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Transition of Metabolisms in Living Poplar Bark from Growing to Wintering Stages and Vice Versa

CHANGES IN GLUCOSE 6-PHOSPHATE AND 6-PHOSPHOGLUCONATE DEHYDROGENASE ACTIVITIES AND IN THE LEVELS OF SUGAR PHOSPHATES[1]

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ABSTRACT

Activities of glucose 6-phosphate, 6-phosphogluconate, and isocitrate dehydrogenases, together with intermediate levels of the glycolytic pathway and the pentose phosphate cycle, were measured throughout a year in the living bark of poplar (*Populus gelrica*). Shoots, immediately after budding (early May), contained very high levels of the three enzyme activities, which fell gradually by early or mid-July to a level, roughly equivalent to budding (May) or growing (July) 2-year-old twigs. In September, the former two dehydrogenase activities of the new shoots and 2-year-old twigs began to rise, while the latter activity started to decrease. The rise of the two dehydrogenase activities continued until late November (or early December). The high level of the two dehydrogenase activities lasted until early in April of the following year and then the decrease in the activities began prior to the onset of budding, reaching a low, basal level in early May. The profile of changes in the two dehydrogenase activities appeared to coincide with the increase and decrease of soluble proteins.

Normal concentrations of total hexose phosphates in the glycolytic pathway plus 6-phosphogluconate were found to be 288 to 895 μmoles/kilogram dry weight. During the metabolism transition (September and April), a transient and striking increase of 6-phosphogluconate was observed. In September, 6-phosphogluconate reached a level on the order of 10^{-4} M and was 4 times that of fructose 6-phosphate. The increase in 6-phosphogluconate coincided with the increase in the glucose 6-phosphate dehydrogenase activity. Coincidentally, with the change of 6-phosphogluconate level, a large deviation of the *in vivo* ratio of fructose 6-phosphate to glucose 6-phosphate from the known equilibrium constant was observed, showing the relation of pentose phosphate cycle enzyme activity to the control of glycolysis. The ratio of glucose 6-phosphate to glucose 1-phosphate deviated from that predicted. These ratios fluctuated throughout the year and were affected by the growth phases. The levels of pentose phosphate cycle metabolites, except for 6-phosphogluconate, in the bark were extremely low.

The level of inorganic phosphate in the living bark throughout the year was dependent upon the growth phases, suggesting the presence of a regulatory mechanism to maintain inorganic phosphate at a given level as the growth phase changed.

Sugar phosphate levels of popular twigs or potato tubers (*Solanum tuberosum*) remained constant after they were stored for 2 weeks at low temperatures, whereas in sweet potato roots (*Ipomoea batatas*), the level rose to about 9-fold of the control, indicating the presence of a strict regulatory system for the synthesis and catabolism of sugar phosphate in the former two.

Previous studies (28) have shown that glucose-6-P and 6-P-gluconate dehydrogenase activities were higher in the wintering poplar xylem than in the budding ones and that the changes in the activities occurred in fall and spring during the transition of the growth phase; *i.e.* from growing to wintering and vice versa. The rise and fall of the enzyme activities in the xylem were accompanied by the increase and decrease of soluble protein. During the phase transition, a number of enzymes appeared to be synthesized and degraded at the same time within a very restricted time over 1 year of cell cycle in the xylem (28).

In the living bark of perennials, the increase and decrease of nitrogenous materials in fall and spring were studied from 1927 through to 1933 by several workers (18) and later in 1949 the same was studied in some detail by Siminovitch and Briggs (30). The results of these experiments imply that metabolic changes were taking place also in the living bark and that the changes in the cell function occur during the period of the phase transition.

Since the changes in the enzyme activities and in the compositions of sugar phosphates and free amino acids were observed in the xylem as the phase transition proceeded (28, 29), the relations of the growth phase transition of poplar to activity changes of glucose-6-P, 6-P-gluconate dehydrogenases, and relative levels of sugar phosphate intermediates were examined in the living bark, and the results are presented in this paper.

