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Decrease of Glucose 6-Phosphate and 6-Phosphogluconate Dehydrogenase Activities in the Xylem of *Populus gelrica* on Budding[1]

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ABSTRACT

The activities of glucose 6-phosphate and 6-phosphogluconate dehydrogenases, transketolase, phosphoglucose isomerase, and fructose 6-phosphate kinase were studied in extracts of wintering poplar (*Populus gelrica*) xylem. The xylem of wintering poplar showed high levels of transketolase, glucose 6-phosphate, and 6-phosphogluconate dehydrogenases. On recommencement of growth, the two dehydrogenase activities decreased. The three remaining enzymes appeared to be unchanged. In spring and early summer, glucose 6-phosphate dehydrogenase of the xylem was extremely low. On the other hand, 6-phosphogluconate dehydrogenase, which also became lower during the metabolic shift from winter to spring, was readily detected, and was several times higher than glucose 6-phosphate dehydrogenase throughout the year. The low dehydrogenase activities lasted into late October and then appeared to resume their original activity. A shift of metabolism at the beginning of growth was also observed by measuring the amount of sugar phosphates, soluble amino acids and amides, and proteins in the xylem. In contrast to the decrease of the two dehydrogenases and soluble proteins at the time of budding, incorporation of lysine-U-^{14}C into the xylem protein ramained constant. A method to transfuse radioactive compounds into a section of stem was described.

The accumulation of various sugars in wintering plants and the cryobiological significance of these compounds, with particular reference to freezing resistance, have been studied by a number of workers (17, 18). However, little attention has been given to the biochemistry of wintering plants, especially that of the stem of perennials. Higher plants require a number of sugars to synthesize the structural components of a stem as the growth proceeds (12). In perennial plants, such as poplar (*Populus gelrica*) the requirement for the sugars as the building block seems to vary from one growth phase to another, owing to a metabolic shift occurring in cellular organization. Therefore, when autumn comes and growth ceases, it is conceivable that the cellular metabolism begins to shift to that characteristic of winter. In the spring, with the commencement of growth, the cells resume enzyme organization for growth.

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The metabolism of carbohydrates and the biochemical significance of the pentose phosphate cycle in plants have been reviewed by Gibbs (5). In order to gain an understanding of the regulatory behavior of pentose phosphate cycle enzymes in the xylem, activity levels of glucose-6-P and 6-P-gluconate dehydrogenases, transketolase, glucose-6-P isomerase, and fructose-6-P kinase were studied. Evidence presented in this report shows that the xylem of wintering poplar has a high level of pentose-P cycle enzymes and that the differences in the activities of the two dehydrogenases were conspicuous in winter and spring, suggesting the existence of a distinct regulatory process at the branch point of glucose-6-P metabolism in the xylem.

MATERIALS AND METHODS

Chemicals and Enzymes. The following sugar phosphates, nucleotides, and enzymes were products of Boehringer u. Soehne GmbH: erythrose-4-P, ribose-5-P, fructose-6-P, glucose-6-P, fructose-1,6-diP, $NADP^+$, UTP, glucose-6-P and 6-P-gluconate dehydrogenases, phosphoglucomutase, and glucose-6-P isomerase. Erythrose-4-P was also prepared from glucose-6-P by lead tetraacetate oxidation (2) and freed from glucose-6-P by incubating the solution with excess glucose-6-P dehydrogenase, lactic dehydrogenase, pyruvate, $NADP^+$, and tris-HCl buffer, pH 7.6, as described by Horecker (6). L-Lysine-U-^{14}C, glucose-1-^{14}C, glucose-6-^{14}C, and glucose-U-^{14}C were purchased from the Daiichi Chemical Co. and glucose-6-P-U-^{14}C was prepared by the methods of Lowenstein (10). A preparation of an equilibrated mixture (21) of ribose-5-P, xylulose-5-P, and ribulose-5-P using a popular bark extract was described previously (16). Partial purification of xylem glucose-6-P dehydrogenase was carried out by the method described by Horecker *et al.* (8).

Plant Material. *P. gelrica* was grown in the field or in pots. One- or two-year-old twigs were collected and used either on the same day or stored in a polyethylene bag at −6 C. The potted plants wintering in the field until mid January were stored at 4 C for 20 days and were then placed in a green house.

