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Review

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Formate: an essential metabolite, a biomarker, or more?

Abstract

Plasma and urinary formate concentrations were recently found to be elevated during vitamin B12 and folate deficiencies. It was proposed that formate may be a valuable biomarker of impaired one-carbon metabolism. Formate is an essential intermediary metabolite in folate-mediated one-carbon metabolism and, despite its importance, our knowledge of its metabolism is limited. Formate can be produced from several substrates (e.g., methanol, branched chain fatty acids, amino acids), some reactions being folate-dependent while others are not. Formate removal proceeds via two pathways; the major one being folate-dependent. Formate is a potentially toxic molecule and we suggest that formate may play a role in some of the pathologies associated with defective one-carbon metabolism.

Keywords: folic acid; formic acid; vitamin B2; vitamin B6; vitamin B12.

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The importance of formate in intermediary metabolism

Formic acid and its conjugate base formate are essential endogenous one-carbon metabolites in virtually all living organisms. Formic acid is sometimes called methanoic acid and with its chemical formula, HCOOH, it is the simplest carboxylic acid. The name formic acid takes its origin from *formica*, the latin for ants from which it was first distilled by Hulse and Fisher after they observed that the ants secreted a substance that caused blue flowers to turn red [1]. For some time this acid was believed by chemists to be acetic acid, malic acid or a mixture of both. However, after thorough examination, it was demonstrated that it differed from acetic and malic acids on the basis of its specific gravity, its reaction with alkali, its metallic salts and its chemical affinities [2]. Formic acid has a pKa of 3.77 and, as a result, most of it occurs in the body as the formate anion.

The importance of formate in intermediary metabolism was recognized over six decades ago when it was shown that the carbon of formate is incorporated into nucleic acids and into the glucogenic amino acid serine [3–6]. The incorporation of formate into nucleic acids and serine requires its activation by tetrahydrofolate (THF) [7] and therefore the metabolism of formate and folate are tightly related. Our current understanding of cellular folate metabolism involves two parallel pools of folate, one mitochondrial and the other cytosolic, connected by one-carbon donors such as glycine, serine and formate [8]. For instance, mitochondria can take up serine, oxidize two of its three carbons to formate and export this to the cytosol where it re-enters the folate pool to participate in methylation reactions or nucleotide synthesis (reviewed by Tibbetts [8]). These observations, combining the roles of formate in amino and nucleic acid metabolism, illustrate the central and essential role of formate in one-carbon metabolism.

Despite the importance of formate in intermediary metabolism, our knowledge of its metabolism is limited. Annison [9] showed that formate represents 10%–30% of the total volatile fatty acids in the blood of numerous animals, but most importantly, that its concentration is similar in goats, cattle, horses, dogs, cats and humans. This observation, combined with that of formate production by perfused goat liver preparation by McCarthy et al. [10] motivated Annison and White [11] to conduct the first and only available constant-infusion study of formate turnover. They reported a production rate of approximately 4.6 mg (100 μmol)/kg/h; a rate that is very similar

to the one we measure in rats in our laboratory (unpublished data). Formate removal has been explored in more detail, principally because of the implication of elevated levels of formic acid in methanol toxicity.

In this short review article, we will first look at the folate-dependent and -independent sources and sinks of formate. We will then discuss the usefulness of formate as a biomarker during vitamin B deficiencies and its potential toxic actions.

Folate-dependent production of formate

Folate-mediated production of formate from common one-carbon donors involves the entry of the donors into mitochondria where their labile carbon is incorporated into the folate pool to form 5,10-methylene-tetrahydrofolate; this is subsequently oxidized to produce formate and THF (Figure 1).

Serine

The catabolism of dietary serine has the potential to yield two one-carbon groups. Serine and THF can be converted, via serine hydroxymethyltransferase (SHMT), to 5,10-methylene THF and glycine. The mitochondrial catabolism of glycine has the potential to yield the second one-carbon group (see below). Two separate isoforms of SHMT are coded for in the nuclear DNA, one of which is expressed in the cytoplasm (SHMT1) and one of which is expressed in mitochondria (SHMT2) [12, 13]. Davis et al. [14], using a stable-isotope approach to delineate quantitative contributions to the one-carbon pool, suggest that serine catabolism is responsible for the majority of one-carbon units utilized by the cytoplasmic transmethylation cycle. Beaudin et al. [15] used a mouse knockout model of cytoplasmic SHMT to investigate this enzyme's role in thymidylate synthesis. They found that the homozygous *SHMT1* knockout fed a folate deficient diet had diminished thymidylate production together with neural tube defects in developing mouse embryos. This demonstrated the importance of serine-derived one-carbon groups in thymidylate biosynthesis. Further work by Anderson and Stover [16] demonstrated that the mitochondrial *SHMT2* gene codes for two transcripts, one of which is localized, in lesser quantities, to the cytoplasm and nucleus. Survival of mice with a homozygous *SHMT1* knockout is attributed to the small activity provided by the cytoplasmic and nuclear variants of this alternate *SHMT2* expression.

