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J. Amer. Soc. Hort. Sci. 112(3):529–535. 1987.

Nucleotide Metabolism in 'Washington' Navel Orange Fruit: I. Pathways of Synthesis and Catabolism

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Additional index words. *Citrus sinensis*, adenine nucleotides, uridine nucleotides, fruit crops

Abstract. **The capacity of 'Washington' navel orange fruit [*Citrus sinensis* (L.) Osbeck] to synthesize and catabolize purines and pyrimidines was assessed. *De novo* biosynthesis of purine nucleotide was demonstrated by [^{14}C] bicarbonate incorporation into purine nucleotides, blockage of this process by four known inhibitors, and assimilation of radiolabeled carbon from formate, both carbons of glycine, and carbon-3 of serine into the adenine ring. *De novo* synthesis of pyrimidines via the orotate pathway in young fruit was demonstrated by incorporation of [^{14}C] bicarbonate and [6-^{14}C]orotic acid into uridine nucleotides, release of $^{14}CO_2$ from [7-^{14}C]orotic acid, and blockage of these processes by 6-azauridine. Synthesis of purine and pyrimidine nucleotides via salvage reactions was demonstrated by incorporation of radiolabeled bases and ribonucleosides into nucleotides and into nucleic acids. Release of $^{14}CO_2$ from radiolabeled adenine, adenosine, hypoxanthine, and xanthine, uric acid, urea (purines), uracil, and uridine (pyrimidines) provided evidence the pathways for catabolism (degradation) of purines and pyrimidines in navel orange fruit are similar to those found in microorganisms and animal tissues. To the best of our knowledge, this report is the first to assess the capacity of anabolic and catabolic pathways of purine and pyrimidine nucleotide metabolism in fruit of any species. *De novo* synthetic activities in orange fruit permit increases in the pools of purine and pyrimidine nucleotides using simple precursors. Further, the patterns of salvage and catabolism suggest riboside pools are reused predominantly as nucleotides, while the majority of base pools are degraded to permit recycling of carbon and nitrogen into other metabolites.**

Purine and pyrimidine nucleotides function as substrates, substrate activators, enzyme cofactors, and energy transducers and thus influence nearly every metabolic pathway of a cell. An inverse relationship between ATP and the organic acid content of juice from mature citrus fruit has been demonstrated (4). In addition, flavor is influenced by nucleotides (3, 15). Thus, availability of nucleotides can affect the development and commercial quality of fruit.

Relatively little is known about the capacity of plant cells to provide nucleotides to meet their varied and changing needs. Two types of synthetic pathways have been identified in microorganisms and animal cells: *de novo* (synthesis of the ring from simple precursors), and salvage (reuse of existing bases and ribonucleosides) (19). Nucleotide synthesis through reuse of existing purine (6, 18) and pyrimidine (1, 9, 13, 20) ribonucleosides and bases has been demonstrated in various tissues from several vascular plants. Activity of the entire orotate pathway for *de novo* biosynthesis of pyrimidine nucleotides (14) and of the latter part of this pathway (1, 9, 14) has been demonstrated in plant tissues. *De novo* synthesis of purine nucleotides has been demonstrated in some plant tissues (2, 12, 21).

Nucleotide metabolism has not been studied previously in fruit of any species. In suspension-cultured cells, increased activity of pyrimidine nucleotide biossynthesis has been associated with growth by cell division (9). Growth of sweet orange fruit proceeds in three distinct phases (7), permitting study of nucleotide synthetic and catabolic activities in relation to growth and development. The objective of research reported here was to identify the pathways by which navel orange fruit synthesize and catabolize nucleotides. Changes in the capacities of these pathways in relation to stage of fruit development and rate of fruit growth are reported in a companion paper (22).

Materials and Methods

Fruit were obtained from 20-year-old 'Washington' navel orange budded to 'Troyer' citrange rootstock [*C. sinensis* x *Pon-*

Received for publication 21 July 1986. This paper represents a portion of the dissertation submitted by P.T.T. in partial fulfillment of requirements for the PhD degree in Botany at Univ. of California. Supported by a Univ. of California Chancellor's Patent Fund grant to P.T.T. and a Statewide Critical Applied Research grant to C.J.L. Use of a company or product name does not imply approval or recommendation of the product to the exclusion of others which may also be suitable. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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cirus trifoliata (L.) Raf.]. Trees were located at the Citrus Research Center and Agricultural Experiment Station, Univ. of California, Riverside. Rooted cuttings were initiated from the most recently matured growth flush of the orchard trees (17) to increase the availability of fruit throughout the year. Cuttings were induced to flower and set fruit in growth chambers as described by Moss (16, 17).

A minimum of eight fruit (transverse diameter specified in text and tables) were taken at random around the orchard trees or from cuttings for each replicate. Fruit were washed in cool tap water containing liquid dishwashing detergent, disinfested 20 to 30 min in 5% chlorine bleach solution, rinsed well with distilled water, and sectioned under 50 mM Hepes buffer, pH 8.5. Stem and stylar ends of each fruit were discarded. The remaining midsection or peel tissue isolated from it was sliced in the radial plane to obtain whole-fruit or peel tissue, respectively (Fig. 1). Tissue slices were pooled during slicing procedures (30 to 45 min). Aliquots of 500 mg (fresh weight) (five to eight slices) were quickly blotted, weighed, and remoistened with buffer until all aliquots were weighed.