MATERIALS AND METHODS

Chemicals and Enzymes. The following sugar phosphates, nucleotides, and enzymes were products of Boehringer Mannheim GmbH; glucose-6-P, fructose-6-P, glucose-1-P, fructose-1,6-diP, 6-P-gluconate, ribose-5-P, erythrose-4-P, glyceraldehyde-3-P, glycerate-3-P, $NADP^+$, NADPH, NAD^+, NADH, ATP, all dehydrogenases, triose-P and glucose-6-P isomerases, phosphoglucomutase, and aldolase. Fructose-6-P kinase was obtained from Sigma, isocitrate from Nutritional Biochemicals Corp., polyclar AT (insoluble polyvinylpyrrolidone) from General Aniline and Film Corp., glucose-6-^{14}C and -1-^{14}C from

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the Daiichi Chemical Co., sedoheptulose-7-P was prepared by the procedure of Wood and Poon (33). Transaldolase (31), transketolase (6), ribose-5-P isomerase (6), and ribulose-5-P epimerase were prepared from yeast (32).

Plant Material and Crude Enzyme. Poplar twigs (*Populus gelrica*) were sampled from trees growing in the field. Portions of the 1- or 2-year-old materials located at about the middle to two-thirds towards the apex were used on the same day. To obtain an active crude enzyme from poplar bark, it was found necessary to add polyclar AT (21) to the homogenization mixture. Very recently, this polymer has became available commercially in our country and difficulties in obtaining a reliable crude bark enzyme were overcome. Addition of Dowex 1 (19) to the mixture did not stabilize the enzyme activities. Homogenization mixtures contained 1.0 g of small excised pieces of bark, 0.5 g of polyclar AT, 1.0 g of sea sand, and 3.5 ml of tris-HCl, pH 7.6. Grinding of the mixture was carried out automatically in a cooled mortar for about 3 to 4 min. A turbid solution was obtained by pressing the slurry through gauze. This was followed promptly by centrifugation at 12,000*g* for 5 min. Measurements of the activities of glucose-6-P, 6-P-gluconate, and isocitrate dehydrogenases in the supernatant were accomplished within 30 min after the centrifugation.

Enzyme Activity Measurement and Analysis of Respiratory CO_2. Glucose-6-P and 6-P-gluconate dehydrogenase activities were measured as described previously (28). Isocitrate dehydrogenase activity was assayed by the procedure described by Kornberg (17) in the following mixture, which contained 0.26 μmole of $NADP^+$, 6 μmoles of $MgCl_2$, 2.5 μmoles of DL-isocitrate, 75 μmoles of tris-HCl buffer, pH 7.6, and 0.1 ml of the bark enzyme solution in a total volume of 1.5 ml. For the analysis of respiratory CO_2 of the bark, a twig sample, 5 to 6 cm in length and 1.5 to 2 mm in thickness, in the bark tissue was excised freshly before use. To the bark of one cut end, a notch was made with a sterilized blade and small pieces of the torn bark sample were prepared. The torn bark, freed from the xylem tissue, weighed 0.2 g wet weight, and was used for the following experiment. The specific radioactivity of the glucose used was 10 mCi/mmole both for glucose-1-^{14}C and glucose-6-^{14}C. Three μl containing 0.06 μmole of the glucose were smeared quantitatively onto the torn surface of the bark, followed by 5 to 10 μl of sterilized water. The bark sample was then placed in a glass tube as described before (28) and respiratory CO_2 was measured.

Protein determination was carried out by amino acid analysis after hydrolysis with 6 N HCl for 22 hr and BSA was used as the standard (28). The water content was measured after drying the materials for 2 days over H_2SO_4 in a vacuum.

