91. SACCHARASE ACTIVITY IN THE BARLEY PLANT

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It has long been recognized that most plant tissues have the capacity of inverting sucrose, but intensive study of saccharase action has hitherto been almost exclusively concerned with the saccharase of yeast [O'Sullivan & Tompson, 1890; Hudson, 1908, 1, 2; Michaelis & Menten, 1913; Nelson & Hitchcock, 1921; von Euler & Josephson, 1923; Willstätter & Kuhn, 1923; Weidenhagen, 1930, and many other papers by these authors and their collaborators]. The information regarding the saccharase of higher plants is frequently confined to records of its presence and of the optimum pH for its action [Kastle & Clark, 1903; Bailey, 1912; Colin, 1915; Maestrini, 1921; McGuire & Falk, 1920; Traegel, 1923; Kondo *et al.* 1929; Astruc & Mousseron, 1927; Blagoveschenski & Sossiedov, 1925; Matsushima, 1937]. Some attention has been paid to possible differences in the course of sucrose hydrolysis by saccharases from different plants [McGuire & Falk, 1923] and the effect of nutrient ions, particularly potassium, has been studied by Doby & Hibbard [1926; 1927], Harrt [1929] and Cattle [1933], while Vinson [1908] and Emiliani [1938] record variations of activity with age in the date and vine respectively. Finally a group of Russian workers [Arassimovitsch, 1939; Kursanov, 1936, 1, 2; Kursanov & Kriukova, 1937, 1, 2; Oparin, 1937, 1, 2; Rubin, 1936; Sisakyan, 1937] have quite recently investigated problems of saccharase action by infiltration methods.

The present paper records the changes in saccharase activity of the organs of the barley plant during the later half of the vegetative stage of growth and throughout the phases of stem elongation and ear development. The purpose of the investigation, which forms part of a study of the physiology of the barley plant in progress in this laboratory, was to ascertain whether the potential saccharase activity, as measured by the action of killed tissue on sucrose, could in any circumstances be a limiting factor in the interconversion of the sugars. It may be stated at once that no such evidence was found. Potential activity was always far in excess of all possible requirements, and the experiments merely emphasize the complexity of the problems involved in the mechanism of saccharase action. Nevertheless the results form a fairly complete record of the changes occurring in the barley plant with age, and for this reason it has been considered worth while to publish them.

The preliminary work necessary to ascertain appropriate conditions for making comparisons of activities at different times is first discussed.

Preliminary experiments on the saccharase of barley; methods of determining saccharase activity

Activity of the barley samples was determined by their action on a known amount of pure sucrose under standard conditions, the amount of sucrose inverted being estimated polarimetrically. Relative activity may be measured

by comparing either the initial velocities of inversion, or the time taken to invert a given, relatively large, percentage of the sucrose. The latter procedure has been adopted here as it requires fewer readings than the accurate determination of initial velocity. It was particularly necessary to minimize the number of observations to be made on each sample as it was found essential to use as inverting agent the ground up material rather than an extract. In these circumstances the usual method of removing aliquots from the digestion flask at appropriate time intervals was inapplicable, since the suspended solid matter could not be removed in proportion to the liquid. A separate sub-sample had therefore to be weighed out for every reading.

For the measurement of times taken to effect a given change Oppenheimer & Pincussen [1929] recommend observation of the times required for an inverting sucrose solution to reach zero rotation (75.93 % hydrolysis). Detailed figures have been published for the course of hydrolysis by yeast saccharase of a 4.75 % sucrose solution, maintained at 30° and pH 4.6, from which such times can be calculated if the angle of rotation be observed at some point between 30 and 70 % hydrolysis. On the arbitrary time scale given by these authors zero rotation is reached at time 13.55, and 50 % hydrolysis (1.88°) at time 7.10, while corresponding time factors are recorded for every 0.03 degrees. Thus if it is found that 50 % hydrolysis is reached in, say, 2 hr., the time taken to reach zero rotation is given by the equation $T = \frac{13.55}{7.10} \times 2 = 3.8$ hr., and the saccharase activity will be inversely proportional to the values of T so found. Two readings are taken (preferably between 40 and 70 % hydrolysis) for each sample, and the values of T should agree, unless the course of hydrolysis is different from that reported by Oppenheimer & Pincussen for their yeast preparations. For yeast saccharase (British Drug Houses) Oppenheimer & Pincussen's values were confirmed.