Determination of radioisotopic content and statistical analysis. Samples, prepared as described later, were diluted with 6.5 ml Liquiscint (scintillation cocktail; National Diagnostics, Somerville, N.J.) per 2-ml sample and counted on a Beckman LS-100 liquid scintillation spectrometer. Each datum was corrected for quenching by an external standardization system of quench analysis.

Data are presented as mean ± SE with number of replicates (N) given in parentheses. Each datum represents net incorporation over nonenzymic (background) incorporation measured using heat-inactivated tissue. All data are expressed on a dry-weight basis.

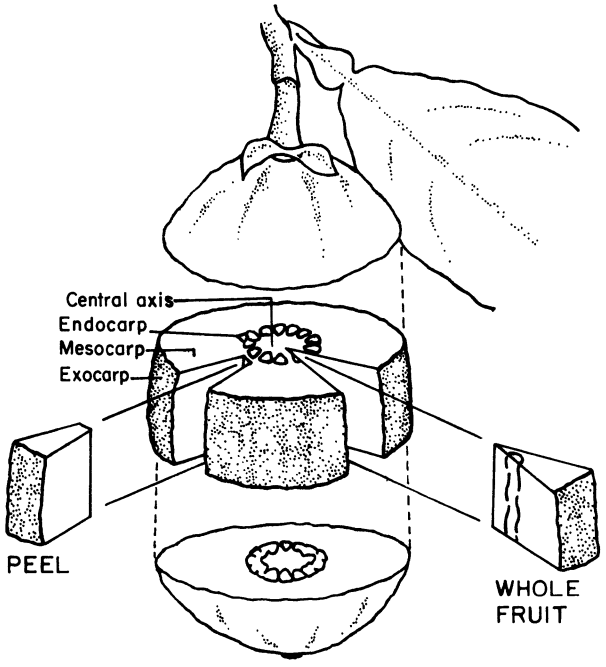

Fig. 1. Diagram of young 'Washington' navel orange fruit, ≈20 mm diameter showing tissues used in assays. Peel tissue contained outer mesocarp and exocarp tissues of the fruit midsection; whole-fruit tissue contained all tissues (central axis, endocarp, mesocarp, and exocarp) of fruit midsection in proportion to their cross-sectional representation in fruit. The same tissues are present in different proportions in fruit 55 mm in diameter.

Incorporation of radiolabeled precursors. Tissue aliquots were incubated either 3 or 5 hr at 30°C in sealed 25-ml Erlenmeyer flasks containing 5 ml of 50 mM Hepes buffer (pH 8.5) plus supplements (see below). Units accompanying each datum indicate incubation duration. Three-hour incubations were preceded by a 2-hr preincubation in 5 ml of the incubation media minus the ^{14}C substrate. Incubations used fresh buffer supplemented with one of the following radiolabeled precursors at the final concentration and specific radioactivity indicated: 5 or 20 mM $NaH^{14}CO_3$ (15,000 or 3200 dpm/nmol, respectively); 5 mM $Na^{14}COOH$ (4000 dpm/nmol); 5 mM [1- or 2-^{14}C]glycine (450 dpm/nmol); 5mM [3-^{14}C]serine (450 dpm/nmol); 2 mM [8- or 6-^{14}C]adenine (1200 dpm/nmol); 2 mM [8-^{14}C]adenosine (1200 dpm/nmol); 2 mM [8-^{14}C]hypoxanthine (840 dpm/nmol); 0.4 mM [8-^{14}C]xanthine (6000 dpm/nmol); 0.4 mM [2-^{14}C]uric acid (270 dpm/nmol); 2 mM [^{14}C]urea (315 dpm/nmol); 2 mM [2-^{14}C]uracil (1770 dpm/nmol); 2 mM [2-^{14}C]uridine (1100 dpm/nmol); 2 mM [6-^{14}C]orotic acid (1200 dpm/nmol); or 2 mM [7-^{14}C]orotic acid (20 to 200 dpm/nmol). Radiolabeled compounds were purchased from ICN (Irvine, Calif.), except [7-^{14}C]orotic acid, which was purchased from New England Nuclear.

The effect of glucose, glutamine, and nucleotide synthesis inhibitors on incorporation of precursors into nucleotide products was determined by supplementing preincubation and incubation media with the treatment compound at the final concentration given in the text or tables. Perchloric acid was purchased from Mallinckrodt. All other chemicals were purchased from Sigma, except for diaza-*oxo*-norleucine, which was a generous gift from a colleague.

Each reaction was terminated by injecting 1 ml of 6 N $HClO_4$ into the flask. Radiolabeled CO_2 distilling from the acidified incubation mixture was trapped (for quantification or safe disposal of unreacted [^{14}C]bicarbonate) in a plastic center well (Kontes Glassware, Vineland, N.J.) containing a filter paper wick and KOH as described previously (14).

