Folate-Dependent Purine Nucleotide Biosynthesis in Humans

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ABSTRACT

Purine nucleotide biosynthesis de novo (PNB) requires 2 folate-dependent transformylases—5'-phosphoribosyl-glycinamide (GAR) and 5'-phosphoribosyl-5-aminoimidazole-4-carboxamide (AICAR) transformylases—to introduce carbon 8 (C8) and carbon 2 (C2) into the purine ring. Both transformylases utilize 10-formyltetrahydrofolate (10-formyl-H4folate), where the formyl-carbon sources include ring-2-C of histidine, 3-C of serine, 2-C of glycine, and formate. Our findings in human studies indicate that glycine provides the carbon for GAR transformylase (exclusively C8), whereas histidine and formate are the predominant carbon sources for AICAR transformylase (C2). Contrary to the previous notion, these carbon sources may not supply a general 10-formyl-H4folate pool, which was believed to equally provide carbons to C8 and C2. To explain these phenomena, we postulate that GAR transformylase is in a complex with the trifunctional folate-metabolizing enzyme (TFM) and serine hydroxymethyltransferase to channel carbons of glycine and serine to C8. There is no evidence for channeling carbons of histidine and formate to AICAR transformylase (C2). GAR transformylase may require the TFM to furnish 10-formyl-H4folate immediately after its production from serine to protect its oxidation to 10-formyldihydrofolate (10-formyl-H2folate), whereas AICAR transformylase can utilize both 10-formyl-H2folate and 10-formyl-H4folate. Human liver may supply AICAR to AICAR transformylase in erythrocytes/erythroblasts. Incorporation of ring-2-C of histidine and formate into C2 of urinary uric acid presented a circadian rhythm with a peak in the morning, which corresponds to the maximum DNA synthesis in the bone marrow, and it may be useful in the timing of the administration of drugs that block PNB for the treatment of cancer and autoimmune disease.

Introduction

In purine nucleotide biosynthesis de novo (PNB), carbons 8 and 2 (C8 and C2, respectively) of the purine ring are derived from 10-formyl-5,6,7,8-tetrahydrofolate (10-formyl-H4folate) (1). The process involves 2 enzymes: 5'-phosphoribosyl glycaminamide (GAR) transformylase for C8 to produce 5'-phosphoribosylformylglycinamide and 5'-phosphoribosyl-5-aminoimidazole-4-carboxamide (AICAR) transformylase for C2 to produce 5'-phosphoribosyl-5-formamidoimidazol-4-carboxamide. Figure 1 shows the biochemical synthetic process of the first purine nucleotide, inosine monophosphate (IMP), involving these 2 folate-dependent reactions. PNB is a vital process because there has been no case report of an inborn deficiency of enzymes involved in the process, and dietary purines are not an alternative source. The final catabolic product of purine is uric acid (1), and this catabolic process does not alter the positions of the carbons in the purine ring (2, 3).

The carbon of the formyl group in 10-formyl-H4folate originates from ring-2-carbon (-C) of histidine, 2-C of glycine, 3-C of serine, and formate (Figure 2). We developed a liquid chromatography–mass spectroscopy method for determining whether 13C-labeled sources enrich C2 alone or C8 (plus C5) of uric acid (5). We now hypothesize that it is unlikely that all one-carbon sources enrich the C2 and C8 positions equally. This was based on reports that 1) GAR transformylase is associated with other folate-metabolizing enzymes [trifunctional folate-metabolizing enzyme (TFM) and serine hydroxymethyltransferase (SHMT)] in chicken liver (6, 7); 2) AICAR transformylase, but not GAR transformylase, can utilize 10-formyl-7,8-dihydrofolate (10-formyl-H2folate)
as a substrate in vitro (8, 9); 3) the unnatural isomer, \([6R]-5\)-formyltetrahydrofolate (5-formyl-\(H_4\)folate), is inactive as a substrate for folate-metabolizing enzymes in vitro but bioactive in vivo in humans, suggesting its conversion to 10-formyl-\(H_4\)folate in vivo (10); and 4) the kinetics of \(C_2\) and \(C_8\) enrichment of uric acid in humans by \([6S]-5\)-13C-formyl-\(H_4\)folate are different, suggesting that GAR and AICAR transformylases do not utilize the same 10-formyl-\(H_4\)folate pool.

