CLXXXVII. THE CARBOHYDRATE ENZYMES OF SOME STARCH-FREE MONOCOTYLEDONS.

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INVESTIGATIONS on the carbohydrates of plant tissues have hitherto almost neglected one method of attack, namely, the examination of the enzymes present in the plant. If the generally accepted theory of enzyme action be correct, that they catalyse both synthesis and hydrolysis of the higher carbohydrates, then the presence or absence of one or more of the chain of enzymes involved in the series of reactions which result in the formation of starch and other polysaccharides from hexose sugars, may afford valuable evidence why different plants produce different carbohydrates. Especially might this be the case for "starch" and "sugar" leaves.

Starch is hydrolysed to glucose by a series of reactions catalysed by amylase, dextrinase and maltase; the synthesis of starch from glucose presumably requires the same chain of enzymes. If one of them be missing, the synthesis of the full series of carbohydrates, maltose, dextrin and starch, should be impossible, and instead of starch as the end-product some other substance would be formed.

The carbohydrates of plant tissues have only been thoroughly worked out in a few cases, *e.g.* the classical researches of Brown and Morris on the foliage leaves of *Tropaeolum majus* [1893], the work of Parkin [1911] on the green leaf of the snowdrop, and the series of researches by Davis, Daish and Sawyer [1915] on the leaves of the starch-containing potato and the starch-free mangold. Davis and his co-workers strongly criticised the results of Brown and Morris, on the grounds that their methods of preparing the leaves for analysis, by drying in air at super-normal temperatures, would afford opportunity for enzyme action to take place, and state that the maltose which Brown and Morris found present was due to hydrolysis of starch. On the other hand, they consider Parkin's results trustworthy. He found glucose, laevulose and sucrose present in considerable quantity, but no maltose, dextrin or starch, the snowdrop being a typical "sugar" plant. Priestley [1913] had stated that the starch-free snowdrop contained diastase but no maltase, but this was based on only qualitative experiments. It was therefore thought that the green foliage leaves of the snowdrop (*Galanthus nivalis*) would form

an excellent subject for a detailed investigation into the presence or absence of the enzymes concerned in carbohydrate synthesis. Two other typical "sugar" plants, the onion (*Allium cepa*) and the closely-related leek (*Allium porrum*) were also examined, together with a typical "starch" plant, a common dock (*Rumex obtusifolius*), as a control.

MATERIAL AND METHODS.

The method finally elaborated, after various vain efforts to use fresh leaves, was to use dried leaf-powder. The fresh green leaves were usually received by post on the day after collection, and were at once spread out to dry on papers in a well-ventilated room, screened from the direct rays of the sun, which might have had a harmful effect on any enzymes present, especially maltase. When the leaves were quite dry and crisp, they were crumbled by hand and then ground as finely as possible in a mill. The leaf-powder was kept in tightly-corked bottles, screened from light by black paper.

The experiments were conducted in two parallel series, one being a control which had been heated at least 15 minutes in boiling water to destroy all the enzymes present. The usual procedure was as follows: 2.00 g. leaf-powder was put into a clean, dry flask of about 70 cc. capacity, 10 cc. water added and the control put in boiling water; 50 cc. of the substrate and 2 cc. toluene were added, and the flasks incubated at $39^{\circ} \pm 0.5^{\circ}$. The loss of water from the control during the inactivation of the enzymes by heat was prevented as far as possible by leaving the stopper of the flask loosely inserted in the mouth. A test showed an actual loss equivalent to an increase of 0.0035° rotation with a 2 % solution of maltose in a 100 mm. tube. This is about the size of the usual experimental error, and is not significant.

The substrates used were Kahlbaum's soluble starch, dextrin and maltose. The latter ran out in the middle of the work, and was replaced by maltose from Messrs Reynolds and Branson, which gave a pure maltosazone, free from glucosazone, and a rotation corresponding to more than 99 % maltose. Recrystallised sucrose from British Drug Houses and amygdalin prepared by myself were also used.

The tests used were partly qualitative and partly quantitative. The latter was the optical rotation, as measured by a Hilger polarimeter reading to 0.01° . The usual probable error was less than 0.010° , and in no case was it more than 0.038° . The actual tests were the following:

Amylase. Tests with 1 % iodine in potassium iodide for the disappearance of starch and the appearance of dextrin, and phenylosazone tests for the appearance of maltose. 1/24,000 of starch gave a definite coloration with iodine.

Dextrinase. As for starch. 1/10,000 of dextrin gave a coloration with iodine. Polarimetric measurements were also made. Complete hydrolysis of 1 % solution of dextrin to maltose shows a change in rotation from $+1.95^{\circ}$ to $+1.45^{\circ}$ (*i.e.* -0.50°); to glucose from $+1.95^{\circ}$ to $+0.59^{\circ}$ (*i.e.* -1.36°).

