Polyamine Anabolism in Germinating Glycine max (L.) Seeds

DYNAMICS OF CADAVERINE AND PUTRESCINE FORMATION IN THE EMBRYONIC AXIS

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ABSTRACT

Active polyamine biosynthesis occurs in the embryonic axis, but not in the cotyledons, during germination of Glycine max (L.) cv Williams seeds and subsequent growth of the young seedlings. The hypocotyl and radicle synthesize and accumulate considerable amounts of cadaverine (Cad) and putrescine (Put) during the early stages of growth. Most of the amino acid precursors for the diamines are supplied from breakdown of the cotyledonary protein.

In the axis, Cad synthesis which is catalyzed by L-lysine decarboxylase precedes the onset of growth (dry weight accumulation) of the axis and its accumulation continues as the growth progresses. After 2 days of imbibition, Cad is synthesized and accumulated at 37.4 nanomoles per axis per hour for at least 6 days. The rate then gradually decreases as the supply of free L-lysine from the cotyledons diminishes. Approximately 40 to 50% of the lysine resulting from breakdown of the cotyledonary protein ends up in Cad in the hypocotyl and radicle. Cad represents about 3.5% of the nitrogen derived from the cotyledons, which is equivalent to about 50% of the lysine content (~7%) of the seed protein.

The synthesis and accumulation of Put in the axis also precedes the onset of growth of the axis. Both L-arginine decarboxylase and L-ornithine decarboxylase are involved in catalyzing the Put formation. The increased content of spermidine (Spd), but not spermine (Spm), parallels growth of the axis. Spm is maintained at a nearly undetectable level. After 2 days of imbibition, Put and Spd are synthesized and accumulated at 0.94 and 0.17 nanomoles per axis per hour, respectively. The rate of Put accumulation, like that of Cad, decreases about 8 days after imbibition. The hypocotyl and radicle contain millimolar concentrations of Cad and Put which are primarily associated with the elongated zones. In contrast, Spd level or the molar ratio of Spd:Put appears to decrease as cell differentiation or elongation progresses.

In recent years, increasing evidence suggests that the naturally occurring polyamines Put², Spd, and Spm may act as modulators of some cellular and physiological processes during plant growth and development (6). Changes in the polyamine content or the activities of enzymes involved in Put biosynthesis have been demonstrated in a variety of plant species during early stages of seed germination or subsequent seedling growth (1, 2, 14, 17, 19). Though some of the changes are somehow correlated with the metabolism of macromolecules (e.g., proteins and nucleic acids), the physiological and biochemical significance of polyamine metabolism in plant growth and development needs to be further elucidated. To understand the potential roles of polyamines in cellular metabolism, it is necessary to clarify whether changes in the content of polyamines in general or a specific polyamine is causally linked to, or merely a consequence of, plant growth and development. In this study, polyamine levels and complexities were determined in the cotyledon, hypocotyl, hook, hypocotyl, and radicle of soybean (Glycine max L. Merr.) cv Williams during and after seed germination. Experiments were also carried out to characterize Cad biosynthesis in the embryonic axis during seed germination and growth of the young seedling.

MATERIALS AND METHODS

Plant Material. Seeds of the soybean (Glycine max L. Merr.) cv Williams harvested in 1982 from Spindletop Experimental Farm of the University of Kentucky were used in this study. Unless otherwise noted, seeds were allowed to germinate at 23°C in the dark on water-moistened Anchor seed germination paper (Anchor Paper Co., St. Paul, MN). Samples were taken at intervals throughout germination and the cotyledons and germinated embryonic axes were manually separated. Unless otherwise stated, each of the embryonic axes was divided into three parts (radicle, hypocotyl, and hypocotyl hook) for determination of fresh weight, dry weight, and polyamine content. The axes could be clearly divided into the above three distinguishable parts by 2 d after the seeds were placed on the water-moistened germination paper. The axes from dry seed or seed that had been on the germination paper for up to 1.5 d were divided by length with one-quarter the length from the apex designated as the radicle, the following one-quarter length as the hypocotyl, and the remaining part as the hypocotyl hook. The separation of the hypocotyl hook from the hypocotyl is based on the observation that the hypocotyl hook portion turns greenish in color within hours after exposure to light of detached embryonic axes cultured in a nutrient agar medium (20). The hook portion in dark-germinated seedlings is yellowish in color.

