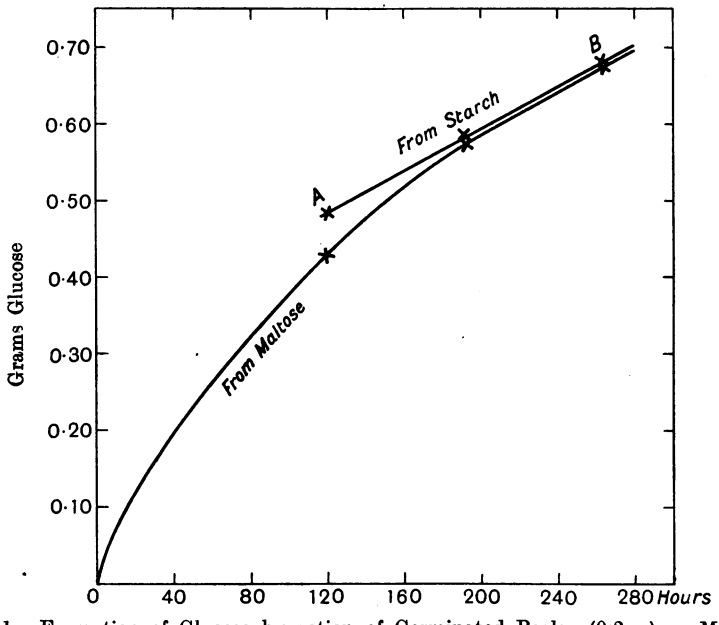

Fig. 1. Formation of Glucose by action of Germinated Barley (0·2 g.) on Maltose and Starch at 38°.

There is therefore no reason to believe that the glucose formed in the transformation of starch ever arises directly from the starch *or in any other way than by the action of maltase on pre-formed maltose.* The action of the germinated barley in this respect is exactly similar to that of taka-diastase or "koji." Beyerinck's [1895] results which were supposed to prove the contrary can be explained by the view that his precipitated "glucase," *which attacked starch and dextrin,* contained sufficient of the ordinary amylo-clastic enzymes to produce the maltose on which the "glucase" could act. That this was the case appears clearly from his statement [Beyerinck, 1895, p. 335] that the formation of glucose from soluble starch or dextrins is less rapid than from maltose. In his purified "glucase" the proportion of the

ordinary amyloclastic enzymes which form maltose was relatively less than that of maltase, so that the rate of formation of glucose in the case of the starch was limited by the small rate of production of maltose. Maize indeed seems to contain the liquefying and maltose-forming enzymes in relatively small amount as compared with other cereals[1] such as barley and the maltase in relative excess, although, as the results of Wierzchowski [1913, 1] show,

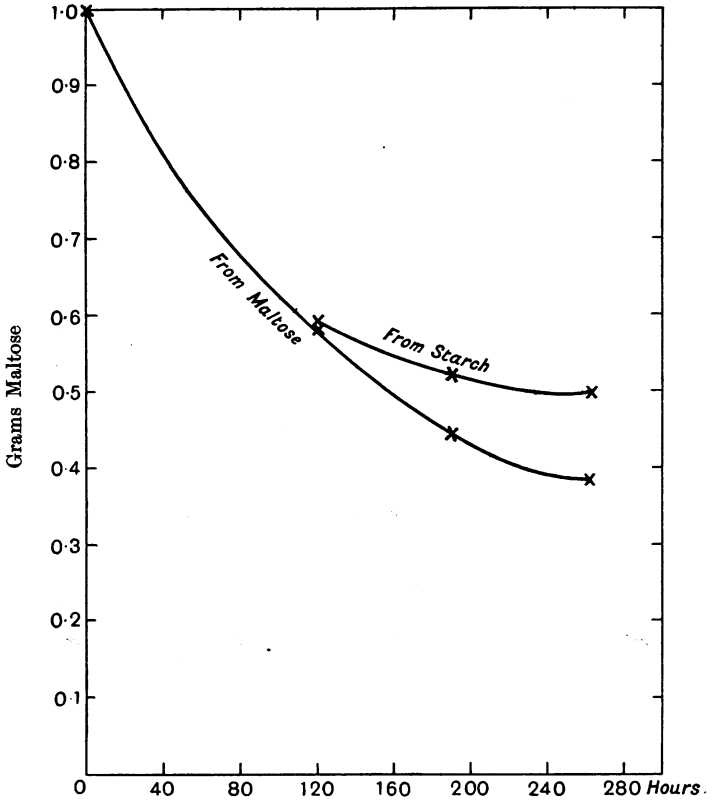

Fig. 2. Fall of Maltose by action of Germinated Barley on Starch and Maltose at 38°.

finely ground maize meal which has *not been first extracted with water* to remove these enzymes contains sufficient of the ordinary diastases to form maltose faster than it can be dealt with by the maltase.