Contents of the flask were homogenized with a Polytron tissue homogenizer equipped with a 2-cm probe (PCU-2, Brinkmann). The probe was rinsed with 1 N $HClO_4$. Acid-insoluble material was removed from the combined sample plus rinse by centrifugation at 12,000× *g* for 20 min at 0°C.

Incorporation of radiolabel from [6- or 8-^{14}C]adenine or [8-^{14}C]adenosine into acid-insoluble material provided an estimate of salvage plus subsequent incorporation into nucleic acids. Acid-insoluble material from the incubation mixtures employing these precursors was washed with 1 N $HClO_4$, extracted 1 hr at 4°C with acetone and collected by suction filtration. The radioisotopic content of insoluble material plus filter disk was determined in 4 ml of 0.5 N HCl.

Isolation of radiolabeled nucleotide products by co-crystallization. Acid-soluble fractions were neutralized with KOH and the resulting precipitate ($KClO_4$) removed by centrifugation prior to isolation procedures. Adenine nucleotides and adenosine synthesized *de novo* from radiolabeled precursors by fruit tissues were converted to adenine (ΣAde) by acid-hydrolysis at 100°C and isolated by co-crystallization from an aqueous solution saturated at the boiling point with commercial adenine (12). Inosine-monophosphate (IMP) synthesized by fruit tissue from [^{14}C]bicarbonate was converted to hypoxanthine and isolated with carrier hypoxanthine similarly (C.J. Lovatt, personal communication). Uridine nucleotides were converted by acid-hydrolysis to the monophosphate form (ΣUMP) and isolated from an ethanolic solution with carrier disodium UMP (14). Orotic acid was isolated by co-crystallization from an aqueous solution

with carrier sodium orotate (14). Metabolites isolated by co-crystallization with carrier were recrystallized to constant specific radioactivity.

Reliability of the co-crystallization procedures to assess pathway activity was verified by identifying [^{14}C]bicarbonate metabolites isolated by co-crystallization with carrier adenine, hypoxanthine, and UMP. Descending chromatography of ΣAde and ΣUMP crystals resulted in <10% change in the specific radioactivity of the crystals. Removal of hypoxanthine by treatment with commercial xanthine oxidase (E.C. 1.2.3.1; Sigma grade IV, from milk) followed by co-crystallization resulted in 94% less radioactivity compared to equivalent samples not enzymically treated. Further, known additions of commercial radiolabeled ATP, adenine, UTP, UMP, IMP, and hypoxanthine were recovered from incubation samples by co-crystallization with the appropriate carrier (adenine, UMP, or hypoxanthine) following acid hydrolysis.

Isolation of [^{14}C]adenine nucleotides by thin layer chromatography (TLC). Adenine nucleotides synthesized from radiolabeled adenosine or adenine were isolated from the neutralized acid-soluble supernatant fraction without prior hydrolysis by TLC on Eastman Kodak #13254 plastic-backed cellulose sheets using 1 M ammonium acetate (pH 7.5) (8). Standards run concurrently with the samples were localized with UV light and areas of adenine, adenosine, and total adenine nucleotides removed to vials containing 2 ml of 0.5 N HCl for determination of radioisotopic content. Reliability of this method was verified by use of a second solvent, 100 0.1 M phosphate buffer (pH 6.8) : 60 saturated ammonium sulfate : 2 *n*-propanol (by volume) (5). The radioisotopic content of the total adenine nucleotides isolated from duplicate samples developed in these two solvent systems differed by only 0.02%.

Results

Demonstration of *de novo* biosynthesis in citrus tissues

Purines. Fruit tissues incorporated into ΣAde radiolabel from bicarbonate, formate, glycine, and serine—the molecules providing the purine ring carbons in other organisms (Table 1). Data obtained for peel and for whole-fruit tissues were combined to permit statistical presentation. No consistent differences in the data were observed between the two types of tissues.

Table 1. Incorporation of precursors of the "classical" pathway for purine *de novo* synthesis into ΣAde[z] by 'Washington' navel orange tissues.[y]

Precursor	Incorporation (nmoles ΣAde/g dry wt during 3 hr)
[^{14}C]bicarbonate	37 ± 5 (8)[x]
[^{14}C]formate	18 ± 9 (4)
[1-^{14}C]glycine	503 ± 136 (3)
[2-^{14}C]glycine	742 ± 429 (3)
[3-^{14}C]serine	396 ± 232 (4)

[z]Sum of adenine nucleotides and adenosine acid-hydrolyzed to and isolated as adenine.

[y]Data pooled from experiments employing either whole-fruit tissue from fruit 10 to 25 mm in diameter or peel tissue isolated from fruit 55 mm in diameter.

[x]Mean ± SE of (N) replicates. Background (nonenzymic) incorporation, subtracted in each experiment, averaged 3%, 25%, 34%, 71%, and 59% of gross incorporation for each of the five precursors, respectively.

