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Sucrose Metabolism in Lima Bean Seeds[1]

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ABSTRACT

Developing and germinating lima bean (*Phaseolus lunatus* var Cangreen) seeds were used for testing the sucrose synthase pathway, to examine the competition for uridine diphosphate (UDP) and pyrophosphate (PPi), and to identify adaptive and maintenance-type enzymes in glycolysis and gluconeogenesis. In developing seeds, sucrose breakdown was dominated by the sucrose synthase pathway; but in the seedling embryos, both the sucrose synthase pathway and acid invertase were active. UDPase activity was low and seemingly insufficient to compete for UDP during sucrose metabolism in seed development or germination. In contrast, both an acid and alkaline pyrophosphatase were active in seed development and germination. The set of adaptive enzymes identified in developing seeds were sucrose synthase, PPi-dependent phosphofructokinase, plus acid and alkaline pyrophosphatase; and, the adaptive enzymes identified in germinating seeds included the same set of enzymes plus acid invertase. The set of maintenance enzymes identified during development, in the dry seed, and during germination were UDP-glucopyrophosphorylase, neutral invertase, ATP and UTP-dependent fructokinase, glucokinase, phosphoglucomutase, ATP and UTP-dependent phosphofructokinase and sucrose-P synthase.

Sucrose is a primary nutrient for essentially all higher plant cells. Recently, a new pathway of sucrose breakdown was proposed which is dependent upon UDP and PPi and that involves a cyclic series of reactions to produce the required UDP and PPi (4, 13, 23, 31). When sucrose is broken down by this pathway, which is called the sucrose synthase pathway, both hexoses from sucrose can feed into glycolysis. In addition, we have proposed that in the plant cell cytoplasm, alternative enzymes are present at various steps in glycolysis and gluconeogenesis for the interconversion of sucrose and pyruvate such that they form a network of reactions rather than the classical textbook-type pathways (3, 19, 24). In view of these and other recent discoveries such as a substrate level pool of PPi and fructose 2,6-P_2 regulation (3, 4, 10, 21, 30), we have reassessed all of plant glycolysis and gluconeogenesis, beginning with sucrose (24).

Because the glycolytic and the gluconeogenic metabolism of sucrose are at a central junction in plant metabolism (7, 12, 28), we have worked with various types of plants and tissues to characterize this fundamental portion of cellular carbon nutrition. Work focused on these aspects of sucrose metabolism is reported here with both the developing and the germinating lima bean seed. Conceptually, the developing lima bean seed is a sucrose sink on the intact plant that, during germination, becomes a source plus a sink as the cotyledons produce sucrose and the young embryonic plant consumes sucrose. The enzyme activity work presented here pivots around understanding plant sucrose metabolism. To approach this goal the work partially tests the sucrose synthase pathway *versus* the invertase pathways (13, 19, 23, 24), examines competing processes such as the metabolism of UDP and PPi, and addresses the proposal (4, 19, 24) of adaptive *versus* maintenance enzymes in plant glycolysis and gluconeogenesis.

MATERIALS AND METHODS

All studies were conducted with lima beans, *Phaseolus lunatus* var Cangreen, purchased locally and grown with good cultural practices either in field plots or in the greenhouse. The studies were conducted over the last 3 years with both field and greenhouse grown plants. Each study was repeated in at least three similar tests and the data presented are illustrative of developmental patterns, recognizing that variations occurred, *e.g.* seedling emergence in a given study might occur on d 5 or 6, etc. Lima bean has an indeterminate flower production; hence, seed development work based on freshly harvested weights was highly facilitated. And seedling development work also was facilitated because the lima bean cotyledon is senescent. Dried commercial seeds purchased fresh annually were used in the germination tests.

To prepare enzyme extracts all plant tissues were freshly harvested, powdered, and homogenized in liquid N_2 with a mortar and pestle. The extraction solution contained 200 mM Hepes/NaOH (pH 7.5), 3 mM Mg acetate, 5 mM DDT, 2% (v/v) glycerol, and 1% (w/v) insoluble PVP. The ratio of tissue fresh weight (g) to extraction solution (mL) was either 1:5 or 1:3 in various tests. The homogenate was passed through one layer of Miracloth and centrifuged at 34,000*g* for 20 min at 4°C. This initial extraction procedure was devised and then used routinely after a number of preliminary tests for maximum enzyme activity recoveries. The supernatant then was fractioned with 30 to 70% $(NH_4)_2SO_4$. The 70% $(NH_4)_2SO_4$ pellet was resuspended in a solution of: 10 mM Hepes/NaOH (pH 7.5), 2 mM DTT, and 2 mM Mg acetate. A Sephadex G-25 column was used for desalting all samples. This $(NH_4)_2SO_4$ fraction procedure was carefully checked for recovery of all enzymes we assayed and between 85 and 100% of all activities were recovered compared to the crude extracts.

The assay for sucrose synthase which initiates the sucrose synthase pathway was previously described in detail (31) even

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with lima bean which we then called by another of its common names, butter bean (23). Two endogenous plant enzymes were involved in this multienzyme assay, *i.e.* sucrose synthase and UDP-glucopyrophosphorylase. The 1 mL reaction mixture contained 100 mM Hepes/NaOH (pH 7.5), 3 mM Mg acetate, 5 mM DTT, 0.02 mM glucose-1,6-P_2, and 0.5 mM NAD. Sucrose (50 mM), UDP (1 mM), and PPi (1 mM) were added to start the reactions. The glucose-1-P produced was coupled via phosphoglucomutase (2 units) and *Leuconostoc* glucose-6-P dehydrogenase (2 units) to form 6-phosphogluconate and NADH. NADH production was monitored spectrophotometrically at 340 nm with a Beckman DU-7. Since UDP-glucopyrophosphorylase activities usually were over 1 unit/mg protein in plant extracts, basically the limiting activity measured was sucrose synthase. If UDP-glucophyrophosphorylase was less than 1 unit in an extract, we routinely added 1 unit of the commercial enzyme.

Invertases were assayed at pH 5.0 and 7.0 for acid and neutral invertase, respectively. Sucrose, 50 mM, was added to the incubation buffer (70 mM K_2HPO_4/40 mM citrate for acid invertase and 160 mM K_2HPO_4/20 mM citrate for neutral invertase) containing plant extracts. The standard incubation time was 15 min at 25°C and reactions were stopped by boiling. Aliquots of incubation mixture were added to a hexose assay mixture consisting of: 100 mM Hepes/NaOH (pH 7.5), 3 mM Mg acetate, 5 mM DTT, 0.02 mM glucose-1,6-P_2, 0.5 mM NAD, with hexokinase (2 units) and *Leuconostoc* glucose-6-P dehydrogenase (2 units) as coupling enzymes. ATP, 1 mM, was added to start the reaction and the reaction was completed when there was no more absorbance increase at 340 nm. All other enzyme assays have been described in more detail earlier (4, 6, 14, 19, 20, 22, 29, 31). Pyrophosphatases were assayed at pH 5.0 (100 mM Mes) and 7.8 (100 mM Tris/acetate) for acid and alkaline pyrophosphatase, respectively (11, 23). UDPase was assayed as alkaline pyrophosphatase with 1 mM UDP as the substrate; phosphate production was measured according to Taussky and Shorr (25). NTP-PFK[2] was assayed with two nucleotides, *e.g.* with UTP or with ATP at 1 mM (19). Fructokinase also was assayed with either UTP or ATP (27). With each enzyme, care was taken to ensure proper substrate levels, protein amounts, and other conditions necessary to measure maximum activities. Extract protein contents were determined using the Bradford procedure with BSA as the standard protein.

