High-dose Therapy with Ascorbate, Niacin, Folate and B_{12}: Pauling was Right but for the Wrong Reason

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***Abstract**: Pauling suggested that responses to high-dose vitamin therapy were due primarily to small increases in response due to lack of complete saturation of enzyme targets. He also suggested that they may be due, in part to "local vitamin deficiencies" although the origin of such deficiencies were unclear. Ames suggested that such therapy might be explained by enzyme polymorphisms involving mutants with lowered Michaelis constants, and while this is an explanation in some cases, this mechanism does not explain any effectiveness of in the broader population of diseased patients. Responses to four vitamins advocated by Pauling can be best explained by the effects of these vitamins on lowering the nitric oxide (NO)/peroxynitrite (ONOO-) cycle, a possible generic mechanism for many different chronic inflammatory diseases. Ascorbate lowers three aspects of the central couplet of the cycle, acting as a peroxynitrite scavenger, restoring tetrahydrobiopterin (BH4) by reducing an oxidized form and inducing increased de novo BH4 synthesis. The nicotinamide form of niacin inhibits poly adenosine diphosphate-ribosylation, thus sparing nicotine adenine dinucleotide (NAD), as well as supplying niacin for synthesis of NAD/NADH, thus helping restore mitochondrial function in NO/ONOO- cycle diseases. Folate in the form of 5-methyltetrahydrofolate is a potent peroxynitrite scavenger, thus lowering the NO/ONOO- cycle in that way. Vitamin B_{12} as hydroxocobalamin lowers the cycle by acting as a nitric oxide scavenger. Each of these responses involve mechanisms that are distinct from the classic functions of these vitamins and they all require supraphysiological levels in order to be effective. Thus they provide explanations for each of the four high-dose therapy vitamins that Pauling suggested and for Hoffer's responses to niacin therapy.*

Introduction

Pauling advocated high-dose therapy involving one or more of four vitamins for a variety of diseases. These vitamins are ascorbate (vitamin C), niacin, folate and vitamin B_{12}.[1,2] Hoffer focused his attention for treatment of schizophrenia and other diseases on high-dose therapy using niacin and to a lesser extent ascorbate.[3-5] However there has not been a plausible explanation, in my judgment, for any substantial efficacy for these four agents which requires the use of such high doses.

Pauling's main suggestion for mechanism was that the normal physiological range of pools of the active form of these vitamins might provide only perhaps 90% of the maximum response and that high-dose therapy might give a small but perhaps physiologically important improvement and might explain responses to high-dose therapy.[1,2] Many scientists including the author have found this explanation unconvincing and have therefore been skeptical about this interpretation of high-dose therapy. Pauling provided a second explanation, that there may be local deficiencies in these vitamins and, where appropriate, their active cofac-

tors and that allaying such local deficiencies might explain the efficacy of such high-dose therapy. While there is some evidence for such local deficiencies, this second explanation failed to explain their origin or how they may fit into the overall etiology of the diseases involved. It is the author's view that allaying local deficiencies provides a partial explanation but that most of the explanation lies elsewhere.

Ames suggested a third explanation for high-dose therapy,[6] following an earlier suggestion also made by Pauling.[1] Ames and coworkers suggested that enzyme polymorphisms may involve alleles encoding enzymes with increased Michaelis constant (Km) values for vitamins or their active cofactors and that high-dose therapy could be effective in the treatment of people carrying such polymorphisms.[6] There is no question that people carrying some such polymorphic genes will respond to high-dose therapy due to this mechanism, but it is questionable whether this could be a more general explanation for any efficacy of high-dose therapy treatments for common diseases.

Hoffer had a specific interpretation[3-5,7] of the mechanism of high-dose therapy with niacin that is discussed below, which also may be questioned.

The apparent efficacy of very high dose intravenous ascorbate in the treatment of some cancer patients has been ascribed to the action of ascorbate in reducing molecular oxygen to hydrogen peroxide and the sensitivity of the cells of some types of cancer to hydrogen peroxide.[8-10] Thus the apparent action of very high dose IV ascorbate here seems to be completely unrelated to either of Pauling's two explanations or Ames' explanation.

The NO/ONOO- Cycle as an Explanation of Many Chronic Diseases

The NO/ONOO- cycle, is a complex biochemical vicious cycle that was first developed as an explanation for the etiology of such related and often comorbid multisystem diseases chronic fatigue syndrome/myalgia encephalomyelitis (CFS/ME), multiple chemical sensitivity (MCS), fibromyalgia (FM) and post-traumatic stress disorder (PTSD).[11-20] It is named for two of its elements, nitric oxide (NO) and peroxynitrite (ONOO-) but contains many other elements, each of which is important to the etiology of most diseases caused by the cycle (**Figure. 1,** p.31).