Preparation and Analysis of Sugar Phosphates. Fresh twig samples from the same tree were selected, usually around the 3rd week of the month and between 10 AM and 1 PM. Other procedures for the preparation of partially purified sugar phosphate mixtures were the same as described previously (28). (*a*) Assay of glucose-6-P, fructose-6-P, glucose-1-P, glyceraldehyde-3-P (dihydroxyacetone-P), fructose-1,6-diP, and sedoheptulose-7-P. The assay mixture consisted of 75 μmoles of tris-HCl, 6 μmoles of $MgCl_2$, 0.26 μmole of $NADP^+$, and an appropriate amount (*e.g.* 0.15 ml) of the sample in a total volume of 1.5 ml. The analytical sequence (28) was initiated by adding 1.4 units of glucose-6-P dehydrogenase (glucose-6-P assay), followed by 7 units of glucose-6-P isomerase (fructose-6-P assay), and then 4 units of phosphoglucomutase (glucose-1-P assay). To the cuvette was then added 30 μl of 6 N HCl and the cuvette was left standing for 5 min at room temperature. After the decomposition of NADPH was completed, the pH of the solution was brought to about 7.4 by an addition of solid $KHCO_3$, and to this 4 μmoles of EDTA and 0.1 μmole of NADH were added. Glyceraldehyde-3-P (dihydroxyacetone-P) was then assayed by adding 20 μg of protein of a mixture of glycerol-3-P dehydrogenase and triose-P isomerase, by the procedure reported earlier (5). After the reaction was completed, fructose-1,6-diP was assayed (15) by an addition of 0.9 unit of aldolase. Finally, sedoheptulose-7-P was assayed by an addition of 5 units of fructose-6-P kinase and 0.5 μmole of ATP, according to the method of Wood and Poon (33); (*b*) 6-P-gluconate and 3-P-glycerate. The assay mixture was the same as (*a*) and 6-P-gluconate was assayed with an addition of 0.24 unit of 6-P-gluconate dehydrogenase (28). After the reaction was completed, 4 μmoles of EDTA, 2 μmoles of ATP, 0.1 μmole of NADH, and 4 units of glyceraldehyde-3-P dehydrogenase were added, and 3-P-glycerate was measured by the NADH decrease upon addition of 40 units of 3-P-glycerate kinase (15). (*c*) Erythrose-4-P and pentose-5-P. The assay mixture contained 150 μmoles of tris-HCl, 30 μmoles of $MgCl_2$, 0.2 μmole of NADH, 3.2 units of glycerol-3-P dehydrogenase, 4.8 units of triose-P isomerase, 4 μmoles of EDTA, 0.2 to 0.5 ml of test sample, and water in a total volume of 3.0 ml. After endogenous glyceraldehyde-3-P and dihydroxyacetone-P had reacted with NADH by the action of the added dehydrogenase and isomerase, erythrose-4-P was assayed by adding 2.5 μmoles of fructose-6-P and 0.05 unit of transaldolase, by the procedure of Cooper *et al.* (5). In this assay system, aldoses such as erythrose-4-P and glyceraldehyde-3-P were known to be the most efficient acceptors for the 3-C moiety, whereas ribose-5-P was utilized slowly (25). Furthermore, since the concentration of ribose-5-P in the sample was extremely low (Table I), a small amount of erythrose-4-P, as low as 0.01 μmole in the reaction mixture, could be detected by the procedure. After the erythrose-4-P assay was completed, then 0.1 unit of ribose-5-P isomerase and 0.01 unit of ribulose-5-P epimerase were added and the total pentose-P was assayed (14) by the NADH decrease as a result of the glyceraldehyde-3-P formation upon addition of 0.05 unit of transketolase. Results were calculated from the standard curves prepared from authentic preparations of erythrose-4-P and ribose-5-P.

Table I. *Sugar Phosphate Levels in the Living Bark of Poplar Twigs throughout the year*

The materials used were 2-year-old (from January to August) and both 1- and 2-year-old (after August) twigs.