Analysis of Polyamine Content. Routinely, the fresh tissue derived from 30 embryonic axes (300 g fresh weight) per assay was homogenized in 5 ml of cold 5% HClO₄ with a Polytron tissue homogenizer. Polyamines in the acid-soluble and -insoluble fractions were qualitatively and quantitatively determined by a HPLC system as described previously (10). In some experiments, 5 to 15 seedlings (after 3 d of imbibition) were used per assay and the seedlings were homogenized as described. Authentic polyamines were used for calibration of polyamine content in the extracts.

Injection of Radioactive Substances into the Cotyledons of Soybean Seedlings. After 2.5 d of imbibition in the dark on water-moistened Anchor germination paper, soybean seedlings were transferred and placed in 9-cm-diameter Petri dishes (six seedlings/Petri dish) containing 2 Whatman No. 1 filter papers. The seed coats were removed prior to injection of the following

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1 This paper (No. 84-3-114) is published with the approval of the director of the Kentucky Agricultural Experiment Station.
2 Abbreviations: Put, putrescine; Spd, spermidine; Spm, spermine; Cad, cadaverine; Agm, agmatine; PMSF, phenylmethylsulfonyl fluoride.
radioactive substances into the cotyledons: L-[U-\(^{14}\)C]arginine (300 mCi/mmole) from Amersham, and L-[U-\(^{14}\)C]lysine (336 mCi/mmole), L-[N-4,5-\(^{3}H\)]lysine (100.1 Ci/mmole), and [1,5-\(^{14}\)C]cadaverine (104.9 mCi/mmole) from New England Nuclear. The radioactive substances were injected directly into the cotyledons with a Hamilton microliter syringe. Unless otherwise stated, each cotyledon received a single injection of 5 \(\mu\)l of the radioactive solution containing 0.5 \(\mu\)Ci. After injection, the seedlings were placed back in the Petri dish to which 2 ml of distilled H\(_2\)O was slowly added along the edge of the Petri dish to keep the filter paper wet throughout the experiments. To prevent the radioactive solution from leaking from the cotyledons onto the filter paper, the seedlings were placed with the injection sites on cotyledons away from the filter paper. The seedlings were further incubated in the dark at 23°C for various times. After incubation, the seedlings were transferred to a paper towel and the embryonic axes manually detached. The axes were washed briefly with distilled H\(_2\)O and divided into radicles, hypocotyls, and hypocotyl hooks. The cotyledons were also washed with distilled H\(_2\)O. Polyamines in the plant parts were qualitatively and quantitatively determined as described. Fractions, 10 drops or 0.18 ml each, eluted from HPLC were collected in scintillation vials, and to each vial 15 ml of Aquassure scintillation fluid (New England Nuclear) was added. The radioactivity was determined by liquid scintillation spectrophotometry.

**Enzyme Assay.** Plant parts of 4-d-old soybean seedlings were homogenized with a Polytron tissue homogenizer in 3 volumes (w/v) of a buffer containing 50 mm Hepes (pH 7.4), 5 mm DTT, 0.01 mm pyridoxal-5-phosphate, 1 mm PMSF, and 5 mm EDTA. After centrifugation at 20,000 g for 20 min, the supernatant (10 ml) was chromatographed on a Sephadex G-25 column (1.5 x 45 cm) and eluted with the above buffer without EDTA. The protein fractions were assayed for the activities of L-lysine decarboxylase, L-arginine decarboxylase, and L-ornithine decarboxylase as described (3) and with some modifications. Briefly, the assay was carried out in a 0.2-ml reaction mixture inside a 1.5 ml polypropylene Eppendorf vial (without cap). The reaction mixture contained 25 mm Hepes (pH 7.4), 2.5 mm DTT, 0.005 mm pyridoxal-5-phosphate, 0.2 mm L-[U-\(^{14}\)C]lysine or L-[U-\(^{14}\)C]arginine or L-[1-\(^{14}\)C]ornithine (Amersham), and 0.1 ml of enzyme solution. The specific activities of \(^{14}\)C-amino acid in the reaction mixtures were 1 x 10^6 cpm/nmole. The vial was placed inside a 20-ml screw-cup glass liquid scintillation vial. A 0.5- x 3.0-cm strip of Whatman No. 1 filter paper containing 0.2 ml of Protosol (0.5 m hyamine hydroxide) from New England Nuclear was placed on the bottom of the glass vial. The reaction was carried out at 30°C for 1 h and terminated by the addition of 0.2 ml of 20% HClO\(_4\) to the Eppendorf vial. The glass vial was uncapped one at a time, recapped, and reincubated for 30 min. After the second incubation, the Eppendorf vial was lifted halfway off from the scintillation counting vial. The outside surface of the bottom portion of Eppendorf vial was rinsed prior to being discarded with 15 ml of the toluene-base scintillation fluid (0.35 g/l of 1,4-bis-2-[5-phenylloxazoyl]benzene and 7 g/l of 2,5-diphenyloxazole). The rinsed fluid was collected in the counting vial and the amounts of \(^{14}\)CO\(_2\) trapped in Protosol were determined by liquid scintillation spectrophotometry at 7% gain. Under the experimental conditions, the routine assays gave a background of about 40 to 60 cpm/assay with a heat-denatured enzyme preparation. The procedure provides a reliable measurement of \(^{14}\)CO\(_2\) release from \(^{14}\)Ccarboxyl-labeled amino acids.

