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PATHWAYS OF CARBOHYDRATE OXIDATION IN LEAVES OF *PISUM SATIVUM* AND *TRITICUM AESTIVUM*

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Key Word Index—*Pisum sativum*; Leguminosae; pea; *Triticum aestivum*; Gramineae; wheat; leaves; glycolysis; pentose phosphate pathway; relative activities.

Abstract—The aim of this work was to establish the pathways of carbohydrate oxidation used in the dark by leaves of *Pisum sativum* and *Triticum aestivum*. Segments of young and mature leaves of pea released the carbons of glucose-[^{14}C] as $^{14}CO_2$ in the order 3,4 > 1 > 2 > 6 whereas in segments of young and mature leaves of wheat the order was 3,4 > 1 > 6 > 2. The detailed labelling of the constituents of mature leaves of wheat by glucose-[1-^{14}C], -[2-^{14}C], -[3,4-^{14}C], and -[6-^{14}C] was determined and showed that the high yield of CO_2 from C-6 relative to that from C-2 was due to release of C-6 during pentan synthesis. Estimates were made of the maximum catalytic activities of phosphofructokinase and glucose-6-phosphate dehydrogenase in pea and wheat leaves of three ages. The results of all the above investigations strongly indicate that both pea and wheat leaves in the dark oxidize carbohydrate via glycolysis and the pentose phosphate pathway with the latter accounting for no more than a third of the total. No evidence was obtained of any major change in the relative activities of the two pathways during the development of either type of leaf.

INTRODUCTION

The routes of carbohydrate oxidation in non-photosynthetic cells of higher plants are reasonably well known. Glycolysis predominates but there is a significant contribution from the pentose phosphate pathway. These pathways are closely related, and most of the pentose phosphate produced in the latter is eventually metabolized to pyruvate [1]. The manner in which photosynthetic cells oxidize carbohydrate in the dark has not been established. Although it is likely that both the above pathways contribute to such oxidation, very little is known about their inter-relationship and relative activities in photosynthetic cells. The small amount of data that is available might be taken to indicate that, relative to glycolysis, the pentose phosphate pathway is quantitatively more important in photosynthetic than in non-photosynthetic cells. C_6/C_1 ratios of leaves are particularly low [2]. There is evidence that the activity of the pentose phosphate pathway in *Chlorella* is increased on darkening, and Bassham has suggested that the principal energy metabolism of *Chlorella* in the dark is via this pathway [3]. The very few available estimates of the activities of phosphofructokinase [4, 5] and glucose-6-phosphate dehydrogenase [6–8] in leaf extracts give higher relative values for the latter enzyme than are normally found in extracts of non-photosynthetic cells. In contrast, in rice leaves the pattern of $^{14}CO_2$ production from specifically labelled glucose-[^{14}C] suggests a dominance of glycolysis [9]. Finally, we do not know whether the relative activities of the two pathways change during development. Gibbs and Beevers suggested that there is a shift towards the pentose phosphate pathway during leaf development [2]. More recently there have been suggestions that the activity of glucose-6-phosphate dehydrogenase decreases as leaves mature [8, 10].

The aim of the present work was to establish the pathways of carbohydrate oxidation in the dark in photosynthetic tissues of two unrelated species, pea and wheat. We paid particular attention to the relationship between the pathways, and to their relative activities during development. Thus we investigated both young and mature leaves of each species. The methods used were those found satisfactory for the study of non-photosynthetic cells of plants [11]. These are the determination of the pattern of $^{14}CO_2$ production, and the detailed labelling of cell constituents, after supplying specifically labelled glucose-[^{14}C]; and measurement of the activities of phosphofructokinase (EC 2.7.1.11) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49), key and diagnostic enzymes of their respective pathways. We appreciate that none of these methods is conclusive on its own; in particular, compartmentation of the leaf may complicate interpretation of the experiments with isotopes. None the less, we suggest that close agreement between results obtained by different methods may be taken as a valid indication of the routes of carbohydrate oxidation.