Incubation procedures were optimized only for [^{14}C]bicarbonate incorporation, hence comparison among precursors is not valid. Adenine nucleotides and adenosine synthesized from the five precursors were subjected to acid hydrolysis and isolated as adenine. Therefore, results presented in Table 1 confirm incorporation into the adenine ring. In addition, fruit tissue incorporated [^{14}C]bicarbonate into IMP, the first true purine nucleotide; i.e., in two separate experiments, 8 and 16 nmol of [^{14}C]bicarbonate were incorporated into IMP per g dry weight during 5 hr. Since [^{14}C]bicarbonate resulted in only a low level of background incorporation and is relatively inexpensive, all subsequent experiments employed this precursor.

Addition of known inhibitors of *de novo* purine biosynthesis effectively reduced incorporation of [^{14}C]bicarbonate into ΣAde by fruit tissue (Table 2). Azaserine (1 mM) also reduced [^{14}C]bicarbonate incorporation into IMP (88% and 100% inhibition). Azaserine and 6-diazo-5-*oxo*-L-norleucine inhibit one or both glutamine dependent reactions of the *de novo* pathway. Aminopterin and amethopterin inhibit synthesis of the single carbon carrier compound that donates carbons at two points in the pathway.

Pyrimidines. Fruit tissue incorporated [^{14}C]bicarbonate (precursor) and [6-^{14}C]orotic acid (late intermediate in orotate pathway) into ΣUMP and released $^{14}CO_2$ from [7-^{14}C]orotic acid (Table 3). In addition, orotic acid was isolated after incubation with [^{14}C]bicarbonate; i.e., in two separate experiments, 6 and 10 nmol [^{14}C]bicarbonate were incorporated into orotic acid per g dry weight during 5 hr. Addition of 6-azauridine, an inhibitor of *de novo* pyrimidine biosynthesis, effectively reduced incorporation of both [^{14}C]bicarbonate and [6-^{14}C]orotic acid into ΣUMP and release of $^{14}CO_2$ from [7-^{14}C]orotic acid (Table 3). Radiolabel isolated with carrier UMP was demonstrated to occur in the pyrimidine ring (data not shown).

Optimal conditions for measuring the incorporation of [^{14}C]-bicarbonate into ΣAde and ΣUMP. Assay flasks routinely contained 500 mg fresh weight tissue in 5 ml 50 mM Hepes buffer (pH 8.5). Both the pH and the molarity of the Hepes buffer used in the incubation medium were optimized (data not shown).

Table 2. Inhibition of [^{14}C]bicarbonate incorporation into adenine nucleotides plus adenosine converted to and isolated as adenine by known inhibitors of *de novo* purine synthesis.[z]

Inhibitor added	Concn (mM)	Inhibition (%)
Azaserine	1	77 ± 11 (6)[y]
	5	90 ± 4 (2)
	10	90 ± 4 (2)
	20	90 (1)
Diazo-*oxo*-norleucine	1	70 (1)
	2.5	87 (1)
	5	90 (1)
	10	98 (1)
Aminopterin	10	54 (1)
Amethopterin	26	65 (1)

[z]Data pooled from experiments employing either whole-fruit tissue from 'Washington' navel orange fruit 10 to 25 mm in diameter or peel tissue from fruit 55 mm in diameter. The average control value (no inhibitor added) was 64 ± 15 (N = 7) nmol [^{14}C]bicarbonate incorporated into adenine nucleotides plus adenosine acid hydrolyzed to and isolated as adenine per gram dry weight during 3 hr.

[y]Mean ± SE % inhibition of (N) replicates.

Table 3. Inhibition by 6-azauridine of [^{14}C]bicarbonate (precursor) and [6-^{14}C]orotic acid (intermediate) incorporation into ΣUMP[z] (product), and generation of $^{14}CO_2$ (product) from [7-^{14}C]orotic acid in 'Washington' navel orange fruit tissues.[y]

Additions	Precursor → product: [^{14}C]bicarbonate → ΣUMP	[6-^{14}C]orotic acid → ΣUMP	[7-^{14}C]orotic acid → CO_2
	Nmoles precursor incorporated into product/g dry wt during 3 or 5 hr[x]		
None (control)	42 ± 5 (9)[w]	237 ± 63 (4)	849 ± 112 (3)
	Inhibition (%)		
6-azauridine (mM)			
1	---[v]	---	9 ± 7 (3)
5	---	---	19 ± 7 (2)
10	19 (1)	76 ± 10 (3)	32 (1)
20	68 ± 23 (2)	79 ± 11 (3)	---

[z]Total of uridine nucleotides acid-hydrolyzed to and isolated as UMP.
[y]Data pooled from experiments employing peel or whole-fruit tissue from fruit 10 to 20 mm in diameter.
[x]Three hours incubation for [^{14}C]orotic acid, 5 hr for [^{14}C]bicarbonate.
[w]Mean ± SE of (N) replicates.
[v]Not determined.