It is further stated by the above authors that saccharase will withstand the temperature of liquid air, and even short exposures to -197°, without undergoing change. Freezing with solid CO₂ immediately upon gathering therefore offered a simple method of preserving the material. For the preliminary work extracts were preferred to whole tissue because of the greater convenience of manipulation, although less than half the total saccharase was removed by the method of extraction used. To prepare extracts the frozen tissue was coarsely ground, and placed at 1° overnight, then warmed to room temperature and the sap pressed out with a hand press. Aliquots (25-30 ml.) of the green colloidal suspension obtained were measured out into 100 ml. flasks and 10 ml. of acetate buffer added. The flasks were placed in a large water bath at 30°, and after 5 min. 50 ml. of a 9.5 % sucrose solution, previously warmed to 30°, were run in and the volume made up to 100 ml. and the flask shaken as quickly as possible. The time was noted when the pipette was half empty. After a suitable time interval 25 ml. of the digest were removed and run into 5 ml. of 2.5 % Na₂CO₃, and the time again noted when the pipette was half empty. This amount of alkali neutralizes the buffer and leaves sufficient excess to stop the enzyme action instantaneously [Hudson, 1908, 1]. After standing 10 min. to complete mutarotation the solution was polarized in a 2 dm. tube at 20°, using a sodium vapour lamp as a light source. This procedure only differs from that of Oppenheimer & Pincussen in that Na₂CO₃ was used to stop the reaction instead of NaOH, in order to avoid the risk of lowering the rotation of the remaining sucrose by excess alkali.

Clarification of barley extracts for polarization

After the addition of the buffer solution, the colloidal material gradually coagulated, leaving a clear brown supernatant liquid, whose colour was intensified by the alkali. As a result the colour was frequently too deep for satisfactory polarimeter readings. All extracts were therefore cleared with normal lead acetate; for this purpose the amount of Na_2CO_3 was added in a solution of such concentration that when a suitable amount of 20% lead acetate was used the volume of 25 ml. removed from the flask was still only increased to 30 ml. The addition of an amount of lead just insufficient to precipitate all the carbonate left, on filtration, a pale yellow liquid quite clear enough for polarization; addition of excess lead and its subsequent removal can therefore be avoided. To test whether sugar, and particularly fructose, was lost during clarification, sucrose solutions, hydrolysed by yeast saccharase, were examined at various stages of hydrolysis, and in addition observations were made on a barley extract sufficiently pale to allow of polarization without clarification. The results of these tests are given in Table 1, from which it is clear that the differences in the readings between cleared and uncleared solutions are negligible.

Table 1. *Rotation of partially hydrolysed sucrose solutions before and after clarification with normal lead acetate*

Initial concentration of sucrose in polarized solution 3.98%					
Time of reaction min.	(a) Hydrolysis by yeast saccharase		Time of reaction hr.	(b) Hydrolysis by extract of barley plants	
	Uncleared degrees	Cleared degrees		Uncleared degrees	Cleared degrees
0	5.24	5.25	20	3.18	3.19
15	1.46	1.52	20	3.22	3.19
30	0.50	0.51	20	3.25	3.25
45	-0.47	-0.49	20	3.29	3.22
1440	-1.64	-1.65			
			Mean	3.24	3.23

Uniformity of saccharase action

The problem of uniformity of saccharase action has been discussed by Nelson & Hitchcock [1921] who showed that for three samples of yeast saccharase the hydrolysis curves were superposable, while very slight differences were found for a fourth sample. On the other hand Nelson & Cohn [1924] found that for honey saccharase the course of hydrolysis for concentrations of sucrose below 4% was similar to that for yeast, but at higher concentrations there was an increase of velocity until 10 or 15% of the sucrose had been inverted. The optimum pH was, moreover, different from that of yeast. Ambard & Trautmann [1937] found that rates of sucrose hydrolysis by saccharases from man, rabbit, dog, snail, bee and yeast were all different, while Weidenhagen & Dey [1928] report differences in behaviour between taka-invertase and yeast invertase. McGuire & Falk [1923] observed an increase in the saccharase activity of banana extracts on standing of the order of 40–100%, which they show could not be due to spontaneous changes of pH. It cannot therefore be assumed that saccharases from all sources will behave in precisely the same way as that from yeast, and it became necessary to test the applicability of the Oppenheimer & Pincussen time factors to hydrolysis of sucrose by barley samples, more especially as the times taken to reach zero rotation when samples of reasonable size were used were far longer than the 2–3 hr. found for the yeast sample.

Using the procedure described above, including the lead clarification, two values of T , the time taken to reach zero rotation, were found for each of a number of barley extracts. In eight instances one pair of values is available from each extract, and in the other two a number of replicate observations were made. The results are set out in Table 2. The values of T ranged from 12 to 66 hr., and the mean difference between the pairs of observations was 1.2%. A statistical test (method of t for 20 pairs of observations gave $t=1$ [Fisher, 1938]) demonstrated that this mean difference was quite insignificant. In these samples therefore the course of hydrolysis of the sucrose was substantially the same as that reported for yeast by Oppenheimer & Pincussen, although it must be emphasized that small differences in the shape of the hydrolysis curve are not excluded. For example in sample 1 of Table 2, where the percentage hydrolysis at the first observation time (t_1) was only 21%, the values calculated for T are all higher than the corresponding values found at the second time (t_2) when 40% of the sucrose had been hydrolysed. This suggests that the rate of hydrolysis at the start is rather slower than the Oppenheimer & Pincussen curve demands.