RESULTS

Metabolism of glucose-[^{14}C]

$^{14}CO_2$ production from glucose-[^{14}C] supplied to segments of leaves of pea and wheat is illustrated in Table 1. Similar results were obtained in duplicate experiments. We also determined the pattern for segments of the first leaf of 7-day-old wheat and found that it was similar to that of leaves from 13-day-old wheat. The above patterns are sufficiently similar to be

Table 1. Release of $^{14}CO_2$ from specifically labelled glucose-[^{14}C] supplied to segments of pea and wheat leaves

Tissue	Position of ^{14}C in glucose-[^{14}C]	Percentage ($\times 10^{-3}$) of added glucose-[^{14}C] that was recovered as $^{14}CO_2$ in: 30	60	90	120	180	240 min*
Young leaflet	1	17.6 ± 1.4	63.1 ± 12.5	132.3 ± 34.5	215 ± 74	420 ± 113	780 ± 274
(fr. wt 8–12 mg)	2	5.8 ± 1.6	26.2 ± 0.3	65.6 ± 10.1	117 ± 3	297 ± 63	547 ± 113
of peas	3, 4	24.4 ± 2.1	133.2 ± 21.2	292.2 ± 29.2	503 ± 37	965 ± 94	1649 ± 194
	6	5.0 ± 1.4	24.0 ± 3.0	48.1 ± 5.8	77 ± 27	152 ± 63	280 ± 129
Mature leaflet	1	26.8 ± 9.9	109.4 ± 5.8	216.9 ± 18.9	354 ± 26		
(fr. wt 22–28 mg)	2	6.9 ± 2.2	36.9 ± 7.5	87.4 ± 15.3	163 ± 35		
of peas	3, 4	11.8 ± 1.9	82.9 ± 10.8	224.1 ± 30.5	464 ± 60		
	6	6.7 ± 2.1	34.6 ± 4.6	84.4 ± 16.9	153 ± 24		
First leaf of	1	27.1 ± 4.7	196.3 ± 49.3	484.3 ± 88.3	966 ± 202	2205 ± 216	4009 ± 348
13-day-old wheat	2	9.8 ± 4.5	73.8 ± 8.5	209.9 ± 28.9	426 ± 28	1025 ± 168	1932 ± 220
	3, 4	19 1 ± 4.5	174.9 ± 29.9	536.1 ± 46.0	1110 ± 109	2653 ± 158	4825 ± 587
	6	19.2 ± 9.1	126.8 ± 49.5	340.1 ± 99.0	629 ± 179	1431 ± 420	2495 ± 727

* Minutes from addition of glucose-[^{14}C] to samples Values are means ±90% confidence limits of estimates from triplicate samples.

Table 2. Distribution of ^{14}C in segments of mature leaves of wheat supplied with specifically labelled glucose-[^{14}C]

Cell fraction	^{14}C recovered per fraction as % uptake of glucose-[^{14}C] labelled in: C-1	C-2	C-3,4	C-6
CO_2	19.0	9.9	22.5	13.8
Water-soluble substances	67.9	75.1	63.4	78.1
Neutral components	20.6	21.5	24.8	16.9
Sucrose	15.5	15.1	18.6	11.2
Glucose	2.5	2 5	3.5	2.6
Fructose	0.9	1.0	1.3	1.1
Oligosaccharides	1 5	1.5	1.3	1.0
Acidic components	18.3	19.5	14.5	19.8
Citrate	1.8	1.9	1.0	2 2
Malate	7 3	6.5	4.8	8.0
α-Ketoglutarate	0.2	0.2	0 1	0.2
Succinate	0.8	0.8	0.4	1.4
Fumarate	0.2	0.1	0.1	0.1
Sugar phosphates	5.4	8.9	5.7	5.3
Basic compounds	26.8	29.8	19.1	34.0
Glutamate	9.3	11.2	4.1	13.8
Aspartate	3.0	3.0	2.7	6.6
Serine	1.3	2.2	1.9	1.9
Alanine + glutamine*	6.9	9.7	8.9	9.9
Glycine + lysine + threonine + asparagine	0 2	0.3	0.3	0.5
Valine + histidine	1.6	2.5	1 9	2.9
Water-insoluble substances	14.7	15.0	14.1	8.0
Protein	2.4	2.7	2.2	2.3
Polyuronides	0.5	0.4	0.6	0 4
Neutral polysaccharides	11.0	10.1	9.4	4.7
Glucose	1.7	1.8	1.9	1.6
Galactose	1.7	1.5	1.5	1.5
Mannose	0.2	0.2	0.1	0.1
Arabinose	2.3	2.0	2.0	0 2
Xylose	3.4	2.9	3.5	0 3
Oligosaccharides	1.1	1.1	0.7	0 7
Uptake as dpm $\times 10^{-3}$	284.6	357.9	209.0	330.3
as % ^{14}C supplied	24.6	26.5	25.2	28 1