Month	G1P[1]	G6P	F6P	FDP	6-PG	R5P	S7P	E4P	GA3P	3-PG
	μmoles/kg dry wt									
Jan	12	259	70	12	14	8	8	5	10	0
Feb	20	380	100	16	20	14			9	0
Mar	40	605	137	8	58	11	13	8	20	13
Apr	21	371	80	8	72	6	0	17	8	0
May 1	35	467	111	21	40	8	19	0	21	8
May 17	47	645	150	21	32	0	12	0	13	0
June	48	283	81	21	6	0	12	0	3	17
July	27	221	73	10	16		11		3	10
Aug	21	174	73	7	13		8		0	22
Sept	18	392	48	0	209		0		6	0
Oct	19	266	73	4	26	0		3	4	0
Nov	25	383	106	5	32	0	14	10	34	21
Dec	14	300	90	10	14	5	21	8	22	10

[1] G1P: glucose-1-P; G6P: glucose-6-P; F6P: fructose-6-P; FDP: fructose-1,6-diP; 6-PG: 6-P-gluconate; R5P: ribose-5-P; S7P: sedoheptulose-7-P; E4P: erythrose-4-P; GA3P: glyceraldehyde-3-P; 3-PG: 3-P-glycerate.

Measurements were carried out with a Beckman DB-GT spectrophotometer connected to a recorder.

Phosphoric Acid Assay. For the assay of P_i, the homogenization and deproteinization of the sample were carried out in a mixture that consisted of 5 g of bark cut with scissors, 5 g of sea sand, and 20 ml of 0.1 N H_2SO_4 for 10 min in a mortar by adaptation of the method described by Korkes (16). The slurry was centrifuged in the cold at 12,000*g* for 10 min and P_i assay in the supernatant, after treatment with Darco G-60 (50 mg/0.3 g of wet weight sample), was carried out by the method of Allen (1).

RESULTS AND DISCUSSION

Measurements of the activities of glucose-6-P, 6-P-gluconate, and isocitrate dehydrogenases were carried out throughout the year and the results are summarized in Figure 1. A comparison of the wintering and growing phase indicated that the living bark of wintering poplar contained high levels of the former two dehydrogenases.

In early May, new shoots contained very high levels of the three dehydrogenase activities. As the growth proceeded, the enzyme activities in the new shoots (1-year-old twigs) decreased gradually until early July to a level similar to those of growing 2-year-old twigs. The lowered activities of glucose-6-P and 6-P-gluconate dehydrogenases lasted until mid-September and then the increase in the activities from the low level of the growing phase to the high level of wintering phase commenced both in the new shoots and 2-year-old twigs. The increase of the two dehydrogenase activities continued until late November or early December and the high level of the activities remained relatively constant throughout the winter. In contrast to the former two dehydrogenase activities, the latter activity started to decrease.

In the following April, the decrease in the glucose-6-P and 6-P-gluconate dehydrogenase activities began prior to the onset of budding and in May, the activities of the two dehydrogennases in the bark both in the 2- (new shoots in the preceding year) and 3-year-old twigs (2-year-old twigs in the preceding year) again reached the basal low levels.

The change in soluble protein concentrations throughout the year is shown in Figure 2. Soon after budding in early May, the new shoots contained a relatively high level of soluble proteins and there was a slow decrease in the amount during the following 2 months, reaching the basal, low level of growing 2-year-old twigs. The low level lasted until September and the soluble protein level started to rise markedly from the end of September both in the new shoots and 2-year-old twigs. The second rise in protein concentration in the new shoot reached a maximum in late November or early December. The high level of soluble protein lasted until early April of the next year and the decrease in the level began prior to the onset of budding, reaching finally the basal low level in May.

The profile of the decrease and increase in the soluble protein level in the new shoots appeared to coincide with the change in the glucose-6-P and 6-P-gluconate dehydrogenase activities, while in September at the time of second rise of the protein concentration, only the activity of isocitrate dehydrogenase began to decrease, as seen in Figures 1 and 2. These results suggested that the metabolism of wintering poplar bark (and perhaps organellar structures, too) is different, at least in part, from that of growing ones.