Crude Enzyme. The xylem with the bark and pith removed was used for the study of enzyme activity determination. Crude enzyme solutions were prepared daily by grinding 1.0 g of excised small pieces of the xylem in a mortar for about 5 min in the presence of 1.0 g of sea sand and 3.0 ml of 50 mM tris-HCl, pH 7.6. A turbid solution was obtained by pressing the slurry through gauze. This was followed by decantation of the extract to remove sea sand and cell debris. The resulting solution was dialyzed for 90 min against three changes of a 20-ml portion of the above buffer. Since activities of the two

dehydrogenases (and transketolase) in the poplar xylem were found only in the soluble fraction, the supernatant was centrifuged at 12,000*g* for 10 min. Glucose-6-P isomerase and fructose-6-P kinase activities, found both in the soluble and insoluble fractions were measured with the dialyzed enzyme without centrifugation.

Enzyme Activity Measurement. Glucose-6-P and 6-P-gluconate dehydrogenase assays were carried out by conventional spectrophotometric methods (8) in the following system. The reaction mixture contained 0.26 μmole of $NADP^+$, 30 μmoles of $MgCl_2$, 0.2 μmole of glucose-6-P or 0.5 μmole of 6-P-gluconate, 150 μmoles of tris-HCl buffer of pH 7.6, and the xylem enzyme solution in a total volume of 3.0 ml.

Owing to the presence of aldolase and yellow-brown materials in the crude enzyme solution, the assay of transketolase activity based on the determination of glyceraldehyde-3-P was not applicable to the crude plant extract. The reaction system used in this paper was essentially the same as reported by Racker *et al.* (4, 21). Their procedure was modified in that erythrose-4-P was used as the acceptor sugar phosphate as in the following. The mixture consisted of 2.8 μmoles of erythrose-4-P, an equilibrium mixture (21) of xylulose-5-P, ribulose-5-P, and ribose-5-P (5.4 μmoles in total in which the latter was 3.4 μmoles), 20 μmoles of tris-HCl buffer of pH 7.4, and 0.1 to 0.3 ml of the xylem enzyme in a total volume of 0.5 ml. Incubation was carried out for 15 min at 25 C. A 0.1 ml portion of the mixture was sampled at 5-min intervals and the reaction was terminated by adding 0.1 ml of 0.2 N $HClO_4$. This was followed by an addition of 0.1 ml of 10% Darco G-60 and water brought to a total volume of 0.4 ml. Colored materials which disturb the spectrophotometric determination of the sugar phosphate were removed by this charcoal treatment. After centrifugation, a 0.2-ml aliquot of the supernatant was neutralized with solid $KHCO_3$ and fructose-6-P formed by the transketolase reaction was determined in the following mixture; 0.32 μmole of $NADP^+$, 0.5 unit of glucose-6-P dehydrogenase, 1.5 units of glucose-6-P isomerase, 30 μmoles of $MgCl_2$, and 150 μmoles of tris-HCl buffer of pH 7.6 in a total volume of 3.0 ml.

Glucose-6-P isomerase was assayed by a slight modification of the procedure of Slein (19). The reaction mixture consisted of 5.0 μmoles of fructose-6-P, 150 μmoles of tris-HCl buffer of pH 7.6, and the xylem enzyme in a total volume of 0.5 ml. Incubation, sampling, deproteinization, and removal of colored materials were carried out as described for the transketolase assay. A 0.2-ml aliquot of the decolorized solution was assayed for the glucose-6-P formed by the poplar enzyme in the following mixture; 0.32 μmole of $NADP^+$, 0.5 unit of glucose-6-P dehydrogenase, 30 μmoles of $MgCl_2$, and 150 μmoles of tris-HCl buffer of pH 7.6 in a total volume of 3.0 ml.

The procedure for the fructose-6-P kinase assay of the plant enzyme (1) was applied to poplar enzyme as in the following. The reaction mixture consisted of 0.21 μmole of glucose-6-P-U-^{14}C (1.38×10^6 cpm), 0.5 μmole of UTP, 1.2 μmoles of $MgCl_2$, 1 unit of glucose-6-P isomerase, 5 μmoles of tris-HCl buffer of pH 7.2, and the xylem enzyme in a total volume of 0.125 ml. After incubation for 90 min at 20 C, the test tubes were chilled on ice and the reaction was terminated by an addition of 0.4 ml of 0.1 N $HClO_4$. After centrifugation for 5 min at 3000 rpm, the supernatant was neutralized by solid $KHCO_3$ and the pH of the solution was then brought to 8 with 2 N NH_4OH. By the chromatography (3) on Dowex 1-X4(Cl^- type, 3 cm long $\times$ 0.31 cm^2), glucose-6-P-U-^{14}C and fructose-6-P-U-^{14}C were eluted with 40 ml of 0.01 N HCl and fructose-1,6-diP was then eluted with 50 ml of 0.02 N HCl containing 0.05 M NH_4Cl. The radioactivity in the eluate was counted and corrections were made for nonenzymatic incubation.