Samples (0.5 g fr. wt) of segments of leaves of 13-day-old plants were incubated in 0.3 mM glucose-[^{14}C] in the dark at 20° for 4 hr The ^{14}C recovered as $^{14}CO_2$, and in the water-soluble substances, and water-insoluble substances, was summed to give total uptake of ^{14}C The polysaccharide fraction was hydrolysed and the labelling of the individual sugars in the hydrolysate was determined: that recovered as oligosaccharides is attributed to incomplete hydrolysis.

*Most of the label was in alanine

discussed as one. Four points are made. First, for all types of leaf at all sampling times there was a significant but not overwhelming excess of C-1 over C-6 in the respired $^{14}CO_2$. However during the course of each experiment the ratio of the yield of $^{14}CO_2$ from C-1 to the yields from each of the other carbons fell. Second, apart from a few of the initial sampling times, carbons 3 and 4 were converted to $^{14}CO_2$ in greater amounts than any of the other carbons. Third, in pea leaves the release of C-2 exceeded that from C-6, although the difference was small in the mature leaf. In contrast, in wheat the yield from C-6 was greater than that from C-2 at every stage in every experiment. Finally, in neither pea nor wheat leaves was there any clear evidence that maturation was accompanied by significant changes in the patterns of $^{14}CO_2$ production.

Because of the similarity between the above patterns of $^{14}CO_2$ production, we investigated the detailed distribution of label amongst cellular components for only one of the types of leaves. We chose the mature leaf of wheat as here the anomalous excess of C-6 over C-2 in the respired $^{14}CO_2$ was most marked. Replicate samples of segments of mature leaves of wheat were incubated in specifically labelled glucose-[^{14}C] in the dark for 4 hr. Then the isotope was washed out of the free space of the segments which were immediately killed and then analysed. The results are given in Table 2. For each sample the label recovered as $^{14}CO_2$, in the water-soluble and in the water-insoluble substances was summed and is used as an estimate of the uptake of glucose-[^{14}C]. The estimates obtained for the different samples agree closely. The soluble substances were fractionated by ion-exchange and paper chromatography. The insoluble material was fractionated into protein and polysaccharides: the latter were hydrolysed and the individual monomers were isolated. The identity of isolated compounds was checked by co-chromatography in at least two solvents. Individual sugar phosphates were not isolated but label was assigned to them on the basis of similar chromatographic mobility to those described for potato tissue [12]. Comparison of the label recovered in the isolated compounds with the present in the unfractionated water-soluble and water-insoluble fractions shows that the analyses were achieved without serious loss: at least 90% of the ^{14}C absorbed by each sample was accounted for.

The above analysis (Table 2) reveals the following. The absorbed sugar must have been rapidly metabolized as little label remained as glucose-[^{14}C]. None the less the sugar fractions were extensively labelled: mostly in sucrose, to which each isotope of glucose contributed more or less equally. There was appreciable labelling of polysaccharide with each isotope contributing equally to the polyuronides and the hexose units of the neutral polymers. In contrast, the considerable labelling of pentans was marked by equal contributions from C-1, C-3,4 and C-2, and an almost negligible contribution from C-6. Analysis of the acidic components of the water-soluble substances indicated similar labelling of the sugar phosphates by each carbon, and the rest of the label was recovered in Krebs cycle acids with the contributions of the individual glucose carbons being in the order C-6 > C-1 > C-2 > C-3,4. The amino acids derived from glycolytic intermediates, alanine and serine, were labelled to about the same extent by C-2, C-3,4 and C-6 of glucose: those derived from the Krebs cycle were labelled in the order C-6 > C-2 > C-1 > C-3,4.

