Activation of the *de Novo* Pathway for Pyridine Nucleotide Biosynthesis Prior to Ricinine Biosynthesis in Castor Beans[1]

DAVID F. MANN[2] AND RICHARD U. BYERRUM
Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824

ABSTRACT

The ricinine content of etiolated seedlings of *Ricinus communis* increased nearly 12-fold over a 4-day period. In plants quinolinic acid is an intermediate in the *de novo* pathway for the synthesis of pyridine nucleotides. The only known enzyme in the *de novo* pathway for pyridine nucleotide biosynthesis, quinolinic acid phosphoribosyltransferase, increased 6-fold in activity over a 4-day period which preceded the onset of ricinine biosynthesis by 1 day. The activity of the remainder of the pyridine nucleotide cycle enzymes in the seedlings, as monitored by the specific activity of nicotinic acid phosphoribosyltransferase and nicotinamide deamidase, was similar to that found in the mature green plant. In the roots of *Nicotiana rustica,* where the pyridine alkaloid nicotine is synthesized, the level of quinolinic acid phosphoribosyltransferase was 38-fold higher than the level of nicotinic acid phosphoribosyltransferase, whereas in most other plants examined, the specific activity of quinolinic acid phosphoribosyltransferase was similar to the level of activity of enzymes in the pyridine nucleotide cycle itself. A positive correlation therefore exists between the specific activity of a *de novo* pathway enzyme catalyzing pyridine nucleotide biosynthesis in *Ricinus communis* and *Nicotiana rustica* and the biosynthesis of ricinine and nicotine, respectively.

Numerous *in vivo* studies have established that the pyridine moiety of quinolinic acid, nicotinamide, and nicotinic acid is directly incorporated into the alkaloids ricinine and nicotine produced by the castor bean plant and the tobacco plant, respectively (7, 11, 20, 25, 26). In plants, one metabolic pathway leading to the formation of pyridine nucleotides is the *de novo* pathway, involving initially the condensation of aspartic acid and glyceraldehyde-3-P or closely related metabolites. The condensation product of aspartic acid and glyceraldehyde-3-P undergoes a series of reactions leading to the formation of quinolinic acid which is then converted to pyridine nucleotides. The only known enzyme in this pathway is

quinolinic acid phosphoribosyltransferase, which catalyzes the conversion of quinolinic acid and phosphoribosylpyrophosphate to nicotinic acid mononucleotide (7, 15). The latter is then converted to NAD via nicotinic acid adenine dinucleotide (16). By means of the pyridine nucleotide cycle, the NAD so made in plants is eventually broken down into nicotinamide and nicotinic acid, which in a cyclic process can also be converted to nicotinic acid mononucleotide and subsequently to NAD (6, 22). The known reactions of the *de novo* synthesis of pyridine nucleotides and the reactions of the pyridine nucleotide cycle are shown in Figure 1. Because quinolinic acid is easily converted to nicotinamide and nicotinic acid (7), and because all three compounds are excellent precursors of the pyridine alkaloids, ricinine and nicotine, the pyridine nucleotide cycle could lie between the *de novo* pathway for pyridine nucleotide biosynthesis and the pyridine alkaloids, as first suggested by Leete and Leitz (11) and later supported by Waller and co-workers (22).

No systematic study has been conducted, however, on the biosynthesis of quinolinic acid, nicotinic acid, or nicotinamide in nicotine and ricinine producing plants. It would seem logical that the production of these alkaloids would require a greater *de novo* synthesis of pyridine nucleotide precursors than would be the case in most other plants, inasmuch as ricinine and nicotine comprise about 1% and 5% of the dry weight of *Ricinus communis* and *Nicotiana rustica,* respectively (4, 19). Mizusaki *et al.* (14) have shown that in tobacco roots the enzymes involved in the conversion of ornithine to N-methyl-Δ^1-pyrroline, the precursor of the pyrrolidine ring of nicotine, increase in activity at about the same time that nicotine synthesis increases. Several other plants which do not synthesize pyrrolidine alkaloids contained no detectable ability to convert ornithine to N-methyl-Δ^1-pyrroline. A similar phenomenon has been reported in the fungus *Claviceps fusiformis,* strain SD-58, where tryptophan synthetase activity increases 20- to 25-fold in the period prior to the production of ergot, a tryptophan containing alkaloid (17).

In order to assess the extent of the *de novo* synthesis of pyridine compounds during alkaloid biosynthesis in castor beans and tobacco, the activity of quinolinic acid phosphoribosyltransferase was determined. The activity of the pyridine nucleotide cycle enzymes was monitored by assaying nicotinic acid phosphoribosyltransferase and nicotinamide deamidase activity. The levels of enzyme activity were then correlated with the degree of alkaloid biosynthesis. Castor beans offered an especially good system in which to study the biosynthesis of a pyridine alkaloid, since Weevers (23) and Waller (19) have found that etiolated castor bean seedlings produced large quantities of ricinine in a short period of time.