The cycle involves a whole series of inflammatory aspects (right side, Fig. 1) including activation of the transcription factor NF-kappa B, a series of inflammatory cytokines (upper right box, Fig. 1) and induction of the inducible nitric oxide synthase (iNOS), suggesting that the entire inflammatory cascade is likely to be active in the NO/ONOO- cycle diseases; increased superoxide production both intramitochondrial and extramitochondrial (center, left); elevated levels of peroxynitrite (abbreviated PRN, below center) and depletion of tetrahydrobiopterin (BH4, below center towards the right); changes in certain physiological receptors are also involved, including excitotoxicity and excessive N-methyl-D-aspartic acid (NMDA) activity (top, center). Each of these cycle elements are linked to each other through a series of well-accepted biochemical and physiological mechanisms indicated by the arrows in Figure 1. The cycle can be seen to be made up of multiple interacting cycles, making it difficult to down-regulate, with what is called the central couplet, the reciprocal relationship between peroxynitrite elevation and BH4 depletion at its core.[11,12,19] The cycle is based on five testable principles[11-13,21] that can be summarized as follows:

1. Stressors, especially short-term stressors act to initiate cases of NO/ONOO- cycle diseases by raising levels of elements of the cycle, often leading to NO increases, thus starting the cycle.

2. The cycle is present during the chronic phase of illness, predicting then that each of the elements of the cycle will be elevated.

3. The symptoms and signs of a NO/ONOO- cycle disease must be caused by one or more elements of the cycle.

4. The cycle is primarily local, localized to different tissues in different individuals.

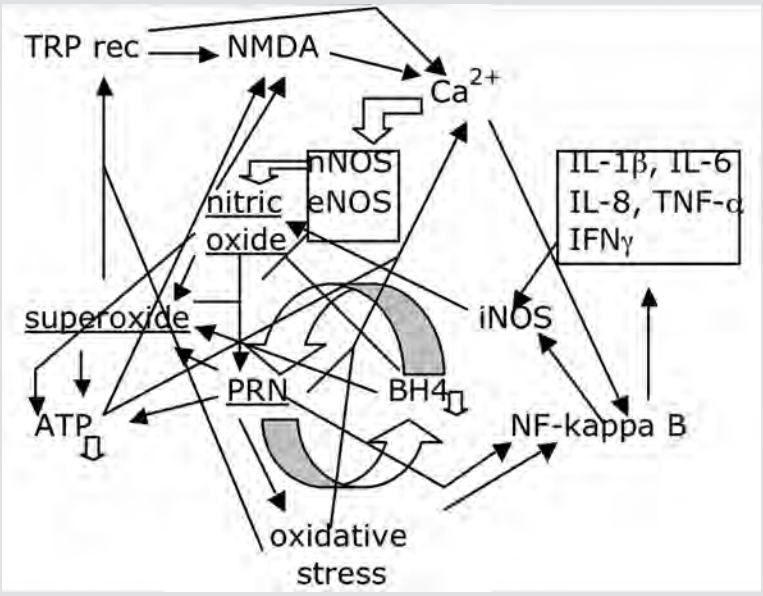
The reason for this is because the three small compounds in the cycle, NO, ONOO- and superoxide all have short half lives in biological tissues and the mechanisms of the cycle, the various arrows shown in Figure 1, all act at the levels of individual cells. Because of its primarily local nature, the cycle is often elevated in different tissues in different individuals, leading to variation in symptoms from one individual to another and often different diagnoses, as well.

5. NO/ONOO- cycle diseases should be treated with agents that down-regulate parts of the cycle. In other words, we should treat the cause, rather than the symptoms.

A good fit to each of these five principles for a specific disease/illness provides a distinct type of evidence for the causality of the cycle. Because of this, if there is a good fit to each of the five principles for a specific disease, this means that that disease is a good candidate to be a NO/ONOO- cycle disease. The five principles serve, for NO/ONOO- cycle diseases, a roughly similar function to what Koch's postulates serve for infectious diseases.[11-13, 21]

Most of the consideration of the NO/ONOO- cycle as a disease mechanism has focused on CFS/ME, MCS, fibromyalgia and PTSD.[11-20] Gulf War syndrome/illness is a combination of the four and is presumably also a NO/ONOO- cycle disease.[13,22] However 14 additional diseases are apparent NO/ONOO- cycle diseases, at least based on a relatively superficial consideration, as follows (Chapter14),[13] and[21,23] tinnitus, post-radiation syndrome, multiple sclerosis, autism, overtraining syndrome, silicone-