Enzyme activities

We investigated the assays in an attempt to determine the maximum catalytic activities of phosphofructokinase and glucose-6-phosphate dehydrogenase. The pH, and the concentration of each component of each reaction mixture, were optimized for the assays of extracts of each leaf at each stage of development. For each type of sample we showed that the activity was linearly related to the amount of extract assayed. The extent to which enzyme activity was lost during extraction of the tissues was investigated as follows. For each species, we prepared duplicate samples that consisted of equal weights of tissue from young and mature leaves. One of each duplicate was extracted and assayed in the usual way. The corresponding samples were extracted in buffer that contained measured amounts of purified phosphofructokinase and glucose-6-phosphate dehydrogenase. Comparison of the activities in the latter extracts with those in the former is taken as a measure of the extent to which the pure enzymes survived the complete process of extraction and assay. For pea leaves our estimates of the recoveries of phosphofructokinase and glucose-6-phosphate dehydrogenase were, respectively, 119 and 102% of the added activity. For wheat, the value for the dehydrogenase was 102%, but those for phosphofructokinase were variable and low. The addition of extract of wheat leaf to a cuvette that contained pure phosphofructokinase caused an immediate inhibition of 20–30%.

There have been reports that the activities of respiratory and photosynthetic enzymes in leaf extracts vary according to whether the leaves, just prior to extraction, or the extracts themselves, are illuminated [13]. We extracted, in complete darkness, leaves from plants that had been kept in the dark for periods varying from 3 to 24 hr. We compared the activities of the two enzymes in such extracts with those in extracts prepared in bright

Table 3. Activities of phosphofructokinase and glucose-6-phosphate dehydrogenase in extracts of pea leaves

Fr. wt of individual leaflets in samples (mg)	No. of samples assayed	Enzyme activity (nkat)* per: g fr. wt		g protein		leaflet	
		PFK	G-6-P DH	PFK	G-6-P DH	PFK	G-6-P DH
1–4	6	19.7 ± 4.0	26.7 ± 4.3	423 ± 88	557 ± 75	0.046 ± 0.023	0.062 ± 0.030
10–20	7	15.5 ± 3.7	26.8 ± 2.3	447 ± 108	750 ± 62	0.223 ± 0.057	0.383 ± 0.082
22–29	5	17.7 ± 5.3	19.3 ± 3.7	682 ± 210	668 ± 27	0.465 ± 0.110	0.467 ± 0.048

* Values are means ±90% confidence limits of estimates from the No. of samples indicated.

Table 4. Activities of phosphofructokinase and glucose-6-phosphate dehydrogenase in extracts of wheat leaves

Age of plants (days)	No. of samples assayed	Enzyme activity (nkat)* per: g fr. wt		g protein	
		PFK	G-6-P DH	PFK	G-6-P DH
5–6	6	12.7 ± 1.7	25.0 ± 1.8	470 ± 53	933 ± 105
7–9	7	13.2 ± 1.5	22.7 ± 1.8	493 ± 48	857 ± 78
12–15	7	10.8 ± 1.3	13.7 ± 1.3	560 ± 68	682 ± 55

* Values are means ±90% confidence limits of estimates from the No. of samples indicated.

light from leaves of plants kept in full daylight. For neither enzyme from either pea or wheat did we detect any significant differences in the activities between the two extracts.