Table 2. *Times taken to reach zero rotation in the hydrolysis of a standard sucrose solution by extracts of stems and leaves of barley at pH 4.8*

Reading before hydrolysis 5.27°. Concentration of sucrose 3.98%.

t_1 and t_2 are the times (in hr.) from the start at which the samples were withdrawn from the digestion flask.

T is the time in hr. to zero rotation calculated by means of Oppenheimer & Pincussen's calibration table, see text.

Saccharase activity $\propto 1/T$

Sample	t_1	Angular rotation	Sucrose hydrolysed %	T	t_2	Angular rotation	Sucrose hydrolysed %	T
1	3	3.75	21.9	15.1	5.5	2.61	38.3	14.2
1	3	3.79	21.3	15.5	5.5	2.46	40.4	13.1
1	3	3.75	21.9	15.1	5.5	2.56	39.1	13.7
1	3	3.69	22.8	14.3	5.5	2.54	39.3	13.8
2	4.5	2.68	37.3	12.0	6.5	1.85	49.2	12.6
3	4.5	2.92	33.9	13.5	7.5	1.39	55.9	12.6
4	4.5	2.65	37.8	11.9	7.5	1.31	57.0	12.4
5	4.5	2.84	35.0	13.0	7.5	1.42	55.5	12.7
5	4.5	2.78	35.8	13.0	7.5	1.54	53.8	13.1
6	4.5	3.25	29.0	16.3	9.0	1.50	54.3	15.6
7	4.5	3.46	26.1	17.9	9.0	1.86	49.1	17.7
8	5.5	3.52	25.2	21.9	10.5	1.94	47.9	21.3
9	24	2.17	44.6	51.9	30	1.58	53.2	53.4
9	24	1.97	47.4	48.3	30	1.39	55.9	50.6
10	20	2.97	33.1	61.7	40	1.20	58.6	62.5
10	20	3.05	31.9	64.5	40	1.18	58.9	62.2
10	20	3.08	31.5	65.6	40	1.24	58.0	63.5
10	20	3.09	31.4	65.9	40	1.18	58.9	62.2
10	20	3.01	32.5	63.0	40	1.28	57.4	64.4
10	20	3.02	32.5	63.4	40	1.26	57.7	63.9
				Mean values of T				
				33.19				
				Mean difference 1.2%.				
				Value of t for 20 observations = 1 ($P=0.05$).				

Possible deviations from the course of hydrolysis found for yeast saccharase indicated in this way require confirmation by detailed study of the early stages of hydrolysis, but it seems clear that any such differences are not sufficiently

great to preclude the use of the calibration table for the purpose of comparing saccharase activities of barley samples provided that the reaction is allowed to continue until at least 30 % of the sucrose has been hydrolysed.

The optimum pH for barley extracts

The determinations of saccharase activity discussed here have all been made at the optimum pH, and therefore represent the maximum potential inverting capacity. The actual capacity will be governed among other factors by the buffer system of the tissues. Plant saccharases have optimum pH values centring round 5. Thus Blagoveschenski & Sossiedov [1925] found a range of 4.5–6.2 for 14 angiosperms using ground air-dried leaves as the inverting agent, while Matsushima [1937] quotes 4.5–5 for tobacco leaves, and McGuire & Falk [1920] 4–5 for potato extracts. The activities of four separate extracts of barley leaves and stems for the range of pH 3.6–6.4 have now been found, two values of T being calculated for each extract at each pH. All four extracts showed optimum activity between pH 4.6 and 4.8 with little change up to pH 5. The results are shown graphically in Fig. 1. Fig. 1 shows for a single extract the mean values of

Fig. 1. Effect of pH on the saccharase activity of extracts of barley leaves and stems. (a) Saccharase activity of a single extract expressed as the time (in hr.) taken for a standard sucrose solution to reach zero rotation. (b) Mean saccharase activity of four extracts expressed as percentages of the mean time to zero rotation for the pH range 4.4–5.2.