RESULTS

Enzyme Specific Activities in the Developing Lima Bean Seed as a Sucrose Sink

When the initial enzymes of sucrose cleavage were assayed for maximum activity in the developing lima bean seed, sucrose synthase was the dominant activity (Fig. 1). Both the acid and neutral invertases were present but at low activities which exhibited little change *versus* seed growth. The decreasing trend in the specific activity of sucrose synthase in Figure 1 will be addressed later.

The enzymes which form hexose-P show three activity patterns during seed development. UDP-glucopyrophosphorylase activity is very high and consistently shows some increase early in seed development and then decreases slightly with seed weight. Phosphoglucomutase shows only a gradual decrease with seed growth (Fig. 2A). These two enzymes are much more active than any others in all of this work. Both fructokinase and glucokinase have much lower specific activity and show about twofold activity changes with seed growth (Fig. 2B). UTP is equally efficient as ATP for the phosphorylation of fructose by fructokinase.

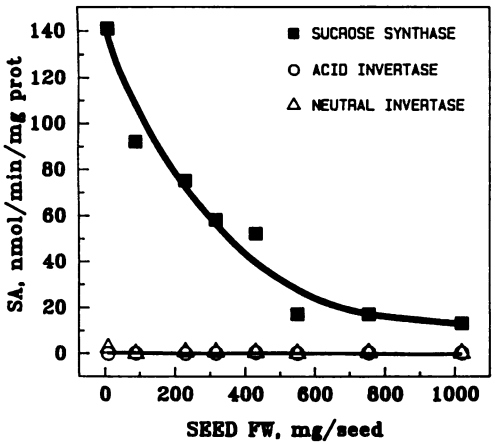

Figure 1. Changes in the specific activities of sucrose cleavage enzymes during lima bean seed development.

When the glycolytic enzymes for converting fructose 6-P to fructose 1,6-P_2 were tested, we found the PPi-PFK to be the major activity (Fig. 3) and, like sucrose synthase, it exhibited a definite pattern of decreasing specific activity as seeds grow. The NTP-dependent PFK showed a similar pattern of activity with both ATP and UTP. There was a tendency for NTP-PFK to rise about twofold early and to decline slowly with seed growth as illustrated in Figure 3.

When enzymes that might compete for the UDP and PPi needed in the sucrose synthase pathway (4, 13, 23) were tested, the UDPase was present at a low but generally constant activity (Fig. 4). However, both the acid and alkaline pyrophosphatase activities were high, rose initially, and then declined with seed growth (Fig. 4). The ability to synthesize sucrose also was tested by assaying sucrose-P synthase and its specific activity varied between 20 and 40 nmol/min/mg protein but showed no discernible pattern with seed weight (data not shown).

From these results on maximum enzyme specific activities *versus* increasing seed weight, we also can observe general patterns for what we have termed adaptive and maintenance enzymes (4, 19, 24). First, the strongly adaptive enzymes seem to be sucrose synthase (Fig. 1), and both pyrophosphatases (Fig. 4). The PPi-PFK also was an adaptive enzyme (Fig. 3). Then we observed maintenance-type enzyme activities which may show changes of several-fold as a tissue grows but with little pattern and/or low specific activities (4, 19, 24). Maintenance-type enzymes in these tests were both invertases (Fig. 1), UDP-glucopyrophosphorylase (Fig. 2), phosphoglucomutase (Fig. 2), both fructokinase activities (Fig. 2), glucokinase

[2] Abbreviation: PFK, phosphofructokinase.

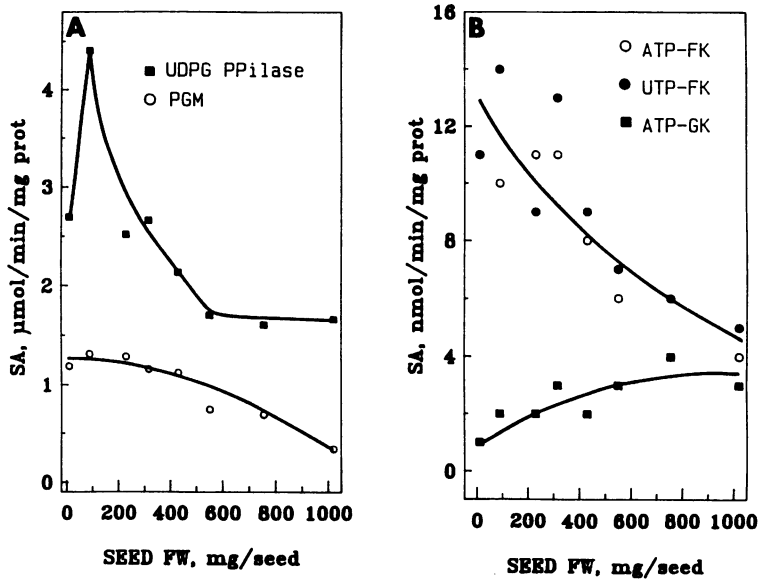

Figure 2. Changes in the specific activities of hexose phosphate forming enzymes during lima bean seed development.

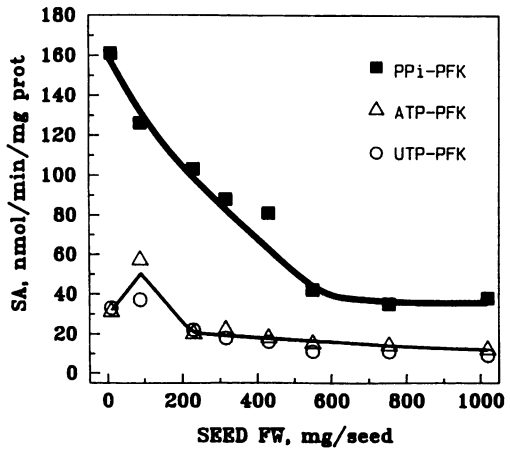

Figure 3. Changes in the specific activities of enzymes converting fructose 6-P to fructose 1,6-P_2 during lima bean seed development.

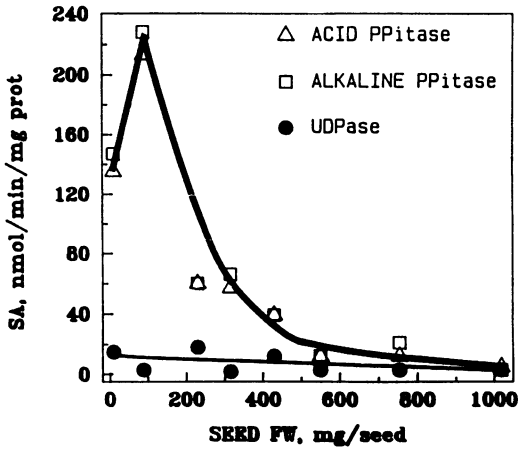

Figure 4. Changes in the specific activities of enzymes that hydrolyze pyrophosphate or uridine diphosphate during lima bean seed development.