The data for pea leaves (Table 3) show that, although the activity of the dehydrogenase was the higher, the difference between the values for the two enzymes was not very marked. There was a slight change in their relative activities, in favour of phosphofructokinase, as the leaves matured. Expression of the activities per leaflet shows that this change did not involve any decline in the activity of glucose-6-phosphate dehydrogenase. The estimates for wheat leaves are in Table 4. Although the values for phosphofructokinase must be regarded as minimal, they are included because they are appreciable in absolute terms, in relation to published values for this enzyme in leaves, and in comparison to the values for the dehydrogenase.

DISCUSSION

The rise in the C_6/C_1 ratios throughout the feeding experiments indicates that our results were not seriously affected by the development of induced respiration in the leaf segments [14]. The fact that these ratios were always less than one is evidence of the operation of the pentose phosphate pathway in both young and mature leaves of pea and wheat. This pathway, operating as a cycle, could release glucose carbons 3 and 4 as CO_2 but not in excess of the yield of CO_2 from carbon 1. Thus the fact that the leaves released C-3 and -4 in greater amounts than C-1 demonstrates the operation of an additional pathway. In peas the relative contributions of C-3,4, -2, and -6 to CO_2 are those expected from substantial oxidation via glycolysis and the Krebs cycle. The higher yields of CO_2 from C-6 than C-2 in wheat leaves are difficult to explain by the straightforward operation of glycolysis or the pentose phosphate pathway. However, the accepted pathway for pentan synthesis [15] involves the release of C-6 of glucose as CO_2. The labelling of the pentans (Table 2) provides very strong evidence for the operation of this pathway in wheat leaves. The difference between the labelling of pentans by C-1, -2, and -3,4 on the one hand, and C-6 on the other, shows that 5% of the absorbed glucose-[^{14}C] was converted to pentan with the loss of C-6. Thus the contribution of glycolysis and the pentose phosphate pathway to $^{14}CO_2$ production from C-6 is given by subtracting this 5% from the yield of $^{14}CO_2$ from this carbon. The value obtained, 8.8%, is less than the yield from C-2 (Table 2). Wheat leaves contain arabinoxylan in which the ratio of arabinose to xylose is similar to that in which they were labelled in our experiments [16]. We suggest that in wheat leaves pentan synthesis is responsible for the high yield of $^{14}CO_2$ from C-6 relative to that from C-2, and that, when this is taken into account, the pattern of $^{14}CO_2$ production is similar to that of pea leaves and to those of non-photosynthetic cells of plants [11, 17, 18], and is indicative of appreciable activity of glycolysis with a smaller contribution from the pentose phosphate pathway.

The yield of $^{14}CO_2$ from glucose-[3,4-^{14}C] has been taken as an indication of glycolytic flux [19]. Our demonstration that glucose-[3,4-^{14}C] labels a range of compounds derived from triose phosphate shows that such an approach could underestimate glycolysis. The appearance of label from C-3 and -4 in compounds such as alanine, that are derived from pyruvate without decarboxylation, requires no comment; but the labelling of the Krebs cycle acids and the amino acids derived therefrom is noteworthy. We attribute the latter to the synthesis of oxaloacetate by phosphoenolpyruvate carboxylase. This view is supported by the fact that the ratios of the contributions of C-3 and -4 to those of C-2, in the following compounds, decrease in the order aspartate, malate, citrate and glutamate. We ascribe the labelling of glutamate to randomization of the label in C-4 acids by fumarase.

The difference between the yields of $^{14}CO_2$ from C-1 (19) and C-6, after correction for pentan synthesis (13.8–5), amounts to 10.2% of the absorbed glucose in the experiment described in Table 2. There is an excess of C-6 over C-1 in the amino acids and Krebs cycle acids that corresponds roughly to the above excess of C-1 over C-6 in the respired CO_2. These analyses indicate that both pathways of carbohydrate oxidation operate in wheat leaves, and that the ribulose-5-phosphate formed in the pentose phosphate pathway is almost all converted to pyruvate via triose phosphate. The situation is similar to that described for non-photosynthetic tissues of plants [11, 17, 20].