[2] Present address: Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Ind. 47907.
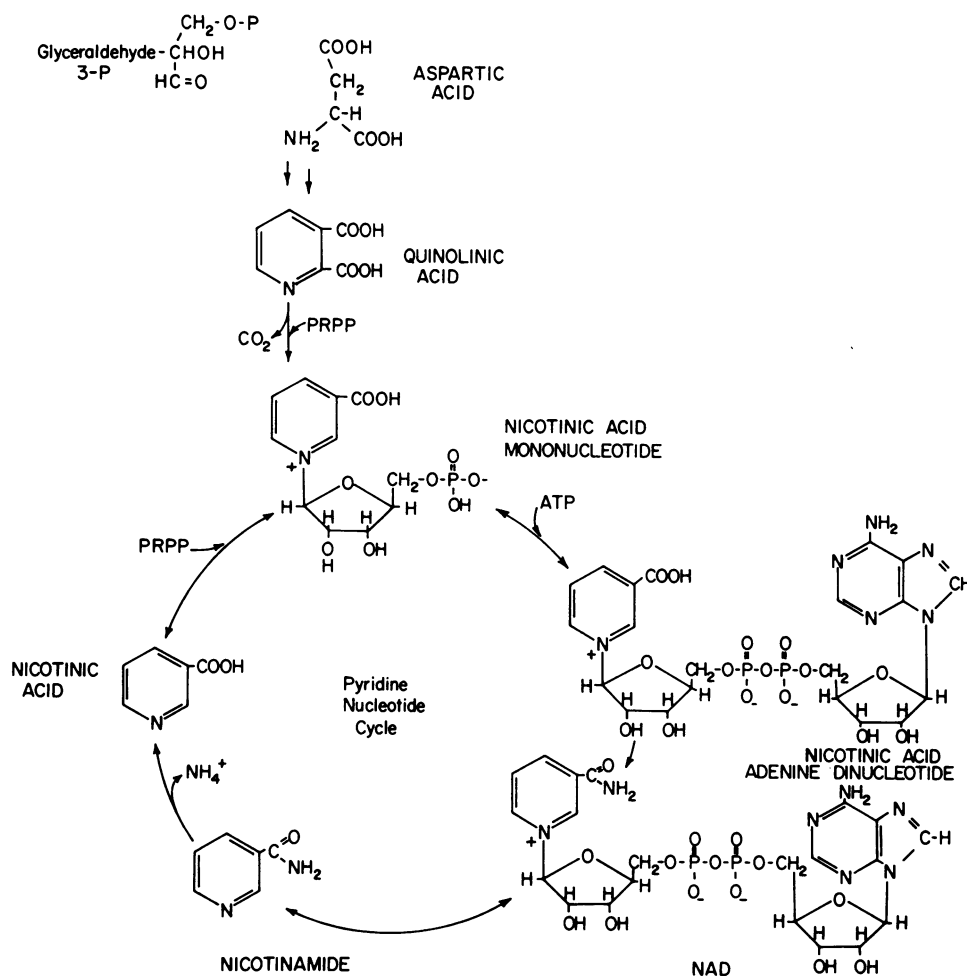
FIG. 1. *De novo* pathway for pyridine nucleotide synthesis and the pyridine nucleotide cycle.

MATERIALS AND METHODS

Seeds of *Ricinus communis*, var. Hale, were treated with a 10% solution of Orthocide, 50% Captan (N-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide), and were germinated at 30 C in vermiculite moistened with either tap water or distilled water. Etiolated seedlings at different stages of physiological development were used in these experiments. The criteria for selection of a seedling of a given physiological age are given in Table I. The green castor beans used in some of the experiments reported here were grown in the greenhouse, and their age was measured from the time they had emerged from the soil in which the seeds were planted. Just prior to flowering, the roots from *Nicotiana rustica* were removed from plants grown in the greenhouse and new roots were allowed to regenerate according to the method of Byerrum and co-workers (8). All other plants were grown in the greenhouse in a soil-sand mixture and were watered as needed.

Enzyme Extraction. For preparation of enzyme extracts, plant material was routinely weighed, washed in distilled water, and dried by blotting with a paper towel. The tissue was then ground in a cold mortar using 2 volumes per g fresh weight of 50 mM potassium phosphate buffer, pH 7.0, containing 10 mM dithioerythritol. After filtering the crude extract through four layers of cheesecloth, the filtrate was centrifuged at 27,000g for 20 min. The supernatant was then used for the various enzyme assays described.

Table I. *Criteria Used to Determine the Physiological Development of the Etiolated Castor Bean Seedlings*

The castor beans were planted in moist vermiculite and germinated in the dark at 30 C. Seedlings were used only if they fit the criteria given below. The primary root was measured from the beginning of the root hairs or secondary roots to the tip of the primary root. The hypocotyl was measured from the top of the curvature in the hypocotyl to the point at which the roots emerged.