Incorporation of L-Lysine-U-^{14}C. A piece of twig sample of poplar, 1.0 g in weight, 0.5 to 0.6 cm in diameter and 5 to 6 cm in length, was excised fresh before use. Both the lower and upper cut ends of the excised sample were cut evenly with a sterilized blade. The sample was held vertically with the original lower cut end upward, and 15 μl of L-lysine-U-^{14}C (0.05 μmole, 3.6×10^5 cpm) was placed and transfused to the cut end. The transfused twig sample was covered with Parafilm and kept for 20 hr at 20 C. At the end of incubation, the samples were chilled on ice and separated into the bark and xylem fractions. This was followed by grinding with 1.0 g of sea sand in the presence of 5 ml of 50 mM tris-HCl buffer of pH 7.6. The slurry was transferred quantitatively to a test tube and centrifuged for 5 min at 2500 rpm. After repeated extraction of the protein with the buffer, further extractions were carried out twice with a 5-ml portion of 2% sodium carbonate, and these extracts were pooled. Aliquots of the pooled extract were treated with 5% trichloroacetic acid and the precipitated protein was collected on a disc of Whatman glass fiber paper. The disc was washed with 5% trichloroacetic acid, ethanol, ethanol-ether, and ether, and the radioactivity was measured. All samples were counted under comparable conditions and no corrections were made for self-absorption.

Preparation and Analysis of Sugar Phosphates. Twigs were sampled fresh before use and the xylem with the bark and pith removed was cut to pieces of about 1 to 2 mm in width with surgical scissors in a cold room. Five grams (wet weight) of the xylem pieces were automatically ground in a mortar in the presence of 5 g of sea sand and 20 ml of cold 5% trichloroacetic acid for 10 min. Sixty grams of the xylem were used for one experiment. The pooled slurry was centrifuged in the cold at 12,000*g* for 10 min and the precipitate was washed once with 40 ml of water. The pooled supernatants were then titrated to pH 6.5 with solid $Ba(OH)_2$ and the sugar phosphates were precipitated by adding 4 volumes of ethanol. The precipitate was allowed to flocculate at 0 C and was collected by centrifugation.

The sugar phosphates in the precipitated barium salt were washed once with ethanol and then extracted four times with a 25-ml portion of 2 N acetic acid. In each extraction, the slurry was triturated well and left standing for 30 min at room temperature. The acetic acid extract was treated twice with ethyl acetate and chloroform, respectively. The remaining chloroform in the water layer was removed by distillation *in vacuo* (water bath temperature was below 40 C) and the solution was treated with Darco G-60 to remove colored material. When the solvent treatment was omitted, it was difficult to remove the colored material from the solution, particularly that of bark material. After removal of charcoal by filtration, the pH of the solution was adjusted to about 6 with 4 N KOH and treated with 4 volumes of ethanol. The precipitate was collected by centrifugation and washed with ethanol. The yield of the crude barium salt, dried *in vacuo* over H_2SO_4, from the wintering and budding xylem was 0.21 and 0.17 g, respectively. For the sugar phosphate measurement, the barium salt, equivalent to 5 g of the dry weight sample was pulverized in a mortar and dissolved in a minimum amount of 2 N acetic acid. The suspension was left overnight at 4 C and centrifuged. The barium salt mixture of the supernatant was decomposed with a slight excess of 0.6 M K_2SO_4 and the barium sulfate was removed by centrifugation. The supernatant solution was brought to a volume of 5 ml with water and used for the sugar phosphate measurement. Recovery experiments with glucose-6-P-U-^{14}C added to the first homogenization step yielded 60% of the added radioactivity.

The solution containing sugar phosphate mixture was neutralized with solid $KHCO_3$ and measurements were carried out by conventional spectrophotometric methods with a Hitachi

UV-VIS spectrophotometer (type 139) or a Beckman DB-GT spectrophotometer. In each run, an appropriate amount (0.1 to 0.5 ml) of the sample was used. The conditions were given below. (*a*) Assay of glucose-6-P, fructose-6-P, and glucose-1-P (8, 11, 19): 0.26 μmole of $NADP^+$; 30 μmoles of $MgCl_2$; 150 μmoles of tris-HCl buffer of pH 7.6; total volume of 3.0 ml. Glucose-6-P dehydrogenase (1.4 units) was added first (glucose-6-P assay), followed by 2 units of glucose-6-P isomerase (fructose-6-P assay), and then 2 units of phosphoglucomutase (glucose-1-P assay). (*b*) Assay of 6-P-gluconate (8): 0.26 μmole of $NADP^+$, 30 μmoles of $MgCl_2$, 150 μmoles of tris-HCl buffer of pH 7.6; 0.24 unit of 6-P-gluconate dehydrogenase; total volume of 3.0 ml.