**General Methods.** Tissue dry weight was determined after drying for 2 d at 70°C. Moisture content was measured by comparing apparent fresh weight with dry weight and expressed on a wet weight basis. Protein concentration was determined by the protein-dye binding method with bovine plasma gamma globulin as standard (4).

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**RESULTS**

Polyamine Titer in Soybean Seed during Imbibition and Germination. Figure 1 shows the increase in dry weight of the embryonic axis over a 6-d period after imbibition. During the first 30 h, the axis is engaged in hydration, resulting in cell expansion and without significant changes in dry weight. After 30 h imbibition, the growth of the embryonic axis is evident as a result of dry weight increases in the radicle and the hypocotyl, but not the hypocotyl hook. Since no exogenous nutrients were supplied during germination, the increases in dry weight of the axis are at the expense of reserve materials stored in the cotyledons. The dry weight of the cotyledons gradually decreases after 1.5 d of imbibition and by days 4 and 6 it is reduced by 26 and 38%, respectively.

The changes which occur in the polyamine level in the cotyledons and embryonic axis over a 6-d period of imbibition are shown in Figure 2, A and B. In the cotyledons, the major polyamine Spd, which is accumulated during seed development (11), decreases sharply after 3 d of imbibition (Fig. 2A). This decrease, however, does not result in any significant changes in the levels of Spm and Put which remain relatively constant over the 6-d period. Significant amounts of Cad are present in the cotyledons of the harvested Williams soybean seeds, but it decreases rapidly upon hydration. Only traces of Cad could be detected in the cotyledons at 2 d after imbibition. Polyamine titer in the embryonic axis following imbibition exhibits an entirely different pattern from that described in the cotyledons. On an axis basis, the contents of Cad, Put, and Spd show linear increases from day 1.5 (Fig. 2B) and the increases parallel the growth of the axis (Fig. 1). The estimated rates of accumulation of Cad, Put, and Spd are approximately 930, 22, and 10 nmol axis\(^{-1}\) day\(^{-1}\), respectively. Cad represents about 1.5% of the dry weight of the axis during the linear growth of the axes from days 2 to 6. In contrast to the other polyamines, Spm in the axis rapidly decreases to a minimum level prior to the onset of the growth (increase in dry weight) of the axis and remains at this low level (<1.0 nmol/axis) throughout the 6-d period. The molar ratio of Put:Spm:Spd in the axis after 6-d imbibition is approximately 1:0.06:0.01.

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**FIG. 1.** Changes in dry weight of the embryonic axis of soybean seed following imbibition in darkness. The hypocotyl hook (Δ), hypocotyl (○), and radicle (□) were separated and their dry weights determined as described in "Materials and Methods".
Figure 3, A to C, shows the changes in the levels of Put, Spd, and Spm in the radicle, hypocotyl, and hypocotyl hook of the embryonic axis during the 6-d period of imbibition. It is evident that the observed increase in Put content in the axes is contributed primarily by the radicle and the hypocotyl. In both the radicle and hypocotyl, the contents of Spd, like Put, increase along with the growth of the plant parts (Fig. 3, A and B). Put and Spd levels in the hypocotyl hook also increase during the early stages of imbibition, but the levels decrease slightly after d 3 (Fig. 3C) as the plant part shows no further increase in dry weight (Fig. 1). Only trace amounts of Spm could be detected in the embryonic axes of germinating soybean seeds or the young seedlings.