Maltase. The chief test was polarimetric. A 2 % solution on hydrolysis changes from $+2.76^{\circ}$ to $+1.11^{\circ}$ (i.e. -1.65°). Osazone tests were also made.

Invertase. As for maltase. Complete hydrolysis shows a change from $+1.33^{\circ}$ to -0.42° (i.e. -1.75°).

Emulsin. A qualitative test only was made, using alkaline picrate paper suspended over the solution. 1/50,000 of hydrocyanic acid gave a strong reaction after about 19 hours at 39° .

1 to 2 cc. toluene were used as antiseptic usually, as Daish [1916] showed that this has little effect on maltase, which gives very variable results with ordinary chloroform and alcohol. Chloroform was used sometimes when testing for diastase or emulsin.

(Other methods which were tried before the foregoing technique was finally evolved may be mentioned briefly. All gave disappointing results. Fresh leaves were frozen in liquid air, or solid carbon dioxide and ether, then thoroughly powdered in a mortar. On testing for maltase, none could be found, though experiments with dried leaves of the same plant (dock) gave positive results. Efforts to extract maltase from fresh leaves, after freezing and powdering, also failed. Attempts to release maltase from the protein-protoplasmic complex of the cell by digestion with trypsin and pepsin with hydrocyanic acid as antiseptic also were unsuccessful.)

After incubation (in experiments where the rotation was required) the solutions were filtered, basic lead acetate added to precipitate proteins, etc., the solution again filtered, solid sodium sulphate added to precipitate the slight excess of lead, and after a drop of 0.880 ammonia had been added to bring the solution to equilibrium, the rotation was measured. The solutions were always rather yellow, but this gave no trouble with a sodium flame except in excess, when the polarimeter was adjusted to let more light through with a resulting slight increase in the probable error. The probable error was worked out by the formulae given by Mellor [1922].

EXPERIMENTAL RESULTS.

Column 4 of the tables gives the rotations corrected to a 100 mm. tube, for dilution by lead acetate and to an original solution of 2.00 % for maltose and sucrose (or 1.00 % for dextrin and starch). A rough attempt has been made to indicate the quantity of osazone precipitated by a standard technique, by the terms very much, much, fair, some and trace.

Emulsin (see Table I). The onion, leek and dock showed a strong reaction for hydrocyanic acid after 5 to 21 hours at 39° ; the snowdrop failed to show it even after 74 hours. There is therefore no emulsin in the snowdrop, but it is present in all the others.

Invertase (see Table II). The leek and dock showed a decrease in rotation when sucrose was present as compared with that when sucrose was absent, of almost exactly the theoretical amount for complete inversion to glucose and

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laevulose; the onion showed rather more, and the snowdrop considerably less decrease, but the results indicate the presence of invertase in all the four leaves. The osazones confirm this.

Table I. *Emulsin*.

0.8 g. leaf + 20 cc. 1 % amygdalin + chloroform.					
Expt. no.	Incubated (hours)	Picrate paper turned	Remarks and conclusions		
<i>Snowdrop</i>					
X 62	74	Yellow	No emulsin present		
<i>Onion</i>					
X 61	21	Red	Emulsin present		
<i>Leek</i>					
X 65	5½	Red	Emulsin present		
<i>Dock</i>					
X 60	21	Deep red	Emulsin present		
<i>Delicacy of the test for Hydrocyanic acid.</i>					
Known strength of solution of HCN		1/1000	1/5000	1/10,000	1/50,000
Incubated for		...	4 mins.	2½ hours	2½ hours
Yellow picrate paper turned		...	Pink	Deep pink	Deep pink
				Deep pink	Deep red

Table II. *Invertase*.

