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Evidence for incorporation of intact dietary pyrimidine (but not purine) nucleosides into hepatic RNA

(*Spirulina platensis*/nucleic acids/poultry/mice)

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ABSTRACT **The absorption and metabolism of dietary nucleic acids have received less attention than those of other organic nutrients, largely because of methodological difficulties. We supplemented the rations of poultry and mice with the edible alga *Spirulina platensis*, which had been uniformly labeled with ^{13}C by hydroponic culture in $^{13}CO_2$. The rations were ingested by a hen for 4 wk and by four mice for 6 days; two mice were fed a normal diet and two were fed a nucleic acid-deficient diet. The animals were killed and nucleosides were isolated from hepatic RNA. The isotopic enrichment of all mass isotopomers of the nucleosides was analyzed by selected ion monitoring of the negative chemical ionization mass spectrum and the labeling pattern was deconvoluted by reference to the enrichment pattern of the tracer material. We found a distinct difference in the ^{13}C enrichment pattern between pyrimidine and purine nucleosides; the isotopic enrichment of uniformly labeled [M + 9] isotopomers of pyrimidines exceeded that of purines [M + 10] by >2 orders of magnitude in the avian nucleic acids and by 7- and 14-fold in the murine nucleic acids. The purines were more enriched in lower mass isotopomers, those less than [M + 3], than the pyrimidines. Our results suggest that large quantities of dietary pyrimidine nucleosides and almost no dietary purine nucleosides are incorporated into hepatic nucleic acids without hydrolytic removal of the ribose moiety. In addition, our results support a potential nutritional role for nucleosides and suggest that pyrimidines are conditionally essential organic nutrients.**

The total daily requirements from all sources for purines and pyrimidines in human adults have been estimated to range between 450 and 700 mg/day (1, 2). The metabolic need for these nucleosides in healthy individuals is presumed to be met by *de novo* synthesis, although there is little *in vivo* information to support this belief. In addition to *de novo* synthesis of both the base and monosaccharide portions of the nucleosides, many cells possess efficient mechanisms for the recovery of nucleosides after hydrolytic breakdown of nucleic acids. The existence of these pathways, in principle, enables the use of nucleotides or nucleosides ultimately derived from the diet. For example, the cells of the intestinal mucosa have been shown to use preformed purine and pyrimidine bases (3), and supplementation of the diet with nucleosides has been shown to advance the growth and maturation of the developing small intestine (4).

Whether there is a dietary "requirement" for nucleosides remains an open question. Little evidence is available to suggest that *de novo* synthesis and salvage pathways are inadequate in healthy well-nourished individuals to replace the daily losses of these molecules. Nevertheless, cellular immunity is decreased in animals that consume purified nucleotide-free diets (5). Resistance to infection in such animals is reduced and tolerance of allografts is increased. The absorption and utilization of nucleosides present in the diet, however, have proved difficult to estimate in a formally quantitative manner.

We have recently used a method based on the ingestion of a uniformly ^{13}C-labeled alga that enables us not only to identify the incorporation of intact dietary organic nutrients but also to detect the synthesis of nutritionally dispensable amino and fatty acids (6). In the present paper, we report the application of this method to investigate the absorption and metabolism of dietary nucleic acids. The results indicate that dietary pyrimidines and purines have different metabolic fates and are incorporated into hepatic nucleic acids to markedly different extents.

MATERIALS AND METHODS

Tracer Material. The prokaryotic blue-green alga, or cyanobacterium, *Spirulina platensis*, is a ubiquitous microorganism (7, 8), which contains 60–65% protein, 11% lipids, 15% carbohydrates, and <5% nucleic acids in its dry matter when grown in the laboratory (8–10). RNA has been reported to represent 2.2–3.5% of the dry weight, whereas DNA represents 0.6–1% (10–12). In the present study, the alga was grown in a closed system bioreactor under conditions in which the sole source of carbon was highly enriched (>99%) $^{13}CO_2$ (11, 12). All organic molecules in the resulting biomass, including the nucleosides, are virtually uniformly ^{13}C labeled.