Wierzchowski [1913, 1] commenced his work on the action of maize-maltase on starch in the hope of being able to split off glucose directly from the latter by rupturing the γ-bonds of Syniewski's formula for starch. He

[1] This appears also from Cuisinier's patent [1885]. In order to facilitate the formation of glucose from maize, the first stages of the action are hastened by the addition of *malt*, which supplies the ordinary diastatic enzymes.

found instead that action occurred, in two stages; in the first maltose was formed and in the second glucose. He correctly attributed this to the presence of the ordinary diastatic enzymes producing dextrins and maltose. In the first stage of the action, maltose is formed more rapidly by these enzymes than it can be converted by the maltase, so that it accumulates[1]. He then extracted the ordinary "diastases" from the meal, completely as he thought, by water and subjected the starch to the action of the extracted meal. He now obtained an entirely different result, glucose apparently being produced without maltose, and considered that he was effecting a pure γ-hydrolysis. The fallacy underlying this view is that in all probability he had failed *completely* to remove the ordinary maltose-forming enzymes by his preliminary extraction with water. What really happened was that these enzymes were left in relatively small proportion to the insoluble maltase present, so that when the *extracted* meal acted on starch, maltose was formed from starch far less rapidly and, *as fast as it was formed,* could be dealt with by the excess of maltase present and transformed into glucose. Consequently maltose did not accumulate. An examination of Wierzchowski's data (glucosazone yields) shows that, even in those experiments in which glucose was thought to be formed directly, some maltose was formed, so that it is clear that he had not entirely removed the ordinary amyloclastic diastases.

In the writer's opinion, there is no reason at present to assume that glucose is ever split off directly from starch or dextrins or that the degradation of starch takes place otherwise than by the series of changes:

Starch $\longrightarrow$ soluble starch $\longrightarrow$ dextrins $\longrightarrow$ maltose $\longrightarrow$ glucose,

each of which changes corresponds with the activity of a special enzyme. The localisation of the different enzymes in the seed is in accord with this view and may be briefly discussed.

Localisation of Maltase in Plant Seeds.

Brown and Morris [1890] at first regarded the scutellum of the embryo as the principal seat of diastatic activity and considered that the aleurone layer was not actively secretive; in later work Brown and Escombe [1898], however, concluded that the aleurone layer has real secretive functions, a

[1] The action of the maize meal is in these respects exactly similar to that of taka-diastase [Davis, 1914]; in both cases the breaking down of starch to maltose gives rise at first to far more maltose than can be immediately transformed. In the case of taka-diastase, dextrin disappears after the first 3 or 4 hours, and after this period the change consists solely in a transformation by maltase of maltose into glucose. The behaviour of taka-diastase towards starch under different conditions is the key to the whole question of the nature of the plant enzymes which effect starch degradation.

view supported by the recent work of Stoward [1911] which tends to show that the aleurone layer has actually a far higher amyloclastic activity than the embryo. In all this work, however, the enzymic activity has been measured on the assumption that only a single sugar—maltose—is formed during the starch transformation; the activity has been expressed in terms of reducing power only, without reference to the actual products of the action. It is unfortunate that Stoward in his recent very detailed work on the barley grain did not realise that in the depletion of the grain the enzyme maltase plays an important part, giving rise to glucose, a sugar with nearly twice the reducing power of maltose. Stoward's digestions to measure the amyloclastic power were carried out at 30°, a temperature at which the maltase could act.

Neither Stoward nor Brown and Escombe have referred to the early but very important work of Beyerinck [1895] on maize, which although giving, as we have shown above, incorrect views as to the nature of starch degradation and the general distribution of maltase, clearly indicates a different place of origin of the different enzymes in the grain. Beyerinck's work is extremely interesting as suggesting that the aleurone layer and embryo secrete in the main *different* enzymes[1]. The scutellum secretes mainly *granulase,* that is the starch-splitting enzyme, especially at the commencement of germination. The aleurone layer is the source of the "glucase," that is the maltose-splitting enzyme. Exactly the same differentiation of function is found in sorghum. According to Wierzchowski [1913, 1] also the "maltase" is mainly localised in the aleurone layer of maize, from which it is best prepared. The embryo, although active in producing the ordinary diastatic enzymes, contains little or no "glucase" (maltase); this enzyme is mainly elaborated by the aleurone cells. It is impossible in the present state of our knowledge to criticise these views, but it is highly probable that different cells secrete the ordinary diastases and the maltase of seeds during germination. It is certain that in future work on the localisation of the amyloclastic enzymes in seeds, the part played by maltase must be more fully recognised than it has been hitherto.