Incorporation of the Ring-2-C-Histidine into the Purine Ring

Histidine is considered to be an essential amino acid in humans (11). Meléndez-Hevia et al. (12) estimated that 4.5 mmol histidine/d is degraded in adults. The major catabolic pathway of histidine is to glutamic acid through urocanic acid (13). The final step of this pathway involves the conversion of formiminoglutamic acid and tetrahydrofolate (\(H_4\)folate) to glutamic acid and 5-formiminotetrahydrofolate (5-formiminoglutamic acid) by the formiminotransferase, where the carbon of the formiminoglutamic acid group is derived from the ring-2-C of histidine. Subsequently, 5-formiminoglutamic acid is metabolized to 10-formyl-\(H_4\)folate by the 5-formiminoglutamic acid cyclodeaminase and the 5,10-methenyltetrahydrofolate (5,10-methenyl-\(H_4\)folate) cyclohydrolase. Presumably, a dietary intake of 4.5 mmol histidine (~0.9 g)/d is sufficient to meet human requirements (12). In terms of folate metabolism, ~30% of 4.5 mmol 10-formyl-\(H_4\)folate/d releases its one-carbon as carbon dioxide, leaving 3.2 mmol/d as a source of one-carbon for PNB and all other anabolic folate-requiring pathways. Adult humans excrete ~2.75 mmol uric acid/d; therefore, PNB requires 5.5 mmol of one-carbon/d (2.75 mmol/d for \(C_8\) and \(C_2\)). Histidine alone probably cannot supply sufficient one-carbon to both GAR and AICAR transformylases (12).

We recently determined the 13C-enrichment of the \(C_8\) and \(C_2\) positions of urinary uric acid after an oral dose of 3.3 mmol...
(0.7 g) [1-ring-2-13C]histidine in humans (4). In the majority of urine voids collected for 3 d, the C2 position of uric acid was predominantly 13C-enriched. The mean percentage enrichments in 2 subjects at C2 were 0.14 and 0.18 (26 and 21 voids in 3 d, respectively), whereas they were −0.005 and 0.008 at C8 (Table 1). The mean percentages of 13C-enrichments at C2 were significantly greater than zero, whereas those of C8 were not. To our knowledge, such a human study has never been reported.

We now discuss some early studies on this issue. Brown et al. (16) subsequently showed that, in rats, 14C from uniformly 14C-labeled histidine was incorporated into uric acid and allantoin. [Ring-2-14C]histidine was reported to label purine 10 times more efficiently than serine or the methyl groups of choline in rats, clearly suggesting that this one-carbon is destined for purine nucleotide biosynthesis rather than supplying the general one-carbon pool (17).

In human studies, 14CO2 production after [ring-2-14C]histidine administration was decreased in folate-deficient patients (18). In addition, 14CO2 production from [ring-2-14C]histidine was not detected in an infant with formiminotransferase deficiency (19). These findings indicate that the ring-2-C of histidine is involved in folate metabolism in humans via the 10-formyl-H4folate dehydrogenase reaction, which catalyzes the formation of carbon dioxide from 10-formyl-H4folate (20). The catabolism of histidine must be important, especially early in life, because the deficiency of formiminotransferase is manifested by growth retardation and neurologic abnormalities (21). In patients with this defect, more urinary 5-aminoimidazole-4-carboxamide (AICA) excretion is observed with (1.7-fold increase) and without (4.9-fold increase) an oral AICA load compared with healthy individuals (22). This indicates that folate-dependent histidine catabolism to produce 10-formyl-H4folate (formed from 5-formimino-H4folate) is apparently limiting to a certain extent the supply of a one-carbon to AICAR transformylase. Because GAR transformylase precedes AICAR transformylase in PNB (Figure 1), elevated AICA excretion in these patients is consistent with ring-2-C of histidine supplying one-carbon primarily to the C2 position. If this were not the case, AICA excretion should have been lower due to a limited supply of one-carbon to GAR transformylase. Collectively, the above data support our findings that the ring-2-C of histidine primarily supplies C2 of purine by AICAR transformylase in humans. Some of the above findings are summarized in Table 1.

### Incorporation of the 2-C of Glycine into the Purine Ring

Glycine is considered to be a nonessential amino acid. The dietary intake of glycine is estimated to be 1.5–3.0 g/d, and an additional ~2.9 g glycine/d is biosynthesized in adult humans. Under normal conditions, 12 g glycine/d turnover occurs with the formation and degradation of collagen (~97 g/d). This is a substantial portion of daily protein turnover of 200–300 g in adult humans (12).