Animal Feeding Trials. *Chicken experiment.* The uniformly labeled *S. platensis* was combined with corn and other dietary components to produce chicken feed. The composition of the feed was as follows (by weight): corn, 77.26%; salt, 0.12%; limestone, 4.016%; dical, 2.72%; vitamin premix, 0.033%; trace mineral premix, 0.033%; choline chloride, 0.087%; soybean oil, 1.87%; *S. platensis* (lyophilized alga), 13.83%; tryptophan, 0.025%. The feed was offered to a laying hen (DeKalb White Leghorn) for 27 days. The average feed intake was 100–120 g per day. At the end of the feeding period, the hen was killed, and its internal organs and carcass were individually deep frozen at −70°C. Data on the labeling of plasma and protein-bound amino acid labeling from this animal have been reported (6).

Mouse experiment. Four mice (CD57, Charles River Breeding Laboratories) (≈20 days of age) had free access for 3 days to feed that contained the following (by weight): basal purified diet (Purina), 89.0%; *S. platensis* (unlabeled), 10.0%; adenosine, guanosine, cytidine, uridine, and thymidine, each 0.2%. For the next 6 days, unlabeled *Spirulina* was replaced by

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Abbreviation: TMS, trimethylsilyl.

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uniformly ^{13}C-labeled *S. platensis*. At the same time, the nucleoside supplementation was removed from the feed of two mice and replaced by an equal amount of the nucleoside-free basal feed. All mice received and completely consumed 3.3 g of feed per day. The composition of the basal diet was as follows (by weight): solca floc, 3.0%; casein, 21%; sucrose, 15%; corn oil, 5%; lard, 5%; choline chloride, 0.2%; dextrin, 43.65%; PMI vitamin mix, 2.0%; PMI mineral mix no. 10, 5.0%; DL-methionine, 0.15%. After 6 days, the animals were killed and their internal organs and carcasses were individually frozen at −70°C.

Isolation of Liver RNA. High molecular weight RNA was isolated essentially as described (13), except that antifoam A (0.3%, vol/vol) was added to the lysis buffer. The initial RNA pellet was redissolved in lysis buffer and the extraction and precipitation steps were repeated. Finally, the RNA pellet was washed twice with ice-cold 75% isopropanol.

Enzymatic Digestion of RNA. Dried RNA pellets were dissolved in autoclaved diethyl pyrocarbonate-treated water, and duplicate 100-μg samples were adjusted to a concentration of 2 μg/ml and digested to nucleotides as described (14). The amounts of nuclease P1 and alkaline phosphatase (Sigma) were decreased 4-fold and digestion time was increased proportionately. Completeness of digestion was assessed by HPLC analysis (15) of a 2-μg aliquot. Samples were dried in a Speed-Vac (Savant).

Trimethylsilylation of Nucleosides and Bases (16). Nucleosides and purine and pyrimidine bases were purchased from Aldrich in the highest purity available (97–99+%). *N,O*-Bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane (BSTFA/1% TMCS) was obtained from Pierce. Pyridine was obtained from Sigma. Standards of known concentration were dissolved in a mixture of BSTFA/1% TMCS and pyridine (5:1, vol/vol), Vortex mixed, and heated for 1 h at 100°C. After cooling, the samples were ready for mass spectrometric analysis. Nucleic acid digests were evaporated to dryness in a Speed-Vac and derivatized in the same manner.

Amino Acid Isotopic Analysis (6). Hepatic protein was isolated by precipitation with 0.5 M perchloric acid. The dried protein pellet was hydrolyzed with 5.4 M HCl at 114°C for 24 h. The amino acids were derivatized with acid isopropanol and heptafluorobutyric anhydride and were analyzed by negative chemical ionization gas chromatography/mass spectrometry.

Gas Chromatography/Mass Spectrometry. Gas chromatography/mass spectrometry was performed on a Hewlett-Packard HP 5988A system equipped with a HP 5890 II series gas chromatograph (Hewlett–Packard). The temperatures of the ion source, transfer line, and glass injection liner were all 250°C. One microliter of derivatization solution was injected (splitless) onto a 30 m × 0.32 mm DB-5 column (film thickness, 1 μm) and eluted with helium as carrier gas. Mass spectrometry was performed by chemical negative ionization using methane as reagent gas at a pressure of 0.6–0.8 torr (1 torr = 133.3 Pa).