The majority of Cad is present in the radicle and the hypocotyl, but not the hypocotyl hook (Fig. 4). During linear growth, Cad in the radicle and the hypocotyl remains relatively constant at about 220 and 150 μmol·g⁻¹ dry weight, respectively. This means that Cad represents about 2% of dry weight in the radicle and 1.5% in the hypocotyl. Kinetically, the rate of Cad accumulation (25 nmol·h⁻¹) in the hypocotyl is about 2 times that observed in the radicle. This is comparable to the observed difference in the growth rates of the two plant parts (Fig. 1).

Distribution of Polyamines in the Root and Hypocotyl of Young Soybean Seedlings. To determine whether selective polyamines are associated with or accumulated in particular types of cells in soybean seedlings during growth and development, the contents of Put, Spd, Spm, and Cad in 1.0-cm segments from the apex of root of 4-d-old seedlings were determined. The distribution of polyamines is uneven along the seedling and a different pattern of distribution exists for Put and Spd or Spm (Fig. 5A). A high level of Put expressed per g dry weight is associated with the differentiated and mature zones of both root and hypocotyl. In contrast, the Spd level gradually decreases from the apex to the base of root or hypocotyl. Thus, a higher molar ratio of Spd:Put exists in the meristematic zones compared to the mature zones (Fig. 5B). The estimated concentrations of Put and Spd in the seedling (hypocotyl and root) are in the ranges of 0.1 to 0.6 mM and 0.05 to 0.35 mM, respectively. Trace amounts of Spm could
Fig. 4. Changes in the Cad content in various parts of the embryonic axis of soybean seed following imbibition in darkness. The Cad content in the radicle (O), the hypocotyl (•), and the hypocotyl hook (©).

Fig. 5. The Put, Spd, and Spm content in the embryonic axis of 4-d-germinated soybean seed. A: The Put (O), Spd (■), and Spm (©) content per g dry weight of each 1.0-cm segment from the apex of root. The arrows indicate the polyamine content in 0.1-cm segment of the root tip. The 10th segment refers to the hypocotyl hook. The black dots shown in the seedling indicate the lateral roots developed from the base of root under the growth conditions. B: the molar ratio of Spd/Put in the segments sliced from the apex of root as described above. The polyamine content and the molar ratio of Spd/Put in the cotyledons are now shown. G indicates the growing point.

be detected only in the root tip and the hypocotyl apex. Similar to Put, the majority of Cad in the seedling is primarily distributed in the differentiated mature zones of the root and hypocotyl (Fig. 6). The estimated concentrations of Cad are in the range of 5 to 18 mm.

The root contains one centrally placed vascular stele which is readily pulled out. The stele contains 147 nmol of Cad, 20 nmol of Put, 9.7 nmol of Spd, and 1.3 nmol of Spm on a per g tissue dry weight basis. The estimated concentrations of polyamines in the stele are about 8.6 mm Cad, 1.2 mm Put, 0.56 mm Spd, and 0.07 mm Spm. The concentration of Cad in the stele is about 40% less than the average value of 13.8 mm estimated for the entire root, indicating that a higher concentration of Cad is present in the cortex and/or the epidermal layer. Unlike Cad, the concentrations of Put, Spd, and Spm in the stele are approximately 2 times greater than in the cortex and/or the epidermal layer as judged by comparison with the concentrations calculated from Figure 5A.