Glycine serves as a source of carbons and 1 nitrogen in the purine ring (1). The carboxyl carbon and 2-C of glycine are directly incorporated into the 4 and 5 positions, respectively. In addition, the 2-C of glycine is metabolized to 5,10-methylene-H4folate (5,10-methylene-H4folate) by the glycine-cleavage system (GCS) (35). This folate is converted to 10-formyl-H4folate by 5,10-methylene-H4folate dehydrogenase followed by 5,10-methylene-H4folate cyclohydrolase. Both of these enzymes are in TFM, where GAR transformylase is closely associated in chicken liver (6, 7), although no such association has been established in humans.

Our study indicates that a 2.5-g oral dose of [2-13C]glycine enriched the C8 plus C5 positions of urinary uric acid, and the mean percentage 13C-enrichments were 0.39 and 0.19 in 2 subjects over a 3-d period (14). The reason for reporting “C8 plus C5” is that our method does not distinguish independent enrichment at C8 and C5 (5). Of 28 voids collected for each subject, >80% were zero or negative for 13C-enrichment at the C2 positions (Table 1).

To our knowledge, there have been only 2 human studies in which the incorporation of the 2-C of glycine into the purine ring was measured. In 1969, Pimstone et al. (23) reported that after an intravenous injection of [2-14C]glycine, the percentages of total 14C in uric acid at the “C2 plus C8” positions fluctuated by 13–23% in patients with porphyria (n = 8) and by

### Table 1

13C-enrichment at the C8 and C5 positions of urinary uric acid by 3 one-carbon sources

<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>C5 + C8</td>
<td>C2</td>
<td>C2</td>
<td>C5 + C8</td>
</tr>
<tr>
<td>A</td>
<td>3</td>
<td>0.008</td>
<td>0.14*</td>
<td>0.39*</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>−0.005</td>
<td>0.18*</td>
<td>0.19*</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>0.016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td>0.05*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td>0.07*</td>
<td></td>
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</tr>
<tr>
<td>F</td>
<td>1</td>
<td>0.00</td>
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</tr>
<tr>
<td>G</td>
<td>1</td>
<td>−0.06</td>
<td></td>
<td>2.00*</td>
</tr>
<tr>
<td>H</td>
<td>1</td>
<td>−0.01</td>
<td></td>
<td>−0.17</td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>0.15*</td>
<td></td>
<td>0.44*</td>
</tr>
</tbody>
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1 Values are mean percentage enrichment of urinary uric acid (all voids combined). The number of voids varied from 5/d to 12/d. *Significantly greater than zero (baseline) with the use of data from all voids and paired t test, *P < 0.05.
2 [2-13C]Glycine can enrich both C4 and C5 positions.
3 Subjects A–C are from references 4 and 14, and subjects D–I are from reference 15.
TABLE 2  Studies including the enrichment or incorporation of tracers into purine and related compounds