Samples were measured in triplicate by selected ion monitoring of the trimethylsilyl ($[TMS]_4$) molecular ions of adenosine and guanosine and the $[M - H]^-$ ions of uridine $[TMS]_3$ and cytidine $[TMS]_4$. Ion intensities were obtained through the full mass range of the compound from the unlabeled isotopomer [M] to the uniformly labeled isotopomer $[M + x]$, where x is the number of carbon atoms of the underivatized molecule.

Data Evaluation. The growth of the photosynthetic alga on $^{13}CO_2$ produced a biomass in which the majority (≈85%) of the nucleosides were uniformly labeled. The present investigation then is, in effect, a multitracer study involving the introduction of $(x + 1)$ labeled species, where x is the number of carbon atoms in the nucleoside in question. Because of the natural abundance of isotopes of the substituent elements, the enrichment of any single ion contains variable contributions of $x + 1$ ions so that expression of the results in terms of molar enrichments (i.e., ratios of tracer/tracee) requires the deconvolution of the overlying mass spectra. This, in its turn, involves the solution of $x + 1$ simultaneous linear equations of the general form

$$I = A_{i,j}x_0 + A_{i,j}x_1 + \ldots + A_{i,j}x_n, \quad [1]$$

where I is the intensity of any given ion, $A_{i,j}$ is the fractional abundance of the ion (i.e., the ratio of ion intensity/the sum of all ion intensities), and $x_{[M+x]}$ is the unknown mole fraction. For example, for a molecule containing 10 carbon atoms the equations are

$$I_{[M]} = A_{1,1}x_{[M]} + A_{1,2}x_{[M+1]} + \ldots + A_{1,10}x_{[M+9]}$$

$$I_{[M+1]} = A_{2,1}x_{[M]} + A_{2,2}x_{[M+1]} + \ldots + A_{2,10}x_{[M+9]}$$

$$\ldots$$

$$I_{[M+10]} = A_{10,1}x_{[M]} + A_{10,2}x_{[M+1]} + \ldots + A_{10,10}x_{[M+9]}. \quad [2]$$

This set can then be expressed in matrix notation as

$$I_{[M+x]} = AX, \quad [3]$$

and the equations are solved for the mole fraction of each isotopomer by the operation inverse of A, transpose A, A transpose I_x

$$X = (A^TA)^{-1}A^TI_{[M+x]}. \quad [4]$$

In theory the construction of this matrix requires information on the relative ion intensities in the mass spectra of the unlabeled compound and of authentic standards containing 1, 2, ... x ^{13}C atoms. However, only unlabeled and uniformly labeled species are available, so in practice we constructed a "design matrix" using the statistical combination program ISOCOM. This uses the elemental formula of the derivative whose mass spectrum has been analyzed together with information on the natural abundances of these elements (in the present case, ^{13}C, ^{15}N, ^{2}H, ^{17}O, ^{18}O, ^{29}Si, and ^{30}Si) and calculates the contribution of any single ion to those whose m/e ratios differ by +1, +2, etc. In the tables, the corrected molar abundances of each isotopomer are shown as excess enrichments after correction for the molar abundances of the various isotopomers of the unenriched TMS derivatives. The software that enables these calculations is available, free of charge, from D. L. Hachey (Children's Nutrition Research Center).

Finally, the contribution of dietary nucleosides to hepatic nucleic acid can be expressed in two ways. (*i*) The contribution of the dietary nucleosides to the total nucleosides can be estimated from the excess molar enrichments of $[M + x]$ and $[M + (x - 1)]$. This measurement, however, does not take into account the dilution of unlabeled nucleic acids present in the liver before introduction of the label. Allowance for this is made by expressing the data in terms of the contribution of the ^{13}C contained in $[M + x]$ and $[M + (x - 1)]$ isotopomers to the total ^{13}C incorporated—namely,

$$\frac{\{(x-1)E_{[M+x-1]}\} + \{xE_{[M+x]}\}}{\{1E_{[M+1]} + 2E_{[M+2]} + \ldots xM_{[M+x]}\}},$$

where x is the number of carbon atoms (10 for purine nucleosides and 9 for pyrimidine nucleosides) and E is the corrected excess molar enrichment (mol of tracer/mol of substance traced). This measurement is an estimate of the contribution of dietary nucleosides to newly synthesized nucleic acid.