Day	Stage of Development
2	Seed coats were cracked open
3	1.8 ± 0.5 cm primary root
4	2.9 ± 0.6 cm primary root
5	0.9 ± 0.3 cm hypocotyl
6	3.0 ± 0.7 cm hypocotyl
7	6.2 ± 0.9 cm hypocotyl
8	7.5 ± 0.7 cm hypocotyl

Enzyme Assays. Quinolinic acid phosphoribosyltransferase activity was determined either by quantitating the $^{14}CO_2$ released from $2,3,7,8$-^{14}C quinolinic acid or by measuring by paper chromatography the formation of 6-^{14}C-quinolinic acid. The two assay procedures gave the same result when compared. The former assay was used only when etiolated castor bean seedlings were the experimental plant, and contained, in a total volume of 0.8 ml in a 25-ml Erlenmeyer flask, the fol-

lowing reagents at the final concentrations given: 1 to 50 units of enzyme (1 unit of enzyme catalyzes the formation of 1 nmole of product/hr·mg protein); 115 mM potassium phosphate, pH 7.0; 5 mM dithioerythritol; 12.5 mM $MgCl_2$; 0.187 mM 2,3,7,8-^{14}C quinolinic acid (0.05 μc/μmole) and 0.375 mM PRPP[3]. Each Erlenmeyer reaction flask was equipped with a rubber septum and a polyethylene hanging well (Kontes Glass Co.) which contained 0.2 ml hydroxide of hyamine 10-X and a folded paper wick. The flasks, minus the PRPP, were equilibrated for 5 min with shaking in a 30 C water bath; the reaction was started by the addition of PRPP. After 20 min, the reaction was stopped by adding 0.3 ml of 10% perchloric acid through the rubber septum. To insure complete absorption of the [14]CO_2 by the hydroxide of hyamine, the flasks were allowed to stand for 1 hr. The well and its contents were then placed in a counting vial with 6 ml of scintillation fluid, which contained 1.265 g of POPOP and 19 g of PPO per 3.79 l of toluene. The ^{14}C in the vials was counted after a period of 4 to 5 hr equilibration in a refrigerated scintillation spectrometer.

The second, more sensitive assay procedure for quinolinic acid phosphoribosyltransferase was used when the enzyme activity was less than 1 unit. For this assay, in which ^{14}C-labeled nicotinic acid mononucleotide formation was measured, a solution was prepared which contained the following reagents in a 25-ml Erlenmeyer flask at the final concentrations given in a total volume of 0.2 ml: 115 mM potassium phosphate, pH 7.0; 5 mM dithioerythritol; 12.5 mM $MgCl_2$; 0.375 mM PRPP; and the enzyme. The reaction was initiated by adding the substrate, 6-^{14}C-quinolinic acid (4.15 μc/μmole), to give a final concentration of 0.3 mM. The reaction was allowed to proceed for 2 hr at 30 C, and was stopped by heating the flask in a boiling water bath for 1 min. Unlabeled quinolinic acid (1.6 μmoles) was added to each assay mixture to serve as a chromatographic standard, and the precipitated protein was removed by centrifugation in a clinical centrifuge. Approximately 10 μl of the assay solution were spotted on Whatman No. 1 paper and developed by descending chromatography in 1-butanol-acetic acid-water (4:1:2, v/v). Quinolinic acid (R_F 0.36) was first detected by a mineralight UVS·II lamp; the ^{14}C-labeled nicotinic acid mononucleotide (R_F 0.14), as well as the quinolinic acid, were then located by scanning 1.5 inch wide strips on a radiochromatogram scanner. Three-inch sections of the strips corresponding to the two radioactive peaks were cut out and placed in counting vials. The vials were filled with scintillation fluid which contained 4 g BBOT per liter of toluene and counted. The percentage of quinolinic acid converted to nicotinic acid mononucleotide was determined by dividing the observed cpm, corrected for background, associated with the product by the total cpm (product and quinolinic acid) on the paper strip. Because the specific activity of the labeled quinolinic acid used and the percentage of conversion of quinolinic acid to nicotinic acid mononucleotide were known, the amount of product formed could be calculated.

Nicotinic acid phosphoribosyltransferase activity was assayed by measuring the formation of ^{14}C nicotinic acid mononucleotide from 7-^{14}C-nicotinic acid. The assay mixture for measuring this enzymatic activity contained the following reagents at the final concentrations given in a total volume of 0.2 ml: 25 mM potassium phosphate, pH 7.0; 4 mM dithioerythritol; 20 mM tris-potassium phosphate, pH 8.0; 60 mM

ATP; 1.24 mM PRPP; and 10 to 30 milliunits of enzyme. The reaction was started by adding 7-^{14}C-nicotinic acid (6.8 μc/μmole) to give a final concentration of 0.148 mM, and was allowed to proceed for 2 hr with shaking in a 30 C water bath. The reaction was stopped by heating the flask in a boiling water bath for 1 min. Authentic nicotinic acid and trigonelline (4 μmoles each) were added to the assay mixture before the precipitated protein was removed by centrifugation. Trigonelline was a side product and had to be accounted for in order to determine the percentage of nicotinic acid mononucleotide formed. From this point on, the assay solutions were treated in the same way as in the chromatographic measurement of quinolinic acid phosphoribosyltransferase activity.

Nicotinamide deamidase activity was determined by measuring the formation of 7-^{14}C-nicotinic acid from 7-^{14}C-nicotinamide. The reaction mixture used to measure this activity contained the following reagents at the final concentrations given in a total volume of 0.15 ml: 36 mM potassium phosphate, pH 7.0; 7.3 mM dithioerythritol; and approximately 1 to 50 units of enzyme. The reaction was started by the addition of 7-^{14}C-nicotinamide (5.13 μc/μmole) to give a final concentration of 0.73 mM and was allowed to proceed for 1 hr in a shaking water bath at 30 C. The reaction was stopped by heating the flask in a boiling water bath for 2 min. Nicotinic acid and nicotinamide (4 μmoles each) were added to the assay mixture and the precipitated protein was removed by centrifugation. Approximately 10 μl of each assay solution was then spotted on Whatman No. 1 paper and developed by descending chromatography in the upper phase of 1-butanol-acetone-water (9:1:10 v/v). The products separated by chromatography were then detected, and the extent of conversion of substrate to product was measured in the same way as was described in the assay procedure for quinolinic acid phosphoribosyltransferase activity.