Polyamine Synthesis and Accumulation in the Embryonic Axes Detached from Soybean Seeds. The marked increase in content of the polyamines in the embryonic axis of soybean seed during germination led to the questions: (a) Is the axis capable of polyamine biosynthesis? and (b) Do the cotyledons provide the substrates for polyamine biosynthesis in the axis during early stages of seed germination or seedling growth? In an effort to answer these questions, polyamine titer in detached embryonic axes following imbibition was determined. The embryonic axes detached either from the 40-h preimbibed seeds or from the dry seeds without preimbibition were used for comparison of polyamine biosynthetic capability. Figure 7A shows that the axes detached from the seeds which have been preimbibed are unable to carry out further Cad synthesis. The rate of Cad synthesis or accumulation (6 nmol .axis \(^{-1} \cdot h^{-1}\)) in the detached axis is approximately 15% of that observed in the intact seeds (36 nmol .axis \(^{-1} \cdot h^{-1}\)). There is no further Cad accumulation in the detached axis after 20 h incubation. The cotyledons detached from the 40-h preimbibed seeds also do not produce significant amounts of Cad over a 33-h period of incubation. Additionally, no appreciable amounts of Cad are released from the detached axes or cotyledons to the media during incubation. In contrast to Cad, the synthesis or accumulation of Put in the axis detached from the preimbibed seed is not much different from that in the axis of intact seed over a 33-h period of incubation. Spd level in the above detached axes is not changed during the incubation.
The embryonic axes detached from the mature dry seeds without preimbibition apparently are capable of synthesis of some Cad at 3.5 nmol·axis⁻¹·h⁻¹, beginning at about 10 h after imbibition (Fig. 7B). After 18 to 20 h or imbibition, the rate of Cad synthesis or accumulation is gradually reduced. At the end of 40-h imbibition, each detached axis accumulates only about 75 nmol of Cad, which is approximately 25% of that observed in the axis of intact seed following the same period of imbibition (Fig. 7A). The hypocotyl hook section representing about one-half of the detached axis may contribute most of the Cad synthesis or accumulation measured. This is based on the observation that the rate of Cad synthesis or accumulation in the axes detached from mature dry seeds is similar to the value of 3 nmol·h⁻¹ obtained for the hypocotyl hook in the intact seed during the early stages of germination (Fig. 4).

A biphasic pattern of changes in Put level occurs in the axis detached from the mature dry seed during 40-h imbibition (Fig. 7B). On an axis basis, the level of Put increases at 1 nmol·h⁻¹ beginning at about 4 to 5 h after imbibition. After 18 to 20 h of imbibition, the rate of Put synthesis or accumulation is double for a short period of time and then gradually decreases. In contrast to Cad and Put, the Spd level shows no significant change until 4 h after imbibition when it gradually decreases. The level of Spm in the detached axis decreases gradually during the imbibition. The changes in the polyamine content described for the detached axis occur primarily in the hypocotyl hook which is engaged in cell expansion and without dry weight increase during imbibition. Evidently, the detached axes are capable of polyamine biosynthesis, but with limited capability.

Conversion of Radioactive Polyamine Precursors Injected into the Cotyledons to Polyamines Synthesized and Accumulated in the Embryonic Axis during and after Seed Germination. Comparison of polyamine synthesis and accumulation, particularly Cad and Put, in detached axes and the axes attached to the cotyledons indicates that the amino acid precursors for the diamine biosynthesis in the axes could be derived from the cotyledons via breakdown of the storage protein following germination. Figure 8 shows that L-[N-4,5-H]lysine and L-[U-¹⁴C]arginine injected directly into the cotyledons of 2.5-d-old soybean seedlings are converted to Cad and Put, respectively, in the embryonic axis after 7 h incubation. Spd and Spm are not labeled under the experimental conditions, indicating that the conversion of radio-labeled lysine and arginine injected directly into the cotyledons to Cad and Put, respectively, in the embryonic axis of germinated soybean seed. L-[N-4,5-H]lysine (80 μCi/seedling; 100 Ci/mmol) and L-[U-¹⁴C]arginine (1.25 μCi/seedling; 300 Ci/mmol) were injected directly into the cotyledons of 2.5-d germinated soybean seeds. After 7 h incubation, polyamines in the embryonic axis (hypocotyl + roots) were extracted by 5% HClO₄, and benzoylated prior to fractionation by HPLC as described in "Materials and Methods". The polyamine profile is shown as are the amounts of radioactivity recovered in or associated with each polyamine fraction.
The soybean seedling axis was injected with [14C]lysine to examine the distribution and metabolism of the polyamines. The [14C]lysine was incorporated into the hypocotyl and root of the seedlings, and the specific activity of L-lysine decarboxylase and the protein content (approximately 1.0 mg/hypocotyl plus radicle) of the 4-d-old seedlings, the estimated rate of Cad synthesis per axis is about 20% of the actual rate of its accumulation in the hypocotyl and radicle following germination (Fig. 4). The apparent rate of Put formation, estimated from the specific activities of both L-arginine and L-ornithine decarboxylases, is 2 times greater than the actual rate of accumulation of Put + Spd + Spm (1.1 nmol·h⁻¹) in the hypocotyl and radicle (Fig. 3, A and B).