Preparation and Analysis of Soluble Amino Acids and Amides. The xylem and bark were cut to pieces immediately and ground at 0 C in 2 ml of 0.2 N $HClO_4$ in a mortar. The slurry was transferred to a test tube and centrifuged for 5 min at 2500 rpm. After a three-time extraction with a 4-ml portion of water, the pooled extract was neutralized to pH 6 with 1 N KOH and was dried *in vacuo* over H_2SO_4. The sample was dissolved in 1.0 ml of H_2O, centrifuged at 100,000*g* for 10 min, and an aliquot of the supernatant was analyzed with a Hitachi Amino Acid Analyzer.

Analysis of Respiratory CO_2. In order to avoid artifacts resulting from the difference in activities between the upper and lower parts of a twig, a piece of twig sample with 1.5 cm in diameter was carefully selected for uniformity and a set of a comparative xylem piece (0.2 g wet weight each) was excised from the same xylem sample. In this way errors due to positional differences were minimized. The specific radioactivity of the glucose used was 10 mc/mmole both for glucose-1-^{14}C and glucose-6-^{14}C. Three microliters containing 0.06 μmole of the glucose were transfused quantitatively to the xylem sample, followed by 3 μl of sterilized water as described above in the incorporation experiment of lysine-^{14}C. The xylem sample was then placed in a glass tube (7 cm long × 0.5 cm^2). CO_2-free air was passed through the tube at a flow rate of 12 ml/min at 20 C. Respiratory CO_2 was trapped in 1.0 ml of 1 N NaOH and the tube containing the alkali was changed every 30 min. After 1 hr, 60 mg of NH_4Cl were added to each of the NaOH samples and the CO_2 was precipitated from the solution by adding 1 ml of 1 M $BaCl_2$. An aliquot of the $BaCO_3$ suspension was collected on a disc of Whatman glass fiber paper and counted with a gas flow counter.

Protein determination was carried out by amino acid analysis (20) after hydrolysis with 6 N HCl for 22 hr and bovine serum albumin was used as the standard.

RESULTS AND DISCUSSION

The levels of glucose-6-P and 6-P-gluconate dehydrogenases were followed throughout the year and summarized in Figure 1. The xylem of wintering poplar contained high levels of the two dehydrogenase activities. Decrease of the two dehydrogenases began at the onset of budding and the activities fell steeply during the following 2 to 3 weeks. In summer, the xylem contained very low glucose-6-P dehydrogenase activity which was hard to measure accurately by spectrophotometric methods. The level of 6-P-gluconate dehydrogenase also became low. However, it could still be detected spectrophotometrically, and was several times higher than the former throughout the year. The low dehydrogenase activities lasted into late October and then, before shifting to a cold environment, appeared to resume their original activities. In contrast to bark extract prepared from the budding twig, which contained a strong inhibitory material for glucose-6-P dehydrogenase (15), xylem extracts contained very little inhibitory materials for the dehydrogenase and more than 90% of the commercial glucose-