[1] In Beyerinck's nomenclature *granulase* is the enzyme which transforms starch into maltose and *achroodextrin*; *maltase* is the enzyme which forms maltose and *erythrodextrin*; *glucase* forms glucose (either from starch, dextrins or maltose). *Dextrinase* converts granulose into "maltodextrin" only. "Amylase" is a collective name for all the starch-splitting enzymes. Beyerinck in general names his enzymes according to the product they form: his *maltase* forms maltose, and must be distinguished from the enzyme now generally called maltase, which is capable of resolving maltose (and α-glucosides) *only* into glucose. This function is given by Beyerinck to "glucase," but as shown above this author is in error in regarding this enzyme as capable of direct action on starch and dextrin. Wierzchowski is equally in error in assuming that his "maltase" (which corresponds more closely to the modern maltase) acts directly on starch or dextrin (see p. 44).

Measurement of Amyloclastic Power (Diastatic Power) of Cereals, Malts and Diastase Preparations.

In order to obtain accurate comparative values of the diastatic activity of plant tissues or enzymic preparations it is necessary to take into account the possible presence of maltase. When maltase is present (air-dried barley, germinated or otherwise, green malts, taka-diastase, pancreas preparations) which gives rise to glucose, a sugar with nearly twice the reducing power of maltose, it is not sufficient to measure the cupric reducing power only after the transformation, but it is necessary to take into account the actual products of the change. Thus in the case of taka-diastase, which contains much active maltase, the measurement of the cupric reducing power only without reference to the specific rotatory power has led to entirely erroneous ideas as to the nature of this enzyme (e.g. by Stone and Wright [1898]). It is true that probably most of the commercial diastase preparations (malt extracts, precipitated diastases) contain very little maltase, owing to its destruction or non-extraction during the process of manufacture; but the recent work of Sherman and Schlesinger [1912] shows that pancreatic preparations, even after the processes of purification and concentration adopted, may contain a considerable amount of maltase which has escaped destruction.

With plant material such as ground barley or malt, the ordinary method of measuring diastatic activity by means of an aqueous extract probably gives a very incorrect idea of the total diastatic enzymes: this is clear from the work of Ford and Guthrie [1908] who showed that by using papain digestion in the preparation of the extracts far higher results (varying from two to sevenfold) were obtained in the case of barley. The ordinary aqueous extraction measures only the *easily soluble diastatic enzymes* which form maltose; papain extraction not only renders soluble the endo-cellular enzymes such as maltase but preserves them in solution. This preservative action is one of the principal functions of the papain in such cases; as was shown by Ford and Guthrie, high values are also obtained by using boiled papain solutions of which the digestive activity had been destroyed. How much of the increased activity in Ford and Guthrie's experiments was due to maltase is not clear: they worked at a temperature of 30°, at which maltase would be active, but they distinctly state that glucose was not formed in their experiments.

The Lintner method of determining diastatic activity at 70° F. (21° C.) would allow maltase to act, but not under optimum conditions. In Sherman, Kendall and Clarke's [1910] method on the other hand the temperature prescribed, viz. 40°, is that of the optimum activity of maltase. In the case

of diastatic agents rich in maltase, such as taka-diastase, the two methods would probably give very different results. Tests carried out at 55° at which the activity of maltase is largely destroyed would again give rise to a totally different measure of diastatic activity.

Fine grinding in the case of barley or malt, as shown on page 37, would increase the diastatic activity by liberating or rendering soluble a larger proportion of endo-cellular enzyme.

Self-digestion of Barley or Malt.

It is clear from Daish's results [1916, 2, Sections i and v] that the self-digestion of germinated barley or malt leads finally to the production of large quantities of glucose. In the early stages dextrins and maltose are also formed, but as time proceeds these give place more and more to glucose, as the action of maltase continues.

Summary.

1. Maltase is probably present universally in plants in which starch degradation occurs. The failure of earlier workers to detect it is due to the fact that this enzyme is endo-cellular and therefore not easily extracted by water; and to its instability. It is easily destroyed by ordinary alcohol or chloroform. Its action, too, is greatly limited or even destroyed at temperatures above 50°.

2. Maltase occurs in considerable quantities in germinated and ungerminated cereals. In malt it may sometimes occur, if the kilning has been at a sufficiently low temperature not to destroy it. In such cases the kilning may actually render the maltase more easily extractable by water. The presence of maltase in malt or malt diastases would explain the formation of glucose from starch which has previously been attributed to other causes.

3. It is shown that the maltase of plants does not act directly on starch or dextrins but only on the maltose, which has been formed by the ordinary diastatic enzymes; no direct splitting off of glucose from starch ever occurs. When such appears to be the case, as in Beyerinck's and Wierzchowski's experiments, maltose has been formed by the previous action of the ordinary diastases and has been then acted on by maltase.

4. The action of the enzymes of germinated barley on starch is very similar to that of taka-diastase, only taka-diastase is richer than the barley in maltose-forming enzymes so that the dextrin stage is passed through more rapidly. In both cases the glucose is formed by the action of a maltase on maltose.

5. It is probable, from Beyerinck's results with maize, that the maltase is localised mainly in the aleurone layer of the endosperm.

6. In determining the diastatic activity of plant material and preparations such as taka-diastase and pancreatins, the presence of maltase should be taken into account.

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