Choline

Choline is unique among the one-carbon precursors in that it has the ability to donate up to four of its constituent carbons to the one-carbon pool. Choline is initially catabolized to betaine aldehyde and subsequently to betaine by choline dehydrogenase and betaine aldehyde dehydrogenase, respectively [17]. Both of these reactions occur in mitochondria. Betaine must then be exported to the cytoplasm where betaine:homocysteine methyltransferase (BHMT) uses one of its methyl groups for the remethylation of homocysteine to methionine, forming dimethylglycine (DMG). This methionine can, of course, be employed for SAM production, as well as other metabolic fates (such as protein synthesis). DMG can be transported into mitochondria, where three sequential enzymatic reactions remove its three carbons (Figure 1). Specifically, DMG dehydrogenase converts DMG to sarcosine, followed by sarcosine dehydrogenase which converts sarcosine to glycine [18]. Finally glycine is catabolized to ammonia and carbon dioxide by the glycine cleavage system [19]. Each of these three

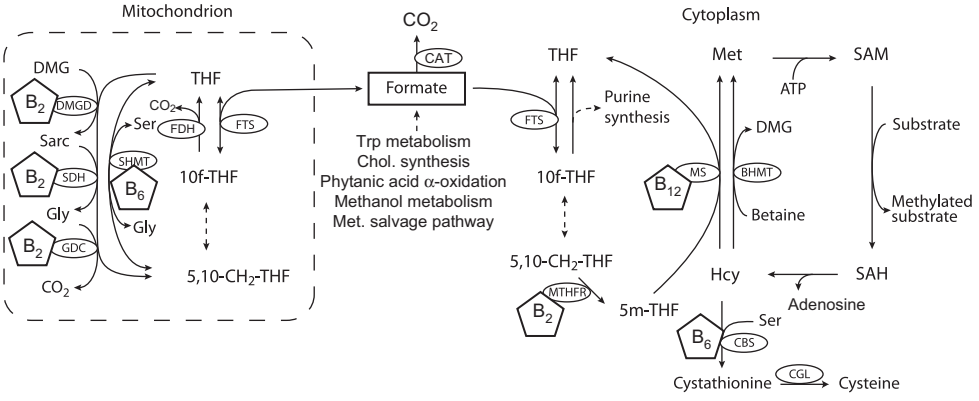

Figure 1 Folate-mediated one-carbon pathway centered on formate.

reactions requires THF as a co-factor and produces a mitochondrial molecule of 5,10-methylene THF. The mitochondrial sarcosine dehydrogenase can convert sarcosine to glycine and formaldehyde in the absence of folate. Whether this occurs in vivo during folate deficiency is not known.

Glycine

The multi-enzyme complex responsible for the catabolism of glycine is only expressed in mitochondria [19]. The Glycine Cleavage System (GCS) consists of four components acting in concert; glycine dehydrogenase, aminomethyltransferase (AMT), GCS protein H and dihydrolipoamide dehydrogenase. The GCS is also involved in the last of several sequential mitochondrial catabolic reactions that remove and activate one-carbon groups from choline. Narisawa et al. [19] used a knockout mouse model in which AMT was eliminated; this resulted in neural tube defects in the developing mouse embryos. This observation highlights the importance of mitochondrial, glycine-derived one-carbon groups to the one-carbon pool.