Adding glutamine as a N source (10 mM) had no appreciable effect on [^{14}C]bicarbonate incorporation into ΣAde (120% ± 6% of control; N = 2) and only a slight effect on incorporation into ΣUMP (140% of control; N = 1). Supplementing the incubation medium with an energy source, 0.1 M glucose, did not stimulate [^{14}C]bicarbonate incorporation into either ΣAde or ΣUMP consistently; i.e., 89% ± 10% (N = 3) and 132% ± 50% (N = 4) of control, respectively. Capacity to incorporate [^{14}C]bicarbonate into ΣAde and ΣUMP was saturated at or below 20 mM bicarbonate (Fig. 2). To permit concurrent measurement of both purine and pyrimidine nucleotide *de novo* synthesis, 20 mM [^{14}C]bicarbonate was routinely used. Incorporation of 20 mM [^{14}C]bicarbonate into ΣAde or ΣUMP was linear for at least 5 hr (Fig. 3).

Salvage and catabolic pathways. Salvage of [^{14}C]purine ribonucleosides and bases into total adenine nucleotides (soluble pool) and nucleic acids (insoluble pool) occurred in young fruit tissue incubated with [6- or 8-^{14}C]adenine, or [8-^{14}C]adenosine (Table 4). By the classical pathway of purine degradation, occurrence of ^{14}C at position 6 results in the release of $^{14}CO_2$; however, radiolabel at position 8 of the purine ring or at position 2 of uric acid would result in radiolabeled urea. Thus, generation of $^{14}CO_2$ from these later positions would necessitate presence of urease activity. Release of $^{14}CO_2$ from [^{14}C]urea confirmed such activity (Table 4).

Salvage of pyrimidine bases and ribonucleosides by fruit was demonstrated by incorporation of [2-^{14}C]uracil and [2-^{14}C]uridine into ΣUMP (table 4). Release of $^{14}CO_2$ from [2-^{14}C]uracil, the substrate of the first committed step in the reductive degradation pathway (23), is consistent with operation of this pathway in fruit tissue (Table 4). Radiolabeled CO_2 was also released from [2-^{14}C]uridine (Table 4).

Uptake of radiolabeled ribonucleosides and bases. Salvage (soluble and insoluble pools) and catabolism of adenine were 2.8- and 340-fold greater, respectively, than those of adenosine (Table 4). Uptake of these two precursors was estimated to determine if either of these differences could be due to lower availability of adenosine in the tissue. It is not possible to prevent catabolism of ribonucleosides and bases during uptake experiments; therefore, the amount of $^{14}CO_2$ released during the 3-hr incubation was added to radioactivity present in the tissue. This sum was used to estimate cumulative uptake. Cumulative uptake of [8-^{14}C]adenine was 2.4-fold greater than that of [8-^{14}C]adenosine: i.e., 4360 nmol·g^{-1} dry weight as compared to 1830 nmol·g^{-1} dry weight, respectively. In contrast, the cumulative uptake of [2-^{14}C]uracil and [2-^{14}C]uridine was similar:

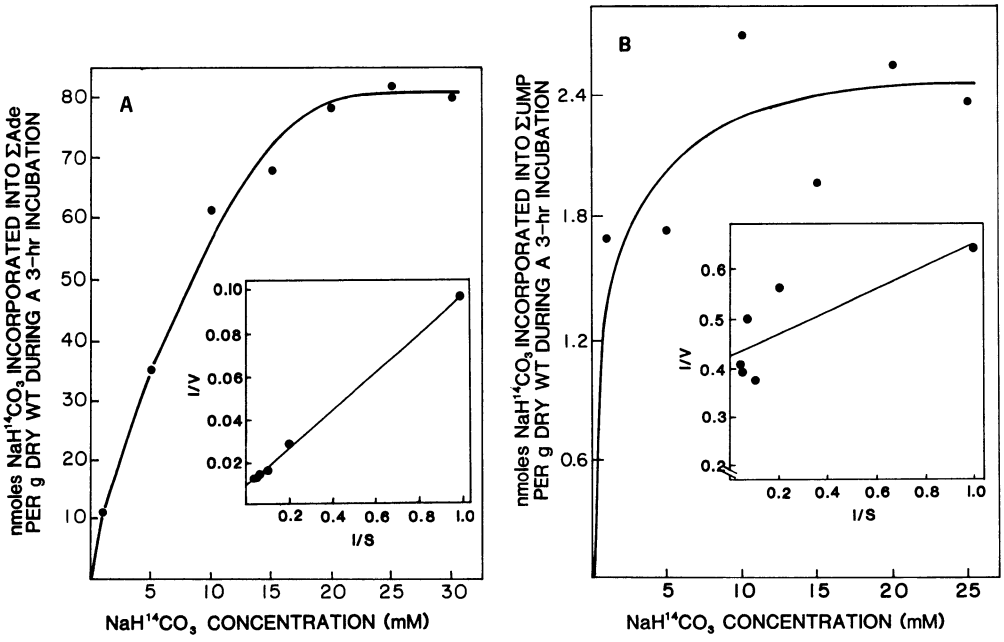

Fig. 2. Incorporation of [^{14}C]bicarbonate into **(A)** ΣAde (total adenine nucleotides plus adenosine converted to and isolated as adenine, a purine) and into **(B)** ΣUMP (total uridine nucleotides converted to and isolated as UMP, a pyrimidine) by whole-fruit tissue from fruit ≈4 mm in diameter, as a function of [^{14}C]bicarbonate concentration. Lineweaver–Burk (double reciprocal; see ref. 10) plots of the data are given in the insets. Linear regression analysis of these data yielded **(A)** $r^2 = 0.999$ ($P < 0.001$) and **(B)** $r^2 = 0.639$ ($P < 0.1$).