(Fig. 2), both NTP-dependent PFKs (Fig. 3), UDPase (Fig. 4), and sucrose-P synthase.

Enzymes of Sucrose Metabolism in the Germinating Lima Bean Seed

In testing sucrose metabolism enzyme maximum activity with these young seedlings we assayed the whole dry seed on d 0. On d 1 we could assay the cotyledons and the embryonic plant separately and by d 3 we divided the embryo into the hypocotyl and epicotyl. We studied only the first week of seed germination. For further orientation purposes the general fresh weight accumulation pattern in the embryonic plants is shown later in the last three figures. Greening of the epicotyl usually was visible on d 5 or d 6 and it increased rapidly. In the first study, we will present separate data for the total embryo axis and for the cotyledons; later, we will present data for the hypocotyl and the epicotyl.

In the germinating embryo axis the specific activity of all three enzymes for breaking down sucrose increased; but little change occurred in the cotyledons (Fig. 5). Note the activities of all three enzymes were very low in the dry seed. Initially, acid invertase and sucrose synthase were at the same low level in embryos, then from d 4 the acid invertase became more active (Fig. 5B). Neutral invertase was the lowest of all, but readily assayed, in the young axis. In this study emergence occurred at d 6, and at d 6 all three enzymes decreased slightly (data on this shown later from another study when emergency occurred at d 5). From these results, both sucrose synthase (Fig. 5A) and acid invertase (Fig. 5B) appear to be major sucrose cleavage enzymes which increase their specific activity in the developing lima bean embryo to support its rapid growth.

Of the three enzymes for forming hexose phosphates, none of them exhibited a clear pattern of changing in the cotyledon (Fig. 6). Again, the UDP-glucopyrophosphorylase is very active in both the embryo and the cotyledon but with no clear

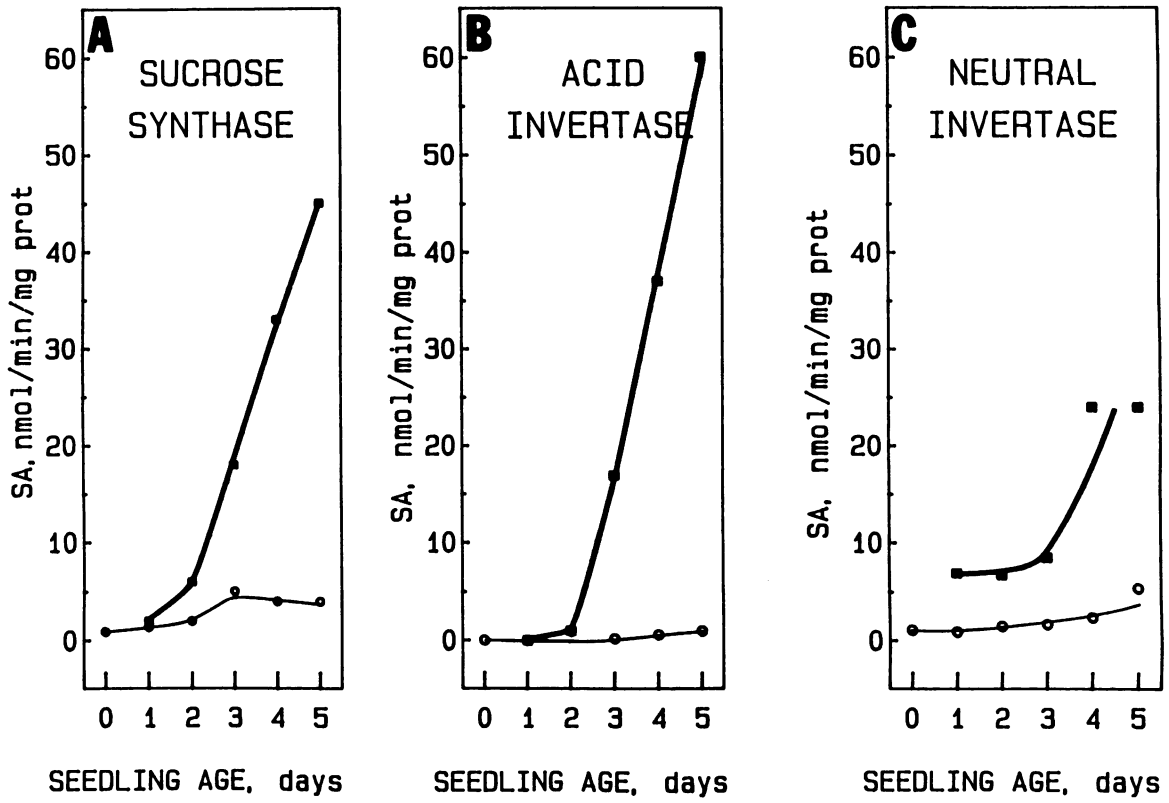

Figure 5. Changes in the specific activities of sucrose cleavage enzymes during lima bean seedling development. (○), Cotyledons; (■), total embryos.

pattern of change with seedling age. In the growing embryo only fructokinase increased (Fig. 6B). Glucokinase activities were very low in either cotyledons or embryos (Fig. 6C). Unlike the sucrose cleavage enzymes (Fig. 5) each of these hexose-P forming enzymes were present at substantial activities in dry lima bean seeds (Fig. 6).

At the step converting fructose 6-P to fructose 1,6-P_2, both the PPi-dependent and the NTP-dependent PFKs increased in the embryo with little change in the cotyledons which again contained substantial activities at d 0 (Fig. 7). The PPi-PFK was the dominant activity each day and increased 1 to 2 d ahead of the NTP-PFK (*cf.* Fig. 7A with B and C).

When enzymes competing for UDP and PPi were tested in young seedlings there was a small increase by UDPase in the embryo but none in the cotyledons (Fig. 8A). However, once again both acid and alkaline pyrophosphatases were quite responsive in the developing plant (acid pyrophosphatase data not shown) (Fig. 8B). But little change was evident in the cotyledons (acid pyrophosphatase data not shown); except as we will show later, an increase in acid pyrophosphatase occurs near cotyledon death. And the synthesis of sucrose, via sucrose-P synthase (Fig. 8C), seemed to remain fairly constant in the cotyledons and also in the embryo. We expected cotyledons to increase this activity, but they did not increase it more than about twofold over that in dry seeds (Fig. 8C). However, later when we examine total sucrose-P synthase activity in cotyledons it will be nearly equivalent to other glycolytic enzymes such as the PPi-PFK.

From these seed germination tests we can sort out that the more adaptive enzymes in embryos are sucrose synthase (Fig. 5A) and acid invertase (Fig. 5B), plus acid and alkaline pyrophosphatase (Fig. 8B). The other enzymes which adapted in the embryos but to a lesser response level were neutral invertase (Fig. 5C), fructokinase (Fig. 6B), the PPi-dependent PFK (Fig. 7A). Much less, more maintenance-type, response was observed in embryos for UDP-glucopyrophosphorylase (Fig. 6A), glucokinase (Fig. 6C), the NTP-PFKs (Fig. 7, B and C), sucrose-P synthase (Fig. 8C), UDPase (Fig. 8A), plus phosphoglucomutase (data not shown).