T plotted against pH, and in Fig. 1b all the results are combined by calculating the activities (times to zero rotation) as percentages of the mean values for the range pH 4.4–5.2. The mean values for each of the four extracts are marked on the vertical lines, together with the means for all extracts. The shape of the curve is very similar to that found for yeast saccharase; it has the same flat top over the range 4.6–5.0, with a rather sharp fall on either side, but especially on the alkaline side of 5.0. The change in activity up to pH 6.4 is about 25 %

of the maximum value. For the present experiments an acetate buffer at pH 4.8 has been used throughout. A few comparisons were made between the activities of unbuffered extracts and those buffered to 4.8; in all cases the unbuffered extracts showed a lower activity than those at pH 4.8, and corresponded approximately to those buffered to between 5.2 and 6. The expressed activity will thus be lower than the potential activity because of the pH, but from the curves of Fig. 1 it is apparent that over the rather wide pH range 3.6–6.4 this effect is only of the order of 25 %. The results of the comparison are shown in Table 3.

Table 3. *The saccharase activity of extracts of barley leaves and stems buffered to pH 4.8 and unbuffered*

Sample	Activity $\propto 1/T$	
	Time to zero rotation (T) in hr.	
	At pH 4.8	Unbuffered
1	12.68	17.91
2	13.03	17.57
3	12.33	13.43

Changes in saccharase activity with standing

In view of Falk & McGuire's [1920] findings on banana extracts, barley extracts were allowed to stand for various times to study the effect on saccharase activity. Only very small changes were found and there was no evidence of a similar situation to that found in the banana. From the data collected it appears that in barley there is a slow fall with time of standing, which is considerably retarded by low temperature, but that extracts can be kept at least 24 hr. at room temperature without measurable loss of activity. A similar slow loss of activity on standing has been found for potato leaf extracts [Doby, 1915]. As a result of these findings, the samples for routine determinations were generally thawed the evening before and kept at 1° overnight. The results of the experiments on time of standing are given in Table 4.

Table 4. *Effect of standing on the saccharase activity of 25 ml. aliquots of extracts of barley stems and leaves*

Sample 1		Sample 2	
Hr. at room temperature	Times to zero rotation (hr.) T	Hr. at room temperature	Times to zero rotation (hr.) T
0	15.6	1	14.7
24	15.9	2	14.4
120	17.8	3.5	14.0
4 days at 1°	15.8	24	14.1

The use of whole tissue as the inverting agent

It was noted by Vinson [1908] that extraction methods failed to remove all the saccharase of dates and that the amount removed increased with the age of the fruits. Falk & McGuire [1920] made a similar observation for bananas, and Mason [1920] has drawn attention to the effects of different methods of extraction on the saccharase of *Galanthus nivalis*. The two former authors suggest that two types of saccharase were present, one soluble (free) and the other insoluble (bound) and that the former increased at the expense of the latter with

age. Grassmann [1928] summarizes the position as regards yeast and makes it clear that only about 10% of the total saccharase goes into a water extract, unless preliminary treatment with proteolytic enzymes, amylases and tannase, is employed, or the yeast is so finely ground that no distinct cell structure remains. He expresses the view that the difference in the state of the saccharase is one of adsorption rather than of the type of enzyme. These observations seem to have been overlooked by other workers on plant saccharases who have used extracts of dried or crushed tissues, or powdered dry material without a satisfactory demonstration of the effectiveness of the extraction methods used [Blagoveschenski & Sossiedov, 1925; Doby & Hibbard, 1927; Harrt, 1929; Cattle, 1933]. It is manifestly impossible to attempt the complete removal of saccharase from a large number of samples, and therefore whole tissue has been used in the present work. This procedure appears to be justifiable for experiments of the present type since Willstätter *et al.* [1922] and Willstätter & Kuhn [1923] have shown that the activity of the enzyme is not affected by purification. Some figures showing the relative amounts of enzyme expressed in the sap from frozen barley plants and that remaining in the press residue, together with a similar experiment on air-dried, ground grass samples are given in Table 5. Only about 1/3 of the saccharase was extracted after fine grinding, and the results are therefore in agreement with those for yeast.

Table 5. *Saccharase activities of sap expressed from duplicate 40 g. samples of frozen barley plants, and of water extracts of 5 g. samples of air-dried grass*

	Expressed sap	Washings after pressing	Residual tissue
	Time (hr.) to zero rotation, <i>T</i>		
Barley 1	52.6	151	18
Barley 2	49.9	138	19
	Water extract		
Grass	—	85	26

In order to deal satisfactorily with a large number of samples some method of preservation was required, as all could not be analysed immediately after collection. The choice lay between drying the samples or preserving them in a frozen condition. A trial of these two methods yielded the results shown in Table 6. The highest activity was found in the frozen samples and there was some loss even when drying was carried out at room temperature, while short exposures to 80° destroyed a large part of the saccharase. Accordingly all samples were preserved in the frozen condition.

Table 6. *The effect of drying on the saccharase activity of grass samples*

- (a) Not dried. Tissue frozen and ground.
 (b) Dried in air at room temperature.
 (c) Dried at 80° for 1 hr.