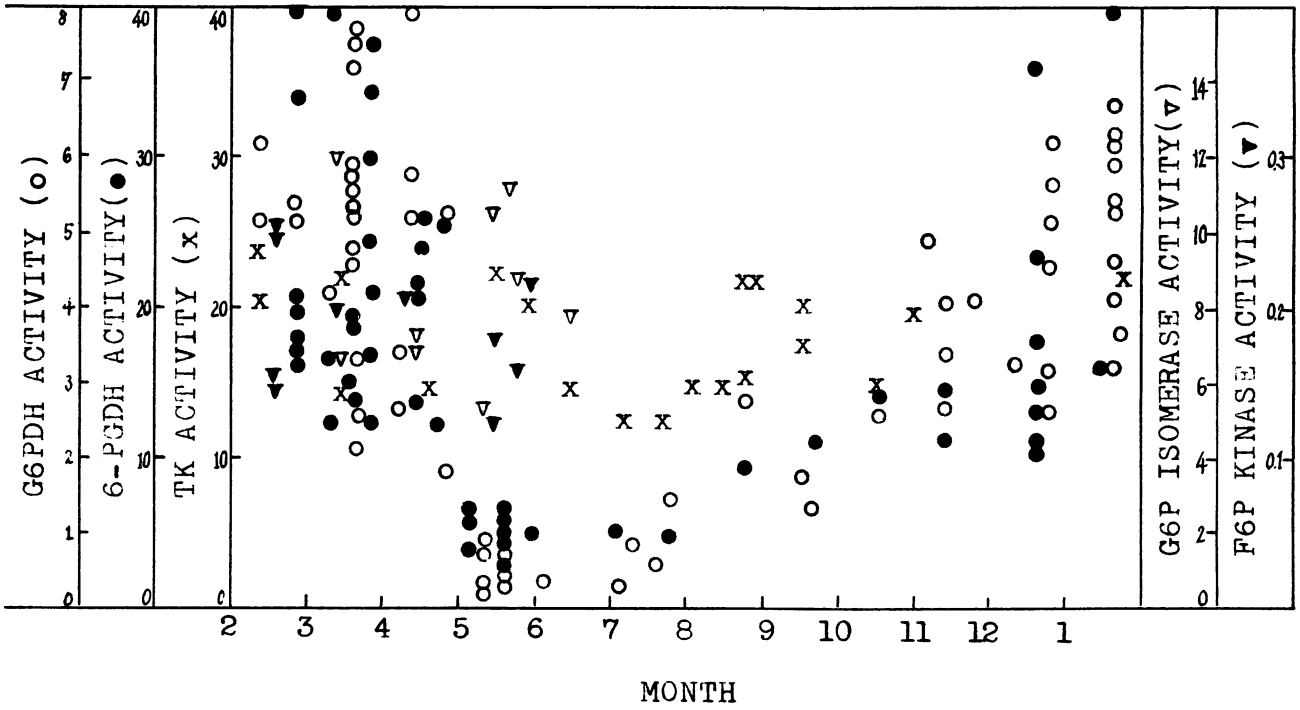

FIG. 1. Changes in the activities of glucose-6-P and 6-P-gluconate dehydrogenases in poplar xylem on budding. The levels of glucose-6-P dehydrogenase(G6PDH), 6-P-gluconate dehydrogenase(6PGDH), and transketolase(TK) are expressed as μmole NADPH formed/g dry wt xylem · hr. Glucose-6-P isomerase and fructose-6-P kinase are expressed as μmole glucose 6-P or fructose-1,6-diP formed/mg protein · hr. Some of the isomerase and kinase remained bound to the insoluble xylem debris, and it was therefore not possible to express as total enzyme activity in a unit weight of the xylem. The bud opening occurred in early May, and the leaves shed in late October.

6-P dehydrogenase activity added to the xylem extract could be recovered.

In order to determine whether the decrease in glucose-6-P dehydrogenase at the onset of budding was due to the appearance of an inhibitor, specific for the poplar enzyme, equal amounts of the two xylem extracts, *i.e.*, wintering and growing, were mixed and assayed. The activity of the mixture was found to be the sum of the separate activities. Furthermore, equal amounts of the scissored xylem sample of the two growth phases were mixed and homogenized as described under "Materials and Methods." The activity of the extract was exactly the expected value from separate analyses. A xylem extract prepared from budding poplar was heated for 10 min at 95 C. After cooling on ice and following centrifugation for 10 min at 11,000*g*, an aliquot of the supernatant, equivalent to 0.1 g wet xylem was mixed with 116 μg of a partially purified glucose-6-P dehydrogenase (activity; 0.043 μmole of NADPH per min·mg of protein) of the wintering xylem, equivalent in activity to 0.1 g wet weight, and assayed. No inhibition of glucose-6-P or of 6-P-gluconate dehydrogenase (activity; 0.145 μmole of NADPH per min·mg of protein) present in the enzyme preparation was observed. Thus, the occurrence of a specific inhibitor for xylem glucose-6-P dehydrogenase, must be excluded.

During dialysis or storage on ice, glucose-6-P dehydrogenase in crude extracts from the wintering xylem slowly decreased. Treatments of the extract with Dowex 1 (19) to remove phenolics, if any, did not stablize the enzyme. Addition of 0.15 mM glucose-6-P, a physiological concentration of poplar xylem (Table I), to the extract, prevented inactivation. However, homogenization of the growing xylem in the presence of the substrate at the same concentration had no effect on inactivation of the enzyme. The possibility of a differential inhibition or activation caused by small molecules, is therefore remote.

Concurrent with the decrease of the two dehydrogenase activities, a series of other events occurred (Tables II and III). The data in Table II show the change of water-soluble protein during the transition from late winter to early spring. A decrease of the soluble protein level was evident both in xylem and bark. This was also true for the insoluble protein fractions found in wintering bark. Analysis of free amino acids and amides in wintering xylem and bark (Table III) indicated that arginine is a major storage amino acid in poplar stem (in xylem 84% and in bark 48% of total). At the onset of budding, a striking change was observed in xylem arginine which decreased to a level of one-fifth of that of winter material. The decrease of arginine and lysine in the xylem and an increase of other amino acids took place during the period, and the glutamine fraction (mostly glutamine) increased to a level 25 times that of winter. In bark, the arginine level remained relatively constant, while the remaining amino acids increased several-fold. These results indicate that there is a substantial change in protein and amino acid at times before visible growth occurs and under these conditions, the poplar becomes more labile to cold temperature. Accumulation of arginine in apple tree was reported by Oland and Yemm (13).