Total Enzyme Activity in the Developing Lima Bean Seed and Adaptive versus Maintenance Enzymes

The proposed (4, 19, 24) adaptive *versus* maintenance aspects of sucrose metabolism can be illustrated further in lima bean seed by plotting the accumulation of total enzyme activity per seed *versus* increasing seed weight. We observed three types of responses with total enzyme activity during seed growth. First, of all the enzymes tested, only sucrose synthase and alkaline pyrophosphatase activities accumulated initially with seed growth and then decreased to a very low level as seed maturation approached (Fig. 9) from experiment to experiment. Some variation was noted in the seed weight where total activities peaked, in the total amounts of activity, and in the level they dropped to near seed maturation. But the patterns in Figures 9 and 10 were observed in each test. Next, the PPi-PFK increased total activity rapidly but did not decline detectably as maturity approached (Fig. 10). In con-

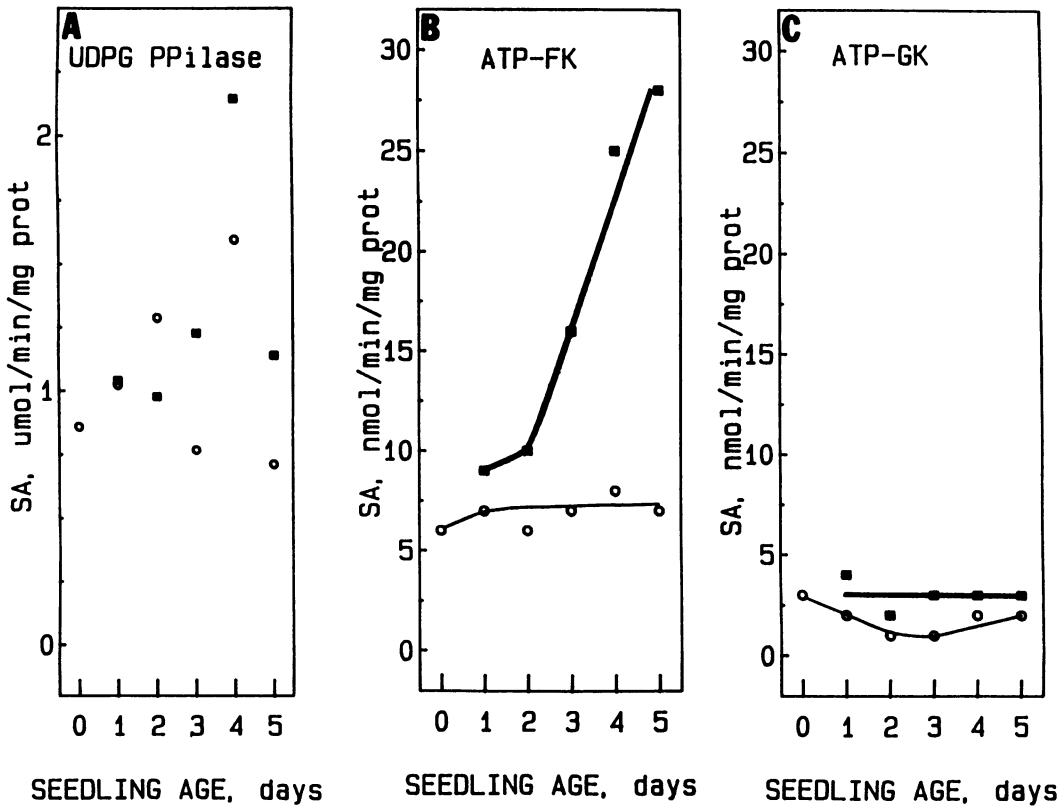

Figure 6. Changes in the specific activities of hexose phosphate forming enzymes during lima bean seedling development. (○), Cotyledons; (■), total embryos.

trast, the rest of the enzymes measured showed continuously accumulating activities with development (Fig. 10). Due to the similar trend of increase, only the acid invertase and NTP-PFK total activities were plotted in Figure 10; however, we have similar data for UDP-glucopyrophosphorylase, fructokinase, glucokinase, neutral invertase, sucrose-P synthase, phosphoglucomutase, and UDPase.

Collectively, these results indicate that sucrose synthase (Figs. 1 and 9) and alkaline pyrophosphatase (Figs. 4 and 9) were the strongly adaptive enzymes in developing lima bean seeds. The PPi-PFK also is an adaptive enzyme (Figs. 3 and 10) but it is not as responsive. The maintenance type enzymes include UDP-glucopyrophosphorylase, both fructokinases, both NTP-PFKs, sucrose-P synthase, both invertases, glucokinase, phosphoglucomutase, and UDPase in germinating seeds.

Thus, when sucrose metabolism feeding carbon through glycolysis and gluconeogenesis is considered in the overall in the seed as a sucrose sink, an initial enzyme of sucrose cleavage, sucrose synthase, is the most responsive or adaptive. But we were surprised also to find pyrophosphatase to be almost equally responsive. At this moment we do not have a hypothesis we can support about the roles of the pyrophosphatase responses but surely expect the roles to relate to the pool of PPi found in plant cells (10, 21) and to polymer synthesis. Possibly, the competition of the pyrophosphatases with the UDP-glucopyrophosphorylase or the PPi-PFK is involved in these changes. We note that, in contrast, the UDPase seems to be fairly constant and seemingly to offer little competition for UDP which the breakdown of sucrose via the sucrose synthase pathway also is dependent upon (23, 31).

Total Enzyme Activities in the Developing Lima Bean Seedling

We studied the lima bean seedling in more detail over the first week of seedling growth because (a) the cotyledons are acting as a sucrose source which is senescensing, while (b) the embryo (hypocotyl, root, and epicotyl) are sucrose sinks, with (c) the epicotyl beginning to green usually by d 5; hence, the epicotyl is developing toward photoautotrophic growth.

If one examines the total enzyme content of lima bean cotyledons (Fig. 11), glycolytic enzymes are present in the dry seed and generally tend to decrease as the cotyledons become senescent (Fig. 11 C, D, and E). The three sucrose cleavage enzymes are low in cotyledons as already noted (Fig. 5) and show little change with seedling growth (Fig. 11B) except an increase in acid invertase just as the cotyledons die. The total sucrose-P synthase activity (data not shown) was approximately half of the PPi-PFK (Fig. 11C) and only declined slightly during the 7 d of seedling growth. The other enzyme which increased appreciably in cotyledons was the acid pyrophosphatase (Fig. 11F); hence, by this analysis the acid pyrophosphatase and the acid invertase were adaptive enzymes late in the life of the cotyledon.