(a)		(b)		(c)	
Time of reaction hr.	Sucrose hydrolysed %	Time of reaction hr.	Sucrose hydrolysed %	Time of reaction hr.	Sucrose hydrolysed %
1.1	2.4	—	—	—	—
4	5.0	—	—	—	—
20	41.8	19	28.8	20	2.7
		43	49.4	47	5.0
76	95	67	73	94	46.9

No change was observed in the activity of material kept frozen with solid CO_2 for 3 months. Some data bearing on this point are shown in Table 7. The mean difference between the values for the longer as compared with the shorter times of storage is negligible, but in some individual instances the differences are rather large.

Table 7. *Saccharase activity of barley leaves after storage in a frozen condition (solid CO_2)*

Sample	Days between freezing and estimation	Activity $\propto 1/T$		Time to zero rotation (T) hr.
		Time to zero rotation (T) hr.	Days between freezing and estimation	
1	15	11.9	73	11.2
1	15	11.8	73	10.7
2	22	12.7	71	10.8
3	20	13.0	69	13.5
4	65	14.6	91	14.7
5	44	13.9	88	16.3
5	44	13.5	88	15.0
Mean		13.1		13.3

The results of these preliminary experiments may be summarized as follows. Hydrolysis of sucrose by barley saccharase follows substantially the same course as that by yeast saccharase, although small differences in the hydrolysis curve are not excluded. The optimum pH for barley saccharase lies between 4.6 and 4.8 and activity falls sharply beyond the values 4.4 and 5.2. Sap expressed from frozen tissue shows a slow decline in activity at room temperature detectable after about 24 hr. standing. When activities at different stages of plant growth are to be compared the whole tissue must be used as the inverting agent, and not an extract therefrom, since a small and variable part only of the saccharase is removed by any simple extraction procedure. Samples may be successfully preserved in the frozen condition by means of solid CO_2 ; this method is recommended in preference to any type of drying since there is a loss of activity even after air drying.

Seasonal changes in the saccharase activity of the barley plant.
Collection of material

The barley used for this investigation was a pure line var. Plumage Archer. The plants were grown in soil in pots in the open at Rothamsted Experimental Station during the summer of 1938. The seeds were sown on 13 May and after germination a dressing of nitrate was given. At the third leaf stage the plants were thinned to three per pot. Collections, begun at 10.30 a.m., were made from the time of emergence of the sixth leaf (27. vi. 38) until the end of September (27. ix. 38), when the plants were ready for harvest. Duplicate samples, usually of three plants, were taken on each occasion. The aerial parts were removed from the roots and weighed, divided into leaves, leaf sheaths, ears and separate internodes of the stem and the fresh weight of each component recorded. Duplicate 5 g. samples of each component were then weighed out and placed in tins (4 in. $\times$ 1½ in.) and the contents frozen instantaneously by placing the tins on a block of solid CO_2 . The roots were washed out of the soil and similarly treated. Parallel samples were weighed out for the determination of dry weight. The samples were transported from the field in a container cooled with CO_2 and finally stored in similarly cooled, insulated chests till required. The frozen material was then removed into a previously cooled mortar and ground to a

powder. The powder, still frozen, was transferred to wide-mouthed, stoppered bottles, and allowed to thaw. If the mortar is cold enough to prevent thawing during grinding, only traces of material adhere to its surface and the losses on transference are negligible. Young tissues can be fairly readily ground in this way, provided that the mortar is first thoroughly cooled in the CO_2 chamber. Difficulties were experienced with older tissues, especially stems, and it is recognized that there is room for much improvement in the disintegration technique, since inefficient grinding reduces the inversion rate at least at the start. Some of the rather large variability found between duplicate determinations may have arisen from this cause, and where inversion rate is very slow a further source of error may be an actual fall in activity during the observation.

When the contents of the bottles reached room temperature 16 ml. of water (on the assumption that each 5 g. sample contains 4 ml. of water) and 5 ml. of buffer solution (pH 4.8) were added. After warming to 30° , 25 ml. of 9.5% sucrose at the same temperature were run in, making a total volume of 50 ml. After an appropriate time at 30° , 25 ml. of liquid were removed from each bottle and run into 3.5 ml. of 3.5% Na_2CO_3 . After 10 min. 1.5 ml. of 20% normal lead acetate were added and the solution filtered and "polarized". Blanks were run for each sample on a parallel 5 g. sub-sample, adding a further 25 ml. of water instead of the sucrose solution.

Experimental results and discussion

The results obtained for the 5 g. samples of fresh material for the several components of the barley plant are shown in Table 8. The saccharase activity is expressed as the reciprocal of the time taken for a standard sucrose solution

Table 8. *Saccharase activities of component parts of the barley plant during growth*

Saccharase activity per 5 g. fresh weight expressed as reciprocal $\times 10$ of time (hr.) taken for a fixed amount of sucrose to reach zero rotation under standard conditions (see text, pp. 756, 757).