Figure 1: Each of the arrows represents one or more mechanisms by which one element of the cycle increases the level of a second element. The various parts of the cycle are discussed further in the text. Abbreviations: TRP, several transfer receptor potential receptors, especially TRPV1, TRPA1 and TRPM2; NMDA is a glutamate receptor that is specifically stimulated by N-methyl-D-aspartate; PRN is peroxynitrite; BH4 is tetrahydrobiopterin; iNOS, nNOS and eNOS are all nitric oxide synthase enzymes. Taken from the author's web site with permission.



implant-associated syndrome, Sudeck's atrophy, postherpetic neuralgia, chronic whiplash-associated disorder, amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, asthma and irritable bowel syndrome. Most of these differ from one another in the critical tissues involved and thus can easily be distinguished from each other by a local mechanism with different tissue distribution. A more detailed and complete consideration of the first two of these as NO/ONOO- cycle diseases, tinnitus[21] and post-radiation syndrome[23] has been published elsewhere.

The NO/ONOO- Cycle as a Generic Explanation of Chronic Inflammatory Disease

The possible role of the cycle in many different diseases suggests that it should be considered as a generic model of chronic inflammatory disease. The following is taken from the author's web site with permission (thetenthparadigm.org/otherdiseases.htm).

One question that should be asked is the following: From first principles, what should a generic model of chronic inflammatory disease look like? I am not going to try to document the following argument but I think that those of you who have a deep familiarity with chronic inflammatory diseases will see its merits.

I argue that it should first of all be a vicious cycle mechanism, otherwise how can so many short-term initiating stressors apparently lead to chronic illness? And it should basically be local in nature, localized in each case to certain regions of the body, but not to other regions. Otherwise how can one explain how a single mechanism can explain many different chronic inflammatory diseases?

It should include, obviously, elevated inflammatory cytokines and other inflammatory markers and oxidative stress and increased NF-kappa B activity, nitric oxide levels and iNOS induction, all common aspects of inflammatory biochemistry. It should also include mitochondrial dysfunction, since this is also reported to occur in many different chronic inflammatory diseases. Because excitotoxicity including excessive NMDA activity is essentially universally found in chronic inflammatory diseases that impact the central nervous system, this may well be another aspect of the mechanism; while such excessive NMDA activity has been much less studied in peripheral chronic inflammatory diseases, the widespread occurrence of NMDA receptors in the peripheral nervous system and in other, non-neural tissues[24,25] suggest that excessive NMDA activity may have a much wider role. BH4 depletion is much less studied than are most of these other NO/ONOO- cycle elements, but is increasingly being reported in various chronic inflammatory conditions and so may also be argued to be a part of such a generic mechanism. After all, it is reported that BH4 depletion has roles in such diseases as Parkinson's disease, Alzheimer's disease, ALS, heart failure, schizophrenia, autism, bipolar disorder, major depression, mast cell activation, chronic renal failure, hypertension, and pulmonary hypertension.

So it may be argued that 'from first principles,' a generic model of chronic inflammatory disease will look very much like the NO/ONOO- cycle mechanism! That does not absolve us from making a detailed case for each disease which may be considered as a possible NO/ONOO- cycle disease. But it does argue that we may be able to explain possible responses to a therapeutic agent in many diseases by looking at its ability to down-regulate one or more aspects of the cycle.

High-Dose Therapy Will Down-Regulate the NO/ONOO- Cycle

Each of the four vitamins discussed above, that Pauling focused on, will when used in high doses, down-regulate important aspects of the cycle and will, therefore, be predicted to lower to some extent the cycle as a whole.

Three of these, ascorbate,[26-28] folate,[29,30] and the hydroxocobalamin form of B_{12}[31,32] have each been shown in high-dose clinical trials to be helpful in the treatment of the

CFS/ME and fibromyalgia group of diseases, suggesting that they act to lower the NO/ONOO- cycle. There are also various clinical observations supporting efficacy of these agents.[13] All three have been used clinically to treat MCS but the only one tested in a clinical trial on that disease was ascorbate,[33] to my knowledge. Let's examine the mechanisms of action for all four vitamins:

Ascorbate. Can act in three distinct ways to lower the central couplet of the NO/ONOO- cycle.[12,34] The central couplet is the reciprocal relationship between peroxynitrite elevation and BH4 depletion, where peroxynitrite acts to oxidize and therefore deplete BH4 and BH4 depletion acts to partially uncouple the nitric oxide synthases and therefore increase peroxynitrite. Ascorbate is a peroxynitrite scavenger but it acts effectively only at high concentrations to effectively lower peroxynitrite levels.[35-37] When peroxynitrite oxidizes BH4, it is converted to BH3, the one electron oxidation product but ascorbate, being a reducing agent can oxidize BH3 back to BH4.[37,39] Because BH3 is itself unstable, it may require high levels of ascorbate to be efficiently reduced before BH3 can be converted to other oxidation products, providing another rationale for the need for high doses. In addition, and this is probably only substantial when using fairly high doses of intravenous ascorbate, ascorbate acts chemically to reduce molecular oxygen to hydrogen peroxide[8-10] and hydrogen peroxide is known to induce the enzyme GTP cyclohydrolase I,[38-40] the first and rate-limiting enzyme in the *de novo* pathway for the synthesis of BH4. In general, then, high-dose therapy will lower the central couplet of the NO/ONOO- cycle via three distinct but interrelated mechanisms. Probably the first two mechanisms can be substantial in levels obtained from oral ascorbate but the hydrogen peroxide-GTP cyclohydrolase I mechanism probably requires the much higher levels that can be produced with IV ascorbate.[12,34] It is possible, then, that high-dose ascorbate may be the most effective single agent in treating NO/ONOO- cycle diseases.[12,34]

Is there empirical evidence that high levels of ascorbate are required to normalize this central couplet? It is well known that BH4 depletion is associated with elevated peroxynitrite in many diseases, so it is reasonable to infer that the levels of ascorbate normally found in these diseases, where blood levels are often in the normal range, is inadequate to lower this central couplet relationship. In CFS/ME and MCS, there is published clinical trial evidence for efficacy of circa 10 g IV ascorbate,[26-28,33] but no clinical trial data or even anecdotal or clinical observations suggest effectiveness for oral ascorbate. Having said that, typically oral ascorbate has been used at doses up to 2 g/day and the much higher doses advocated by Pauling, doses that produce substantially higher blood levels of ascorbate, albeit lower blood levels than are obtained with IV ascorbate,[9] may be more effective. Clearly we need data on such higher dose oral ascorbate.

Niacin. One of the most important mechanisms for producing mitochondrial dysfunction in NO/ONOO- cycle diseases is thought to involve the nicking of deoxyribonucleic acid by peroxynitrite-derived free radicals, leading to major stimulation of poly adenosine diphosphate (ADP)-ribosylation of chromosomal proteins.[41-43] The substrate for ADP ribosyltransferase activity is nicotinamide adenine dincleotide (NAD) and many inflammatory diseases can lead to major depletion of NAD/NADH pools via this mechanism,[43-46] with NADH depletion leading to mitochondrial dysfunction. High concentrations of nicotinamide effectively inhibit poly ADP-ribosylation, leading to restoration of NAD/NADH pools and therefore improved mitochondrial function, also lowering other NO/ONOO- cycle elements.[43-46] Vitamin B_3 as either nicotinamide or nicotinic acid will also help restore those pools by acting as a precursor of NAD biosynthesis. It should be noted that nicotinic acid (niacin) can generate nicotinamide in vivo, and thus may potentially act via both of these mechanisms. Thus high-dose niacin, will be expected to produce substantial improvement in mitochondrial dysfunction,

thus lowering the NO/ONOO- cycle.

Folate. In the form of 5-methyltetrahydrofolate (5-MTHF) is known to be a potent peroxynitrite scavenger, reacting with peroxynitrite in a semi-diffusion controlled manner.[47,48] It also reacts in a semi-diffusion controlled manner with singlet oxygen,[49] another important oxidant. High dose folate is also known to help restore BH4 pools and lower nitric oxide synthase uncoupling;[50-53] the probable mechanism is that by lowering peroxynitrite, high levels of reduced folates, including especially 5-MTHF, will lower peroxynitrite-mediated BH4 oxidation and thus raise BH4 pools. High dose folate has been shown to be useful in the treatment of CFS/ME[29] and the probable mechanism, in my judgment, is the mechanism outlined in this paragraph, acting to restore BH4 levels by scavenging peroxynitrite. Although the reaction between peroxynitrite and 5-MTHF has a very high rate constant,[47,48] the low concentration of 5-MTHF normally present in the body and the much lower concentrations of peroxynitrite together with its short half life, suggests that effective scavenging of peroxynitrite is expected to require relatively high-dose folate therapy needed to achieve supraphysiological levels of 5-MTHF. The same thing is probably also true for scavenging the peroxynitrite product, nitrosoperoxycarbonate (ONOOCO2-), which is probably also scavenged by 5-MTHF due to its weak oxygen-oxygen bond.