Exp. no.	Details of experiment (toluene as antiseptic)	Incubated (hours)	Rotation (corrected)	Change	Remarks
<i>Snowdrop</i>					
X 105	Leaf-powder I + cane-sugar	88½	+0.77° ± 0.002°	-0.71°	No maltosazone; much glucosazone
X 106	Control of X 105 (15 mins. at 100°)		+1.48° ± 0.003°		No maltosazone; good glucosazone
X 123	Leaf-powder III + water	68½	-0.03° ± 0.003°	-0.12°	Trace maltosazone; good glucosazone
X 124	Control of X 123 (15 mins. at 100°)		+0.09° ± 0.002°		" "
X 103	Leaf-powder I + water	308½	-0.16° ± 0.004°	-0.41°	
X 104	Control of X 103 (15 mins. at 100°)		+0.25° ± 0.005°		
<i>Onion</i>					
X 109	Leaf-powder I + cane-sugar	64½	-0.72° ± 0.003°	-2.10°	No maltosazone; very heavy glucosazone
X 110	Control of X 109 (15 mins. at 100°)		+1.38° ± 0.002°		No maltosazone; good glucosazone
O 13	Leaf-powder III + water	45½	-0.02° ± 0.014°	-0.09°	Trace maltosazone; no glucosazone
O 14	Control of O 13 (30 mins. at 100°)		+0.07° ± 0.008°		Trace maltosazone; some glucosazone
O 3	Leaf-powder III + water	234½	-0.14° ± 0.004°	-0.10°	Trace maltosazone; good glucosazone
O 4	Control of O 3 (30 mins. at 100°)		-0.04° ± 0.009°		" "
<i>Leek</i>					
X 107	Leaf-powder I + cane-sugar	237½	-0.33° ± 0.002°	-1.67°	No maltosazone; very heavy glucosazone
X 108	Control of X 107 (15 mins. at 100°)		+1.34° ± 0.003°		No maltosazone; some glucosazone
L 3	Leaf-powder II + water	41½	-0.02° ± 0.006°	-0.10°	No maltosazone; little glucosazone
L 4	Control of L 3 (30 mins. at 100°)		+0.08° ± 0.004°		No maltosazone; trace glucosazone
<i>Dock</i>					
X 111	Leaf-powder I + cane-sugar	262½	-0.40° ± 0.003°	-1.71°	No maltosazone; very heavy glucosazone
X 112	Control of X 111 (15 mins. at 100°)		+1.31° ± 0.002°		? Maltosazone; fair glucosazone
X 3	200 g. fresh leaves (without mid-ribs) frozen with solid CO ₂ and ether. Powdered, shaken 1 hour with 200 cc. tap-water (without antiseptic). 20 cc. filtrate + cane-sugar	71	-0.28°	-1.73°	(Cleared by shaking with 1.4 g. of animal charcoal for half an hour)
X 3a	Control of X 3 (20 mins. at 100°)		+1.45°		(Cleared similarly)
X 21	Leaf-powder I + water	119	± 0.00°	-0.05°	A little glucosazone
X 22	Control of X 21 (20 mins. at 100°)		+0.05°		" "

Table III. *Maltase.*

Exp. no.	Details of experiment (toluene as antiseptic)	Incubated (hours)	Rotation (corrected)	Change	Remarks
<i>Snowdrop</i>					
<i>X 39a</i>	Leaf-powder I+maltose	90	+2.77°	-0.11°	Much maltosazone; no glucosazone
<i>X 40a</i>	Control of <i>X 39a</i> (20 mins. at 100°)		+2.88°		" " "
<i>X 121</i>	Leaf-powder III+maltose	68½	+2.98±0.006°	-0.11°	Good maltosazone; some glucosazone
<i>X 122</i>	Control of <i>X 121</i> (15 mins. at 100°)		+3.09±0.004°		" " "
<i>S 1</i>	Leaf-powder IV+maltose	70½	+2.63±0.023°	-0.35°	Much maltosazone; no glucosazone
<i>S 2</i>	Control of <i>S 1</i> (33 mins. at 100°)		+2.98±0.012°		" " "
<i>S 5</i>	Leaf-powder IV+maltose	141½	+2.54±0.010°	-0.42°	Some maltosazone; no glucosazone
<i>S 6</i>	Control of <i>S 5</i> (33 mins. at 100°)		+2.96±0.019°		Some maltosazone; a little glucosazone
<i>S 11</i>	Leaf-powder IV+maltose	70½	+2.96±0.015°	-0.06°	Good maltosazone; some glucosazone
<i>S 12</i>	Control of <i>S 11</i> (30 mins. at 100°)		+3.02±0.008°		Fair maltosazone; a little glucosazone
<i>X 101</i>	Leaf-powder I+maltose	332	+2.29±0.004°	-0.37°	
<i>X 102</i>	Control of <i>X 101</i> (15 mins. at 100°)		+2.66±0.003°		
<i>X 123</i>	Leaf-powder III+water	68½	-0.03±0.003°	-0.12°	Trace maltosazone; good glucosazone
<i>X 124</i>	Control of <i>X 123</i> (15 mins. at 100°)		+0.09±0.002°		Little maltosazone; good glucosazone
<i>S 9</i>	Leaf-powder IV+water	120	+0.19±0.023°	-0.46°	Trace maltosazone; fair glucosazone
<i>S 10</i>	Control of <i>S 9</i> (30 mins. at 100°)		+0.65±0.014°		Trace maltosazone; some glucosazone
<i>X 103</i>	Leaf-powder I+water	332	-0.16±0.004°	-0.41°	
<i>X 104</i>	Control of <i>X 103</i> (15 mins. at 100°)		+0.25±0.005°		
<i>S 7</i>	No leaf; 10 cc. water+50 cc. 2.00 % maltose+1 cc. toluene	141½	+2.58±0.013°	nil	Much maltosazone; no glucosazone
<i>S 8</i>	Control of <i>S 7</i> (33 mins. at 100°)		+2.58±0.006°		" " "
<i>Onion</i>					
<i>X 39</i>	Leaf-powder I+maltose	190½	+1.16°	-1.37°	Maltosazone; glucosazone
<i>X 40</i>	Control of <i>X 39</i> (15 mins. at 100°)		+2.53°		Maltosazone; no glucosazone
<i>O 3</i>	Leaf-powder III+water	234½	-0.14±0.004°	-0.10°	Trace maltosazone; good glucosazone
<i>O 4</i>	Control of <i>O 3</i> (30 mins. at 100°)		-0.04±0.009°		" " " "
<i>Leek</i>					
<i>X 70</i>	Leaf-powder I+maltose	209	+1.34°	-0.57°	Maltosazone; some glucosazone
<i>X 71</i>	Control of <i>X 70</i> (18 mins. at 100°)		+1.91°		Maltosazone; no glucosazone
<i>L 3</i>	Leaf-powder II+water	41½	-0.02±0.006°	-0.10°	No maltosazone; little glucosazone
<i>L 4</i>	Control of <i>L 3</i> (30 mins. at 100°)		+0.08±0.004°		No maltosazone; trace glucosazone
<i>Dock</i>					
<i>X 17</i>	Leaf-powder I+maltose	240	±0.00°	-1.73°	? Maltosazone; heavy glucosazone
<i>X 18</i>	Control of <i>X 17</i> (20 mins. at 100°)		+1.73°		Maltosazone; no glucosazone
<i>X 19</i>	Leaf-powder I+maltose	119	+2.15°	-0.68°	No maltosazone; glucosazone
<i>X 20</i>	Control of <i>X 19</i> (60 mins. at 100°)		+2.83°		Maltosazone; no glucosazone
<i>X 21</i>	Leaf-powder I+water	119	±0.00°	-0.05°	A little glucosazone
<i>X 22</i>	Control of <i>X 21</i> (20 mins. at 100°)		+0.05°		" "