Ricinine Determination. Ricinine was extracted from castor beans by homogenizing the plant material in a Sorvall Omnimixer with 5 ml hot water (approximately 80 C) per g of tissue for 2 min at top speed. The homogenate was then filtered and the residue was re-extracted with hot water and filtered. The combined filtrates were then extracted with one-fourth volume of diethyl ether at least three times, or until no more lipid could be removed. The aqueous phase was concentrated almost to dryness on a rotary evaporator and then diluted to volume with water in either a 10 or 25 ml volumetric flask. An aliquot (500 μl) was streaked on Whatman No. 1 paper, 22.5 × 8.5 in, and the chromatogram was developed overnight in 1-butanol-acetic acid-water (4-1-2 v/v). After examining the chromatogram with a Mineralight UVS·II lamp, the fluorescent band at the R_F of authentic ricinine was eluted with water by means of spin-thimbles (Reeve Angel Company, New York, N. Y.). The eluted ricinine solution was adjusted to a volume of 10 ml and the absorbance of an aliquot was measured at 260 nm. The amount of ricinine was determined from a Beer's Law plot of varying concentrations of authentic ricinine versus absorbance at 260 nm. The UV spectrum of the isolated material was identical with the spectrum of authentic ricinine. Since paper chromatography was used to isolate the ricinine, interfering ultraviolet absorbing compounds were eliminated.

Protein Determination. Protein determinations were made using the method of Lowry et al. (12).

Synthesis of 2,3,7,8-[14]C Quinolinic Acid. Specifically labeled 2,3,7,8-^{14}C-quinolinic acid was synthesized by the method of Gholson et al., (5) and modified by Fleeker and Byerrum as follows. Glycerol was condensed with uniformly labeled ^{14}C-aniline to form ^{14}C-quinoline, which was then

oxidized to ¹⁴C-quinolinic acid by means of an alkaline permanganate oxidation. The following reagents were added in order: 87 mg of pulverized FeSO₄·7 H₂O, 0.84 ml of anhydrous glycerol, 227 mg of redistilled aniline, 3.23 mg of uniformly labeled ¹⁴C-aniline (0.25 mc), 0.18 ml of redistilled nitrobenzene, and 0.48 ml of concentrated H₂SO₄. The flask, equipped with an air-coiled reflex condenser, was placed in a 140 C Wood's metal bath for 6 hr. The reaction mixture was then steam-distilled to remove all the nitrobenzene. After the addition of 9 ml of 40% KOH, the reaction mixture was steam distilled again. This time the distillate, which contained the ¹⁴C quinoline, was collected in 0.1 N NaOH, and 150 ml of 5% KMnO₄ were added dropwise. The flask containing the KMnO₄ solution was fitted with a water-cooled reflux condenser and placed in a steam bath for 15 hr. The MnO₂ formed was removed by filtration; absolute ethanol was used to convert excess KMnO₄ to MnO₂, which was again removed by filtering the solution. The solution was applied to a Dowex 1X8 (50-100 mesh) formate column (2.7 × 30 cm) and the quinolinic acid was eluted with a 1000 ml linear formic acid gradient (0 to 0.4 M). The quinolinic acid fraction was evaporated to dryness on a rotary evaporator and recrystallized from water to give a 31% yield. One-fourth the total radioactivity was located in the α-carboxyl group of quinolinic acid as was shown by decarboxylation of the quinolinic acid at 165 C in cyclohexanol accompanied by collection, and counting of the ¹⁴CO₂ evolved. The quinolinic acid was radiochemically pure as determined by radioautography of paper chromatograms run in several solvent systems.

RESULTS AND DISCUSSION

Ricinine Biosynthesis. The ricinine content of castor bean seedlings at different stages of development was determined, as seen in Figure 2. Under our growth conditions, ricinine synthesis had started by day 4, and within a 4-day period, an average seedling contained between 4 and 5 mg (24 to 30 µmoles) of ricinine. In fact, 75% of the total ricinine was synthesized in just 2 days (days 6 and 7). Between days 7 and 8 there was a small decrease in the total quantity of ricinine per seedling in this experiment which might indicate that some destruction of the alkaloid occurred during this period. When the concentration of ricinine in the various tissues of the seedlings was examined, however (Fig. 3), it was observed that the concentration in the cotyledon increased daily during the 8-day period. The concentration in endosperm increased slightly
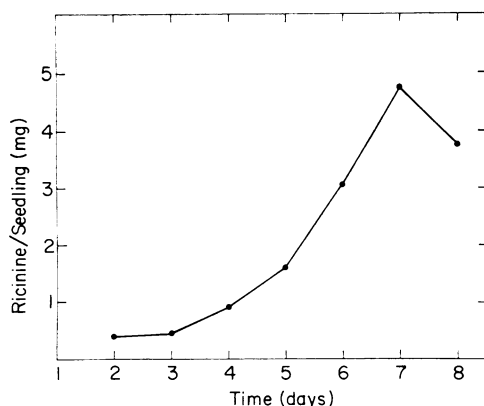