To gain an understanding of the regulatory behavior occurring in the xylem, the levels of transketolase, glucose-6-P isomerase, and fructose-6-P kinase were studied. The levels of these enzymes (Fig. 1) did not seem to change significantly during the period, although a slight variation in the activities was found, but it was difficult to distinguish between the variation of the activities occurring at the beginning of budding and the individual differences. Glucose-6-P dehydrogenase of the wintering poplar bark was very low and difficult to measure quantitatively by the spectrophotometric method as in the case

Table I. *Sugar Phosphate Levels in Xylem of Wintering and Growing Poplar Twigs*

	Wintering Xylem	Growing Xylem[1]
	μmoles/10 g dry wt	
6-P-Gluconate	0.17	0
Glucose-6-P	1.50	1.16
Fructose-6-P	0.33	0.33
Glucose-1-P	0.17	0.17

[1] From twigs with about 7-cm active shoots.

Table II. *Decrease of Water-soluble Protein of Poplar Twigs during the Transition from Winter to Spring*

The xylem or bark (0.1 g wet wt) was ground in the cold in a mortar with 0.3 g of sea sand and then the water-soluble protein was extracted from the fine powder with 5 ml of H_2O.

Date	Xylem Protein		Bark Protein	
	Water-soluble[1]	Water-insoluble	Water-soluble[1]	Water-insoluble
	mg/g dry wt			
March 20	1.8	27.8	19.3	50.7
April 7	2.1	28.8	36.0	34.0
May 15	0.5	33.4	13.8	23.0
May 27	<0.1	33.4	<0.07	21.6

[1] Supernatant centrifuged at 700*g* for 5 min and contains particulate fractions.

Table III. *Changes in the Soluble Amino Acids and Amides in Xylem and Bark during Transition from Winter to Spring*

Poplar trees were grown in pots and were left in the field until mid January. The entire plants were stored at 4 C. These were then placed in a green house and allowed to proceed to budding. Budding began within 2 to 3 weeks after placing the trees in a green house.

Amino Acids and Amides	Xylem		Bark	
	Wintering	Growing[1]	Wintering	Growing[1]
	μmoles/g dry wt			
Lysine	0.15	0.09	0.08	0.29
Histidine	trace	trace	0.09	0.46
Arginine	8.52	1.58	2.54	3.00
Aspartic acid	0.37	0.24	0.52	0.22
Threonine and glutamine	0.25	6.34	0.44	2.87
Serine and asparagine	0.26	1.35	0.18	1.80
Glutamic acid	0.44	0.94	0.70	2.21
Proline	trace	0.32	trace	0.40
Glycine	trace	0.15	0.05	0.33
Alanine	0.19	0.37	0.19	0.72
Valine	trace	0.56	0.06	0.63
Methionine	trace	0.03	0.02	0.05
Isoleucine	trace	0.28	0.05	0.41
Leucine	trace	0.33	0.03	0.58
Tyrosine	trace	0.33	0.12	0.26
Phenylalanine	trace	0.32	0.25	0.53

[1] From twigs with about 7-cm active shoots.

Table IV. *Changes in Metabolism of Glucose-1-^{14}C and Glucose-6-^{14}C during Transition from Winter to Spring*

Xylem Sample	$^{14}CO_2$ Respired[1]		C_6/C_1 Ratio
	C_1	C_6	
	cpm		
Wintering xylem	3740	740	0.20
Growing xylem	2950	1900	0.64

[1] Mean of triplicate samples respired for 1 hr at 20 C.

Table V. *Incorporation of Lysine-U-^{14}C into Xylem and Bark Proteins of Young Poplar Stem at the Different Growth Phases*

L-Lysine-U-^{14}C was transfused to a piece of twig samples at the different growth phases. The transfused twig samples were placed for 20 hr at 20 C. The extracted protein, precipitated by 5% trichloroacetic acid and washed as described under "Materials and Methods," was counted with a gas flow counter. The results in Table V show the total radioactivity found in a 1.0-g wet weight sample. Details were described under "Materials and Methods."

Twig Sample	Radioactivity in		Radioactivity in Xylem
	Xylem protein	Bark protein	
	cpm		%
Before budding[1]	18,400	44,100	29.4
After budding	29,900	34,600	46.3
Growing in summer	40,400	22,900	63.8

[1] Poplar trees were grown in the pots and conditions of budding were as described in Table III.

of the growing poplar xylem, indicating that the wintering xylem contained higher levels of pentose-P cycle enzyme than the bark.