Maltase (see Table III). Three different annual crops of snowdrop leaves agreed in showing no difference in the decrease of rotation with maltose present or absent, that is, there is no maltase in the snowdrop. The onion, leek and dock all showed considerably more decrease when maltose was present than when it was absent, *i.e.* these leaves all contain maltase. The osazones confirmed these results.

Dextrinase (see Table IV). In the snowdrop, leek and dock, the dextrin was hydrolysed, *i.e.* dextrinase is present. In the onion, qualitative and quantitative tests failed to show any hydrolysis, *i.e.* there is no dextrinase in the onion.

Amylase (see Table V). The starch was hydrolysed by all the leaf-powders except the leek, *i.e.* the snowdrop, onion and dock all contain amylase, but it is absent from the leek.

Table IV. *Dextrinase*.

Exp. no.	Details of experiment (chloroform as antiseptic, unless otherwise stated)	Incubated (hours)	Rotation (corrected)	Change	Remarks
<i>Snowdrop</i>					
X 28	Leaf-powder I + dextrin + toluene. Then added an equal volume of 2 % dextrin, and replaced in the incubator. After a further	15 7	— —	— —	Tested with iodine. No colour Repeated iodine test. No colour
X 34	Leaf-powder I shaken 1 hour with water + chloroform. Filtered. Made up to 1 % dextrin	47½	—	—	With iodine test. No colour
<i>Onion</i>					
X 36	Leaf-powder I extracted ½ hour with water + chloroform. Made up to 1 % dextrin	21 40 91	— — —	— — —	With iodine. Brown " " " "
O 5	Leaf-powder III + dextrin + toluene	½	—	—	With iodine. O 5 Brown. O 6 Brown
O 6	Control of O 5 (30 mins. at 100°)	20 44½ 65½ 97½	— — — —	— — — —	" " " " " " " "
O 1	Leaf-powder III + dextrin + toluene	112½	+0.96° ± 0.005°	—0.18°	
O 2	Control of O 1 (30 mins. at 100°)		+1.14° ± 0.004°		
O 13	Leaf-powder III + water + toluene	45½	-0.02° ± 0.014°	-0.09°	No glucosazone; some maltosazone. With iodine. Brown.
O 14	Control of O 13 (30 mins. at 100°)		+0.07° ± 0.009°		Trace glucosazone; fair maltosazone. With iodine. Brown
O 3	Leaf-powder III + water + toluene	234½	-0.14° ± 0.004°	-0.10°	With iodine. Brown
O 4	Control of O 3 (30 mins. at 100°)		-0.04° ± 0.009°		" "
<i>Leek</i>					
X 64	Leaf-powder I + water + chloroform. Shaken 1 hour. Extract made up to 1 % dextrin	112½	—	—	With iodine. Colourless
X 68	Leaf-powder I shaken with 200 cc. water + chloroform. Filtered. Made up to 50 % alcohol. Precipitate dissolved in 70 cc. water. Made up to 0.50 % dextrin	18½ 41½ 73½	— — —	— — —	With iodine. Very pale brown ? Brown Colourless
X 117	Leaf-powder II + dextrin + toluene	336	+0.90° ± 0.004°	-0.25°	With iodine. Colourless
X 118	Control of X 117 (15 mins. at 100°)		+1.15° ± 0.005°		With iodine. Brown
X 119	Leaf-powder II + 0.5 g. CaCO ₃ + dextrin + toluene	336	+0.77° ± 0.004°	-0.35°	With iodine. Colourless
X 120	Control of X 119 (15 mins. at 100°)		+1.12° ± 0.005°		With iodine. Brown
L 3	Leaf-powder III + water + toluene	41½	-0.02° ± 0.006°	-0.10°	With iodine. Colourless
L 4	Control of L 3 (30 mins. at 100°)		+0.08° ± 0.004°		With iodine. Brown
<i>Dock</i>					
D	Leaves II shaken up with water + chloroform for ½ hour. Extract made up to 0.5 % dextrin	70	—	—	With iodine. Colourless
F	Leaves III frozen with CO ₂ and ether. Powdered. Shaken 1 hour with tap-water. Made up to 0.5 % dextrin + toluene. Added more dextrin. Incubated a further	24 22	— —	— —	With iodine. Colourless " "
X 6	Leaf III shaken 1 hour with tap-water + chloroform. Extract made up to 0.5 % dextrin + toluene	20½ 70	— —	— —	With iodine. Very pale brown " Colourless