FIG. 2. Ricinine content of etiolated castor bean seedlings at different days of development. At least six seedlings were used for each determination; the experiment was repeated with similar results.
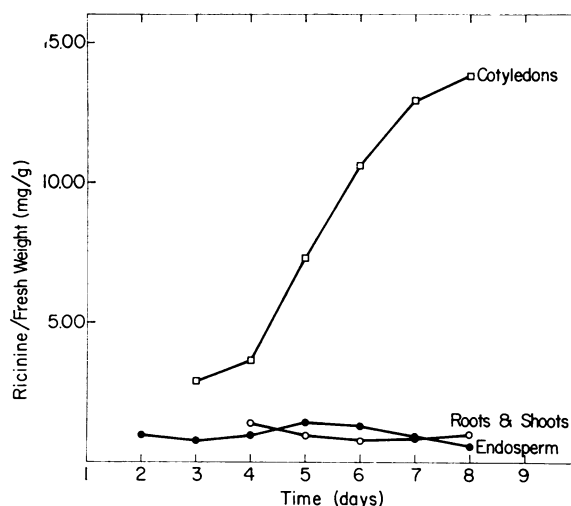

FIG. 3. Ricinine content of various organs of etiolated castor bean seedlings on different days after germination. At least seven seedlings were used for each determination.

between days 4 and 5 and then decreased as senescence of this tissue occurred. The concentration of ricinine in the remainder of the plant stayed at a low, more or less constant level during the experiment. Waller (19) found that ricinine biosynthesis started after 2 or 3 days of germination in tap water; however, he observed that when germination took place in distilled water or in a nutrient medium, the onset of ricinine synthesis was delayed until the 6th day of germination. In the present study ricinine synthesis started at approximately the same time regardless of whether germination had occurred in tap water or distilled water. The large amount of ricinine synthesized in a relatively short period of time suggests that the rate of synthesis of pyridine precursors for ricinine might also be elevated at this time. Evidence was therefore sought to support this hypothesis.

Levels of Quinolinic Acid Phosphoribosyltransferase, Nicotinic Acid Phosphoribosyltransferase, and Nicotinamide Deamidase in Etiolated Castor Bean Seedlings and Green Castor Bean Plants. The data depicted in Figure 4 are those of typical experiments in which only beans of a given state of physiological development were used, based on the criteria given in Table I. As seen in Figure 4A the level of quinolinic acid phosphoribosyltransferase activity increased sharply until the 5th day in the endosperm and, to a lesser degree, until the 6th day in the cotyledons. The activity decreased sharply after the 5th day in the endosperm and after the 6th day in the cotyledons; by the 7th day of development, the activity was approximately the same in both tissues. The decrease in enzyme activity in the endosperm was probably related to the senescence of this tissue, since the endosperm was no longer functional after approximately 6 days of development of the plant. The increase in quinolinic acid phosphoribosyltransferase activity preceded by about a day the rapid formation of ricinine shown in Figure 2.

In contrast, although present at a fairly constant but declining level, the activity of quinolinic acid phosphoribosyltransferase in the adult plant (Table II) was approximately 60-fold lower than in 5-day-old etiolated endosperm. The possible presence of inhibitors in adult plants was assessed by mixing the crude extracts of the adult plant and the etiolated seedlings, and no decrease in activity characteristic of etiolated seedlings was observed. The lower enzyme activity in the adult plant could be due to a diminished need for pyridine
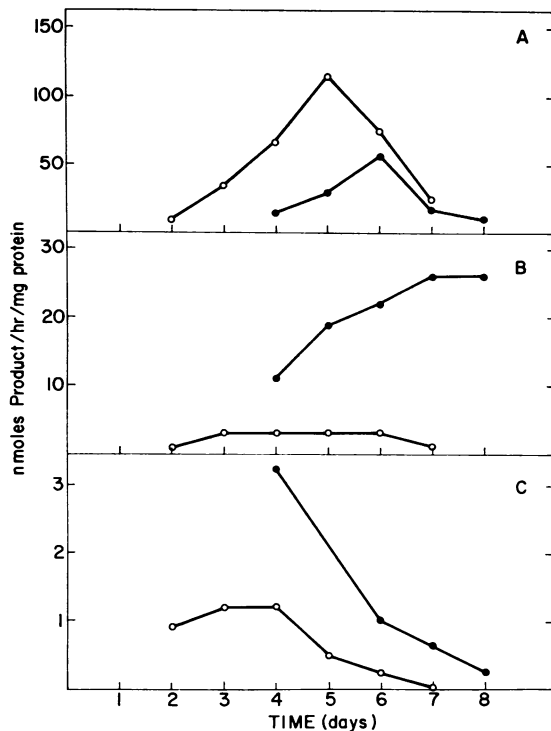
FIG. 4. Specific activity of (A) quinolinic acid phosphoribosyl-transferase, (B) nicotinamide deamidase, and (C) nicotinic acid phosphoribosyltransferase in the cotyledons (●) and endosperm (○) of etiolated castor bean seedlings as a function of days of development. In each case the values for the enzyme activities reported here were determined under conditions in which the enzymatic reaction was linear with respect to time and enzyme concentration. At least seven seedlings were used for each determination and the procedure was repeated twice with similar results.