RESULTS

The provision of $^{13}CO_2$ as the sole carbon source for the photosynthetic organism *S. platensis* resulted in virtually com-

plete and uniform labeling of all algal organic molecules including the nucleosides. Although most of the molecules were uniformly labeled ([M + 9] for pyrimidines, [M + 10] for purines), a small fraction (≈14%) was present as the [M + (x − 1)] isotopomer. The isotopic enrichment of isotopomers of mass < [M + x] was taken into account in calculations of the isotopomer enrichment patterns of the hepatic RNA nucleosides.

The corrected excess enrichment data (mol of tracer per 100 mol of tracee) for the hepatic RNA purine and pyrimidine nucleosides from the chicken (Table 1) showed two completely different labeling patterns. There was essentially no label in isotopomers > [M + 6] in the purines, while 22% of the cytidine and 29% of the uridine molecules were uniformly labeled. Significant isotopic enrichments of [M + 7] and [M + 8] isotopomers of the pyrimidines were also observed. Unlabeled isotopomers ranged from 50% to 60% in both the purines and pyrimidines. The reverse pattern was found in isotopomers < [M + 5]; these were substantially more enriched in the purines. The results suggested that endogenous synthesis accounted for ≈98% of the newly incorporated adenosine and guanosine, whereas 85% of the cytidine and 87% of the uridine incorporated into nucleic acids had not been synthesized by the hen and must have arisen from the diet.

Although the shorter duration of the labeling period resulted in a lower overall isotopic enrichment of the murine than galline nucleic acid nucleosides, the qualitative pattern of labeling was similar (Table 2). Thus, ≈3% of the total ^{13}C incorporated into adenosine and guanosine was in the [M + 9] and [M + 10] isotopomers, while 25% of the total ^{13}C incorporated into cytidine and uridine was in the [M + 8] and [M + 9] isotopomers.

Table 2 also shows the labeling patterns in the nucleic acids of the two mice that ingested a nucleic acid-free diet. The enrichment of the [M + 1] to [M + 3] isotopomers of both the purines and the pyrimidines was higher than those isolated from the nucleic acids of the mice that consumed a conventional nucleic acid-containing diet. There was also a tendency for the [M + 8] and [M + 9] isotopomers of the pyrimidines to be higher in the nucleic acids isolated from the mice that received the nucleic acid-free diet. There was still no significant incorporation of uniformly labeled purine nucleosides.

Table 3 shows the isotopic enrichments of all mass isotopomers of hepatic aspartate and alanine. The uniformly labeled isotopomers of both amino acids were the most highly enriched. The molar isotopic enrichment of [M + 2] glycine was much higher than that of [M + 4] aspartate. Nevertheless, there was readily measurable incorporation of ^{13}C into the lower mass isotopomers.

DISCUSSION

Intracellular nucleosides and nucleotides used as precursors for nucleic acid synthesis, sources of "high-energy" phosphate, intracellular monosaccharide carriers, and intracellular regulatory molecules can, in principle, be derived from four sources: (*i*) incorporation of preformed nucleosides or bases transported from the extracellular space and, hence, possibly from the diet; (*ii*) recycled nucleotides released after nucleic acid degradation; (*iii*) resynthesis of purine nucleosides via the so-called salvage pathway; and (*iv*) incorporation from pools of bases and ribose synthesized *de novo* within the cell.