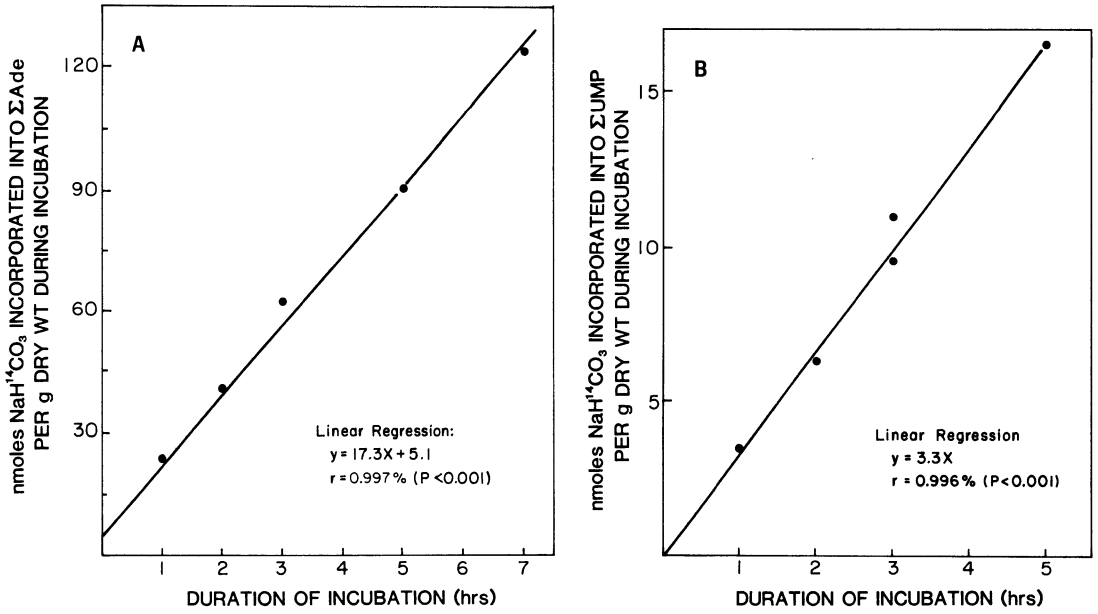

Fig. 3. Incorporation of 20 mM [^{14}C]bicarbonate into **(A)** ΣAde (total adenine nucleotides plus adenosine converted to and isolated as adenine) and **(B)** ΣUMP (total uridine nucleotides converted to and isolated as UMP) by whole-fruit tissue from fruit ≈4 mm in diameter, as a function of incubation duration.

Table 4. Salvage (resynthesis of nucleotides) and catabolism (breakdown, measured as release of CO_2) of purine and pyrimidine bases and ribonucleosides by whole-fruit tissue from 'Washington' navel orange fruit, 10 to 15 mm in diameter.

	Nmoles precursor incorporated into product/g dry wt during 5 hr		
	Product		
Precursor	Nucleotides[z]	Nucleic acids	CO_2
Purines			
[6-^{14}C]adenine	1177 ± 243 (2)[y]	846 ± 48 (2)	332 ± 70 (7)
[8-^{14}C]adenine	964 ± 156 (3)	646 ± 71 (3)	1610 ± 394 (3)
[8-^{14}C]adenosine	278 ± 13 (3)	291 ± 58 (3)	4.7 ± 3.2 (3)
[8-^{14}C]hypoxanthine	---	---	154 ± 30 (3)
[8-^{14}C]xanthine	---	---	7.3 ± 3.3 (3)
[2-^{14}C]uric acid	---	---	22 ± 2.4 (3)
[^{14}C]urea	---	---	1037 ± 99 (3)
Pyrimidines			
[2-^{14}C]uracil	56 ± 6 (3)	---	673 ± 17 (3)
[2-^{14}C]uridine	210 ± 40 (3)	---	149 ± 22 (3)

[z]Total adenine nucleotides for purine precursors; total uridine nucleotides acid-hydrolyzed to and isolated as UMP for pyrimidine precursors.
[y]Mean ± SE of (N) replicates.

i.e., 2242 nmol·g^{-1} dry weight as compared to 1713 nmol·g^{-1} dry weight, respectively.