The levels of glucose-6-P, fructose-6-P, and 6-P-gluconate in the bark of poplar twigs were measured at monthly intervals throughout the year (Table I). The bark contained an appreciable amount of hexose-P throughout the year and from February to May the levels of total hexose-P existing in the bark were higher than those of the growing period (from June to August). The normal level of the sum of hexose-P (including 6-P-gluconate) seemed to be between 288 (August) and 895 μmoles (May)/kg dry weight and no large changes seemed to appear in the levels of total hexose-P throughout the year, suggesting that a regulatory mechanism in the synthesis of hexose-P may be involved in the perennials. To investigate the regulatory situations, a piece of poplar twig (sampled after budding), potato tubers, and sweet potato roots (both obtained from a local market in February) were kept at 0 or 4 C in a polyethylene bag for 2 weeks and analyses were carried out for hexose-P (Table II). A striking change of the hexose-P

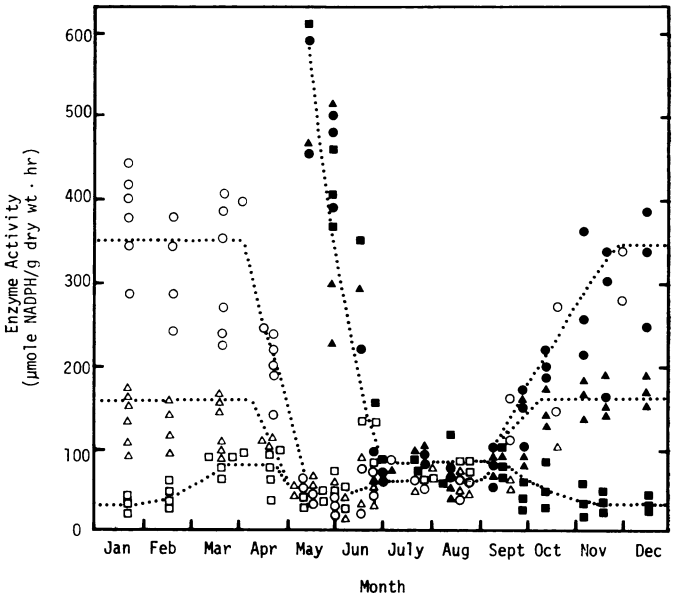

FIG. 1. Changes in the activities of glucose-6-P (●, ○), 6-P-gluconate (▲, △) and isocitrate (■, □) dehydrogenase activities in poplar bark. Enzyme activities in new shoots (closed symbols) and 2-year-old twigs (open symbols).

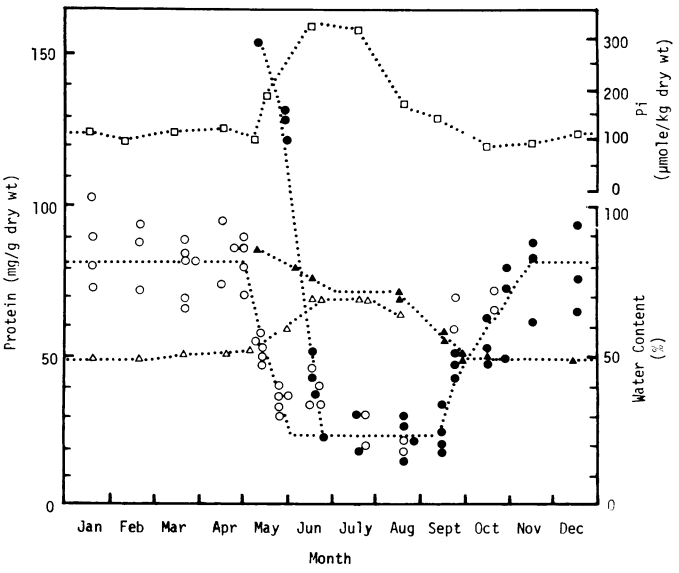

FIG. 2. Changes in the protein (●, ○), inorganic phosphate (□) and water (▲, △) content in poplar bark. Values of new shoots (closed symbols) and 2-year-old twigs (open symbols).

(about 9-fold) was observed in sweet potato roots, a cold sensitive plant, whereas the hexose-P in the potato tubers and the poplar twigs remained near control levels. These results indicate that low temperatures close to 0 C are detrimental to the organizational system of sugar-P or high energy synthesis in sweet potato roots and that cold-resistant plants possess a strict regulatory system in the metabolism of sugar-P under low temperatures.