Digestion of dietary nucleic acids leads to generation of nucleotides, nucleosides, and free bases (17, 18), and there is evidence for the existence of specific transport systems for absorption of both bases and nucleosides (19, 20). The present results demonstrate that significant quantities of pyrimidine nucleosides derived from the diet were incorporated into hepatic nucleic acids. In the chicken, at least 30% of the pyrimidines in the hepatic nucleic acid pool had been derived from the diet and, because 80% of the ^{13}C in the [M + 8] and [M + 9] isotopomers of the pyrimidines was present as the [M + 9] form, they had been incorporated without hydrolytic cleavage of the base from the ribose moiety. This finding was reported many years ago, although the data were less formally quantitative (21).

This method of expression, however, underestimates the contribution of dietary nucleosides to new nucleic acid synthesis because at the start of the experiment the nucleic acids were unlabeled. An estimate of this can be obtained from the contribution of the ^{13}C in these two isotopomers to total ^{13}C incorporated. The result of this calculation suggested that in the hen ≈85% of the pyrimidine nucleotides incorporated into newly synthesized nucleic acid had derived from dietary nucleosides. A somewhat lower (22–27%) proportion of the murine nucleic acid synthesis had utilized dietary pyrimidine nucleosides, but their contribution was nutritionally significant. In contrast to these results, virtually none of the purine nucleosides in nucleic acid were present as [M + 9] and [M + 10] isotopomers, even after 27 days of labeling. At most these isotopomers accounted for 3% of the total incorporation of ^{13}C. This result is in keeping with earlier and more indirect methods based either on the excretion of purine metabolites after ingestion of purine nucleosides (22) or on the use of ^{15}N from yeast nucleic acids (23).

While incorporation of the [M + 8], [M + 9], and [M + 10] isotopomers allows unequivocal identification of the incorporation of dietary nucleosides, the labeling patterns in lower mass isotopomers enable us to draw tentative conclusions with respect to the relative contributions of the alternative pathways by which purines and pyrimidine nucleotides are produced.

The first concerns incorporation of dietary bases and ribose, as opposed to nucleosides. Because both the purine bases and the ribose in the spirulina nucleic acids were present largely as the [M + 5] isotopomers, entry from the diet of either precursor into the nucleosides via the salvage pathway (24) would lead to the appearance of [M + 5] isotopomers of the purine nucleosides. These isotopomers were labeled in both the avian and murine nucleic acids, although the enrichment was relatively low. ^{13}C in [M + 5] isotopomers of adenosine and guanosine accounted for only 6% and 4% of the total ^{13}C incorporated into the avian hepatic nucleic acids. The apparent contribution of dietary bases and/or ribose via the salvage pathway was somewhat higher in the mice and it appeared that more guanosine had derived from the salvage pathway (17% and 9% of total ^{13}C incorporation in the N^+ and N^- groups,

Table 1. Mass isotopomer excess abundances (mol of isotopomer per 100 mol of nucleoside) in hepatic nucleic acid nucleosides of a hen after a 27-day period in which the bird received *S. platensis* uniformly labeled with ^{13}C

	Mass isotopomer*											
Nucleoside	[M]	[M + 1]	[M + 2]	[M + 3]	[M + 4]	[M + 5]	[M + 6]	[M + 7]	[M + 8]	[M + 9]	[M + 10]	% ^{13}C from diet
Adenosine	50.7	22.2	13.3	7.86	3.32	1.19	0.39	0.43	0.36	0.20	0.12	2.9
Guanosine	60.2	25.1	10.2	3.05	0.53	0.39	0.21	0.10	0.07	0.06	0.07	2.0
Cytidine	57.4	2.48	4.71	4.54	1.12	0.07	0.46	1.57	5.67	21.9	—	84.6
Uridine	48.2	5.39	2.51	1.51	2.00	1.00	0.96	2.20	7.38	28.8	—	86.6

*Values are normalized to give the sum of all ions = 100%.

