QUINOLINIC ACID AS A PRECURSOR OF NICOTINIC ACID AND ITS DERIVATIVES IN PLANTS[1]

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The conversion of tryptophan to "niacin" (more accurately - niacin derivatives) is well established in mammals (Krehl, Teply, Sarma and Elvehjem, 1945) and in Neurospora (Beadle, Mitchell and Nyc, 1947). Recent data (Nishizuka and Hayaishi, 1963 and Gholson, Ueda, Ogasawara and Henderson, 1963) indicate that quinolinic acid is an intermediate in the conversion. In contrast, tryptophan is not a precursor of niacin or its derivatives in plants (Henderson, Someroski, Rao, Wu, Griffith and Byerrum, 1959) or in E. coli and Bacillus subtilis (Yanofsky, 1954).

In castor plants succinic acid and glycerol are precursors of the ring carbons of the pyridine alkaloid ricinine (Juby and Marion, 1963; Essery, Juby, Marion and Trumbull, 1962; Waller and Yang, 1963) as is nicotinic acid (Leete and Leitz, 1957). Glycerol and succinic acid are also precursors of the pyridine ring of niacin in E. coli (Ortega and Brown, 1960). The labeling patterns found in ricinine formed from succinate-C^{14} and glycerol-C^{14} suggested that the condensation of a three carbon unit with a four carbon dicarboxylic acid might form quinolinic acid; which could then be decarboxylated to form niacin and ultimately ricinine (Yang, 1963). Accordingly, an investigation of the metabolism of quinolinic acid in plants was begun. While this work was in progress a report of the formation of nicotinic acid mononucleotide from quinolinic acid by E. coli appeared (Andreoli, Ikeda, Nishizuka and Hayaishi, 1963).

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EXPERIMENTS IN VIVO

Franklin Yellow Dent corn was germinated in one liter Ehrlenmeyer flasks (Henderson, Someroski, Rao, Wu, Griffith and Byerrum, 1959). After germination, quinolinic acid-2,3,7,8-C^{14}[2], 1.67 mg (10^6 cpm) in 5 ml of water was added to each flask containing 15 kernels of corn. Four days later the embryos were separated from the seed, washed and lyophilized. Twenty embryos were powdered in a mortar with liquid nitrogen; the powder was extracted for 15 minutes with each of two 50 ml volumes of boiling absolute ethanol. The ethanol was removed under vacuum, the residue taken up in 50 ml of water, the pH adjusted to 7.4 and the solution placed on a Dowex-1 X 8 formate column (2.5 X 20 cm). Upon elution with increasing concentrations of formic acid, four major radioactive peaks were obtained (Figure 1): Peak I was trigonelline as indicated by the addition of carrier, the formation of trigonelline picrate and recrystallization to constant specific activity[3]. Peak II is as yet unidentified. Peaks III and IV were nicotinic acid and quinolinic acid, respectively, as shown by paper chromatography using known compounds and five different solvent systems[4]. These data indicate that quinolinic acid can be converted to niacin in corn.

Mature castor plants[5] (Ricinus communis) were injected with quinolinic acid-2,3,7,8-C^{14} and ricinine isolated. Twenty-five percent of the administered quinolinic acid was incorporated into the alkaloid with 814 fold dilution. Nicotinic acid-7-C^{14} under the same conditions was

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2. The synthesis of quinolinic acid-2,3,7,8-C^{14} containing one fourth the total activity in the β carboxyl is described elsewhere (Gholson, Ueda, Ogasawara, and Henderson, 1963).

3. Trigonelline picrate was recrystallized five times from absolute ethanol to constant specific activity, 99, 82, 85, 75, 80 mμc/mmole.

4. (A) 85% Isopropanol, (B) 60% propanol, (C) Butanol:water:acetic acid (25:25:6), (D) Butanol saturated with 15% NH_4OH and (E) 7 parts of 95% ethanol to 3 parts of 1M ammonium acetate, adjusted to pH 5.0 with HCl.

5. Yang, K. S. and G. R. Waller, unpublished observations.

diluted about 15,000 fold with 8,0 percent of the administered nicotinic acid incorporated. These results suggest that quinolinic acid is incorporated into ricinine without going through free nicotinic acid.