Table V. *Amylase*.

Exp. no.	Details of experiment (chloroform or toluene as antiseptic)	Incu- bated (hours)	Rotation (corrected)	Change	Remarks
<i>Snowdrop</i>					
X 27	Leaf-powder I + starch	15	—	—	With iodine. Colourless
X 33	Leaf-powder I + water + chloro- form. Shaken $\frac{1}{2}$ hour. Extract made up to 1 % starch	47	—	—	With iodine. Colourless
S 19	Leaf-powder IV + starch	72	$+0.83^\circ \pm 0.016^\circ$	-0.64°	With iodine. Colourless. Maltosazone, trace glucosazone
S 20	Control of S 19 (40 mins. at 100°)		$+1.47^\circ \pm 0.038^\circ$		With iodine. Blue. No maltosazone, trace glucosazone
S 21	Leaf-powder IV + starch	1433	$+0.51^\circ \pm 0.019^\circ$	-1.05°	With iodine. Colourless
S 22	Control of S 21 (40 mins. at 100°)		$+1.56^\circ \pm 0.027^\circ$		" Blue
S 9	Leaf-powder IV + water	120	$+0.19^\circ \pm 0.023^\circ$	-0.46°	Some maltosazone; fair glucosazone
S 10	Control of S 9 (30 mins. at 100°)		$+0.65^\circ \pm 0.014^\circ$		Trace maltosazone; some glucosazone
X 103	Leaf-powder I + water	332	$-0.16^\circ \pm 0.004^\circ$	-0.41°	
X 104	Control of X 103 (15 mins. at 100°)		$+0.25^\circ \pm 0.005^\circ$		
<i>Onion</i>					
X 35	Leaf-powder I + water + chloro- form. Shaken $\frac{1}{2}$ hour. Extract made up to 1 % starch	21 40 91 624	— — — —	— — — —	With iodine. Blue Purple Brown "
O 11	Leaf-powder III + starch	45 $\frac{1}{2}$	$+0.70^\circ \pm 0.016^\circ$	—	With iodine. Brown
O 12	Control of O 11 (30 mins. at 100°)		—		" Blue. (Could not measure rotation, because solution would not filter.)
O 3	Leaf-powder III + water	234 $\frac{1}{2}$	$-0.14^\circ \pm 0.004^\circ$	-0.10°	
O 4	Control of O 3 (30 mins. at 100°)		$-0.04^\circ \pm 0.009^\circ$		
O 9	Leaf-powder III + starch	1362	$+1.25^\circ \pm 0.010^\circ$	-0.15°	With iodine. Brown
O 10	Control of O 9 (30 mins. at 100°)		$+1.40^\circ \pm 0.012^\circ$		" Blue } No maltosazone much glucosazone
<i>Leek</i>					
X 63	Leaf-powder I + water + chloro- form. Shaken 1 hour. Extract made up to 1 % starch	13 47 112 $\frac{1}{2}$	— — —	— — —	With iodine. Blue " "
X 66	Leaf-powder I + water + chloro- form. Shaken 1 hour. Filtered. Precipitated with 50 % alcohol. Precipitate dissolved in water. Made up to 0.5 % starch	18 $\frac{1}{2}$ 41 $\frac{1}{2}$ 73 $\frac{1}{2}$ 166 192 $\frac{1}{2}$ 456	— — — — — —	— — — — — —	With iodine. Blue " " " " "
X 67	As X 66, but made up to 1.25 % starch	18 $\frac{1}{2}$ 192 $\frac{1}{2}$ 456	— — —	— — —	With iodine. Blue " "
X 113	Leaf-powder II + starch	573 $\frac{1}{2}$	$+0.91^\circ \pm 0.005^\circ$	-0.12°	With iodine. Blue
X 114	Control of X 113 (15 mins. at 100°)		$+1.03^\circ \pm 0.011^\circ$		" " } Maltosazone; much glucosazone
X 115	Leaf-powder II + starch + 0.5 g. CaCO ₃	598	$+0.90^\circ \pm 0.004^\circ$	-0.13°	With iodine. Blue. Much glucosazone
X 116	Control of X 115 (15 mins. at 100°)		$+1.03^\circ \pm 0.023^\circ$		" " } Maltosazone, glu- cosazone
L 7	Leaf-powder III + starch	72	—	—	With iodine. Blue
L 8	Control of L 7 (40 mins. at 100°)		—	—	" " } Could not filter after adding lead acetate
L 9	Leaf-powder III + starch	1410	$+0.87^\circ \pm 0.036^\circ$	-0.23°	With iodine. Blue. Fair maltosazone, very much glucosazone
L 10	Control of L 9 (40 mins. at 100°)		$+1.10^\circ \pm 0.010^\circ$		With iodine. Blue. Much maltosazone, much glucosazone
L 3	Leaf-powder III + water	41 $\frac{1}{2}$	$-0.02^\circ \pm 0.006^\circ$	-0.10°	Trace maltosazone, little glucosazone
L 4	Control of L 3 (30 mins. at 100°)		$+0.08^\circ \pm 0.004^\circ$		Trace glucosazone
<i>Dock</i>					
A	Fresh leaf I; solidified by liquid air, powdered. Pulp extracted with water + chloroform. Extract made up to 1 % starch	19 $\frac{1}{2}$	—	—	With iodine. Colourless