In September, there was a striking and transient rise in the level of 6-P-gluconate. The concentration reached about 0.2 mM and after September the low level of the ester did not change significantly until February (Table I). In May and April, prior to the onset of budding, there was again a rise in the level of the ester. Apparently, a transient change in the level occurred during or prior to the transition of a growth phase: *i.e.* from growing to wintering phase (September) and vice versa (April and May). Measurements of the levels of fructose-1,6-diP, glyceraldehyde-3-P (dihydroxyacetone-P), erythrose-4-P, pentose-5-P, sedoheptulose-7-P, and 3-P-glycerate were also carried out throughout the year (Table I). In general, the amounts of these phosphates in the bark were very low (the order was about 20 μM or less) and close to the limit of detection, particularly, those of pentose-5-P and erythrose-4-P. The very low level of pentose-5-P and sedoheptulose-7-P in the bark, regardless of the growth phase, indicates that the intermediate phosphates after the 6-P-gluconate step were drained into hexose-P pool, as shown earlier by Dische and Igals (7), Axelrod *et al.* (2), Horecker *et al.* (11), and Gibbs and Horecker (9), and that the reactions after ribulose-5-P proceed at rates faster than the rate at which ribulose-5-P is produced. When ribose-5-P was incubated with a cell free extract of wintering poplar bark at 0 or 12 C and the mixture was chromatographed on a Dowex 1 column, a formation of a rapid and significant amount of xylulose-5-P and sedoheptulose-7-P was observed, followed by fructose-1,6-diP and fructose-6-P (27), consistent with the result of Gibbs and Horecker (9) obtained from pea leaf and root preparations. The very low level of erythrose-4-P in the bark also suggests the formation of fructose-6-P from xylulose-5-P and erythrose-4-P by transketolase reaction. Concentrations of fructose-1,6-diP shown in Table I were the sum of the fructose-1,6-diP and sedoheptulose-1,7-diP, if any, since the analytical method could not distinguish between the two compounds. From May to August, the level of fructose-1,6-diP was about one-fourth to one-tenth of that of fructose-6-P, in other words, the ratio of fructose-6-P to fructose-1,6-diP remained fairly constant during the particular period. After the shoot growth ceased, the level of the fructose-1,6-diP dropped below the limit of detection (from August to November). This indicates that the level of fructose-1,6-diP was dependent upon the growth phase

Table II. *Sugar Phosphate Levels in Potato Tuber, Sweet Potato Roots, and Poplar Twigs after Storage at Low Temperature for 2 Weeks*

	Potato Tuber		Sweet Potato Root		Poplar Twig[1]	
	0 C	20 C	0 C	20 C	4 C	20 C
	μmole/kg wet wt				*μmole/kg dry wt bark*	
Glucose-6-P	108	108	857	95	330	220
Fructose-6-P	23	25	136	19	50	50
Glucose-1-P	5	3	44	4	30	40
6-P-Gluconate	<0.8	<0.8	<0.8	<0.8		

[1] After budding (May).