Vitamin B_{12}. The hydroxocobalamin form of B_{12} and the rapidly interconvertable aquacobalamin form have been shown to be potent nitric oxide scavengers, lowering the effects of nitric oxide both in vivo and in cell culture or other *in vitro* situations.[13,32] This mechanism is sufficiently well documented such that hydroxocobalamin has been used as an agent to demonstrate roles of nitric oxide in biological processes.[13,32] In order to undergo this reaction, hydroxocobalamin/aquacobalamin must be reduced to the cobalt II form, a process that occurs readily in the presence of physiological levels of ascorbate. In general, supraphysiological levels of hydroxocobalamin must be present in order to produce substantial lowering of nitric oxide effects.[13,31,32]

It can be seen from this that high-dose therapy for all four of the vitamins that Pauling proposed for use in such therapy[1,2] can be explained by their action in down-regulating the NO/ONOO- cycle.

What About Psychiatric Diseases?

Pauling[1,2,50] and Hoffer[2-5,7,55,56] both focused to a great extent on high-dose therapy of psychiatric disease and their interests led to the founding of the *Journal of Orthomolecular Psychiatry,* later changed to the *Journal of Orthomolecular Medicine.* They were specifically interested in schizophrenia treatment, but also had some interest in other psychiatric diseases. Are psychiatric diseases NO/ONOO- cycle diseases?

A detailed case for one psychiatric disease, PTSD, has already been published.[13,22] The author is in the process of finishing a paper arguing that schizophrenia is another NO/ONOO- cycle disease, caused by the impact of the cycle on regions of the prefrontal cortex, specifically focused on the dorsolateral prefrontal cortex. It is possible that others, specifically including major depression and bipolar disorder may involve cycle impact on other regions of the brain as elements of the cycle are well documented to have roles in both.

It might be argued that psychiatric diseases may be etiologically diverse and thus it may be implausible that such diseases as schizophrenia, bipolar disorder and major depression might have a common etiology produced by the impact of a local mechanism on different regions of the brain. However, these three diseases have each been reported to have elevation of such NO/ONOO- cycle elements as oxidative stress, nitric oxide, peroxynitrite, inflammatory cytokines, NF-kappa beta, mitochondrial dysfunction and BH4 depletion, suggesting but not proving a NO/ONOO- cycle etiology.

What about therapy? Do agents predicted to lower the NO/ONOO- cycle produce clinical improvements in psychiatric diseases? Here the literature is dominated by studies

of pharmaceuticals, rather than nutritional agents. However there are a number of studies that support this prediction, in addition to those of Hoffer and Pauling. For example, high dose folate including 5-MTHF have been reported to produce substantial clinical improvements in schizophrenia[57] as well as major depression and bipolar disorder.[57-61] It should be noted that these studies have usually been interpreted in terms of the ability of 5-MTHF in stimulating methylation activity, but its role as a peroxynitrite scavenger provides an equally viable interpretation.

Newbold reported that high doses of the hydroxocobalamin form of B_{12} produced substantial measurable improvements in a group of psychiatric patients.[62]

Hoffer's interpretation of mechanism with regard to high-dose niacin therapy of schizophrenia was that schizophrenia may be caused by excessive adrenochrome, an oxidation product of adrenaline, and that the role of niacin was to serve as a substrate for methylation and thus possibly lowering the methylation that is required to produce the adrenaline precursor of adrenochrome. However, Hoffer was clearly frustrated that the evidence for efficacy of high-dose niacin was not being considered independently of this possible interpretation. For example Hoffer and Osmond[4] stated about opponents of high-dose niacin treatment that: "They believed that orthomolecular treatment was inextricably bound to the adrenochrome hypothesis; that if they accepted one, they would have to accept the other. This of course was nonsense. We used the adrenochrome hypothesis to lead us to the vitamins, but we might have come upon it serendipitously. The adrenochrome hypothesis may be completely wrong, but this has no bearing on whether vitamin B_3 is therapeutic for schizophrenics."

Adrenochrome is produced by the oxidation of adrenaline by superoxide.[63,64] It can be argued, therefore, that the best way to reduce the levels of adrenochrome in schizophrenia, whatever its role in causing the disease, is to lower the NO/ONOO- cycle because of the role of the cycle in raising superoxide levels.

Perhaps the interpretation of mechanism of action of high-dose therapy using these four vitamins, provided in this paper, will lead to a reconsideration of high-dose therapy of not only niacin, but also ascorbate, folates and vitamin B_{12}, not only for schizophrenia and other psychiatric diseases, but also for a wide range of other chronic inflammatory diseases.

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