The hypocotyl, of course, developed prior to the epicotyl but it passed peak growth by about d 5 (Fig. 12A). The total

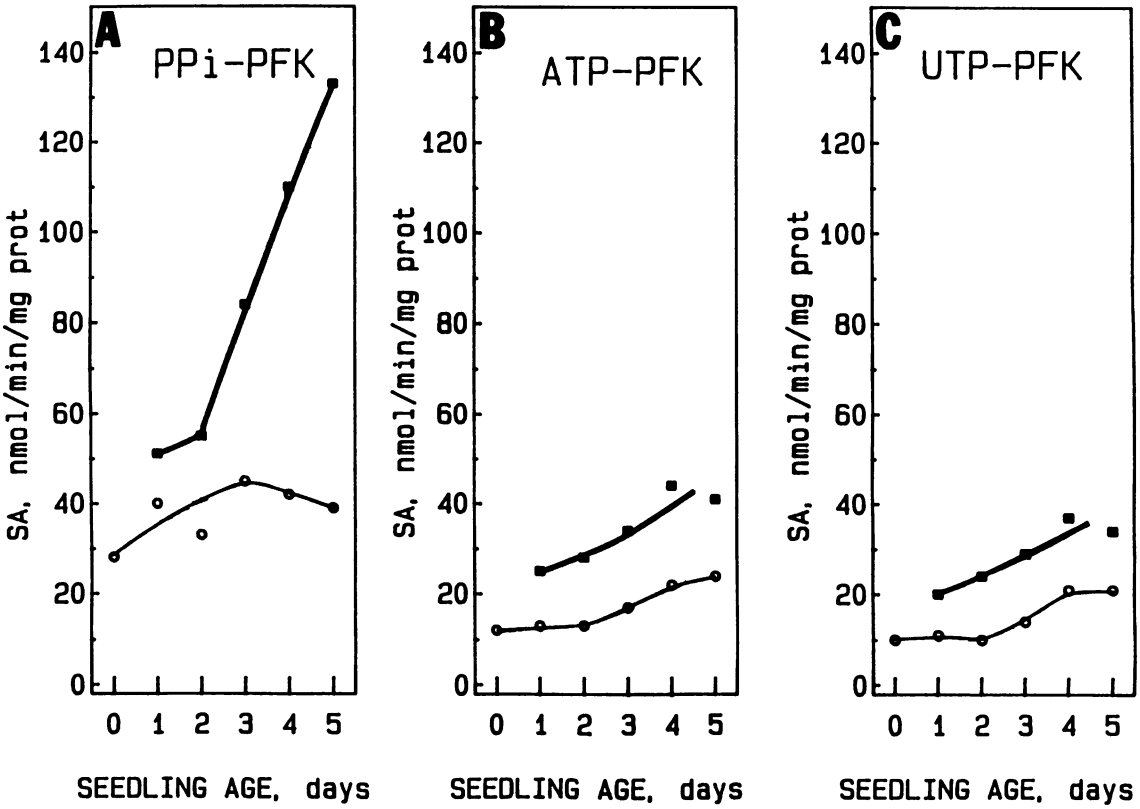

Figure 7. Changes in the specific activities of enzymes converting fructose 6-P to fructose 1,6-P_2 during lima bean seedling development. (○), Cotyledons; (■), total embryos.

activities of sucrose cleavage enzymes (Fig. 12B) coincide with hypocotyl growth (Fig. 12A). Indeed, we can again find evidence for their adaptive response either in the decrease in total invertase or in the slower accumulation of sucrose synthase activity after about d 3 to 4 of seedling growth (Fig. 12B). In contrast, other enzymes generally increased total activity as the seedlings grew (Fig. 12 C, D, E, and F) with little indication of decline or cessation up to d 7.

In the epicotyl, growth noticeably lagged at emergence (d 5) and most of the enzyme accumulation patterns reflected this (Fig. 13). But again, as in Figure 5 with the whole embryo, the enzymes of sucrose cleavage increased, with the acid invertase dominating in the epicotyl (Fig. 13B). As the epicotyl greened, the sucrose synthase activity was lagging noticeably as we expect in plant tissues as they become autotropic (12, 28). Other enzymes in the epicotyl simply increased in total amounts as the tissue continued to grow (Fig. 13 C, D, E, and F).

DISCUSSION

We have conducted this work focused around the objective of understanding the central role of sucrose metabolism in plants knowing, as an example, that there is a wealth of published data showing that sucrose is the major carbon source for seed development and that sucrose is the principal breakdown product of seed storage materials, *i.e.* lipids, proteins, and carbohydrates, to feed a developing embryo. For instance, there are many published reasons to accept that at least parts of glycolysis and gluconeogenesis are ready to operate early in germination, indeed even in the dry seed (1, 5, 8, 9, 16, 22, 26–29), and many studies have been reported on enzyme changes during seed development and germination (2, 15–18, 20). In seeds, the pivotal segment between sucrose and triose-P surely must be functional either to feed sugar into cellular metabolism or for sucrose translocation to occur. We are not attempting here to exactly place our results in the complete sequence of events which occur during seed formation or germination nor will we make extensive comparisons with the published literature on seed maturation and germination. Rather, we are using lima bean seed metabolism as a model in learning about plant sucrose metabolism for feeding hexoses into glycolysis. Our results are interpreted also knowing the following limitations or reservations: (a) the enzyme activities are only extractable maximum activities, even though we technically guarded against problems, such as proteases and inhibitors, by using known precautions; (b) we recognize that strongly bound enzymes, such as a cell wall invertase or mitochondrial hexokinases, are not likely to be fully extracted; (c) we assume these maximum activities represent the total tissue, *i.e.* vacuole, nucleus, plastid, and cytoplasm, and that these enzymes can act in the expected glycolytic or gluconeogenic sequences; (d) we know isozymes exist for many enzymes and their cellular localization, *e.g.* cytoplasm *versus* plastid, must be identified; and (e) we do not know the sucrose flux rates in each of these tissues. These reservations are topics of our current research; but even with these recognizable limitations, we propose that these *in vitro*

