How Can We Cure NO/ONOO- Cycle Diseases? Approaches to Curing Chronic Fatigue Syndrome/Myalgic Encephalomyelitis, Fibromyalgia, Multiple Chemical Sensitivity, Gulf War Syndrome and Possibly Many Others

by Martin L. Pall, Ph.D.
Professor Emeritus of Biochemistry and Basic Medical Sciences,
Washington State University and
Research Director, The Tenth Paradigm Research Group

Abstract:

The NO/ONOO- cycle is a biochemical vicious cycle that is thought to cause such diseases as chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME), multiple chemical sensitivity (MCS), fibromyalgia (FM) and possibly a large number of other chronic inflammatory diseases. The chemistry/biochemistry of the cycle predicts that the primary mechanism is local such the depending on where it is localized in the body, it may cause a variety of different diseases. Previous studies have shown that agents that lower such cycle elements as oxidative stress, nitric oxide, inflammatory responses, mitochondrial dysfunction, tetrahydrobiopterin (BH4) depletion and NMDA activity produce clinical improvements in CFS/ME and FM patients, consistent with the predictions of the cycle mechanism. Multiagent protocols lowering several aspects of the cycle appear to be the most promising approaches to therapy. These include an entirely over-the-counter nutritional support protocol developed by the author in conjunction with the Allergy Research Group. However such mulitagent protocols to date, have not produced any substantial numbers of cures of these presumed NO/ONOO- cycle disease. Why is that? This paper argues that what is called the central couplet of the cycle, the reciprocal relation between peroxynitrite elevation and BH4 depletion, is not being adequately down-regulated by these multiagent protocols. Ten agents/classes of agents are available, each of which down-regulates one or the other end of this central couplet. It is suggested, then, that treatments that simultaneously effectively down regulate both ends to the central couplet, when used along with multiagent protocols lowering other aspects of the cycle and avoidance of stressors that otherwise up-regulate the cycle, will lead to substantial numbers of cures of these chronic diseases.

The basic concept of the NO/ONOO- vicious cycle mechanism is simple. It is that various short term stressors can initiate this cycle which, like all vicious cycles, propagates itself over time. The cycle then, depending on where it is located in the body, causes various chronic diseases. But in order to treat chronic diseases caused by the NO/ONOO- cycle and hopefully cure them, one needs to understand the details of the cycle mechanism. And that is where things becomes much more complex.

The NO/ONOO- cycle* is a primarily local biochemical vicious cycle that appears to be the central cause of such multisystem diseases as chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME), multiple chemical sensitivity (MCS), fibromyalgia (FM)

---

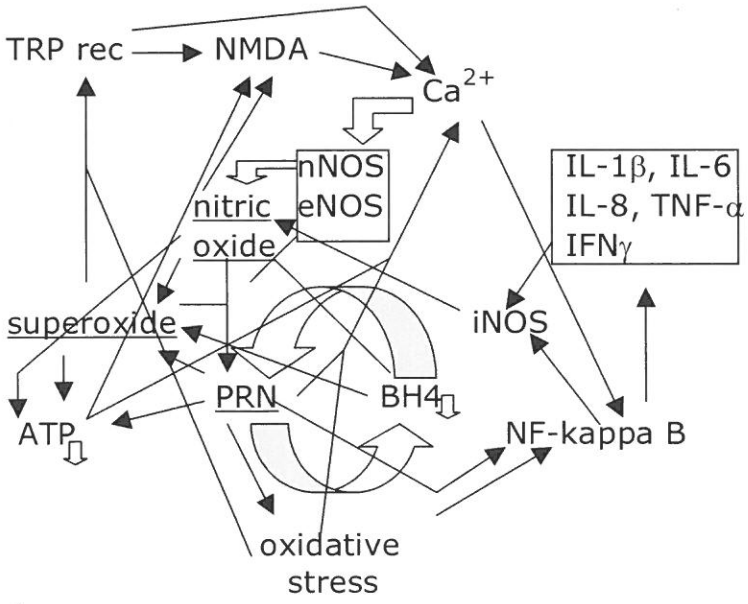
* The NO/ONOO- cycle is named for two of its many elements, nitric oxide (NO) and peroxynitrite (ONOO-) and is pronounced no, oh no.

and post-traumatic stress disorder (PTSD) (1-7). Cases of all four of these share many symptoms and signs and are each highly variable from one patient to another (1-4). These diseases often occur together in specific patients, that is they are comorbid (1-4,6,7). The variations among different patients is explained by the primarily local nature of the cycle, such that the different tissue localization of the NO/ONOO- cycle from one case to another produces different tissue impact and therefore different symptoms and often different diagnoses (1-3).

The NO/ONOO- cycle is diagrammed in Fig. 1. Each of the arrows shown in Fig. 1 represents one or more mechanisms by which one element of the cycle raises the levels of another cycle element. There are now a total of 30 specific mechanisms involved here, most of which are well documented, well accepted biochemistry and physiology (1-3). The three mechanisms that were least documented at the time my book was published (1) are now substantially better documented (2). Consequently, there is very little that is truly original about the NO/ONOO- cycle mechanism, except that when taken together, the individual mechanisms act to produce multiple, interacting vicious cycles which explain the chronic nature of these diseases, the challenges in treating them and many other important features (1-7).