<table>
<thead>
<tr>
<th>Authors (ref)</th>
<th>Model</th>
<th>Tracer</th>
<th>Measurement</th>
<th>Purine labeling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamura and Baggott (4)</td>
<td>Human</td>
<td>[Ring-2-14C]histidine</td>
<td>Urinary uric acid</td>
<td>See Table 1</td>
</tr>
<tr>
<td>Brown et al. (16)</td>
<td>Monkey and rat</td>
<td>[U-14C]histidine</td>
<td>Urinary uric acid</td>
<td>ND</td>
</tr>
<tr>
<td>Sprinson and Rittenberg (17)</td>
<td>Rat</td>
<td>[Ring-2-14C]histidine</td>
<td>Purine and RNA in visceral organs</td>
<td>14CO2, ND</td>
</tr>
<tr>
<td>Fish et al. (18)</td>
<td>Human with folate or B-12 deficiency</td>
<td>[Ring-2-14C]histidine</td>
<td>Urinary uric acid</td>
<td>See Table 1</td>
</tr>
<tr>
<td>Baggott et al. (14)</td>
<td>Human</td>
<td>[2-14C]Glycine</td>
<td>Urinary uric acid</td>
<td>C4 + C6/C2 + C8 = 3.3–6.4</td>
</tr>
<tr>
<td>Pimstone et al. (23)</td>
<td>Human with/without porphyria</td>
<td>[2,14C]Glycine</td>
<td>Urinary uric acid</td>
<td>ND</td>
</tr>
<tr>
<td>Heinrich and Wilson (26)</td>
<td>Rat</td>
<td>[1,2-14C]Glycine</td>
<td>Purine in carcass</td>
<td>C2/C8 = 0%/100%</td>
</tr>
<tr>
<td>Elwyn and Sprinson (27)</td>
<td>Rat</td>
<td>[2-14C]Glycine</td>
<td>Guanine in visceral organs</td>
<td>C2/C8 = 43%/57%</td>
</tr>
<tr>
<td>DeGrazia et al. (28)</td>
<td>Human with folate or B-12 deficiency</td>
<td>[3-14C]Serine</td>
<td>14CO2</td>
<td>ND</td>
</tr>
<tr>
<td>Baggott et al. (14)</td>
<td>Human</td>
<td>[14C]Formate</td>
<td>Urinary uric acid</td>
<td>See Table 1</td>
</tr>
<tr>
<td>Buchanan and Rollins (29)</td>
<td>Human with gout</td>
<td>[14C]Formate</td>
<td>Urinary uric acid</td>
<td>27–105 cpm/mg</td>
</tr>
<tr>
<td>Stahelin et al. (30)</td>
<td>Human with/without folate deficiency</td>
<td>[14C]Formate</td>
<td>Urinary uric acid</td>
<td>44–177 cpm/mg</td>
</tr>
<tr>
<td>Wagner and Levitch (31)</td>
<td>Human</td>
<td>[14C]Formate</td>
<td>In vitro incorporation into IMP in erythrocytes</td>
<td>9–978 nmol of [14C]IMP/mL of erythrocytes</td>
</tr>
<tr>
<td>Drysdale et al. (32)</td>
<td>Rat</td>
<td>[14C]Formate</td>
<td>Purines in liver and other visceral organs</td>
<td>C2 = 26–52%</td>
</tr>
<tr>
<td>Shuster and Goldin (33)</td>
<td>Mouse</td>
<td>[14C]Formate</td>
<td>Adenine in liver</td>
<td>C2/C8 = 1.7–2.0</td>
</tr>
<tr>
<td>Dowdle et al. (34)</td>
<td>Human with/without porphyria</td>
<td>[5-14C]8-Aminolevulinic acid</td>
<td>Urinary uric acid</td>
<td>C4 + C8 = 91–105%</td>
</tr>
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</table>

1-12 vitamin 8-12, cpm, counts per minute; IMP, inosine monophosphate; ND, not determined; ref, reference.

19–20% in 14 d in healthy subjects (n = 3). The total [2-14C] glycine incorporated into uric acid varied by 2.1-fold. It is necessary to note that their method did not distinguish the independent incorporation between C2 and C8. In 1 patient, the percentage of 14C incorporated into both C2 plus C8 positions declined from 28% to 20% from the first to the third day after the dose of [2-14C]glycine (23). Gutman et al. (24, 25) performed 2 studies of oral administration of [2-14C]glycine in patients with gout, where the percentage of the dose in C8 position, whereas it was not detectable at the C2 position of the purine uric acid. Using the data of their method did not distinguish the independent incorporation between C2 and C8. In 1 patient, the percentage of 14C incorporated into both C2 plus C8 positions varied by 2.1-fold.

In 1950, Heinrich and Wilson (26) examined the 14C incorporation of [1,2-14C]glycine into purine of a rat carcass. This [1,2-14C]glycine could label the C2, C4, C5, and C8 positions of the purine ring. They independently analyzed by using a degradation method the 14C labeling at the C2 and C8 positions and observed that 14C labeled the C4 and C8 positions as expected. Furthermore, they found labeling at the C6 position, whereas it was not detectable at the C2 position of the purine ring. With the use of their data, 18% of the total radioactivity in purine was found in C8. In 1954, Elwyn and Sprinson (27) reported that purine in rat visceral organs was labeled by [2-14C]glycine at the C2 plus C8 positions. By using a degradation analysis, 57% and 43% were in the C8 and C2 positions, respectively, suggesting that some of this one-carbon was utilized by AICAR transformylase. The above studies are shown in Table 2.