Date 1938	Stage of plant growth		Leaves	Leaf sheaths	Ears	Stems	Roots
27. vi.	6th leaf expanded	Leaf development	1.21	1.07	—	—	—
4. vii.			0.72	1.13	—	—	0.38
11. vii.			0.47	1.02	—	—	0.47
18. vii.			0.75	0.51	—	0.41	0.32
20. vii.	10th leaf expanded	Stem elongation	0.68	0.54	0.23	0.26	0.36
22. vii.			0.91	0.50	0.46	—	0.34
25. vii.			0.83	0.37	—	0.25	0.29
27. vii.	Awns showing		0.80	0.39	1.29	0.30	0.40
29. vii.			0.74	0.29	—	0.24	0.36
2. viii.	Ear emergence		0.73	—	1.64	0.25	0.27
4. viii.			0.71	0.42	3.33	0.21	0.32
6. viii.	3 green leaves left		0.70	0.65	2.33	0.22	0.40
10. viii.		Ear development	0.56	0.55	1.72	0.17	0.41
16. viii.			0.78	0.55	0.76	0.20	0.36
22. viii.			0.65	0.68	0.43	0.14	0.22
29. viii.			0.86	0.49	0.33	0.18	0.18
5. ix.	All leaves dead	Matura- tion without growth	—	—	0.27	0.19	0.23
12. ix.			1.16	0.51	0.22	0.18	0.18
19. ix.			1.52	0.54	0.20	0.11	0.26
27. ix.			1.28	—	—	0.21	0.25

to reach zero rotation under the conditions described. All parts of the plant showed considerable saccharase activity throughout the period studied, and although there was a general decline towards harvest, death of the tissues was

not accompanied by disappearance of enzyme. This decline was least in the leaves, where on a concentration basis dead leaves actually showed higher values than green ones, although the absolute amounts per leaf were the same. The occurrence of saccharase in the roots may perhaps be emphasized, since neither Colin [1915] nor Leonard [1938] found any in the contrasting type of storage root of beet.

In terms of the fresh weight the level of activity is lowest in the stems, with rates only slightly higher in the roots, and in both cases there is a decline to a more or less constant value soon after ear emergence (16. vii. 38). In the ears,

Fig. 2.

Fig. 2. Change in saccharase activity of the barley plant from the time of emergence of the sixth leaf until harvest. Saccharase activity expressed as the reciprocal $\times 10$ of the time (in hr.) taken for a standard sucrose solution to reach zero rotation. (a) Total activity of whole plant. (b) Activity per plant of the ears. Dotted lines show corresponding fresh weights.

Fig. 3.

Fig. 3. Change in saccharase activity, per plant, of the leaves, leaf sheaths, stems and roots of the barley plant from the time of emergence of the sixth leaf until harvest. Saccharase activity expressed as reciprocal $\times 10$ of the time (in hr.) taken for a standard sucrose solution to reach zero rotation. Dotted vertical lines show position of maximum fresh weight of each component.

activity rises sharply until emergence and then falls at first rapidly and later more slowly, reaching a final low level comparable with that of the stems and roots. The saccharase activity developed by the ear as it emerges is three times

as great as that shown at the same time by any other part of the plant, but, as will be shown later, it is likely that the high value is an expression of the ear's relative immaturity rather than of a greater capacity to form saccharase. In the leaves and sheaths the values are about twice as great as those of the stems and roots, and again activity tends at first to fall with time, but about the end of July for sheaths, and end of August for leaves, there is a secondary rise. This rise is coincident with rapid desiccation and death and only indicates the high rate of water loss relative to the slow loss of saccharase.

Figs. 2 and 3 show the saccharase activity of the whole plant and of its components calculated as the amounts per plant from the data of Table 8. In Fig. 2 the fresh weights are also included. As growth proceeds there is a rise in the absolute activity of the whole plant (Fig. 2*a*), but after the maximum weight is reached there is a rapid decline, largely due to changes in the ear (Fig. 2*b*) accompanied by only small losses of weight. In the case of the various parts (Fig. 2*b* and Fig. 3) a similar sequence of changes is found in the leaves, sheaths and stems, namely a rise to a maximum as each reaches its maximum weight (marked by dotted line in Fig. 3) followed by a fall to a more or less constant low value. In the roots the time of the maximum weight is doubtful and was probably prior to the start of the collections. Only the general decline is therefore indicated with certainty; moreover, too much weight must not be placed on the rather sudden drop at the end of August, since this is due to a similar fall in fresh weight, which was mainly brought about by increasing brittleness of the roots resulting in very imperfect removal from the soil at this stage. In the ear the maximum value occurred rather earlier than the maximum weight. This difference is doubtless due to the fact that the later increase in weight is largely the result of starch storage and not of cell division or expansion. Fig. 2 also emphasizes that the ear as it develops is contributing a rapidly increasing proportion of the total activity, for at this time all the other parts have passed their maximum weights, and saccharase activity is declining. The percentage contributions of the several parts as each reaches its maximum weight are shown in Table 9. At first nearly half the total amount is contributed by the leaves, but later the contribution of the ear rises to 60 %, while that of the stems is throughout of the order of 10 %.