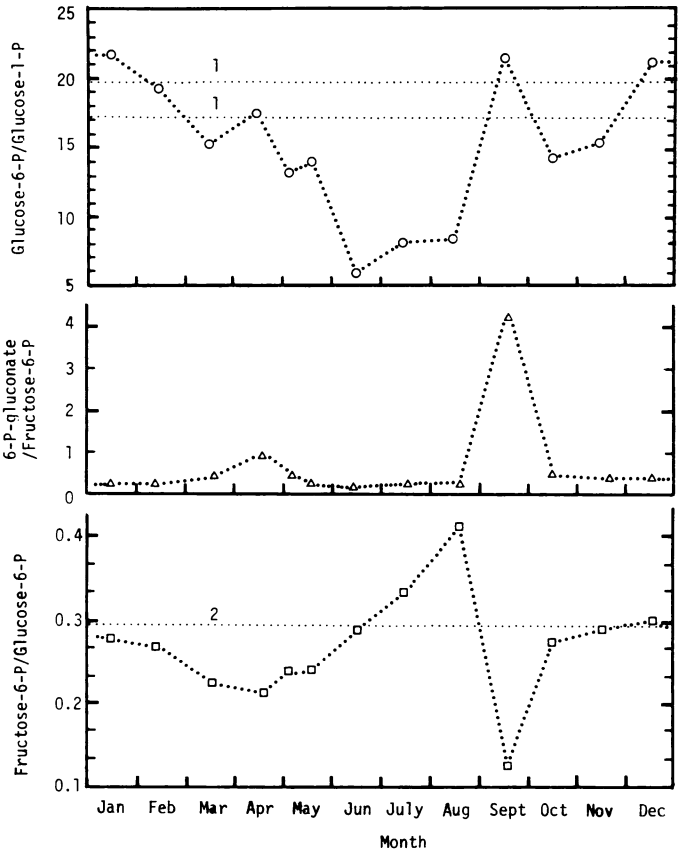

FIG. 3. Changes in the ratios of fructose-6-P to glucose-6-P, 6-P-gluconate to fructose-6-P and glucose-6-P to glucose-1-P in poplar bark throughout the year. 1: The *in vitro* thermodynamic equilibrium at 20 and 30 C was reported to be 19.8 and 17.2, respectively (23). 2: The *in vitro* thermodynamic equilibrium was reported to be 0.298 (13).

and that there was a restriction in the cellular intermediate level in a sequence of glycolytic reactions by which a preferable level in the bark was maintained.

The ratios of fructose-6-P to glucose-6-P, glucose-6-P to glucose-1-P, and 6-P-gluconate to fructose-6-P, calculated from Table I, are shown in Figure 3. The fructose-6-P-glucose-6-P minima, which were concurrent with 6-P-gluconate-fructose-6-P maxima, occurred twice in April and September. From Table I and Figure 3, it seems likely that 6-P-gluconate-fructose-6-P maxima were accompanied by a very low level of fructose-1,6-diP. Under these conditions, glyceraldehyde-3-P was found to exist at a concentration of 20 μmoles/kg of materials in March, and 6 μmoles in September, suggesting that the triose-P, produced from the pentose-P cycle at that particular time, was not readily equilibrated with the fructose-1,6-diP pool of the glycolytic system. Accumulation of the triose-P in November and December indicated that the pentose-P cycle is operating in midwinter.

Relative concentrations of the hexose-P were different from those predicted. Apparently, the ratios were affected by the growth phase of the poplar tree. The ratio of fructose-6-P to glucose-6-P, for example, reached only in June and December (Fig. 3) to a value of about 0.3, which is close to that predicted from the equilibrium constant of glucose-6-P isomerase (13). Coincidentally with the marked decrease of 6-P-gluconate, fructose-, and glucose-6-phosphate resumed their predicted levels. In July and August, the ratios, 0.33 and 0.42, of fructose-6-P-glucose-6-P were obtained and the results indicated a

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

	$^{14}CO_2$ Respired[1]		C_6/C_1
	C-1	C-6	
	cpm		
Wintering bark	34,040	15,940	0.47
Growing bark[2]	25,180	16,420	0.65

[1] Mean of duplicate samples (0.2 g wet wt) respired for 1 hr at 20 C.

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

marked decrease in glucose-6-P level as compared with that predicted from the equilibrium constant.

The departure of the ratio of fructose-6-P to glucose-6-P from the equilibrium of the reaction could be explained partly by the inhibition of glucose-6-P isomerase by 6-P-gluconate (24) and erythrose-4-P (10), because moderate concentrations of the two esters that partially inhibited the isomerase were found to occur *in vivo* in September and April in the bark as shown in Table I. The K_i values for 6-P-gluconate, and erythrose-4-P of the poplar enzyme were found to be 0.19 mM, and 8 μM (unpublished data), respectively. (Significant amounts of sedoheptulose-7-P did not accumulate in the bark.) However, the large departure of the ratio (*e.g.* 0.42 in August, 0.12 in September) from the equilibrium of the reaction could be the result of the inhibitory effect of 6-P-gluconate and erythrose-4-P, because the 6-P-gluconate level found in August was very low (the order of 10 μM) and the departure observed in September was higher than the value predicted. Therefore, it appeared pertinent to assume that another mechanism is involved in the control of hexose-P level in the bark.