Incorporation of the 3-C of Serine into the Purine Ring

Serine originates from dietary sources and the biosynthesis from intermediates in glycolysis and glycogenogenesis. Dietary intake is estimated to be ~3.7 g/d (12). In the presence of H4folate, the 3-C of serine is transferred to the methylene moiety of 5,10-methylene-H4folate via SHMT. This is metabolized to the methenyl moiety of 5,10-methenyl-H4folate, then to 10-formyl-H4folate by 5,10-methylene-H4folate dehydrogenase and 5,10-methenyl-H4folate cyclohydrolase, respectively. Elwyn and Sprinson (27) reported that [3-14C]serine equally labeled the C2 and C8 positions of purine of rat visceral organs, suggesting that both GAR and AICAR transformylases utilize this one-carbon.
In humans, to our knowledge, only 1 study has been published in which [3-14C]serine was used to evaluate the 14CO2 production in adult patients with folate or vitamin B-12 deficiency (28). A marked decrease in the rate of oxidation of [3-14C]serine was observed in folate deficiency. Therefore, a major metabolic pathway for the 3-C of serine involves its conversion to 10-formyl-H4folate and subsequent carbon dioxide production through 10-formyl-H4folate dehydrogenase. The independent incorporation of radio- or stable-isotope-labeled 3-C of serine into the C2 and C8 positions of the purine ring has never been evaluated in humans. However, [2-13C]glycine produces [3-13C]serine in vitro and in vivo by the GCS reactions along with 13C-exchange by SHMT; thus, it is likely that [3-13C]serine enriches principally C8 (35, 37). In adults, ~54% of an oral glycine dose is converted to serine in 60 h (38). Therefore, it may be impossible to measure only 2-14C of glycine labeling without a simultaneous measurement of 3-14C of serine labeling in vivo. These findings suggest that [10-13C]formyl-H4folate originating from [3-13C]serine is channeled to GAR transformylase in a complex containing TFM and SHMT (6, 7). The above may be speculative, and human experiments should be performed with the use of [3-13C]serine. These studies that used labeled serine are summarized in Table 2.

Incorporation of the Formate Carbon into the Purine Ring

In humans, the normal plasma formate concentration is low (20–250 μmol/L) and increases within 30 min of an oral formate dose (39). A low formate concentration probably is maintained because excess formate is toxic. Formate is generated through folate-requiring pathways, which involves the removal of formate from 10-formyl-H4folate, where one-carbon originates from ring-2-C of histidine, 3-C of serine, and 2-C of glycine as well as from methyl groups of choline and methionine (40). Formate is generated from a wide variety of non–folate-requiring pathways as well. These include the oxidation of methanol and formaldehyde, α-oxidation of FAs, and its production from tryptophan catabolism, cholesterol biosynthesis, as well as the metabolism of δ-aminolevulinic acid and methythaedoxenosine (34, 41). In humans, formate generated from 14C-δ-aminolevulinic acid labeled primarily the C2 plus C8 positions of uric acid, with only 0.7–4.4% found in the other carbons (Table 2) (34). In adult humans, it was estimated that 0.5–1.0 mmol formate/d is generated from methythaedoxenosine metabolism (41). Although it is known that formate is generated in mitochondria (42), it remains to be determined as to how important such formation is in relation to all other formate sources as noted above. In humans, to our knowledge, mitochondrial formate generation has not been well established, and the formate production from methythaedoxenosine is the only quantitative estimate (41).

In our study, a 1.0-g oral dose of sodium [14C]formate primarily enriched the C2 position of urinary uric acid in subjects A–C in Table 1 (14). In 2 of these 3, little or no enrichment was observed at the C8 position; however, in 1 subject, C2 enrichment was ~3 times that of C8 (14). In an additional 6 subjects in our later study, 3 showed only C2 enrichment and little or no enrichment at C8 (15). One subject showed C2 enrichment, which was, again, ~3 times that of C8. In the remaining 2 subjects, little or no enrichment above background was detected at both C2 and C8 (Table 1). Thus, total [14C]formate labeling of uric acid varied widely among individuals.