Methionine

Methionine contributes to the cytoplasmic one-carbon pool indirectly through its incorporation into S-adenosylmethionine (SAM) by methionine adenosyltransferase (Figure 1). SAM is the key substrate for the process of transmethylation whereby numerous methyltransferases transfer methionine's methyl group to a variety of nucleophilic substrates, to produce methylated products [20] and SAH (S-adenosyl-L-homocysteine). Glycine-N-methyltransferase (GNMT) serves as an overflow mechanism such that if the cell generates more SAM than it requires for methylation and other reactions, the methyl group may be transferred to glycine, creating sarcosine [21]. Sarcosine (also a catabolite of choline) is subsequently catabolized through the choline degradation pathway, thereby contributing directly to the mitochondrial 5,10-methylene THF pool (Figure 1). The SAH produced by methyltransferase reactions is hydrolyzed to adenosine and homocysteine. Re-methylation of homocysteine regenerates methionine with methyl groups provided either by 5-methyl THF (catalyzed by methionine synthase) or betaine (catalyzed by BHMT). While methionine synthase is widely distributed in tissues, BHMT has a rather restricted distribution in humans, BHMT only occurs in the eye, kidney and liver. This remethylation of homocysteine to methionine requires an appropriate one-carbon unit; this may be provided by choline via betaine to BHMT [14] and by serine (via cytoplasmic SHMT) [22], or cytoplasmic formate (via incorporation into the cytoplasmic folate pool by 10-formyl THF synthase) to methionine synthase.

Folate-independent production of formate

Formate production does not always require folate. The metabolism of methanol is perhaps the most obvious example of formate production without the involvement of folate; formate is also produced in the metabolism of tryptophan and phytanic acid and in the synthesis of cholesterol. We are not aware of any data on the relative contributions of folate-independent and folate-dependent pathways for formate production.

Methanol

Methanol poisoning was practically unknown before 1890, at which time an inexpensive method for producing pure methanol was discovered. Methanol toxicity is characterized by a latent period of many hours followed by an acute metabolic acidosis and ocular toxicity. Methanol is converted to formaldehyde by alcohol dehydrogenase and formaldehyde is further converted to formic acid by aldehyde dehydrogenase. It is worth noting that in rodents, the first step of methanol metabolism is catalyzed by the catalase-peroxidase system rather than by alcohol dehydrogenase [23]. Formic acid has long been regarded as the peccant substance in methanol poisoning [24]; however, it was not until the work of Martin-Amat et al. [25] and Tephly et al. [26] that the involvement of formate in both the acidosis and ocular toxicity was established. Tephly [23] has reviewed the comparative aspects of methanol toxicity. The toxicity of methanol is evident in humans and monkeys while it is not observed in lower animals such as mice and rats. The accumulation of formate in humans and monkeys is attributed to their low capacity for formate removal owing to their relatively low concentration of hepatic THF and 10-formyl-THF dehydrogenase activity. Human blood contains a low endogenous concentration of methanol. The origin of this methanol remains controversial; however it is clear that some of it comes from the fermentation in the gastro-intestinal tract of pectin-containing fruits [27]. In addition, alcoholic beverages inevitably contain small quantities of methanol that is produced as a congener of ethanol [28]. While these sources of methanol indubitably

contribute to the pool of formate their relative contribution to formate metabolism remains unknown. There are also reports of methanol as a trace product of mammalian intermediary metabolism [29]; however, its net contribution to the formate pool may be limited since methanol also seems to be produced from SAM [30].

Tryptophan

Tryptophan catabolism involves an initial oxidation step, catalyzed by tryptophan dioxygenase. This produces *N*-formylkynurenine which is in turn converted to kynurenine and formate in a folate-independent manner by kynurenine formamidase [31].

Cholesterol

The production of cholesterol (27 carbons) from its precursor lanosterol (30 carbons) involves a number of enzymatically-catalyzed reactions, including three demethylations [32]. One of these carbons is released as formate, through the action of lanosterol 14-demethylase, a cytochrome P450 enzyme which cleaves the initial methyl group from lanosterol [33].

Branched chain fatty acids

The metabolism of branched chain fatty acids through α-oxidation [34], which occurs in peroxisomes, also produces formate. Phytanic acid, a tetramethyl, branched-chain fatty acid is produced from phytol, which anchors chlorophyll in the chloroplast membrane. Of dietary origin, it is found at low concentration in human plasma. Work by Poulos et al. [35] using skin fibroblasts demonstrated that, when incubated with labeled phytanic acid, significant quantities of labeled formate were generated. Labeled CO_2 was also found and assumed to result from the incorporation of labeled formic acid into the folate pool, and subsequent oxidation. The production of formate from phytanic acid was further validated in vivo [36]. More recently, the α-oxidation of 2-hydroxylated straight chain fatty acids was also shown to produce formate in rat C6 glial cells and human fibroblasts [37].