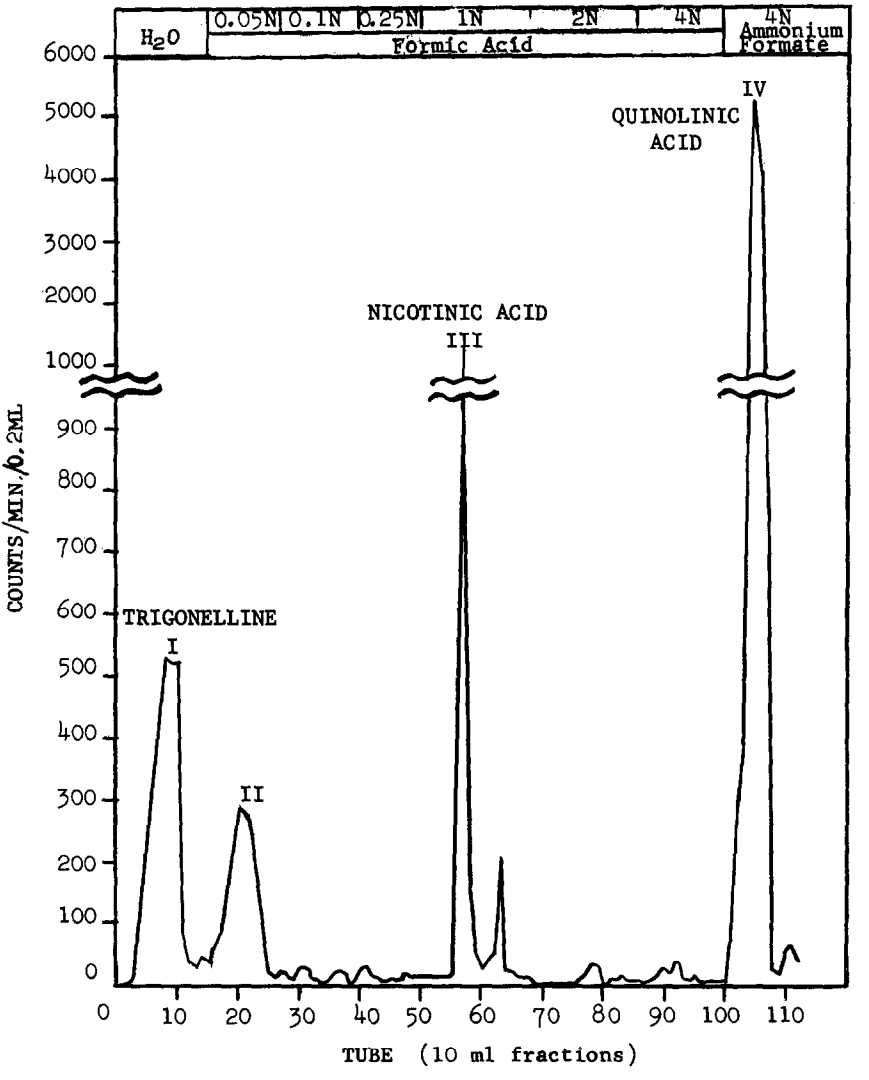

Fig. 1. Quinolinic acid-2,3,7,8-C^{14} and products of its in vivo conversion in corn seedlings separated on a Dowex-1 X 8 column.

EXPERIMENTS IN VITRO

In homogenates of castor seedlings, quinolinic acid is converted to nicotinic acid mononucleotide in a 5-phosphoribosyl-1-pyrophosphate (PRPP) dependent reaction.

Castor seedlings were grown in sterile sand for six days, the cotyledons harvested, and the following steps performed at 0-4° C. Twenty grams of cotyledon tissue were ground with sand in a mortar and extracted with 35 ml of 0.01M potassium phosphate pH 7.4. This preparation was filtered through cheesecloth, adjusted to pH 7.4 and centrifuged at 27,000 X g for 20 minutes; the enzyme activity remained in the supernatant fraction. The reactants (see Table I) were incubated in evacuated Thunberg tubes whose side arms contained 1N NaOH as a CO_2 trap. The reaction was stopped with 0.3 ml of 30% perchloric acid and the tubes allowed to stand for one hour, aliquots of the NaOH were removed and assayed in a Packard Tricarb liquid scintillation spectrometer. The data in Table I show that PRPP is required for decarboxylation of quinolinate and that ATP inhibits this decarboxylation.

TABLE I

Factors Affecting Decarboxylation of Quinolinic Acid

One ml of the enzyme preparation was incubated for 1 hour at 30° C in a total volume of 1.6 ml containing in μmoles: phosphate buffer pH 7.4, 600; quinolinic acid-2,3,7,8-C^{14} (4.9 X 10^4 cpm) 0.31; and additions as noted.

Additions in μmoles	$C^{14}O_2$ counts/min
1. $MgCl_2$ (1)	0
2. PRPP* (0.6)	6,633
3. PRPP* (0.6) + $MgCl_2$ (1)	7,119
4. PRPP* (0.6) + $MgCl_2$ (1) + Nicotinic Acid (3)	6,363
5. PRPP* (0.6) + $MgCl_2$ (1) + ATP (40)	1,638

*Magnesium salt

The product(s) of this PRPP dependent decarboxylation of quinolinate were identified by use of a large scale reaction mixture subjected to a Dowex-1 X 10 formate column (Figure 2). Three radioactive peaks were found