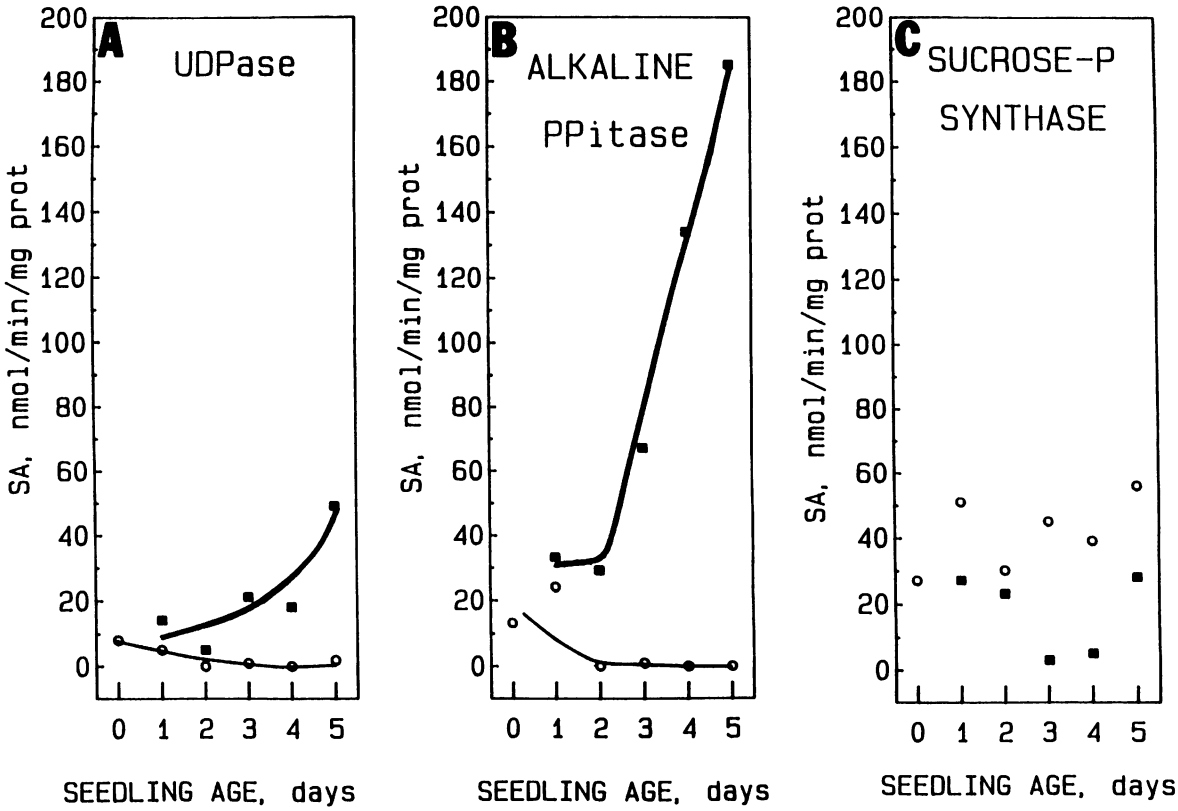

Figure 8. Changes in the specific activities of UDPase, alkaline pyrophosphatase, and sucrose-P synthase during lima bean seedling development. (○), Cotyledons; (■), total embryos.

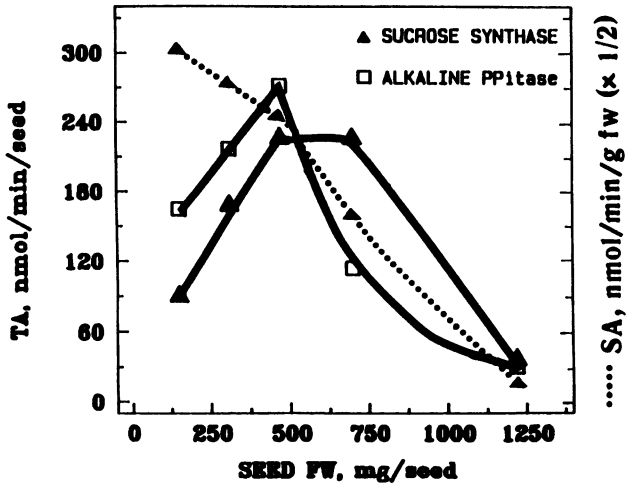

Figure 9. Patterns in the total activity per seed during lima bean seed development for highly adaptive enzymes. Sucrose synthase data are given on both a seed and fresh weight basis.

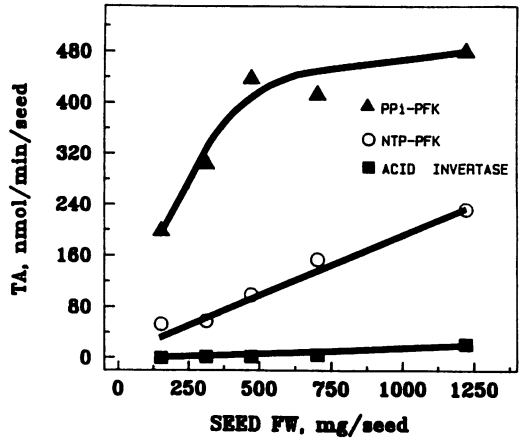

Figure 10. Patterns in the total enzyme activity per seed during lima bean seed development for the PPi-PFK and for the set of maintenance enzymes.

lima bean enzyme studies reveal new features about sucrose nutrition in plant cells.

If we first question our enzyme rates we find they are compatible with estimations in other tissues (no lima bean data are available) of sucrose uptake rates near 17 nmol/g fresh weight/min with celery cells (7), 52 to 118 nmol/g fresh weight/min with pea embryos (8, 9); or with a starch accumulation rate of 47 nmol of hexose unit/g fresh weight/min in sucrose utilizing potato tuber; or with a sucrose synthesis rate of 300 to 400 nmol/g fresh weight/min in the maize scutellum (7). In Figure 9, comparable data on a gram fresh weight basis for sucrose synthase are presented. During seed development, sucrose synthase is more than sufficient (values of nearly 600 in Fig. 9) for the expected sucrose metabolism; but invertase is insufficient, a maximum of only 7 nmol/g

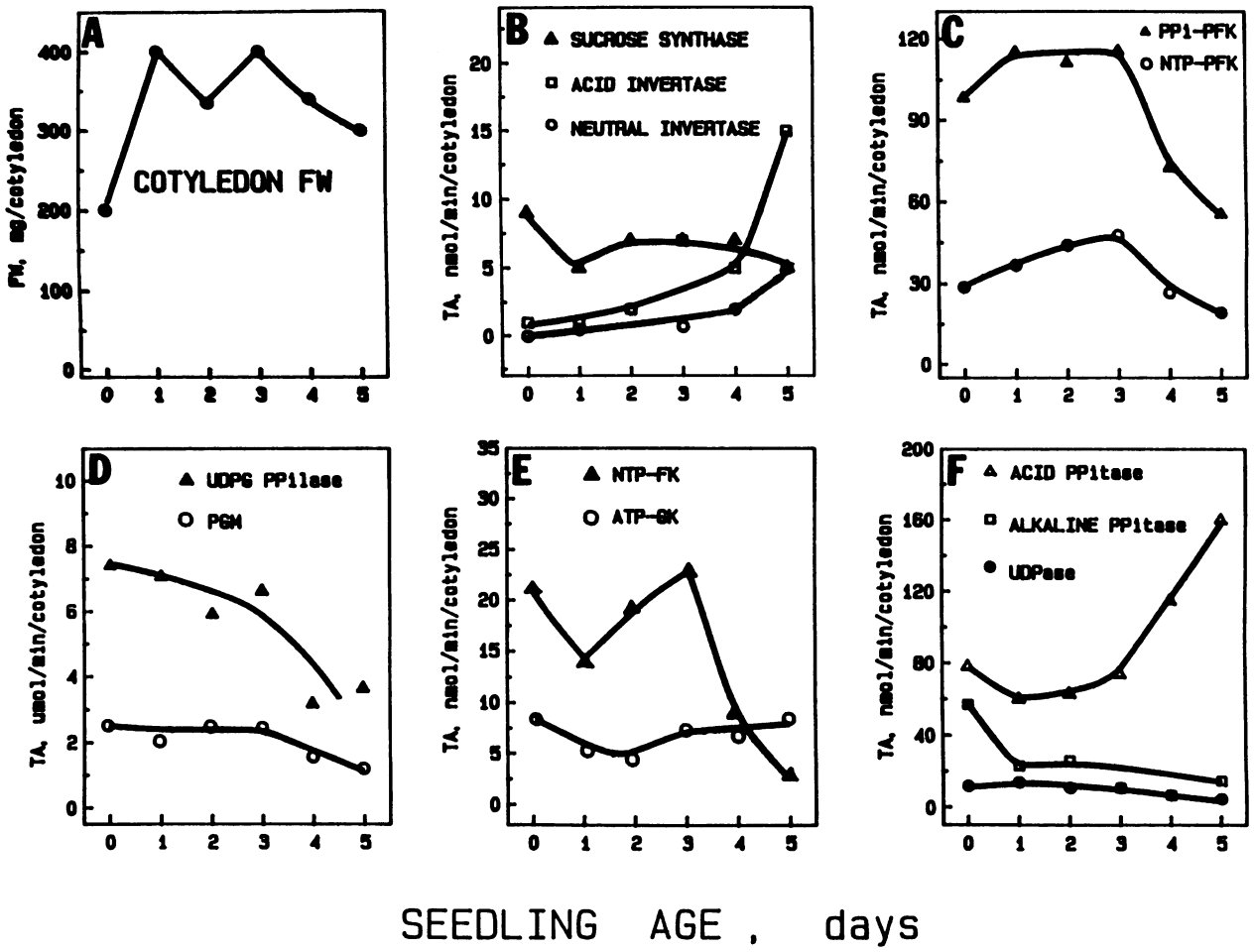

Figure 11. Changes in the total amount of enzyme activities per cotyledon during lima bean seedling growth.

fresh weight/min was observed during early seed development in the same study (data not given).