The potted poplars left in the field until mid January and stored at 4 C for 20 days, were placed in a green house and allowed to proceed to budding without any control in the photoperiod. Changes in the level of the two dehydrogenases of the xylem followed. Within 2 weeks after the beginning of budding, most of the dehydrogenase activities disappeared, regardless of the natural budding season. Thus, the beginning of the budding period of the entire poplar plants was accompanied by a rapid and significant decrease of the two enzymes.

The data in Figure 1 show that the glucose-6-P dehydrogenase activity gradually became more active toward late autumn, and as the temperature became lower and poplar trees shed their leaves, the activity appeared to resume its original activity. The increase in activity would then appear to be associated with the increased demand of NADPH for required reactions for wintering. When midwinter comes, the active synthetic reactions coupled with NADPH oxidation cease, resulting in a decreased rate of glucose-6-P oxidation. Analysis of 6-P-gluconate, glucose-6-P, glucose-1-P, and fructose-6-P in xylem was carried out (Table I) and corrections were made for recovery as determined by glucose-6-P-U-^{14}C. A difference was observed in the concentration of 6-P-gluconate, which was present in the wintering xylem at a concentration of approximately 1.7 μM and disappeared at the onset of growth. The physiological concentration of the glucose-1-P and fructose-6-P remained relatively constant before and after budding, while the glucose-6-P level decreased slightly during the period of budding. The disappearance of 6-P-gluconate in a budding xylem is consistent with the decreased level of glucose-6-P dehydrogenase on budding.

A comparison of glucose-1-^{14}C and glucose-6-^{14}C was carried out (Table IV) in order to measure the oxidative pentose-P cycle. There were considerable changes in the metabolism of glucose in the xylem before and after budding. The increase in C_6/C_1 ratio after budding resulted from increased $^{14}CO_2$ evolved from glucose-6-^{14}C. Inhibition of glucose-6-P isomerase by 6-P-gluconate was reported earlier by Parr (14) and Kohana *et al.* (7) and the Ki was reported to be 5 μM (7). It could account for the increased $^{14}CO_2$ from glucose-6-^{14}C. In the condition under which 6-P-gluconate is accumulating, the coenzyme remained mainly in a reduced form, resulting in a decreased rate of $^{14}CO_2$ production from glucose-1-^{14}C. Since only wintering xylem contained an appreciable amount of 6-P-gluconate (1.7 nmoles/0.1 g dry weight of xylem) and less than 2% of radioactivity was recovered from the ^{14}C-glucose (1.2 nmoles from given 60 nmoles) for 1 hr, the total amount of CO_2 produced from C-1 of glucose in the wintering xylem would be more than twice as much as that shown in Table IV. After budding, there were essentially no increase in $^{14}CO_2$ production from glucose-1-^{14}C.

Incorporation of L-lysine-U-^{14}C into stem protein was studied and the results are shown in Table V. The level of the lysine pool size in wintering and growing poplar was shown in Table III. In poplar twigs, the free lysine pool in the xylem declined on budding to a level of 64% of the winter, whereas in the budding bark lysine was present at 3.8 times of the concentration of winter. There was an inverse relationship in the level of the lysine pool, and the difference in the incorporation of labeled lysine between the xylem and the bark might be due to the different degrees of dilution (Table V). At the end of incubation, about 40 and 50% of the labeled lysine were present in the xylem and the bark, respectively. These results show that in contrast to the distinct decrease of the soluble protein and of the levels of the two dehydrogenases, incorporation of lysine-U-^{14}C into xylem protein appeared to remain relatively constant during the period. A selective breakdown and a synthesis of proteins appeared to be taking place at the same time during the period of budding of the entire poplar plants.

Thus, the distinct decrease and the increase of the two dehydrogenase activities appears to be a characteristic event, regulated by the over-all developmental process. The changes were completed in a 1-year cycle. The question as to how the two dehydrogenases function in potentiating the stem is obscure, but it is nevertheless clear from my results that the changes of the two dehydrogenases depend on the metabolic phase of the life process.

The findings described in this paper pose questions as to the biochemical functions of xylem in the life process of perennial plants as well as poplar, and seem to have bearing on the two aspects of stem metabolism. An obvious implication of these results is that at the onset of budding enzymatic activity of the first step of the oxidative pentose-P cycle in the xylem cells of poplar stem will be controlled to such an extent that it is permitted to proceed at a rate commensurate with the demands of the cell. Another aspect of the results might be related to the functional consequence of the dehydrogenases in stem cells to provide energy and materials for a fundamental demand, since in a 5-year-old poplar stem (10 cm in diameter), the annual ring of the 5th year still contained an appreciable amount of glucose-6-P dehydrogenase activity (2.6 μmoles of NADPH per g dry weight of xylem·hr), although a considerable decrease in its activity was present as compared with the newly synthesized xylem (6.7 μmoles of NADPH per g dry weight of xylem·hr).