Total [14C]formate labeling of uric acid varied 6.6-fold in patients with and without gout (29) and 6.4-fold in subjects who were folate deficient and later repleted (30). Similarly, total 14C in uric acid from [5-14C]δ-aminolevulinic acid varied ~3.8-fold (34). This variability is greater than that in total labeling of uric acid by [2-14C]glycine (23). Our subjects who did not utilize formate may represent one end of this spectrum of variation. Wagner and Levitch (31) observed a 100-fold variation in the ex vivo ability of RBCs to biosynthesize IMP from AICAR and [14C]formate among 16 subjects (Table 2). It is possible that, in certain individuals, PNB is completed (i.e., AICAR to IMP) in erythrocytes and erythroblasts (see below), whereas other individuals may utilize ring-2-C of histidine to complete PNB. It is important to note that formate can be oxidized to carbon dioxide in both folate- and nonfolate-dependent pathways, and the extent to which this occurs may limit its utilization in PNB (40).

[14C]Formate was reported to label equally both C2 and C8 positions of purine or uric acid in rats (32), whereas the ratio (C8:C2) of hepatic radioactivity incorporation was reported to be 1.7–2.0 in mice (33). It is obvious that our findings of 13C-enrichment at C2 and C8 in humans are not in complete agreement with those in experimental animals. This could be because of fundamental differences in one-carbon metabolism and/or methodologic discrepancies among the studies (Table 2).

Mechanistic Explanation of the Predominant Enrichment at the C2 Position by [13C]Formate in Humans

We postulated that the liver stops purine biosynthesis de novo at AICAR (14, 15). This is consistent with the low capacity of mammalian hepatocytes or liver slices to synthesize purines in vitro from AICAR, formate, or serine (43–46). Therefore, to explain our data, we tried to identify cells that 1) predominately utilize formate as a source of one-carbon, 2) have AICAR transformylase and 10-formyl-H4folate synthetase, and 3) have an external supply of AICAR. We identified erythrocytes and presumably erythroblasts in the bone marrow as being a prime candidate that fulfills all of these requirements because erythrocytes 1) possess the above 2 key enzymes but neither GAR transformylase, GCS, nor SHMT (35, 47, 48), and 2) are exposed to AICAR-ribose, or its base in the circulation, because AICA is a normal constituent of human urine (49). Therefore, in erythrocytes with no GAR transformylase, formate cannot enrich the C8 position.

Marie et al. (50) reported on a girl with severe physical and neurologic anomalies who had a congenital defect of AICAR transformylase. The patient excreted a massive amount of urinary AICA-ribose nearly equal to that of normal uric acid.
A circadian rhythm in the incorporation of \(^{13}\text{C}\)histidine into the C\(_2\) position of urinary uric acid was observed with the morning \(^{13}\text{C}\) incorporation peak in 2 subjects (4). In 7 subjects, a similar enrichment peak in the C\(_2\) position was detected after the dose of \(^{13}\text{C}\)formate (15). This is consistent with the peak in circulating uric acid concentrations in humans (52). It has been proposed that the erythroblasts complete PNB (see above) in certain individuals by converting AICAR to IMP at a normal rate. Therefore, we hypothesized that the formation of IMP from AICAR with formate and \(\text{H}_4\text{folate}\) occurs in erythrocytes and erythroblasts.

Lowy et al. (48) and Wagner and Levitch (31) reported that the in vitro incubation of intact erythrocytes with \(^{14}\text{C}\)formate and AICA-riboside led to the formation of IMP, and that this metabolism required \(\text{H}_4\text{folate}\), ATP, and the formation of AICAR from AICA-riboside. The mass of human erythrocytes plus erythroblasts, both almost equal to that of the liver, should not be underestimated in its ability to metabolize purines (51).

Our data suggest that \(^{13}\text{C}\) from formate and 2-\(^{13}\text{C}\) from glycine are metabolized differently; therefore, the incorporation of \(^{13}\text{C}\) of formate and 2-\(^{13}\text{C}\) of glycine into purines may require metabolic synchronization, which could occur at the molecular level as well as interactions at the organ level (i.e., erythrocytes/erythroblasts and liver).

**Circadian Rhythm**

A circadian rhythm in the incorporation of [ring-2-\(^{13}\text{C}\)]histidine into the C\(_2\) position of urinary uric acid was observed with the morning \(^{13}\text{C}\) incorporation peak in 2 subjects (4). In 7 subjects, a similar enrichment peak in the C\(_2\) position was detected after the dose of \(^{13}\text{C}\)formate (15). This is consistent with the peak in circulating uric acid concentrations in humans (52). It has been proposed that the erythroblasts complete PNB (see above) in certain individuals by converting AICAR to IMP (14, 15). If this is true, it explains why DNA synthesis in the bone marrow peaks at 800–1600 h in humans (53). With the use of a mouse model, Fustin et al. (54) reported a circadian rhythm with peak concentrations of adenosine, guanosine, and inosine nucleotides appearing in the liver in the morning, which is consistent with our results.