Discussion

De novo synthesis. 'Washington' navel orange fruit were shown to provide purine and pyrmidine nucleotides through *de novo* synthesis in a manner consistent with operation of the "classical pathway" for *de novo* synthesis of purine nucleotides and the orotate pathway for *de novo* synthesis of pyrimidine nucleotides in fruit. The low level of incorporation of [^{14}C]bicarbonate into IMP compared to ΣAde and apparent greater sensitivity of the former incorporation to inhibition by azaserine are consistent with the occurrence of IMP as an intermediate in *de novo* synthesis with faster turnover rate and/or smaller pool size than ΣAde.

Activity of the pathway for *de novo* synthesis of purine nucleotides in 'Washington' navel orange fruit falls within the broad range of activities reported for this pathway in other plant tissues (2, 12, 18). Fruit tissue incorporated 20 nmol of [^{14}C]bicarbonate into ΣAde per g fresh weight during 5 hr. In contrast, the capacity of roots excised from 2-day-old *Cucurbita pepo* L. seedlings to incorporate [^{14}C]bicarbonate into ΣAde assessed by the same methods, was 244 nmol/g fresh weight during 3 hr (12). Cell-free extracts of nodules of nitrogen-fixing *Vigna unguiculata* (L.) Walp. displayed activity two orders of magnitude greater than that reported here (2). Rates reported for nodules from an amide-exporting legume (2) and for cotyledons and embryonic aces from 1- and 2-day-old *Phaseolus (Vigna) mungo* L. seedlings (18) are similar to those reported here.

Activity of pyrimidine *de novo* biosynthesis in fruit tissue, expressed as [^{14}C]bicarbonate assimilation into ΣUMP averaged 9.5 nmol/g fresh weight during 5 hr. In contrast, the capacity of roots excised from 2-day-old *C. pepo* seedlings, assessed by the same method, was 144 nmol/g fresh weight during 3 hr (14). However, [^{14}C]bicarbonate incorporation into ΣUMP by the distal 3 cm of the roots during hydroponic culture of 2-day-old *C. pepo* seedlings for 24 hr declined to 2.4 nmol/g fresh weight during 3 hr in a manner that directly paralleled the decline in root glucose content (11). Further, adding glucose to the incubation media stimulated orotate pathway activity (11). Pyrimidine nucleotide *de novo* biosynthetic activity in orange fruit tissue is similar to that for hydroponically grown squash roots, but is not stimulated by the addition of glucose.

Activity of the later part of the orotate pathway in cotyledons and embryonic axes from 2-day-old *P. mungo* seedlings was 0.3 to 0.4 nmol of [6-^{14}C]orotic acid assimilated into uridine nucleotides per cotyledon or axis (≈75 mg fresh weight) during 4 hr (1). In contrast, young navel orange fruit tissue incorporated 68 nmol of [6-^{14}C]orotic acid into ΣUMP per g fresh weight during 3 hr. Roots excised from 2-day-old *C. pepo* seedlings displayed even greater activity, 496 nmol/g fresh weight during 3 hr (14).

Salvage and catabolism. Purine salvage activity, assessed by incorporation of adenine and adenosine into total adenine nucleotides and into nucleic acids, was similar in magnitude to that reported for other tissues (6, 18). Differences in cumulative uptake of adenosine and adenine was similar to and thus may account for the differences observed in salvage activity using these two precursors. Activity of pyrimidine salvage, assessed by the incorporation of uracil and uridine into ΣUMP, was similar to that reported for roots excised from 6-day-old hydroponically grown *C. pepo* seedlings (13). Additional evidence for functioning of enzymic machinery for salvage of pyrimidine ribonucleosides was provided by 6-azauridine inhibition of the orotate pathway, because inhibition is dependent on the capability of tissue to transform this ribonucleoside to the nucleotide form (19). Incorporation of purine and pyrimidine ribonucleosides (adenosine and uridine, respectively) into nucleotides suggests adenosine and uridine kinases, which phosphorylate the ribonucleoside to form the nucleotide, are active in navel orange fruit.

Synthesis of nucleotides from adenine and uracil may occur by sequential ribosylation and phosphorylation or by direct phosphoribosylation. Conversion of orotic acid to UMP via the orotate pathway involves a direct phosphoribosylation. In peel tissue, 10 mM adenine inhibited incorporation of [6-^{14}C]orotic acid and [2-^{14}C]uracil into ΣUMP (data not shown). This inhibition suggests that purine and pyrimidine bases can be converted to nucleotides by direct phosphoribosylation, but does not rule out involvement of the sequential mechanism in the salvage of bases. Activity of enzymes involved in both mechanisms for the salvage of purine and pyrimidine bases have been reported in other plant tissues (9, 18–20).

Degradation of [8-^{14}C]adenine was 2 orders of magnitude greater than that of [8-^{14}C]adenosine, even after adjustment for differences in their cumulative uptake. This difference suggests that adenosine phosphorylase and/or hydrolase activity, which removes the ribose moiety to form the base, is minimal in navel orange fruit. The greater anabolism relative to catabolism of uridine suggests activity removing the ribose moiety from pyrimidine ribonucleosides is also low in navel orange fruit. Similar results have been reported for other plant tissues (1, 13, 19). Taken together, the patterns of salvage and degradation suggest ribonucleoside pools are predominantly reused as nucleotides; pools of bases may be degraded to permit recycling of carbon and nitrogen into other metabolites.