Methionine salvage pathway

The principal fate of SAM is its utilization in biological methylations; however decarboxylated SAM can also transfer aminopropyl groups, e.g., during polyamines synthesis. 5′-methylthioadenosine (MTA), formed during such aminopropyl transfer reactions, is converted to methionine via the methionine salvage pathway. One of the enzymes of this pathway, catalyzed by the enzyme aci-reductone dioxygenase produces a formate molecule [38].

Metabolic fates of formate

Damian and Rabbe [39] have shown that formate is rapidly eliminated by the perfused rat liver; this elimination followed Michaelis-Menten kinetics and gave a Vmax of 10 μmol/min at a Km of 1.3 mM. This is a very high rate and, obviously, the rate of disposal will be much lower at the physiological plasma concentration of about 50 μM. Damian and Rabbe [39] also showed that formate may be eliminated in the urine, but this is a minor route compared to metabolic disposition. When rats were provided with quite high (1 mM) concentrations of formate almost 20% of it was eliminated in the urine. We have found rat urinary [formate] to be approximately 10-fold that of plasma [formate] [40], also suggesting that renal elimination of formate occurs but that it is likely to be a minor route. It appears that formate is reabsorbed by the rat kidney, although there is some controversy as to whether this is passive [41] or active [39].

Metabolic formate elimination appears to occur via its oxidation to carbon dioxide. There are a number of mechanisms by which this can occur. Catalase can oxidize formate, a process that requires hydrogen peroxide, according to the equation:

$$H_2O_2+H^++HCOO^-\rightarrow 2H_2O+CO_2$$

The extent to which catalase plays a role in formate disposition is uncertain, and may involve species differences. Administration of the catalase inhibitor, 3-aminotriazole, to mice at doses sufficient to inhibit the enzyme by 95% did not affect the oxidation of formate to carbon dioxide at low concentrations of formate; however, 3-aminotriazole did decrease formate oxidation at high formate concentrations [42]. These results would suggest that catalase is not required for the oxidation of formate at physiological formate concentrations but may become important at the elevated [formate] that occurs upon methanol intoxication. However, 3-aminotriazole does inhibit formate oxidation in rats made moderately folate deficient [43], suggesting the occurrence of a folate-dependent mechanism for formate oxidation. Such a

mechanism is provided by the combined actions of 10-formyltetrahydrofolate synthetase and 10-formyltetrahydrofolate dehydrogenase which, respectively, catalyze the following reactions:

$$\text{Formate+THF+ATP}\rightarrow\text{10-formyltetrahydrofolate+ADP+Pi},$$

$$\text{10-formyltetrahydrofolate+NADP}^{+}\rightarrow\text{THF+CO}_2\text{+NADPH}$$

There are both cytosolic and mitochondrial isoforms of both of these enzymes. Cook et al. [42] investigated the role of these enzymes in the cytoplasm in NEUT2 mice which lack cytosolic 10-formyltetrahydrofolate dehydrogenase. The oxidation of low doses of formate to carbon dioxide by these mutant mice occurred at about half the rate of that found in normal mice. However, 3-aminotriazole treatment did not affect the residual formate oxidation seen in NEUT2 mice, indicating that this is not brought about by catalase. These results suggest the existence of a third, as yet undefined, mechanism whereby formate may be oxidized to carbon dioxide [42].

Formate as a biomarker of vitamin B deficiencies

Folate-mediated one-carbon metabolism depends on B-vitamins as cofactors for many of the key reactions involved. Any deficiency of these key vitamins impairs the pathway, in whole or in part, and is generally accompanied by an elevation of homocysteine concentration. We have recently shown that both the plasma concentration and urinary excretion of formate are elevated in folate deficient rats [40]. The elevation in urinary excretion of formate in this study confirms work done in the 1950s that showed elevated urinary formate excretion in folate deficient rats [44]. Elevations in plasma formate levels due to folate deficiency are most likely due to continued production of formate by folate-independent reactions, while the incorporation of cytosolic formate into the folate one-carbon pool is impaired.