We suggest that, phosphofructokinase in wheat apart, our estimates reflect the maximum catalytic activities of the leaves. The values for glucose-6-phosphate dehydrogenase are comparable to those reported for other leaves [8, 10] but those for phosphofructokinase are appreciably higher than previously reported estimates [4]. In both absolute and relative terms our estimates do not differ greatly from the activities reported for non-photosynthetic tissues [21]. In neither leaf is there any evidence of restricted capacity for glycolysis. By assuming that half of the hexose that enters the respiratory pathways is used for biosynthesis, we estimate, from the rates of respiration [22, 23], that the rates at which pea and wheat leaves oxidize carbohydrate in the dark are in the region of 60–140, and 50 nmol per min/g fr. wt, res-

pectively. These rates are so far below the values reported in Tables 3 and 4 that we may conclude that the activities of both enzymes are more than adequate to support the observed carbohydrate oxidation in pea and wheat.

Apart from the complicating effects of pentan synthesis, we found little difference between leaves of different ages or between leaves of the two species. The different experimental approaches all gave similar indications. We therefore conclude that, in the dark, pea and wheat leaves oxidize carbohydrate via glycolysis and the pentose phosphate pathway. The ribulose-5-phosphate formed in the latter is largely converted to pyruvate via the non-oxidative branch of the pathway. and glycolysis. There is no evidence that leaf development is accompanied by marked changes in the relative activities of the two pathways. Finally, we suggest that in pea and wheat leaves in the dark, as in most non-photosynthetic tissues, glycolysis predominates over the pentose phosphate pathway. As shown above for the experiment reported in Table 2, the difference between the contribution to $^{14}CO_2$ of C-1 and C-6 of glucose-[^{14}C] that entered glycolysis and the pentose phosphate pathway amounted to 10.2% of the absorbed glucose-[^{14}C]. This difference may be attributed to the activity of the pentose phosphate pathway. An estimate of the total amount of glucose-[^{14}C] that entered the two pathways may be obtained by summing the contribution of glucose-[3,4-^{14}C] to CO_2, Krebs cycle acids, amino acids, and protein. The value obtained from Table 2 is 50.7% of the absorbed glucose-[^{14}C]. These estimates are approximate because they do not take into account re-cycling in the pentose phosphate pathway or differences in pool size and specific activities of the various intermediates. None the less, it seems most unlikely that the pentose phosphate pathway accounts for any more than 30% of the carbohydrate oxidation in wheat leaves in the dark.

EXPERIMENTAL

Materials. We obtained glucose-[3,4-^{14}C] from NEN Chemicals, Dreieichenhain, West Germany; the other isotopes from the Radiochemical Centre, Amersham; and co-factors and enzymes from Boehringer. Seeds of *Pisum sativum* L. cv Kelvedon Wonder were soaked in 10% (v/v) HOCl for 15 min, washed in running H_2O for 24 hr, planted in sterilized mica saturated with H_2O, and watered daily thereafter. Seeds of *Triticum aestivum* L. cv Maris Huntsman were spread over sifted and dampened compost (John Innes No. 2), covered with a thin layer of compost, watered × 2 daily for 2 days and daily thereafter. All plants were grown at 25° with a 16 hr photoperiod in light of intensity 58 × 10^{-3} J cm^{-2} min^{-1} at the soil surface. Pea leaflets were taken from 7- to 18-day-old plants and fr. wt was used as a measure of the age of the leaflets. The first leaves of 5- to 14-day-old wheat plants were used and the age of plants was taken as a measure of the age of the leaves. The basal 3.5 cm of each wheat leaf was discarded.