**Polyamine Titer in the Greenhouse-Grown Soybean Seedlings.** Table III shows the changes in polyamine content in various parts of soybean seedlings at various times after imbibition in vermiculite and germination in the greenhouse. In general, the complexity and content of polyamine in the radicle, hypocotyl, and hypocotyl hook during early stages of seedling growth is similar to that described for the seedlings of dark-germinated soybean. During 8-d growing period, the levels of Cad in the radicle and hypocotyl represent about 1.5 and 0.7% of the dry weight of the plant parts, respectively. At 15 d after imbibition, the Cad levels in all parts of the seedlings except the leaf decrease substantially. It is noteworthy that the secondary roots also contain high levels of Cad during early stages of growth and that the Put level rapidly decreases at about 8 d after imbibition.

**DISCUSSION**

The results of this study demonstrate that active polyamine anabolism, particularly the synthesis and accumulation of Cad and Put, occurs in the embryonic axis during the early stages of seed germination and the growth of young seedlings of soybean cv Williams. The cotyledons apparently play an important role in the determination of polyamine biosynthesis in the embryonic axis by providing most of the amino acid precursors for Cad and Put.

Comparison of polyamine titer in the embryonic axis during seed development and germination (Fig. 10, A and B) shows that the rate of polyamine synthesis or accumulation in general is greatly accelerated as the hydration and germination of the seed proceeds. Thus, the activation of polyamine synthesis, particularly Cad, following imbibition is not merely a continuation of the polyamine metabolism occurring in the embryonic axis of the developing seed prior to dehydration during seed maturation.

**Table I. Distribution of [14C] in the Acid-Insoluble Acid-Soluble and Cad Fractions of Embryonic Axes of Germinated Soybean cv Williams Seeds at Various Times after Supplying L-[U-14C]Lysine to the Cotyledons**

<table>
<thead>
<tr>
<th>Time of Incubation</th>
<th>Part of Embryonic Axis</th>
<th>Acid-Insoluble Fraction</th>
<th>Acid-Soluble Fraction</th>
<th>[14C] Recovered as Cad</th>
<th>Incorporation into Cad³</th>
</tr>
</thead>
<tbody>
<tr>
<td>h</td>
<td></td>
<td>[14C] cpm × 10⁻³/plant part</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Cotyledons</td>
<td>626</td>
<td>72.7</td>
<td>553</td>
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<tr>
<td></td>
<td>Hypocotyl hook</td>
<td>25.3</td>
<td>10.0</td>
<td>15.3</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>Hypocotyl + root</td>
<td>36.8</td>
<td>15.0</td>
<td>21.8</td>
<td>5.80</td>
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<tr>
<td>4.5</td>
<td>Cotyledons</td>
<td>659</td>
<td>169</td>
<td>490</td>
<td>9.40</td>
</tr>
<tr>
<td></td>
<td>Hypocotyl hook</td>
<td>59.0</td>
<td>31.3</td>
<td>27.7</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td>Hypocotyl + root</td>
<td>72.3</td>
<td>33.3</td>
<td>39.0</td>
<td>22.40</td>
</tr>
<tr>
<td>7</td>
<td>Cotyledons</td>
<td>633</td>
<td>143</td>
<td>490</td>
<td>9.10</td>
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<tr>
<td></td>
<td>Hypocotyl hook</td>
<td>51.3</td>
<td>31.5</td>
<td>19.8</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>Hypocotyl + root</td>
<td>66.9</td>
<td>25.7</td>
<td>41.2</td>
<td>32.00</td>
</tr>
</tbody>
</table>

*The percent incorporation into Cad = the amount of [14C] cpm recovered as Cad × 100/the total amount of [14C] cpm in the plant part.*
the incorporation of L-[U-14C]lysine (injected directly into the cotyledons) to Cad in the embryonic axes of germinating soybean seeds. Details of the experiments are described in Table I and also in "Materials and Methods". The amounts of Cad synthesized and accumulated (■—■) and the amounts of 14C recovered as Cad (○—○) per axis (hypocotyl + root) were determined. The specific activities of Cad synthesized and accumulated in the axis at various times of incubation are shown in the inserted figure.