Figure 3 shows that the ratio of glucose-6-P to glucose-1-P was reduced significantly in association with the onset of the budding and growing until August. This phenomenon may partly be interpretable by the temperature effect on the equilibrium of the reaction (23), but, at present, no explanations can be found for the large departure from the equilibrium of the reaction, as seen in Fig. 3. The rise in the ratio of 6-P-gluconate to fructose-6-P (about 1 in April and 4 in September) appeared to occur at times when the transition of the metabolic organization is taking place, emphasizing the relative level of glucose-6-P dehydrogenase activity on the control of glucose metabolism in perennials.

The fructose-1,6-diP level in the bark was found to be below 20 μM. Triose-P also existed at concentrations of about 20 μM or less and did not accumulate as much as would be expected if P_i were, at any time, the limiting factor (12, 22, 26). The level of P_i found in the bark was low (the order was 10^{-5}–10^{-4} M) (Fig. 2) and was less than that of glucose-6-P, except for June and July. However, from the very low level of triose-P found in the bark, the P_i level did not seem to exert a controlling influence on the metabolism. Since the P_i level in the wintering bark was lower than in the growing ones and when the tree became more active in growth, the P_i level rose abruptly several-fold, the P_i level itself appeared to be controlled preferentially to a given level dependent upon the growth phases.

An increase in the activity of glucose-6-P dehydrogenase in the bark was accompanied by an increased rate of CO_2 production from C-1 of glucose as measured by glucose-1- and -6-^{14}C (Table III), indicating that the lowering of the fructose-6-P level apparently reduced the rate of carbon flow through the glycolytic system.

Thus, based on the enzyme activity changes and the substrate level analysis, the rate of glucose metabolism in poplar would be controlled at the four steps; *i.e.* (*a*) glucose-1-P to glucose-6-P and vice versa, (*b*) glucose-6-P to 6-P-gluconate (and further to ribulose-5-P), (*c*) glucose-6-P to fructose-6-P and vice versa, and (*d*) fructose-6-P to fructose-1,6-diP and vice versa, preventing an excessive metabolism of hexose and an excessive accumulation of the metabolites. The oxidation steps of glucose-6-P and 6-P-gluconate were regulated by the change of the respective enzyme activity and also by $NADP^+$ levels. The two enzyme activities were geared virtually to the recycling of the oxidation and reduction mediated by $NADP^+$. From midNovember to the end of April the flow of hexose through the pentose-P cycle increased when the two dehydrogenase activities increased. Also, an abrupt increase for the demand of NADPH appeared to occur in midNovember and April, as visualized by the accumulation of the large amount of 6-P-gluconate. However, from May to August (and also in October), the hexose flow through the glycolytic pathway increased with the increase of the ratio of fructose-6-P to glucose-6-P, concurrent with the lowered glucose-6-P dehydrogenase activity.

There are a number of reports dealing with a simultaneous analysis of the sugar phosphate intermediates and coenzymes in plants under particular conditions (3, 4, 8, 15, 20). A special feature of this paper was a strict temporal control imposed on the synthesis and degradation of the glucose-6-P and 6-P-gluconate dehydrogenases during the 1-year-cell cycle of living poplar bark. Another feature was a characteristic appearance of 6-P-gluconate as a result of enhanced glucose-6-P dehydrogenase activity at times when the metabolism transition is taking place in a natural environment. Furthermore, measurements of the concentration of the glycolytic intermediates in different states of growth showed that a large deviation of the ratio of fructose-6-P to glucose-6-P from the equilibrium of glucose-6-P isomerase reaction occurred at a particular time in the cell cycle. These results demonstrated that one of the strong regulatory steps of glycolysis exists at the step of glucose-6-P isomerase. Also, it seems clear that a control of pentose-P cycle activity is geared to a regulatory process operative in the glycolytic pathway.

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