The rhythmicity of PNB could be the result of changes in substrate availability, enzyme activities, chain lengths of folate polyglutamates, and feedback inhibition. There was no apparent circadian rhythm for the enrichment of the C\(_8\) position of urinary uric acid after the dose of 2-\(^{13}\text{C}\)glycine in our study. However, collagen turnover, substantial dietary intake, and biosynthesis of glycine could have obscured a rhythm. Furthermore, the flux of one-carbon units through GAR transformylase may not noticeably fluctuate.

Regardless of the exact metabolic mechanism of PNB, the circadian rhythm could be useful in timing doses of drugs, which are known to block PNB. For example, an oral methotrexate dose for the treatment of an autoimmune disease could be more effective when given before bedtime rather than in the morning. For example, doses were given in the evening to maximize the effect of methotrexate (55). Furthermore, the reduction in dietary histidine and tryptophan (to reduce formate production) intake in concert with the administration of cancer chemotherapy before the time of maximum PNB may potentiate the efficacy of anticancer drugs.

**Comparison of GAR and AICAR Transformylases: A Hypothesis**

If our findings are true, AICAR transformylase is at a metabolic disadvantage compared with GAR transformylase. The capacity to biosynthesize serine and glycine must be large, because they originate from the 3-phosphoglycerate intermediate in glycolysis and glycogenesis. In contrast, histidine biosynthesis (if any) must be low, and circulating and intracellular formate concentrations must be kept to a minimum to avoid acidosis. If, however, the coenzyme for AICAR transformylase is 10-formyl-\(\text{H}_4\text{folate}\) instead of 10-formyl-\(\text{H}_2\text{folate}\) (9), a portion of 10-formyl-\(\text{H}_4\text{folate}\), which leaks away from the TFM and becomes oxidized to 10-formyl-\(\text{H}_2\text{folate}\), could supplement the low one-carbon pool (compared with serine and glycine pools) originating from histidine. This assumption may be reasonable, because 10-formyl-\(\text{H}_4\text{folate}\) cannot be utilized by GAR transformylase. It should be noted that folate produced by GCS and SHMT is 5,10-methylene-\(\text{H}_4\text{folate}\), of which the pteridine ring is not labile to oxidation. Once converted to 10-formyl-\(\text{H}_4\text{folate}\) by TFM, however, the ring becomes labile to oxidation to 10-formyl-\(\text{H}_2\text{folate}\). If TFM is closely associated with GAR transformylase in humans (6, 7), this enzyme complex may “protect” the labile 10-formyl-\(\text{H}_4\text{folate}\) from oxidation by immediately furnishing it to GAR transformylase. In contrast, AICAR transformylase can utilize both 10-formyl-\(\text{H}_4\text{folate}\) as well as 10-formyl-\(\text{H}_2\text{folate}\) and does not require 10-formyl-\(\text{H}_2\text{folate}\) to be “protected” (9).

**Conclusions**

We reviewed the data indicating that the one-carbon sources for the C\(_8\) position of the purine ring are 2-C of glycine (and most probably 3-C of serine) and those for the C\(_2\) position are 5-C of histidine and formate in humans (4, 14, 15). These findings are unexpected considering the previously held notion that the formyl carbon of 10-formyl-\(\text{H}_4\text{folate}\) is equally incorporated into both C\(_8\) and C\(_2\) positions of the purine ring, regardless of its sources.

Folate-dependent PNB is a fundamental biological process; therefore, it is the target for drugs used for cancer chemotherapy as well as for the treatment of autoimmune disease. This area is important in terms of basic research as well as in clinical settings. Many scientists consider that they already know a great deal about folate-dependent PNB in humans; however, this may not be true. Furthermore, this area of research with the use of human subjects has largely been neglected for the past several decades. This should be reestablished. Considering that folate-dependent PNB in humans may be accomplished utilizing various organs depending on one-carbon precursors, it is evident that useful data will be obtained only by performing in vivo experiments in humans.
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References