Table 2. Mass isotopomer excess abundances (mol of isotopomer per 100 mol of nucleoside) in hepatic nucleic acids of mice that received *S. platensis* uniformly labeled with ^{13}C for 6 days

Nucleoside	Mass isotopomer [M]	[M + 1]	[M + 2]	[M + 3]	[M + 4]	[M + 5]	[M + 6]	[M + 7]	[M + 8]	[M + 9]	[M + 10]	% ^{13}C from diet
Adenosine												
Diet N^+	67.6	14.3	11.0	4.02	1.55	0.62	0.45	0.19	0.10	0.11	0.14	3.6
	± 0.7	± 2.2	± 0.9	± 0.39	± 0.03	± 0.49	± 0.24	± 0.03	± 0.02	± 0.04	± 0.00	
Diet N^-	62.0	18.4	11.3	4.90	1.65	0.84	0.28	0.20	0.12	0.12	0.12	3.1
	± 3.5	± 4.0	± 1.3	± 0.65	± 0.09	± 0.31	± 0.17	± 0.03	± 0.02	± 0.02	± 0.01	
Guanosine												
Diet N^+	76.5	10.4	7.37	2.38	0.78	1.78	0.32	0.20	0.11	0.09	0.10	3.6
	± 0.3	± 0.4	± 0.15	± 0.26	± 0.11	± 0.87	± 0.22	± 0.09	± 0.03	± 0.01	± 0.02	
Diet N^-	74.6	11.4	8.17	3.15	1.14	0.96	0.16	0.15	0.09	0.06	0.09	2.8
	± 1.4	± 0.6	± 0.48	± 0.47	± 0.28	± 0.18	± 0.16	± 0.07	± 0.03	± 0.03	± 0.03	
Cytidine												
Diet N^+	81.8	5.08	4.97	6.48	0.18	0	0.30	0.30	0.36	0.96		22.8
	± 1.0	± 0.36	± 1.05	± 0.39	± 0.05		± 0.04	± 0.05	± 0.06	± 0.01		
Diet N^-	78.9	5.78	6.69	6.38	0	0	0.39	0.39	0.40	1.25		24.9
	± 1.9	± 0.49	± 1.17	± 0.27			± 0.04	± 0.24	± 0.10	± 0.19		
Uridine												
Diet N^+	85.0	8.80	0.75	1.84	1.04	0.56	0.32	0.43	0.42	0.80		27.4
	± 0.34	± 0.20	± 0.17	± 0.01	± 0.04	± 0.03	± 0.00	± 0.01	± 0.04	± 0.00		
Diet N^-	82.3	9.80	1.58	2.18	1.09	0.56	0.48	0.59	0.45	1.00		27.3
	± 1.6	± 0.70	± 0.52	± 0.21	± 0.04	± 0.02	± 0.11	± 0.08	± 0.04	± 0.12		

Animals received either a basal, nucleotide-free diet (N^-) or the same diet supplemented with RNA. Data are mean values ± SEM for two animals in each feeding group.

respectively) than adenosine (5% and 6% for the N^+ and N^- groups). Even so, the majority (83–94% in the hen and 80–94% in mice) of both the base and the ribose of the purine nucleosides have been synthesized *de novo*.

Consideration of labeling of the [M + 4] and [M + 5] isotopomers also proved to be of interest in some other respects. First, because the pyrimidine bases contain only four carbons, the presence of label in the [M + 5] isotopomer of these nucleosides could arise only from the incorporation of 5-phospho[U-^{13}C]ribose 1-pyrophosphoric acid (PRPP). Strikingly, there were significant quantities of [M + 5] uridine present in the hepatic nucleic acids of both species but we did not find any [M + 5] cytidine. This provides evidence for compartmentation of the PRPP used in synthesis of uridine and cytidine, a surprising result considering their generally presumed precursor product relationship.

The idea that the pathways of uridine and cytidine are compartmentalized is also supported by the observation that in the chicken the enrichment of [M + 4] uridine was twice that of [M + 4] cytidine, while in mice we detected [M + 4] uridine but not [M + 4] cytidine. These results suggest that in both species ribose and uracil derived from the diet are incorporated into tissue nucleic acids. This conclusion would be in keeping with the recent observation that the immunological benefit of the inclusion of nucleic acid in the diet can be reproduced merely by addition of uracil alone (25).

We were also surprised to detect label in the [M + 4] isotopomers of the purine nucleosides. The likelihood that [M + 4] purines could arise from synthesis *de novo* is very low and we believe that the majority of the [M + 4] isotopomer represents incorporation of labeled ribose. We come to this conclusion by the following reasoning.