Testing the Sucrose Synthase Pathway

Collectively, we interpret these results as firm support for the sucrose synthase pathway of feeding sucrose into glycolysis (4, 23, 24); hence, we will conclude that this pathway is a major component of seed maturation and embryo development. Much of the sucrose fed to the developing seed enters via the sucrose synthase (Figs. 1 and 9) with invertases being secondary (discussed in the previous section). In contrast, in the growing seedling both the sucrose synthase pathway and acid invertase are active (Fig. 5 A and B). Certainly, in the growing seedling we know that nucleotide sugars are a major component of cellular metabolism (2, 12, 15) so we expect competition for UDP-glucose. Unfortunately, we cannot quantitate how much UDP-glucose is fed into nucleotide sugar metabolism *versus* the subject to pyrophosphorolysis. But, seemingly, the growing seedling ensures sucrose feeding by both invertase and sucrose synthase (Fig. 5) which agrees with the recent hypothesis that glycolysis may be initiated by one of three enzymes during sucrose nutrition (24).

The PPi-PFK which produces PPi as part of the cyclic PPi metabolism in this pathway (31) also is active at comparable levels to sucrose synthase and somewhat follows the same pattern during development as the sucrose synthase (*cf.* Figs. 1 *versus* 3 or 5 *versus* 7). The cyclic production of UDP seemingly would require both the UTP-PFK (4, 23) and the UTP-dependent fructokinase (13) to be as active as the sucrose synthase (*cf.* Figs. 1 *versus* 2B and 3 or Figs. 5 *versus* 6B and 7 B and C). There seems to be no indication that either UDP-glucopyrophosphorylase or phosphoglucomutase activities (Figs. 2A, 6A, 12D, or 13D) would limit the sucrose synthase pathway or glycolysis in general. Therefore, we conclude that these data along with our previous work (23, 24, 31) are strong support for the sucrose synthase pathway as a major route for feeding sucrose into plant cellular metabolism.

Competition in the Metabolism of UDP and PPi

We obtained no evidence that the UDPase, which might compete for UDP, is a component which changes during seed sucrose metabolism (Figs. 4, 8A, 12F, 13F). Hence, it is unlikely that UDPase competes well with sucrose synthase for UDP.

But we think the competition for PPi is a component of sucrose metabolism. The pyrophosphatases were quite adaptable enzymes in both seed development and germination (Figs. 4, 8B, 9, 11F, or 13F). Plant pyrophosphatases are

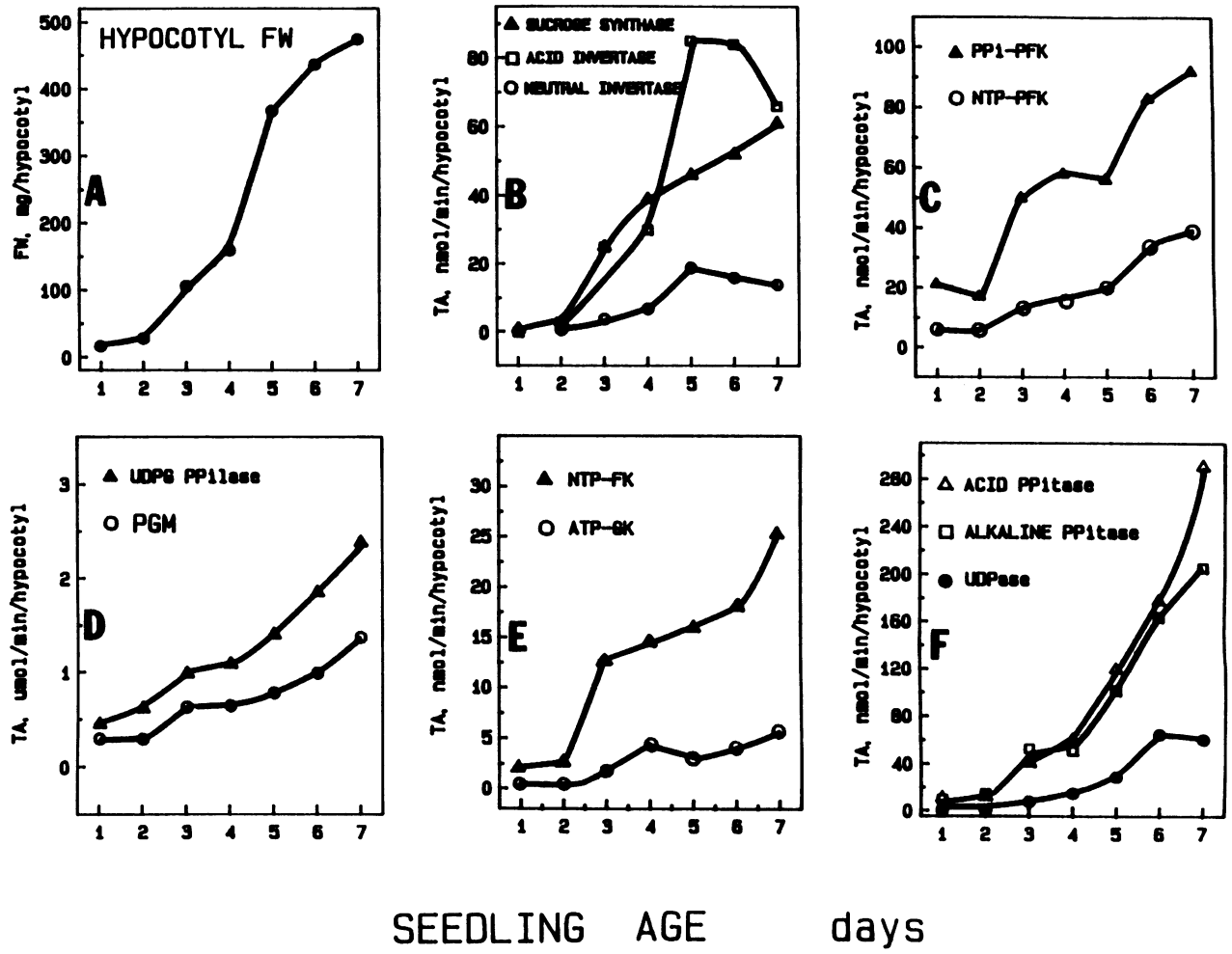

Figure 12. Changes in the total amount of enzyme activities per hypocotyl during lima bean seedling growth.

known to occur in the plastid and in the vacuole (11, 23). Though the cellular location of these pyrophosphatases is unknown in lima beans, we expect them to be in these cellular locations along with the nucleus and the cytoplasm. However, more research is required to determine the roles of these pyrophosphatases in PPi metabolism and if they compete with the sucrose synthase pathway. We could speculate that the increase in acid pyrophosphatase (Fig. 11F) is related to the almost complete translocation of Pi from senescing cotyledons (Fig. 6.21A in Ref. 2).