Table 9. *Percentages of the total saccharase activity contributed by the various parts of the barley plant*

T is the time in hr. taken to reach zero rotation under standard conditions

At the stage of maximum fresh weight of	Total activity $1/T$	% of the total activity (round numbers)				
		Leaf	Leaf sheaths	Ear	Stem	Root
Root (18. vii. 38)	0.472	45	18	0	12	25
Leaf (20. vii. 38)	0.452	44	12	4	10	30
Stem (10. viii. 38)	0.893	16	6	47	12	19
Whole plant (10. viii. 38)	0.920	15	5	60	10	10
Ear (29. viii. 38)	0.414	29	8	35	17	11

Since the total saccharase content increases at approximately the same rate as that of the ear during ear development, while changes in other components are both relatively and absolutely small, there is no evidence of enzyme transference from one part of the plant to another; the conclusion to be drawn is rather that new enzyme is synthesized in each successive growing region.

In terms of the dry weight (Fig. 4), the early values are high in every instance, and there follows a fall at first rapid and then slow. The low level reached by the

stems and ears is less than that of the other components, probably as the result of the high proportion of structural tissues in the stems and of starch in the ear. Increase in saccharase activity relative to dry weight was only recorded for the ear, but in the other parts the very early stages of development are not included. It is likely that at correspondingly early stages of development the leaves, leaf sheaths etc. would show a corresponding rise, especially as the earliest recorded result for sheaths (7th leaf stage) in which the unexpanded leaves are included is of the same order as that of the ear as it reaches its maximum value. Values for the separate internodes of the stem are shown in Fig. 5.

Fig. 4.

Fig. 4. Change in saccharase activity per g. dry weight of the barley plant and its components from the time of emergence of the sixth leaf until harvest. Saccharase activity expressed as the reciprocal $\times 10$ of the time (in hr.) taken for a standard sucrose solution to reach zero rotation.

Fig. 5.

Fig. 5. Change in saccharase activity per g. dry weight of the stem internodes of the barley plant. Saccharase activity expressed as the reciprocal $\times 10$ of the time (in hr.) taken for a standard sucrose solution to reach zero rotation.

The level of activity is about the same for them all and there is no indication of a gradient along the stem. The large top internode has a high initial value, due to its relative immaturity at the first analysis (18. vii.), and all show the decline in activity towards harvest. There is evidence that high values occur early in the development of the bottom (4th) internode and of the peduncle, and the same is no doubt true of the intermediate ones.

From the above account it would seem that saccharase formation is very closely associated with growth, in the sense of cell division and expansion, and in all probability especially the former. Consideration of the absolute amounts

present show that saccharase formation ceased immediately growth was complete, and this was true not only for the plant as a whole, but for each component as the succession of maximum weights was reached. The continuous fall relative to dry weight emphasizes the relatively high rate of production in the early stage of growth, and the course of saccharase accumulation appears to be very similar to that already well established for nitrogen, although nitrogen can of course be transferred from one part of the plant to another.

The results reported here are in general agreement with what is already known of saccharase changes. Thus Doby & Hibbard [1927] found a higher saccharase content in young than in old beet leaves, and Cattle [1933] found, in terms of leaf area, a falling activity from the first to the eighth leaf of bean plants comparable with the fall here noted with age in terms of the dry weight.

If potential saccharase activity be considered in relation to the supply of assimilate to the various parts of the plant it becomes apparent that the maximum possible requirements can always be met. For consideration of the saccharase activity in this relation it is necessary to make an estimate of the initial velocities of inversion. These have been calculated on the assumption that sucrose is hydrolysed according to the Oppenheimer & Pincussen curve, an assumption which has been shown to be sufficiently close to the facts to ensure that the calculated velocities are certainly of the right order. The maximum possible requirements have been calculated from the dry weight values on the assumption that all the assimilate arrives at (or departs from) any given organ as sucrose, and that it must be hydrolysed before it is further metabolized. Dry weight increases (or decreases) per hour per plant will then give a measure of the requirements.