In the present experiments, labeled ribose arises from two sources: absorption from the diet and synthesis from glucose. The latter pathway will yield ribose with a mass isotopomer distribution that is a reflection of that of glucose. Thus, [M + 5] ribose will arise as a result of the metabolism of [M + 6] glucose derived from the labeled *Spirulina* carbohydrate, while the [M + 1]–[M + 3] (but not [M + 4]) isotopomers of ribose will be synthesized via gluconeogenesis.

However, entry of [U-^{13}C]glucose into the pentose phosphate pathway can also lead to formation of [M + 4] ribose via label equilibration between erythrose 4-phosphate and ribose 5-phosphate. If label equilibration between all the intermediates in the pentose monophosphate pathway is complete, then the isotopic enrichment of [M + 5] ribose and [M + 4] ribose should be essentially the same. This was true for cytidine in the avian nucleic acids, but it was not true for adenosine, guanosine, and uridine, in which isotopic enrichment of the [M + 4] isotopomer exceeded that of [M + 5]. With the purines, excess enrichment of the [M + 4] isotopomer over that of the [M + 5] isotopomer is compatible with random association of the highly enriched [M + 1]–[M + 3] bases and ribose. However, this does not account for the excess enrichment of uridine, and we conclude that the observation reflects incorporation of [M + 4] dietary uracil.

As glycine contributes two carbons to the purine bases, while aspartate contributes three of the four carbons in the pyrimidine ring, as a first approximation the relative enrichments of [M + 2] and [M + 1] of the purines should reflect the relative enrichments of [M + 2] and [M + 1] glycine, while the enrichments of [M + 3], [M + 2], and [M + 1] isotopomers of the pyrimidines should reflect those of [M + 4], [M + 3], and [M + 2] of aspartate. However, the inferences that can be

Table 3. Mass isotopomer excess abundances (mol of isotopomer per 100 mol of amino acid) of hepatic protein-bound aspartate and glycine after ingestion of *S. platensis* uniformly labeled with ^{13}C for 27 (hen) or 6 (mouse) days

	[M]	[M + 1]	[M + 2]	[M + 3]	[M + 4]
			Hen		
Aspartate	73.9	10.9	3.05	1.07	11.1
Glycine	82.6	7.83	9.59		
			Mouse		
Aspartate					
Diet N^+	71.0	4.76	1.76	0.87	21.6
Diet N^-	74.2	4.43	1.62	0.75	19.0
Glycine					
Diet N^+	60.5	2.41	37.1		
Diet N^-	63.6	2.48	33.9		

drawn from the labeling patterns of the isotopomers < [M + 3] are strictly limited, because we administered a complex mixture of labeled organic compounds and there was little obvious relationship between the relative enrichments of the low mass isotopomers of the nucleosides with those of their precursor amino acids (Table 3).

The present results suggest that the pathways followed by dietary nucleosides differ markedly between the pyrimidine and purine bases. Dietary pyrimidine nucleosides make a highly significant contribution to the hepatic pyrimidine pool, while apparently little or no dietary purine nucleosides are used for synthesis of hepatic nucleic acids. Furthermore, there appears to be significant incorporation of dietary uracil but not cytosine. Whether the same applies to the nucleic acids of the gut or immune system, however, remains an open question. We propose that the distinct difference may arise from the fact that ATP and GTP, and their metabolites (c)AMP and (c)GMP, are involved in so many key aspects of cellular metabolism and function that their respective pool sizes are strictly regulated and perturbations in these pools arising from differences in dietary composition must be minimized.

Apart from the biochemical insights generated by these results, they have general nutritional importance. Human milk contains large quantities of nucleosides (3), and the mixture contains a disproportionately large contribution of cytidine and uridine (26–28). Provision of preformed nucleosides has been reported to benefit cellular proliferation of the gut (3, 4) and has led to development of infant formulas, which contain preformed nucleotides (29). The present results strongly suggest that adding purine nucleotides to infant diets would have little influence on the purine status of infants. On the other hand, the addition of dietary pyrimidines might have an important effect on the pyrimidine base and nucleoside economy of the growing child.

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