Table II. Specific Activities of L-Lysine, L-Arginine, and L-Ornithine Decarboxylases in 4-Day-Old Seedlings of Soybean cv Williams

The specific activities of decarboxylases in extracts of the root and hypocotyl and the cotyledon were determined as described in "Materials and Methods." The protein fractions from Sephadex G-25 gel filtration were assayed for the enzyme activities.

<table>
<thead>
<tr>
<th>Source</th>
<th>Specific Activity</th>
<th>nmol CO2 released/h mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Lysine</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>L-Ornithine</td>
<td>4.17</td>
<td>1.31</td>
</tr>
</tbody>
</table>

Another striking difference between the developing and germinating soybean seeds is the metabolism of Put which is synthesized and accumulated in substantial amounts only in the axes of germinating seeds. Since the dry weight of the axes remains unchanged during cell hydration, the early increased synthesis and accumulation of Cad and Put in the axes appears not to be a consequence of the axis growth which does not occur until 36 h after imbibition (Fig. 1). It has been suggested that increase of polyamine content in the embryonic axis during imbibition of soybean seeds may be a result of movement of polyamines from the cotyledons (1). This apparently is not the case according to this study: (a) the detached embryonic axes are capable of synthesis of Cad and Put for approximately 30 h during imbibition (Fig. 8B), while the detached cotyledons produce insignificant amounts of the diamines; (b) the supply of the diamine precursors (L-lysine and L-arginine or L-ornithine) from the cotyledons for continuous synthesis of the diamines in the axis is not needed until 25 to 30 h after imbibition when the axis growth begins; and (c) [1,5-14C]Cad injected directly into the cotyledons of young soybean seedlings is not translocated to the growing embryonic axes. Apparently, polyamines in general are not transported in vivo in higher plants (21). Our preliminary observation also indicates that the activities of the diamine biosynthetic enzymes (L-lysine decarboxylase, L-arginine decarboxylase, and L-ornithine decarboxylase) are primarily present in the growing axis, and the activities increase during imbibition. These results strongly argue that the synthesis and accumulation of Cad and Put during the first 24 h of imbibition is carried out in and by the axis itself.

Some correlation exists between polyamine synthesis and the growth of soybean seedlings (shown here) as demonstrated in young seedlings of other plant species (1, 2, 15, 19). In this study, the Cad, Put, and Spd content in the embryonic axis of soybean seeds increases as the growth of axes progresses, and the increases are maintained at maximum rates during the early linear phase of growth of the axes. Both of the activity of L-lysine decarboxylase present in the axis and the ability of L-lysine derived from breakdown of the cotyledonary protein are important factors for the determination of Cad synthesis in the hypocotyl and root of young soybean seedlings. Most of Cad accumulated in the soybean hypocotyl and root appears to be resulting from enzymic decarboxylation of the L-lysine derived directly from the cotyledons. According to Kato and Kitada (9), the embryonic axis accumulates approximately 1 mg of nitrogen axis-1 d-1 during the first 14 d of imbibition of soybean seed under the conditions that no exogenous nutrients are supplied. After 14 d, the supply of nitrogen from the cotyledons decreases to a minimum level as does the accumulation of nitrogen in the axis. This apparently is also true for Cad (and Put), whose synthesis and accumulation in the axis evidently follows the supply of nitrogen (e.g. amino acid precursors for the diamines) from the cotyledons. It is estimated that over the entire course of utiliza-
FIG. 10. Summary of kinetic changes in polyamine content in the embryonic axes of soybean cv Williams seeds during seed maturation and germination. A, Rates of change in polyamine content per axis per d in the seed of soybean grown in the field as described previously (17). PM refers to the physiological maturity of the seed which has maximum dry weight accumulation under the experimental conditions. B, Rates of changes in polyamine content per axis per d in the axis of soybean seed following imbibition in darkness. Polyamines: Cad (©); Put (©); Spd (©); and Spm (A).