Brown and Morris [1893] especially note that in leaves containing much tannin, the action of diastase is inhibited. The leaf-powders were therefore tested for tannin as follows. To a strong infusion of the leaf-powder (prepared by shaking 20 g. for an hour with 100 cc. water) 1 % iron alum was added. There was no trace of precipitate or coloration in any of the leaf-extracts. A further test was made. A freshly-prepared solution of 1 % gelatin in 10 % sodium chloride was added drop by drop to 10 cc. of the leaf-extract, till six drops had been added. There was no trace of precipitate or opalescence in any case. Tannin is therefore absent from all these leaves. (The author is indebted for these tests to Mr F. C. Thompson, of the Department of Leather Industries, Leeds University.)

GENERAL DISCUSSION.

It has long been known that certain plants normally do not contain starch in their green, foliage leaves. Arthur Meyer [1885] divided plants into five series according to the amount of starch contained in their leaves, varying from "very rich" to "very little," and specifically stated that five species of *Allium* which he examined contained no starch at all. Böhm [1883] showed that certain leaves normally starch-free can be made to form starch by floating the leaves on sugar solutions. He found that strong solutions were more efficacious than weak ones, and that on 20 % cane sugar, starch was formed in the leaves of *Galanthus nivalis* and other starch-free leaves. These results were confirmed by Schimper [1885] for certain starch-free leaves, but all efforts to form starch in the leaves of the onion failed, and he concluded that the onion had lost the power to form starch. Meyer also obtained starch formation in leaves which were normally starch free (such as *Iris* and *Arum* species), by exposure to strong light in 3 % carbon dioxide, but failed with *Allium* again. Schimper crushed fresh leaves with water and added the solution thus obtained to 1 % starch paste. With the exception of the onion, all had liquefied the starch-paste in 24 hours, including the iris. Parkin [1899] found that the very young leaves of the onion may contain starch, but that this is absent from the older plants. The mature leaves of both the leek and snowdrop do not normally contain starch. Rendle [1889] also states that starch may be found round the vascular bundles of young leaves but rarely in older ones. He tried to induce starch formation by floating the leaves on 20 % glucose, but failed. Parkin¹ tried similar experiments with 20 % sucrose and also failed. Mature leaves of the snowdrop, floated on 20 % sucrose and other sugars also failed to show any starch formation; young leaves also usually failed to show starch formation, but in a few cases a little was found near the vascular bundles. That is, the mature leaves which were used in these experi-

¹ On p. 53 of Parkin's paper it is stated that onion leaves were floated on "2 % cane sugar." This is apparently a misprint for "20 % cane sugar," as on p. 52 he states that solutions of "from 5 % to 20 %" were employed

ments would not contain starch, and therefore should not contain the full series of enzymes needed for starch formation. Schimper's experiments with starch paste showed that the onion had no diastase, which agrees with the results of Table IV—that dextrinase is lacking in the onion.