Metabolism of glucose-[^{14}C]. Pea leaflets were excised, deribbed and cut into segments 0.2 cm wide; wheat leaves were cut into 0.5 cm segments. Comparisons between different isotopes were always made within a given batch of carefully prepared replicate samples, fr. wt 0.5 g. Each sample was suspended in 4 ml 20 mM KH_2PO_4 (pH 5.2) that contained 0.3 mM glucose-[14C]. The specific activities were 0.63, 0.71, 0 32 and 0.80 μCi/μmol for glucose-[1-^{14}C], -[2-^{14}C], -[3,4-^{14}C], and -[6-^{14}C], respectively, for the expts in Table 1. The corresponding values for the experiments in Table 2 were 0.45, 0.45, 0.28, and 0 45 μCi/μmol. Samples were incubated in the dark in 100 ml flasks at 20°. Respired $^{14}CO_2$ was collected in alkali. For detailed analysis the isotope was removed at the end of the incubation and each sample was washed with 4 successive 7-ml portions of 0.3 mM glucose in 20 mM KH_2PO_4 (pH 5.2) for 10, 15, 60 and 60 sec, respectively. We showed that this effectively removed the glucose-[^{14}C] from the free space. Each sample was then killed with boiling 80% aq. EtOH and extracted successively with boiling 20-ml vols of 50% aq. EtOH, H_2O, 80% aq. EtOH. The residue is called water-insoluble substances. The extracts were combined and reduced to 5–6 ml at 28° to give the water-soluble substances that were divided into their acidic, basic, and neutral components as in ref. [24] except that 4 M HCO_2H was used to elute the anions. Further analysis was by PC on Whatman No. 3 paper with the following solvents: acidic components, PrOH–HCO_2H–H_2O (5:2:5) upper phase, *n*-BuOH–HCO_2H–H_2O (4:1:5) upper phase; basic components, PhOH–H_2O (4:1), PhOH–NH_4OH–H_2O (160:1:40), PhOH–EtOH–H_2O (3:1:1), *n*-BuOH–HOAc–H_2O (12:3:5); neutral components, EtOAc–Py–H_2O (8:2:1). Acidic compounds were revealed with bromocresol green, basic compounds with ninhydrin, and neutral compounds with $AgNO_3$ as in ref. [25]. The water-insoluble substances were dried at 70° for 30 min, ground to a fine powder and suspended in H_2O. Portions of the suspension were used to determine ^{14}C present as in ref. [26], ^{14}C in protein as in ref. [27], and ^{14}C in polysaccharides. For the latter, the suspension was centrifuged, the pellet was dried and then vigorously homogenized in 26 M H_2SO_4 for 3 min. The homogenate was then diluted with H_2O to 0.33 M H_2SO_4 and hydrolysed as in ref. [28]. Uronic acids and sugars were isolated from the hydrolysate as described in ref. [29]. ^{14}C was determined as described in ref. [30].

Assay of enzymes. Samples (fr. wt 0.2–0.8 g) of de-ribbed leaflets of pea, and of the first leaves of wheat, were homogenized in 10–20 vol. 50 mM glycylglycine buffer (pH 7.5), first with a pestle and mortar and then in an all-glass homogenizer. Examination of the homogenates with a microscope revealed very few unbroken cells. Homogenates of pea leaflets were centrifuged at 100 000 *g* for 30 min and the supernatant was assayed at once. The homogenates of wheat leaves were passed through a column (11 × 1.4 cm) of Sephadex (G-25, Coarse) and then assayed immediately. All enzymes were measured at 25° by methods described in ref. [30]. The reaction mixtures contained in 3.0 ml: glucose-6-phosphate dehydrogenase from peas, 0.3 mM NADP, 30 mM $MgCl_2$, 10 mM glucose-6-phosphate and 1 μg (0.012 unit) 6-phosphogluconate dehydrogenase in 50 mM glycylglycine, pH 8.2; for wheat the composition was the same except that it was at pH 8.25 and $MgCl_2$ was 15 mM; phosphofructokinase from peas, 15 mM fructose-6-phosphate, 1 mM ATP, 0.1 mM NADH, 2 mM cysteine, 0.5 mM $MgCl_2$, 15 μg (0.135 unit) aldolase and 6 μg of a mixture of glycerol-3-phosphate dehydrogenase (0.36 unit) and triosephosphate isomerase (0.03 unit) in 50 mM glycylglycine at pH 7.25; the same mixture was used for wheat except that fructose-6-phosphate was 20 mM, ATP 2 mM, cysteine 4 mM, and $MgCl_2$ 2 mM. Protein in the extracts was measured as in ref. [11].

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