Adaptive and Maintenance Enzymes in Plant Glycolysis and Gluconeogenesis

Several years ago we recognized that some plant enzymes in sugar metabolism changed their activities more than others during transitions brought on by development or by environment (4, 19). These were called adaptive and maintenance enzymes; at least two adaptive enzymes were identified in PPi-PFK and sucrose synthase (23, 24). Sucrose synthase is, of course, recognized as playing a major role for the entry of sucrose into plant cellular metabolism. And the PPi-PFK functions at a strong regulatory site internal to glycolysis and gluconeogenesis. The regulation by fructose 2,6-P_2 at the interconversion of fructose 6-P and fructose 1,6-P_2 no doubt is a key site of cytoplasmic regulation in plant cells (3, 29, 30) along with these adaptive traits of PPi-PFK. The work in this manuscript was being conducted during the time period of those discoveries and this report adds to those reports.

This lima bean work also adds other adaptive enzymes in the acid and alkaline pyrophosphatase plus the acid invertase. Indeed, these enzymes along with sucrose synthase seem to be more responsive than the PPi-PFK. Adaptive enzymes are characterized by: (a) fairly large specific activity changes (5- to 10-fold or more) in response to development or environmental stresses (4, 19); (b) high specific activities at maximum levels; and (c) when depressed, they are at very low activities, *e.g.* see the dry seed and cotyledon data in Figure 5.

Maintenance-type enzymes also were identified as the ATP-PFK (4, 19) (Figs. 3 and 7); the UTP-PFK; UDP-glucopyrophosphorylase; UDPase; phosphoglucomutase; neutral invertase; glucokinase, NTP-fructokinase and sucrose-P synthase. Maintenance enzymes are characterized by: (a) small and fairly slow activity changes, perhaps a few percent to threefold maximum, in response to development or environment; and (b) specific activity levels adequate to carry out reasonable metabolic rates, *e.g.* in glycolytic or gluconeogenic sugar

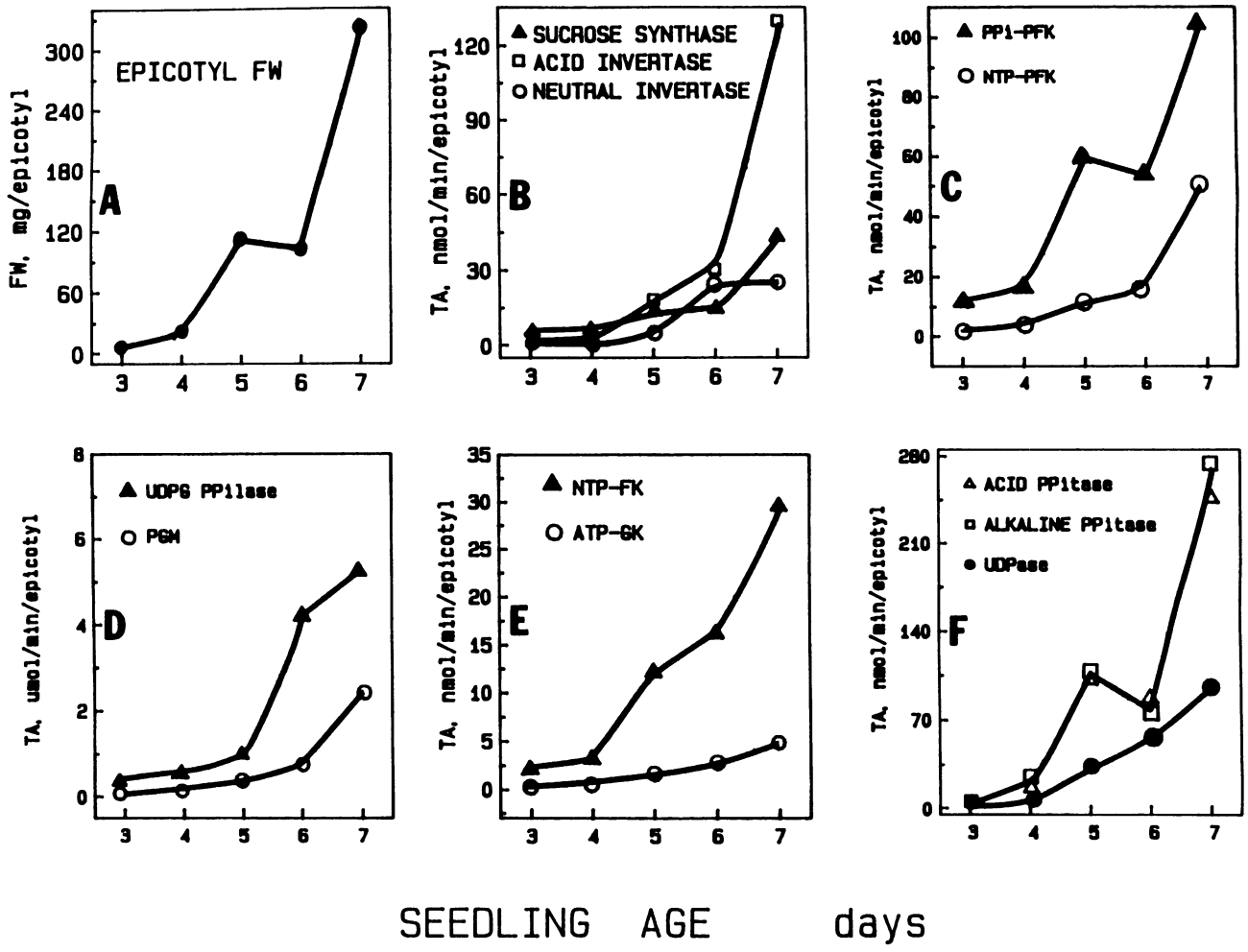

Figure 13. Changes in the total amount of enzyme activities per epicotyl during lima bean seedling growth.

conversions even in dry seed cotyledons or embryos. Indeed, we propose that plant cells maintain these enzymes to ensure survival and stability even in quiescent moments, *e.g.* in dry seeds or in dormant plants or during bud set.

In conclusion, these lima bean seed studies: (a) are supportive of the hypothesis that the UDP- and PPi-dependent sucrose synthase pathway is a major route to feed sucrose into plant metabolism; (b) support the hypothesis that plants have a set of maintenance enzymes to keep segments of glycolysis and gluconeogenesis functional, ten maintenance enzyme activities were identified; (c) support the hypothesis that plants have a set of adaptive enzymes which change sharply during development in specific tissues; and (d) identify the most adaptive set of enzymes in lima bean seeds as sucrose synthase, acid pyrophosphatase, alkaline pyrophosphatase, acid invertase and PPi-PFK.

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