It has been known since at least 1862 that the guard-cells of the stomata contain starch grains which are retained with great tenacity even when the leaf is starved by being kept in darkness. Parkin [1899] found starch in the guard-cells of all the plants he examined, including many starch-free monocotyledons, except species of *Allium*. He correlated the presence of starch with the presence of chloroplasts, and showed that eight species of *Allium* which contain no starch in the guard-cells also have no chloroplasts or any sign of green coloration there: all other plants examined had both starch and chloroplasts in the guard-cells. Normally, 24 hours in darkness is sufficient to remove nearly all the starch from parenchyma cells, and all traces have gone in 48 hours, but Parkin showed that detached leaves of *Narcissus poeticus* retained starch in their guard-cells after seven days, and that even after fifteen days, though the leaves were turning yellow and withering the guard-cell starch had not wholly disappeared. This starch need not be the product of photosynthesis. Plants of *Galanthus nivalis* grown in the dark showed a little starch in the guard-cells of the upper mature part of the leaf, but none in the lower part. Parkin therefore considers that the presence of stomatal starch is to be correlated with the development of the stomata, and that it only appears when these are fully formed and functional.

The work of Böhm, Schimper, Rendle and Parkin in feeding experiments, showing that the mature green leaves of the onion, leek and snowdrop do not form starch when floated on strong sugar solutions, is quite in accordance with the results of Tables III, IV and V, in which it is shown that the complete series of enzymes required for starch formation is not present in any of these leaves.

Schimper's [1885] results seem to indicate that in order to form starch in the leaf, a certain minimum concentration of sugar is necessary. Where this minimum concentration is not normally attained, the plant does not form starch. In certain cases, the formation of starch can be induced by floating the leaf on strong sugar solutions; this will provide the necessary concentration. But in other cases this method failed, *e.g.* the onion, leek and snowdrop, and in these cases the mechanism for the production of starch is lacking, so that the presence of the raw material, sugar, makes no difference.

The guard-cell starch of those plants which do not contain any other starch may be regarded as formed by a mechanism which later disappears. Table III shows that the snowdrop has no maltase. But starch cannot be formed from glucose without maltase (if enzymes are true catalysts), so that presumably in the young leaf, the whole apparatus—maltase, dextrinase and amylase—required to convert glucose into starch is present in the guard-cells. Starch

is formed, and afterwards the maltase disappears. Thus no more starch can be formed, but equally the starch already formed cannot be hydrolysed further than maltose. The series of changes

under the influence of amylase and dextrinase would form an ideal mechanism for controlling the opening of the stomata if the reaction is

when the plant has plenty of water and transpiration is not too intense, and

when transpiration is high and water supplies are failing. But these are exactly the circumstances required, because the full equation is

and a high water content in the leaf would cause the reaction to go to the right. The maltose thus formed would increase the soluble contents of the guard-cells, water would be taken in by osmosis until turgidity was reached, and the stomata would open. If water were lacking, the reaction would go to the left, maltose would be replaced by insoluble starch, the soluble contents of the guard-cells would be lessened, water would be lost by osmosis, the guard-cells would no longer be turgid and the stomata would close. This system of amylase and dextrinase with no maltase might be described as a "chemical appendix," a relic of an earlier state, but in this case the appendix is still functional and useful. Tables IV and V seem to indicate that there is only a small quantity of diastase in the snowdrop, which would be quite compatible with this theory.

Similarly, the onion and the leek cannot form starch in the green leaf, because the complete starch-forming machinery is again lacking; the onion lacks dextrinase and the leek lacks amylase.

The dock can form starch because it contains the full equipment of maltase, dextrinase and amylase.

Brown and Morris determined the "diastatic activity" of a number of plants, defining it as the number of grams of maltose formed from soluble starch by 10 g. of leaf at 30°. These varied from 240.3 for *Pisum sativum* to 0.267 for *Hydrocharis morsus-ranae*. Three members of the Solanaceae, the potato, tobacco and tomato, which were specially mentioned by Arthur Meyer [1885] as forming abundant starch, have values from 6.57 to 8.16, little removed from that of the onion, 3.76. Brown and Morris do not comment at all on this rather curious juxtaposition and do not say that the Solanaceae have any tannin (which might have inhibited the action of the diastase).

It is interesting to note that in the snowdrop, the β -glucoside-splitting enzyme, emulsin, is also absent, as well as the α -glucoside-splitting enzyme, maltase.

The theory stated above, that where starch is normally found, the full equipment of enzymes, maltase, dextrinase and amylase, should be present,

is confirmed by the results of Mason [1916]. He used essentially the same method as was used for the experiments described in this paper, except that his leaves were treated with 95 % alcohol to remove sugars before being dried and powdered, and instead of measuring the rotations of his solutions, he took the copper-reducing power. He found in *Polytrichum commune* glucose, laevulose, sucrose, a little maltose and starch; he also found diastase and maltase present. In *Thuidium tamariscinum* and *Sphagnum cymbifolium*, he found present glucose, laevulose and sucrose, but no maltose or starch; he also found no diastase in these mosses. That is, diastase and maltase were only found in starch-containing leaves.