Table II. *Specific Activity of Quinolinic Acid Phosphoribosyltransferase, Nicotinamide Deamidase, and Nicotinic Acid Phosphoribosyltransferase in Green Castor Bean Plants*

The plants were grown in the greenhouse under natural lighting. The age was based on the date at which the plants emerged from the soil in which they were grown. All of the leaves from two plants were used to prepare the plant homogenate to be used for enzyme assays, except in the case of the 12-week-old plants, in which only two leaves were used.

Enzyme	Enzyme Activity				
	Wk 1	Wk 2	Wk 3	Wk 4	Wk 12
	nmoles product/hr·mg protein				
Quinolinic acid phosphoribo-syltransferase[1]	1.9	1.5	0.7	0.8	0.4
Nicotinamide deamidase[2]	26.5	16.8	40.5	32.0	24.2
Nicotinic acid phosphoribosyl-transferase[1]	1.1	1.0	0.1	0.1	0.1

[1] Values reported are for nicotinic acid mononucleotide formation.

[2] Values reported are for nicotinic acid formation.

compounds, since ricinine is synthesized to a lesser degree in the adult plant. Bogdachevskaya (2) has observed that the concentration of ricinine in the whole green plant (mg ricinine/g wet weight) increased up to 20 days, after which the absolute quantity of ricinine increased but the concentration of ricinine in the plant declined. *In vivo* work of Waller and Henderson (20) has shown that castor bean plants 3 to 5 inches in height were able to incorporate two to four times more radioactively labeled nicotinamide into ricinine than plants 7 to 9 inches in height.

In the endosperm of the etiolated seedling, the specific activity of nicotinamide deamidase (Fig. 4B) remained at a constant low level, which was approximately 8-fold lower than that found in the 7- and 8-day-old cotyledonary tissue as well as in the adult plant (Table II). The specific activity of nicotinamide deamidase in the cotyldeons increased at about the same time that ricinine accumulated in this tissue (Fig. 3). The specific activity remained constant between days 7 and 8 and was at approximately the same level as found in the adult green plant. NAD has been reported as a feedback inhibitor of nicotinamide deamidase in yeast and certain micro-organisms, thereby allowing it to control some of the reactions of the pyridine nucleotide cycle (3). We have also observed a similar inhibition by NAD in castor beans (unpublished data). The low specific activity of nicotinamide deamidase in the seedling at a time when the rate of synthesis of pyridine nucleotides by the *de novo* pathway is high may limit the flow of metabolites through the pyridine nucleotide cycle and result in funneling of prior intermediates in the cycle into ricinine biosynthesis.

The specific activity of nicotinic acid phosphoribosyl-transferase activity (Fig. 4C) in the endosperm remained fairly constant through day 4, but decreased as the tissue became nonfunctional. The specific activity of the enzyme in the cotyledons was highest on day 4; it then also decreased over the next 4-day period. The specific activities of nicotinic acid phosphoribosyltransferase in the endosperm and cotyledons of etiolated seedlings were all similar to the activities found in the green plant (Table II). This observation may indicate that the seedling does not reutilize any more nicotinic acid for pyridine nucleotide synthesis than the adult plant, even though the seedling has the capacity to form, via the *de novo* pathway, a great deal more nicotinic acid mononucleotide than the adult plant. Also, the lower specific activity of nicotinic acid phos-phoribosyltransferase in relation to the levels of quinolinic acid phosphoribosyltransferase activity in both the seedling and the adult plant probably explains the observation of Waller and co-workers (22, 26) that nicotinic acid is less efficient than quinolinic acid as a precursor of ricinine. These same workers (22) have also reported that nicotinic acid phosphoribosyl-transferase was 20-fold lower in specific activity than quinolinic acid phosphoribosyltransferase in the cotyledons of 7-day-old castor bean seedlings.

The large increase in specific activity of quinolinic acid phos-phoribosyltransferase just prior to an increased rate of synthesis of ricinine supports the hypothesis that the *de novo* pathway for pyridine nucleotide biosynthesis has been activated to supply precursors for ricinine biosynthesis. The initial increase in quinolinic acid phosphoribosyltransferase activity preceded the increased synthesis of ricinine by about 1 day, which might be expected if quinolinic acid phosphoribo-syltransferase activity were responsible for supplying a precursor for ricinine biosynthesis. Konno *et al.* (10) have also observed, in nine species of mycobacteria, similarly elevated levels of quinolinic acid phosphoribosyltransferase, which they were able to correlate with the amount of nicotinic acid found in the culture filtrate of these organisms.