During vitamin B12 deficiency, plasma homocysteine and plasma and urinary formate concentrations are also elevated [40]. The accumulation of homocysteine is attributable to the fact that methionine synthase employs 5-methyl cobalmin as its cofactor. Vitamin B12 deficiency results in a functional, intracellular folate deficiency, a phenomenon known as the methyl-folate trap [45, 46]. The cytosolic folate coenzymes largely accumulate as 5-methyl-THF, reducing the concentration of THF and impeding the incorporation of formate in the folate pool with consequent formate accumulation.

Vitamin B6 is a cofactor for cystathionine-β-synthase (CBS); during vitamin B6 deficiency, plasma total homocysteine accumulates as a result of its impaired catabolism. We studied the effect of vitamin B6 deficiency on formate concentration in rats and found that its concentration was not affected during deficiency [40]. FAD, a coenzyme derived from riboflavin (vitamin B2), is required for several reactions in folate-mediated one-carbon metabolism. FAD is a component of MTHFR [47] and of methionine synthase reductase (MTRR), an enzyme which is essential for the reactivation of methionine synthase from its oxidized form [48]. Not surprisingly, vitamin B2 deficiency results in an accumulation of homocysteine [49]. Finally, FAD is a component of the mitochondrial glycine cleavage system [50]. No published data are available on the effects of vitamin B2 deficiency on formate concentration; however work in progress in our laboratory has shown that circulating formate concentrations are lower in vitamin B2 deficient rats compared to control rats (unpublished). This result could be explained by a reduced activity of the glycine cleavage system and ensuing reduced mitochondrial formate production.

Table 1 summarizes the effects of vitamin B deficiencies on homocysteine, formate and MMA concentrations in rats. Adding formate to the commonly measured homocysteine and MMA allows for the discrimination between vitamin B12-, folate-, vitamin B6- and vitamin B2-deficiencies. These findings stress that formate may not just be an important intermediary metabolite but also a valuable biomarker for vitamin B deficiencies. No information is available yet on the effects of relatively common genetic defects in proteins (transporters and enzymes) involved in folate-mediated, one-carbon metabolism (e.g., MTHFR 677C→T) on formate concentrations but we anticipate that formate may become a valuable diagnostic tool for such genetic diseases.

Vitamin deficiency	Hcy	Formate	MMA
Vitamin B12	↑	↑	↑
Folate	↑	↑	↔
Vitamin B6	↑	↔	↔
Riboflavin	↑	↓	ND

Table 1 Plasma formate, Hcy, and MMA levels permit discrimination between four common vitamin B deficiencies. Arrow up means increased concentration, arrow down means decreased concentration and horizontal means no change.
ND, not determined.

The toxicity of formate

Much of the work on the toxicity of formic acid is related to methanol poisoning which involves an acute accumulation of very high concentrations of formate, causing an uncompensated acidosis, coma and eventually death. Here, we are more interested in the potential toxicity of formate at physiologically relevant concentrations; i.e., concentrations that would be encountered during impaired one-carbon metabolism. Is it possible that elevated formate could exert pathological effects? Early work from Nicholls (1975) showed that formate is an inhibitor of cytochrome c oxidase; however its K_i is in the millimolar range [51, 52]. More recently Kapur et al. (2007) reported that a formate concentration as low as 1 mM causes neurotoxicity in rat hippocampal brain slices [53]. As rats have a substantially better capacity to eliminate formate via the folate pathway than do humans, it is conceivable that formate, at lower concentrations, may be a more serious issue in humans. We suggest that further work on the toxicity and pathological action of formate is warranted.

Conclusions

Formate is at the heart of the folate-mediated one-carbon metabolism pathway. As both its production and removal involve the folate pathway, formate is a valuable biomarker of impairments of the pathway originating from vitamin B deficiencies and potentially from genetic defects. Furthermore, formate is a toxic metabolite and the elevation of its concentration during folate- and vitamin B12 deficiencies may elicit some of the pathologies (e.g., neurocognitive and neuropsychiatric) associated with those vitamin deficiencies.

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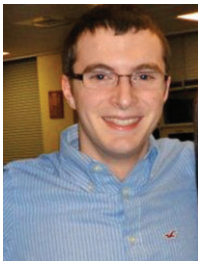

Luke Macmillan recently graduated from Memorial University of Newfoundland with a Bachelor of Science (Hon) in Biochemistry/Nutrition (Memorial University). He is currently enrolled as a Masters student at Memorial University. His current project is dealing with formate turnover in vivo in both healthy and B vitamin deficient animals.

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