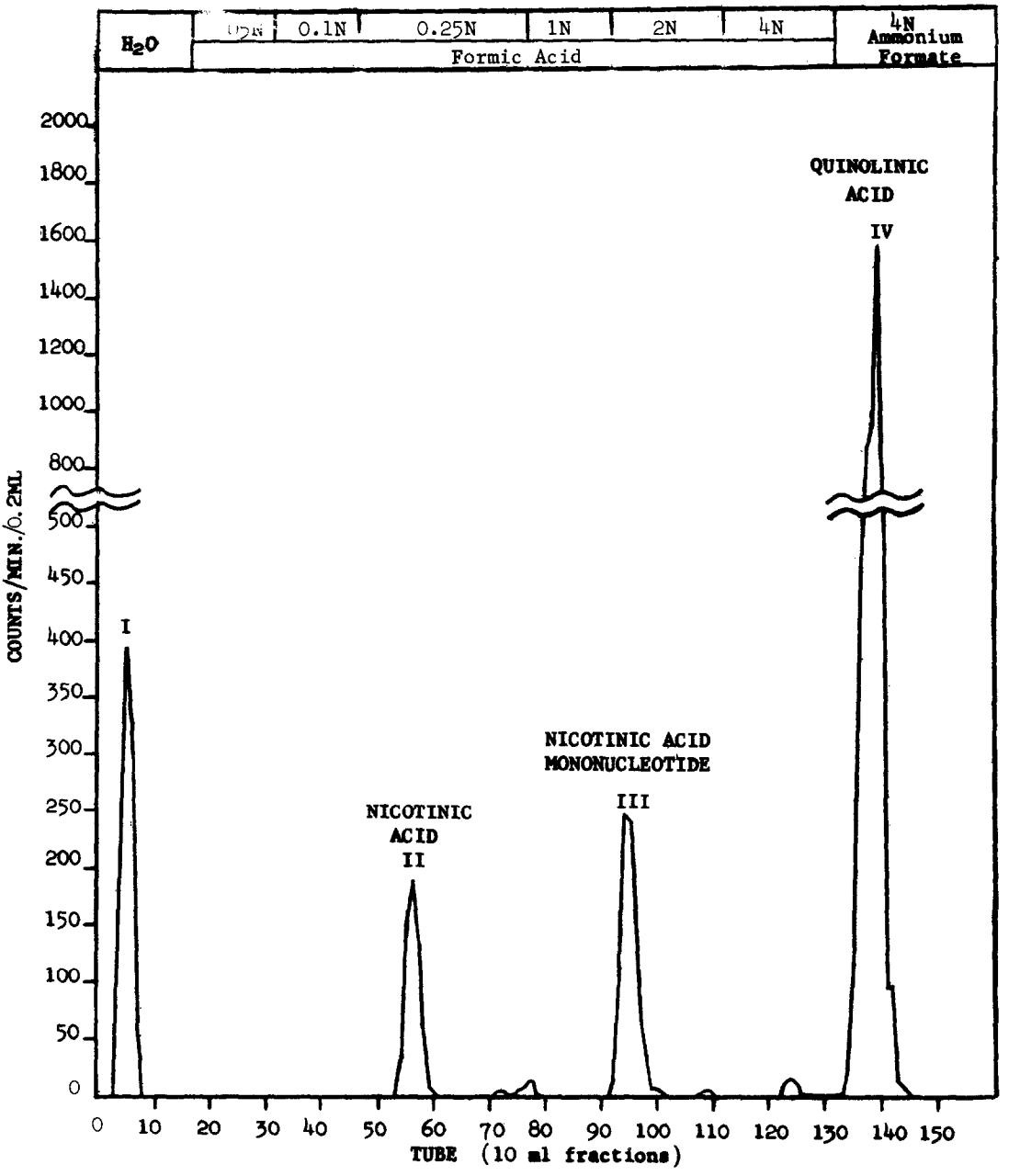

Fig. 2. Dowex 1-chromatography of carbon-^{14} labeled compounds isolated from a reaction mixture containing 3.11 μmole of quinolinic acid-2,3,7,8-C^{14} (4.94 X 10^5 cpm), 3 mmoles of phosphate buffer, pH 7.4, 3 μmoles of PRPP, 5 μmoles of $MgCl_2$ and 10 ml of the enzyme preparation incubated for 4 hours at 30°.

in addition to quinolinic acid. Peak 3 (69,000 cpm) was identified as nicotinic acid mononucleotide by paper chromatography (see footnote 4) and upon

hydrolysis in 0.1N NaOH for 30 minutes at 100° C produced radioactive nicotinic acid. Peak II (30,900 cpm) was nicotinic acid as indicated by paper chromatography. Peak I gave rise to nicotinic acid upon hydrolysis in 0.1N NaOH for 30 minutes at 100° C. With an incubation time of only one hour nicotinic acid mononucleotide was the only major radioactive compound formed. It is therefore assumed that Peaks I and II arise from further reactions of nicotinic acid mononucleotide. The results presented here, together with the previous findings in mammals (Nishizuka and Hayaishi, 1963 and Gholson, Ueda, Ogasawara and Henderson, 1963) and bacteria (Andreoli, Ikeda, Nishizuka and Hayaishi, 1963) suggest that quinolinic acid, although it arises by different pathways in different organisms, may be a universal intermediate in the biosynthesis of pyridine ring compounds.

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