The site of ricinine synthesis in castor bean seedlings is unknown. The alkaloid could be synthesized entirely in the cotyledons, the endosperm, the roots or in a combination of

these. As was seen in Figure 1, each etiolated castor bean seedling produced, on the average, 26 μmoles of ricinine in a 96-hr period—between days 3 and 7 of development—of which 19 μmoles were produced in the last 48 hr of this period. Calculations based on the activity (Table III) of quinolinic acid phosphoribosyltransferase in the cotyledons of a seedling suggest that this tissue can account for only about one-third of the total production of precursor needed for ricinine biosynthesis. However, calculations based on the activity per seedling of this same enzyme in the endosperm over a 3-day period (days 3–5) indicate that this tissue could produce approximately 69 μmoles of precursor during that time, more than enough to account for the ricinine produced.

Therefore if one assumes that a pyridine nucleotide cycle intermediate formed from quinolinic acid is a precursor for ricinine biosynthesis, the endosperm would be the major site of production of this intermediate. On the other hand, the endosperm is probably not the major site for the synthesis of ricinine, since a rapid accumulation of the alkaloid occurs in the cotyledons (Fig. 3) at the time the endosperm tissue is starting to disintegrate. Our data therefore would not seem to agree well with the original suggestion of James (9) that ricinine is translocated from the endosperm to the cotyledons with or without degradation. The observation that a substance accumulates in a given tissue does not necessarily prove, though, that the substance was synthesized in that tissue. Waller and Nakazawa (21) have found that the nicotinic acid content of the endosperm decreases while the nicotinic acid and ricinine content of the cotyledons increases in 7- to 8-day-old seedlings of different sizes. They suggest that nicotinic acid might be transported from the endosperm to the cotyledons where it could be used for ricinine synthesis.

Because of the high rate of de novo synthesis of pyridine compounds in castor bean seedlings, one might expect a large increase in the content of NAD, but the results of Yamamoto (25), who studied 5-day-old castor bean seedlings similar to those used in the present study, indicated that the concentrations of NAD and NADP (both oxidized and reduced forms) in both the endosperm and cotyledons are similar to those of other nonpyridine alkaloid containing seedlings of the same age. Therefore the level of NAD does not appear to be altered by the large increase in activity in the de novo pathways for pyridine nucleotide synthesis, at least in this one instance. The concentration of endogenous quinolinic acid is unknown; however, unpublished data from our laboratory indicate that there is probably a sufficient quantity of ribose 5-phosphate for PRPP biosynthesis to yield the amount of nicotinic acid mononucleotide synthesized (13).

Table III. *Total Activity per Seedling of Quinolinic Acid Phosphoribosyltransferase in the Cotyledons and Endosperm of Etiolated Castor Bean Seedlings*

Day	Activity per Seedling	
	Cotyledons	Endosperm
	nmoles nicotinic acid mononucleotide/hr·seedling	
2		117
3		378
4	18	837
5	57	1670
6	184	675
7	73	95
8	35	

Table IV. *Specific Activity of Quinolinic Acid Phosphoribosyltransferase, Nicotinic Acid Phosphoribosyltransferase, and Nicotinamide Deamidase in Tobacco, Soybeans, Sunflowers, Peas, and Spinach*

The tobacco plants were about 8 weeks old, whereas the soybeans, peas, and spinach were 4 weeks old. The sunflowers were 1 week old. The crude homogenates were prepared from the leaves unless stated otherwise.

Plant Source	Enzyme Activity		
	Quinolinic Acid phosphoribosyltransferase	Nicotinic acid phosphoribosyltransferase	Nicotinamide deamidase
	nmoles product/hr·mg protein		
Tobacco leaves	0.6	0.4	2.7
Tobacco roots	23.9	0.6	13.5
Tobacco leaves and roots	7.5	0.5	13.3
Soybean	0.3	3.1	5.2
Sunflower	0.3	0.2	1.5
Pea	0.2	3.3	14.7
Spinach	0.8	7.9	12.4

Tobacco Plants. Tobacco is another plant which produces a pyridine alkaloid, nicotine; therefore it might also be expected to have an elevated de novo pathway for pyridine nucleotide biosynthesis, as represented by the specific activity of quinolinic acid phosphoribosyltransferase. Dawson and co-workers (4) found that nicotine was produced principally in the roots and that the most rapid period of formation of the alkaloid was prior to flowering. It was therefore at this stage of development that we determined the level of the various enzymes in the roots and leaves of Nicotiana rustica. The results are given in Table IV along with similar measurements in a few other plants. These data show that the roots of tobacco plants contained considerably more quinolinic acid phosphoribosyltransferase activity than tobacco leaves or the leaves of the other plants surveyed. Therefore, in tobacco root tissue there is another correlation between an elevated quinolinic acid phosphoribosyltransferase activity and pyridine alkaloid synthesis. The value given for quinolinic acid phosphoribosyltransferase activity in the tobacco leaf is probably lower than actually exists in vivo, because when the 27,000g supernatant of a leaf extract was used as the grinding medium for the tobacco root enzyme, there was a 68% loss of root enzyme activity. This loss may be due to chlorogenic acid, which is present in high concentrations in tobacco leaves (1). As seen in Table IV, the activity of the de novo pathway for pyridine biosynthesis as represented by quinolinic acid phosphoribosyltransferase is about the same or lower in all plants in comparison with nicotinic acid phosphoribosyltransferase activity except in tobacco and, as was shown previously, in castor bean, two plants which synthesize pyridine alkaloids. It would appear in these instances that the de novo pathway has been activated in order to supply the extra pyridine compounds needed for alkaloid production while maintaining adequate NAD and NADP levels.