Table 10. *The initial velocities of hydrolysis of sucrose by the component parts of the barley plant (after freezing and grinding), and the rates of change of dry weight (i.e. the maximum possible amount of sucrose to be hydrolysed) during various periods of the growth cycle*

	Period 1938	Average rate of increase or decrease* of dry weight	Initial velocities of inversion at pH 4.8	
		mg. per plant per hour	Min.	Max.
Leaves:				
Dry weight increasing	27. vi.–29. vii.	22.7	192	244
Dry weight decreasing rapidly	29. vii.–10. viii.	25.5	208	209
Dry weight decreasing slowly	10. viii.–19. ix.	4.2	188	208
Stems:				
Dry weight increasing	18. vii.–10. viii.	10.1	61	118
Dry weight decreasing	10. viii.–19. ix.	2.5	73	86
Ears:				
Dry weight increasing rapidly	25. vii.–29. viii.	10.2	90	721
Dry weight increasing slowly	29. viii.–19. ix.	2.0	94	94
Roots:				
Dry weight increasing	22. vii.–2. viii.	5.0	110	148
Dry weight decreasing	2. viii.–29. viii.	2.0	54	54

In leaves and roots only a part of the phase of increasing dry weight is included, as observations were not begun until the emergence of the sixth leaf.

The calculations are brought together in Table 10, which gives the rates of change of dry weight for different periods of growth, and the range of initial velocities of inversion given by the samples of material collected during the same

period. Everywhere the velocities of inversion are many times as great as the rate of dry weight change, and therefore all possible sucrose could at all times be readily inverted by all parts of the plant. Accumulation of sucrose is thus never influenced by lack of potential inverting capacity, and it is quite clear that the presence of free sucrose presupposes some mechanism either chemical or physical preventing the exercise of the large inverting capacity. Kursanov [1936, 1] indicated in a general way the existence of some such system by his observation that the activity of living chicory (found by infiltration methods) was only 1/8th of that of the autolysed tissue, while Weidenhagen & Paa Chung Lu [1936] gave data showing the inhibiting effect of ascorbic acid, and Doby & Hibbard [1926] show that Cl ions may paralyse saccharase. From the present data it may be stated that it is extremely unlikely that an unfavourable pH plays an important part in the inhibiting system unless very large local differences of pH exist in the cell. It will be remembered that unbuffered barley extracts showed an activity similar to that found for a pH range of 5.2-6.0, while the fall in activity over the range 3.6-6.8 was of the order of 25%. Reduction of the velocities given in Table 10 by even 30% would still leave an inverting capacity largely in excess of that required for the incoming assimilate.

SUMMARY

Saccharase activity has been determined in the component parts of the barley plant from the time of emergence of the sixth leaf until harvest.

Fresh material was frozen by means of solid CO₂, and the material stored in the frozen condition until required; activity remained unchanged under these conditions. Activity was measured as the reciprocal of the time taken to effect 75.93% hydrolysis (i.e. the time to zero rotation) of a given amount of sucrose under standard conditions, the amount of sucrose inverted being found polarimetrically. It was necessary to clear the solutions with normal lead acetate before polarization.

Tissue, previously ground in the frozen condition, was used as the inverting agent as no simple extraction procedure removed more than a fraction of the saccharase. Drying even at air temperature caused some loss of activity.

The optimum pH for the saccharase of the barley plant was found to be 4.8, and all measurements were made at this pH.

In terms of fresh weight the level of activity was lowest in the stems, slightly higher in the roots, while leaves and leaf sheaths were about twice as active as the stems. In the ear activity rose rapidly with growth and declined again after full emergence. The maximum value in the ear was the highest recorded for any part of the plant.

The total activity of the plant rose until ear emergence, which was coincident with the maximum weight of the plant, and then declined. Each part showed a similar trend of increasing activity with its growth, followed by a fall. Loss of activity with time was least in the leaves, dead leaves being still quite active.

Expressed in terms of the dry weight the activity was high initially, and fell off at first rapidly and then more slowly. The differences in level between the various parts were small, stems and ears showing rather lower values than the other parts of the plant towards harvest, probably owing to the large proportion of structural tissue in the former and storage of starch in the latter.

Saccharase production thus appears to be associated with growth and to cease when growth ceases. No evidence was found of transport of enzyme in the plant. The production is relatively greatest in the very early stages of the

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for Grassmann (1928). *Ergebn. Enzymforsch.* 65

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Enzymforschung, p. 65. Munich.

development of each organ, as is shown by the continuous fall in terms of dry weight.

The potential inverting capacity is always many times as great as the maximum requirement (measured by dry weight increase) in any part of the plant. The existence of free sucrose therefore requires the presence of some inhibiting mechanism. An unfavourable *pH* is unlikely to be a major factor in this mechanism since over the range *pH* 3.4–6.8 the reduction in activity as compared with that at the optimum value is only of the order of 25 %.

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