...tion of cotyledonary protein, each axis (hypocotyl and root) accumulates approximately 1.2 mg of Cad. Thus, the amount of Cad represents about 3.5% of the nitrogen content in the axis or accounts for about 50% of the L-lysine derived from breakdown of the cotyledonary protein (~7% lysine content). This is close to the observed value of 40 to 50% of the L-lysine, which is directly injected into the cotyledons, transported to the axis, and subsequently converted to Cad (Table I). The kinetic studies of L-lysine incorporation also indicate that a large portion of the amino acid which is not converted to Cad is recovered in the acid-insoluble fraction, presumably protein. Unlike most of the other amino acids, L-lysine is a ketogenic amino acid which is not subjected to transamination reaction. The above results suggest that, in young soybean seedlings, there are two main paths in the axes for utilization of L-lysine derived from the cotyledons. One is for newly synthesized proteins in the growing hypocotyl and root. The other is for Cad synthesis. The relationship between the two paths, if any, remains to be studied.

The accumulated Cad may not serve as storage nitrogen for future seedling growth because it represents only a small fraction of the total nitrogen in the hypocotyl and root, and because its turnover appears to be limited. At present, the physiological significance, if any of mass synthesis and accumulation of Cad in the young soybean seedlings is not clear. Attempts have been made to block the synthesis of Cad by using some protein inhibitors of L-lysine decarboxylase (e.g. D-lysine and ε-amino-n-caproic acid). However, none of the compounds tested at 0.5 to 2.0 mm show significant effects on soybean seed germination or the growth of young soybean seedlings (unpublished observation). It has been reported that millimolar concentrations of L-lysine may be toxic to the growth of barley seedlings (13). However, the young soybean seedlings grow normally in the presence of 0.2 to 2 mm L-lysine at pH 6.5. In fact, the levels of free lysine in the hypocotyl and root of 3- to 6-d-old soybean seedlings are maintained at about 0.05 and 0.02 μmol plant−1, respectively (8). The estimated concentrations of free L-lysine in the hypocotyl and root, based on fresh weight of the seedling, are approximately 0.2 to 0.5 mm. Thus, the toxicity of L-lysine to the soybean seedlings could be ruled out. In vitro studies across Cad may affect the activity of urease in soybean radicles (18). Whether the effect occurs in vivo is not known. Cad is not widely distributed in higher plants (12, 17). In view of its high concentration, its limited turnover, and its restricted distribution, Cad appears not to be an ideal candidate for regulatory function in common metabolic processes in higher plants. At present, available evidence from this study and studies of Cad metabolism in germinating seeds of a variety of plant species (12) argue in favor of the suggestion that Cad is either an end product or the precursor for some secondary products in higher plants. However, it is possible that Cad formation (decarboxylation of L-lysine) is a metabolic process by which the embryonic axis adjusts its intracellular pH or utilizes the liberated CO2 for unknown purposes during early stages of seedling growth.

Similar to Cad, the synthesis and accumulation of Put in the hypocotyl and root may be a result of uncharacterized physiological processes by which a portion of L-arginine or L-ornithine not used for normal metabolic requirements is removed. Though the increased content of Put, like Cad, in the embryonic axis of germinating soybean seed may not be crucial for seedling growth, some of the Put is converted to Spd which may be of some importance in cell enlargement and growth (19). High molar ratios of Spd/Put are associated with the cells having high meristematic activity in soybean seedlings (Fig. 5B). Similar results have been previously reported for Zea mays seedlings (5, 16). Anguillesi et al. (1) argue that high levels of Spd/Spm may be correlated with the onset of mitotic activity in the root tip meristems of germinating soybean seed, but not Helianthus and Triticum seeds. The present study, however, indicates that though the Spd/Spm ratio decreases as cell elongation and maturation progresses, the changes are primarily due to decreased Spd content in soybean seedlings (Fig. 5A). Taking these results together, it is evident that the synthesis of Spd, involving transfer of the propylamino group from decarboxylated S-adenosylmethionine to Put via the reaction catalyzed by spermidine synthetase, may be important in plant growth and development.

Further work is needed to see if blocking the synthesis and accumulation of Cad or Put affects the growth and development of soybean seedlings, and if protein synthesis determines the polyamine levels or vice versa. Further work is also needed to characterize polyamine metabolism and its relation to seedling growth and development in other soybean genotypes and other plant species.

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