Robertson, Irving and Dobson [1909] working on the sugar-beet, *Beta vulgaris*, found present in the leaf and root both diastase and maltase, but in the stem only diastase without any maltase. The leaf and root do not contain starch (except in the very young leaf), though in the leaf, at any rate, there appears to be the full enzyme mechanism and the raw material (glucose) for the manufacture of starch. No mention is made of tannin, which might inhibit the action of diastase *in vivo* as it does *in vitro*, and there is no discussion of the reason for the absence of starch from the leaf, though the authors agree with the view that the same enzyme or enzymes should catalyse both in the direction of synthesis or hydrolysis, according to the conditions present. A variety of the same species, the mangold, was investigated by Davis, Daish and Sawyer [1915]. Starch was found in the very young leaf, when the root was small and not available as a storage organ, but as soon as the root becomes sufficiently large to serve as a storage organ, starch disappears from the leaf. Carbohydrates are stored in the root chiefly as sucrose. Daish [1916] showed that maltase was present in a number of starch-forming plants, *Tropaeolum*, potato, dahlia, turnip, mangold and sunflower, although hitherto maltase had been rarely described as present in foliage leaves. Davis [1915] concluded from these results that maltase would be universally found present in starch-forming plants, and that failure to demonstrate its presence in the past has been due to difficulties of extraction, and to its instability. (It is easily destroyed by temperatures above 50°, and by contact with ordinary alcohol and chloroform.) With this conclusion the writer is in complete agreement. Davis, Daish and Sawyer also noted that when proper precautions are taken to avoid the hydrolysis of starch and dextrins, maltose is invariably absent from the leaf. They consider that in the plant, the reactions

proceed under the action of the appropriate enzymes, "liquefying enzymes" (amylase) for the first stage, dextrinase for the second, and maltase for the third. Presumably these enzymes also catalyse the reverse process of synthesis.

A very striking example of the failure of a common plant reaction, when the appropriate mechanism is lacking is given by Davis and Sawyer [1915, p. 357] concerning the enzymes of selective yeasts. The ordinary plant, both

green and parasitic, etc., can quite easily be supplied with carbohydrates as sugar solutions, including maltose and, what is usually the best of all, cane sugar. On the other hand, the yeasts *Saccharomyces anomalous* and *S. exiguus* which do not contain maltase, are unable to assimilate maltose but starve in its presence. Similarly, *Torula monosa*, which does not contain invertase, will starve in the presence of cane sugar.

The apparent anomaly of the sugar-beet and mangold, which though containing maltase and diastase in the green leaf, do not form starch, may be explained by the assumption, that in order for starch to be formed a certain minimum concentration of hexose is necessary. In these plants, before the minimum concentration is reached the hexoses are changed into cane sugar which is translocated to the root. According to Robertson, Irving and Dobson, the root of the sugar-beet contains diastase and maltase but no invertase, whilst the carbohydrate there is cane sugar, both starch and reducing sugars being absent according to Girard [1883, 1884], though Colin [1914] states that small quantities of a reducing sugar are found in the root, varying from 0.178 of the amount of cane sugar in the very young root, to 0.011 of the cane sugar in the older root. Here it would appear that the lack of starch in the root is due to the lack of the raw material, glucose. Such small quantities of glucose as are found there are insufficient to reach the necessary minimal concentration. Also, because of the absence of invertase, glucose cannot be formed by the hydrolysis of the cane sugar.

Hudson and Paine [1914], working with yeast invertase, concluded that the enzyme does not take any part in synthesis, that the equilibrium of the reaction

is not mobile but the change goes to complete hydrolysis, previous workers who stated that they had obtained synthesis of cane sugar having been misled by experimental errors due to the action of hydrochloric acid on laevulose, and other causes. These facts are difficult to reconcile with the current theory of the nature of enzyme action, which cannot be lightly discarded.

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SUMMARY OF RESULTS.

1. The mature green leaf of the snowdrop (*Galanthus nivalis*) contains amylase, dextrinase and invertase, but no maltase or emulsin.
2. The mature green leaf of the onion (*Allium cepa*) contains amylase, maltase, invertase and emulsin, but no dextrinase.

3. The mature green leaf of the leek (*Allium porrum*) contains dextrinase, maltase, invertase and emulsin, but no amylase.
4. The mature green leaf of the dock (*Rumex obtusifolius*) contains amylase, dextrinase, maltase, invertase and emulsin.
5. It is suggested that the failure of the snowdrop, onion and leek to form starch in the mature green leaf is due to the enzyme machinery necessary for starch formation being incomplete in each case.

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