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J. Amer. Soc. Hort. Sci. 112(3):535–539. 1987.

Nucleotide Metabolism in 'Washington' Navel Orange Fruit: II. Pathway Capacities During Development

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Additional index words. *Citrus sinensis*, *de novo* synthesis, nucleotide salvage, catabolism, fruit development, peel

Abstract. **Changes in the capacity of 'Washington' navel orange [*Citrus sinensis* (L.) Osbeck] fruit to synthesize (*de novo* or by salvage) pyrimidine nucleotides, but not purine nucleotides, appears to be related to the stage of fruit development. *De novo* pyrimidine synthesis in whole-fruit tissue increased 6-fold during Stage I of development (cell division phase), from 10 nmol [^{14}C]bicarbonate incorporated into uridine nucleotides during 5 hr per g dry weight whole-fruit tissue from ovaries harvested at flower petal drop to 57 nmol for 2-month-old fruit. Capacity of peel tissue to synthesize pyrimidine nucleotides *de novo* decreased following completion of Stage I, from 43 nmol [14C]bicarbonate incorporated into uridine nucleotides during 5 hr per g dry weight of peel tissue from 2-month-old fruit to 11 nmol for 5-month-old (Stage II) fruit. This decrease was not offset by increased salvage of uridine. Capacity of whole-fruit tissue to synthesize purines *de novo* increased 3-fold during Stage I. Synthetic capacity of peel tissue from Stage I fruit was half that observed for whole-fruit tissue and did not decrease significantly during Stages II (cell enlargement phase) and III (maturation phase). These observations suggest purine synthetic capacity may not be related to stage of development. Changes in protein or glucose contents, or respiratory activity of peel tissue, could not account for the observed reduction in pyrimidine synthetic capacity. Thus, the reduction observed in synthetic activity was specific for pyrimidine nucleotides. The capacity of fast-growing, 1-month-old fruit (high potential to set) to synthesize or catabolize either pyrimidine or purine nucleotides did not differ from that of slow-growing fruit (low potential to set), suggesting that nucleotide synthesis is not limiting to growth.**

'Washington' navel orange fruit growth occurs in three stages, predominated by cell division, cell enlargement, and maturation, respectively (2, 5, 7). Fruit set occurs during Stage I of development, the period from flower petal drop to 2 months past petal drop. Cell enlargement predominates during Stage II. Fruit approach full size during this ≈3-month period. Maturation occurs during Stage III. In this nonclimacteric fruit, maturity is judged against legal/commercial standards.

Increased pyrimidine nucleotide biosynthesis has been reported to occur in pea cotyledons during germination and early development (10), during the early phases of the mitotic cycle of synchronously dividing cells of *Vinca rosea* L. [now, *Catharanthus roseus* (L.) G. Don] (8), and during embryogenesis of *Daucus carota* L. cells in suspension culture (1). In at least the two latter species, it appears that growth by cell division is accompanied by increased pyrimidine biosynthetic activity. Further, activities specifically associated with growth by cell division might be expected to decline during periods of growth by cell enlargement and of maturation. Consistent with this interpretation, Kanamori-Fukuda (8) has reported decreased pyrimidine nucleotide biosynthetic activity of *V. rosea* cells in suspension culture following completion of the cell division phase of growth. The apparent relationship between growth by cell division and rate of pyrimidine nucleotide biosynthesis suggested this metabolic activity might change during the three stages of citrus fruit development.

Differences in growth rate exist among Stage I fruit (9, 13), permitting examination of nucleotide synthetic and catabolic capacities in relation to rate of growth during the cell division phase of growth. Fruit growth rate during early development is one of many factors associated with citrus fruit set (9, 13): Faster-growing fruit have a greater potential to set and survive to harvest, while slower-growing fruit tend to abscise early in their development. An association between rate of growth during Stage I and capacities of purine and pyrimidine nucleotide synthesis and catabolism would provide a physiological mechanism associated with fruit set.

To the best of our knowledge, this is the first study to assess the activities of the pathways for the *de novo* biosynthesis, salvage, and catabolism of purine and pyrimidine nucleotides in relation to stage and rate of growth in fleshy fruit.

Materials and Methods

Fruit were collected from 20-year-old 'Washington' navel orange trees on 'Troyer' citrange rootstocks or from cuttings (1- to 2-years-old) rooted from these scions. Trees were located at the Citrus Research Center and Agricultural Experiment Station, Univ. of California, Riverside. Fruit of known age (months past

Received for publication 11 Sept. 1986. This paper represents a portion of the dissertation submitted by P.T.T. in partial fulfillment of requirements for the PhD degree in Botany at the Univ. of California. Supported by a Univ. of California Chancellor's Patent Fund grant to P.T.T. and a Statewide Critical Applied Research grant to C.J.L. Use of a company or product name does not imply approval or recommendation of the product to the exclusion of others which may also be suitable. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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