LITERATURE CITED

1. ANDERSON, J. W. 1968. Extraction of enzymes and subcellular organelles from plant tissues. Phytochemistry 7: 1973–1988.

2. BOGDACHEVSKAYA, O. V. 1952. Formation of ricinine in the castor plant. Doklady Akad. Nauk. S. S. S. R. 82: 1001–1003.

3. CALBREATH, D. F. AND J. G. JOSHI. 1971. Inhibition of nicotinamidase by nicotinamide adenine dinucleotide. J. Biol. Chem. 246: 4334–4339.

4. DAWSON, R. F. 1948. Alkaloid biogenesis. In: F. F. Nord, ed., Advances in Enzymology, Vol. 8. Interscience Publishers, Inc., New York. pp. 203–251.

5. GHOLSON, R. K., I. UEDA, N. OGASAWARA, AND L. M. HENDERSON. 1964. The enzymatic conversion of quinolinate to nicotinic acid mononucleotide in mammalian liver. J. Biol. Chem. 239: 1208–1214.

6. GODAVARI, H. R. AND E. R. WAYGOOD. 1970. Nicotinamide adenine dinucleotide metabolism in plants. Can. J. Bot. 48: 2267–2278.

7. HADWIGER, L. A., S. E. BADIEI, G. R. WALLER, AND R. K. GHOLSON. 1963. Quinolinic acid as a precursor of nicotinic acid and its derivatives in plants. Biochem. Biophys. Res. Commun. 13: 466–471.

8. HENDERSON, L. M., J. F. SOMEROSKI, D. R. RAO, P. L. WU, T. GRIFFITH, AND R. U. BYERRUM. 1959. Lack of tryptophan-niacin relationship in corn and tobacco. J. Biol. Chem. 234: 93–95.

9. JAMES, W. O. 1950. Alkaloids in the plant. In: R. H. F. Manske and H. L. Holmes, eds., The Alkaloids, Vol. 1. Academic Press, New York. pp. 15–90.

10. KONNO, K., K. OIZUMI, AND S. OKA. 1965. Biosynthesis of niacin ribonucleotide from quinolinic acid by mycobacteria. Nature 205: 874–875.

11. LEETE, E. AND F. H. B. LEITZ. 1957. Biogenesis of ricinine. Chem. Ind. (London) 1957: 1572.

12. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.

13. MANN, D. B. 1973. Aspects of NAD biosynthesis in castor bean endosperm. Master's thesis. Michigan State University, E. Lansing.

14. MIZUSAKI, S., Y. TANABE, M. NOGUCHI, AND E. TAMAKI. 1973. Changes in the activities of ornithine decarboxylase, putrescine N-methyl transferase

and N-methyl putrescine oxidase in tobacco roots in relation to nicotine biosynthesis. Plant Cell Physiol. 14: 103–110.

15. NISHIZUKA, Y. AND O. HAYAISHI. 1963. Enzymatic synthesis of niacin nucleoides from 3-hydroxyanthranilic acid in mammalian liver. J. Biol. Chem. 238: PC483–485.

16. PREISS, J. AND P. HANDLER. 1958. Biosynthesis of diphosphopyridine nucleotide. J. Biol. Chem. 233: 493–500.

17. ROBBERS, J. E., L. W. ROBBERTSON, K. M. HORNEMANN, A. JINDRA, AND H. G. FLOSS. 1972. Physiological studies on ergot. J. Bacteriol. 112: 791–796.

18. RYRIE, I. J., AND K. J. SCOTT. 1969. Nicotinate, quinolinate, and nicotinamide as precursors in the biosynthesis of nicotinamide-adenine dinucleotide in barley. Biochem. J. 115: 679–685.

19. WALLER, G. R. 1961. Biosynthesis of the alkaloid ricinine in the castor bean plant Ricinus communis L. Ph.D. thesis. Oklahoma State University, Stillwater.

20. WALLER, G. R. AND L. M. HENDERSON. 1961. Biosynthesis of the pyridine ring of ricinine. J. Biol. Chem. 236: 1186–1191.

21. WALLER, G. R. AND K. NAKAZAWA. 1963. Nicotinic acid-ricinine relationship in sterile cultures of Ricinus communis L. Plant Physiol. 38: 318–322.

22. WALLER. G. R., K. S. YANG, R. K. GHOLSON, L. A. HADWIGER, AND S. CHAYKIN. 1966. The pyridine nucleotide cycle and its role in the biosynthesis of ricinine by Ricinus communis L. J. Biol. Chem. 241: 4411–4418.

23. WEEVERS, T. 1934. De vorming van alkaloiden in de plant. Chemisch Weekblad. 31: 19–24.

24. YAMAMOTO, Y. 1963. Pyridine nucleotide content in the higher plant. Plant Physiol. 38: 45–54.

25. YANG, K. S., R. K. GHOLSON, AND G. R. WALLER. 1965. Studies on nicotine biosynthesis. J. Amer. Chem. Soc. 87: 4184–4188.

26. YANG, K. S. AND G. R. WALLER. 1965. Biosynthesis of the pyridine ring of ricinine from quinolinic acid, glycerol and aspartic acid. Phytochemistry 4: 881–889.