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LITERATURE CITED

1. Axelrod, B., P. Saltman, R. S. Bandurski, and R. S. Baker. 1952. Phosphofructokinase in higher plants. J. Biol. Chem. 197: 89–96.
2. Ballou, H. O. 1963. Preparation and properties of D-erythrose 4-phosphate. *In:* S. P. Colowick and N. O. Kaplan, eds., Methods in Enzymology, Vol. VI. Academic Press, London. pp. 479–484.
3. Benson, A. A. 1957. Sugar phosphate, paper and column chromatography. *In:* S. P. Colowick and N. O. Kaplan, eds., Methods in Enzymology, Vol. III. Academic Press, New York. pp. 110–129.
4. De La Haba, G., I. G. Leder, and E. Racker. 1955. Crystalline transketolase form bakers' yeast: Isolation and properties. J. Biol. Chem. 214: 409–426.
5. Gibbs, M. 1959. Metabolism of carbon compounds. Annu. Rev. Plant Physiol. 10: 329–378.
6. Horecker, B. L. 1957. Preparation and analysis of ribulose 5-phosphate. *In:* S. P. Colowick and N. O. Kaplan, eds., Methods in Enzymology, Vol. III. Academic Press, New York. pp. 190–192.
7. Kahana, S. E., O. H. Lowry, D. W. Schulz, J. V. Passonneau, and E. J. Crawford. 1960. The kinetics of phosphoglucoisomerase. J. Biol. Chem. 235: 2178–2184.
8. Kornberg, A. and B. L. Horecker. 1955. Glucose-6-phosphate dehydrogenase; Horecker, B. L. and P. Z. Smyrniotis. 6-Phosphogluconic dehydrogenase. *In:* S. P. Colowick and N. O. Kaplan, eds., Methods in Enzymology, Vol. I. Academic Press, New York. pp. 323–327.
9. Lam, T. H. and M. Shaw. 1970. Removal of phenolics from plant extracts by grinding with anion exchange resin. Biochem. Biophys. Res. Commun. 39: 965–968.
10. Lowenstein, J. H. 1963. Preparation of tritium-labeled substances; preparation of glucose-6-phosphate-1-^{3}H. *In:* S. P. Colowick and N. O. Kaplan, eds., Methods in Enzymology, Vol. VI. Academic Press, London. pp. 871–881.
11. Najjar, V. A. 1955. Phosphoglucomutase from muscle. *In:* S. P. Colowick and N. O. Kaplan, eds., Methods in Enzymology, Vol. I. Academic Press, New York. pp. 294–299.
12. Nevins, D. J., P. D. English, and P. Albersheim. 1967. The specific nature of plant cell wall polysaccharides. Plant Physiol. 42: 900–906.
13. Oland, K. and E. Yemm. 1956. Nitrogenous reserves of apple trees. Nature. 178: 219.
14. Parr, C. W. 1956. Inhibition of phosphoglucose isomerase. Nature. 178: 1401.
15. Sagisaki, S. 1969. Regulatory steps of sugar phosphate flow in winter bark of *Populus gelrica*. Low Temp. Sci. Ser. B 27: 81–88.
16. Sagisaki, S. 1969. Methods for the quantitative determination of transketolase and transaldolase activities in plant extracts. Low Temp. Sci. Ser. B 27: 89–96.
17. Sakai, A. 1957. Relation of increase of carbohydrate and water soluble protein to frost-resistance in tree. Low Temp. Sci. Ser. B 15: 17–29.
18. Siminovitch, D., D. M. Wilson, and D. R. Briggs. 1953. Studies on the chemistry of the living bark of the black locust in relation to its frost hardiness. V. Seasonal transformations and variations in the carbohydrates; starch-sucrose interconversions. Plant. Physiol. 28: 383–400.
19. Slein, M. W. 1950. Phosphomannose isomerase. J. Biol. Chem. 186: 753–761.
20. Spies, J. R. 1957. Colorimetric procedures for amino acids. *In:* S. P. Colowick and N. O. Kaplan, eds., Methods in Enzymology, Vol. III. Academic Press, New York. pp. 467–477.
21. Tabachinick, M., P. A. Srere, J. Cooper, and E. Racker. 1958. The oxidative pentose phosphate cycle. III. The interconversion of ribose 5-phosphate, ribulose 5-phosphate and xylulose 5-phosphate. Arch. Biochem. Biophys. 74: 315–325.