Central to the cycle is the reaction of two free radicals in the body, nitric oxide with superoxide to form peroxynitrite (abbreviated PRN in Fig. 1). Peroxynitrite, a potent oxidant, produces oxidative stress (lower center, Fig. 1). On the right side of Fig. 1 are a number of inflammatory responses, including elevation of the transcription factor NF-kappa B, increased production of inflammatory cytokines (upper right box) and also induction of the inducible nitric oxide synthase (iNOS). These predict that much of the inflammatory cascade will be at least modestly elevated in NO/ONOO- cycle diseases.

Nitric oxide synthase activity may be elevated not only from iNOS induction but also from the calcium-dependent elevation of the other two nitric oxide synthases, nNOS and eNOS (upper center). Multiple mechanisms lead to increases in superoxide



(center

Figure 1. NO/ONOO- Cycle diagram

left) both intramitochondrial and extramitochondrial. And mitochondrial dysfunction leads to lowered energy metabolism and depletion of ATP, the energy currency of the cell (lower left). Another important cycle element is the elevated activity of the NMDA receptors which leads, in turn to what has been called excitotoxicity (Fig. 1, top). NMDA receptors have been most studied in the central nervous system but are widely distributed in both neuronal and non-neuronal tissues (8) and may, therefore, have widespread roles in the NO/ONOO- cycle as it impacts various regions of the body.

Probably the most important part of the cycle is what I have called the central couplet, the reciprocal relationship between elevated peroxynitrite (abbreviated PRN) and the depletion of a compound called tetrahydrobiopterin (BH4) (see Fig. 1 center to below center). Peroxynitrite oxidizes BH4 at physiologically relevant concentrations (9,10) leading to a BH4 depletion (9-11). BH4 is a cofactor in nitric oxide synthases, such that when these NOSs are missing BH4, they become uncoupled, producing superoxide in place of nitric oxide. In partial uncoupling this superoxide can react, in turn, with the nitric oxide produced by adjacent coupled enzymes, leading to more peroxynitrite. Because the reaction between superoxide and nitric oxide is extraordinarily rapid, what is called diffusion controlled, the production of both molecules by adjacent enzymes may be particularly effective in raising peroxynitrite levels. Thus, although this partial uncoupling lowers nitric oxide production, it is expected to increase peroxynitrite production, the most central part of the wider NO/ONOO- cycle (1-3,11). The superoxide produced by such partial uncoupling has a special role, then, in producing peroxynitrite. It should be noted that the production of superoxide far away from the production of nitric oxide will be much less effective in raising peroxynitrite levels because there are high amounts of the enzyme superoxide dismutase in cells and in the extracellular fluid, which destroy most of the superoxide before it travels very far from its site of synthesis.

The importance of this reciprocal relationship between peroxynitrite elevation and BH4 depletion (11), what we are calling the central couplet, has also been proposed by Foxton et al (12) in the context of its role in neurodegenerative diseases, diseases that are also proposed to be consequences of the action of the NO/ONOO- cycle (1).

<u>Types of Evidence Supporting the NO/ONOO- Cycle Mechanism</u>

There are multiple types of evidence, each of which provides substantial support for a NO/ONOO- cycle etiology for the multisystem diseases CFS/ME, MCS, FM and PTSD including but not limited to the following:

1. There are a total of 17 distinct short term stressors that are reported to initiate cases of one or more of these diseases, and all 17 are known to be able to stimulate cycle elements and are known or presumed, in turn, to increase subsequent nitric oxide and peroxynitrite (1-3). They are able, therefore, to initiate the cycle via these mechanisms.
2. The various cycle elements have been found to be elevated in the chronic phase of illness in at least one and in most cases all four of these diseases.
3. Several aspects of the cycle are implicated by genetic studies of susceptibility (1-3).
4. The cycle is supported by animal model studies of CFS/ME, PTSD and MCS (1-3,5) with the most extensive such animal model evidence for MCS (2).

5. THE most important types of evidence, from the standpoint of people suffering from these diseases or physicians or other medical care providers trying to produce improvements in them is evidence on efficacy of possible therapeutic agents. Studies of individual agents in clinical trials provide evidence for efficacy of a variety of agents predicted to lower various aspects of the NO/ONOO- cycle (1-3). This evidence is summarized in Table 1.

Table 1. Agents with Favorable Response in Clinical Trials Predicted to Lower Aspects of the NO/ONOO- Cycle

agent(s)	probable mechanism	comments
flavonoids, Ecklonia cava extract, algal supplements	Chain breaking and other antioxidant activity	Some may act as peroxynitrite scavengers
NMDA antagonists, other agents that indirectly lower NMDA activity; magnesium	All act to lower excessive NMDA activity	
acetyl carnitine/carnitine, coenzyme Q10, low hyperbaric or normobaric oxygen	Improved mitochondrial function	Oxygen must be used with caution, particularly in severe cases of CFS/ME
hydroxocobalamin form of vitamin B-12	Reduced *in vivo* to a form that is a potent nitric oxide scavenger	Higher dosage (i.e. 5 to 10 mg) needed than is needed to treat a B-12 deficiency; Typically used via IM injection, as an inhalant or via nasal spray to obtain high blood levels; oral or sublingual should be useful but are clearly suboptimal because of limited absorption
high dose folates	Serves as precursor of 5-methyltetrahydrofolate (5-MTHF), a potent peroxynitrite scavenger	Unclear whether folic acid, folinic acid, 5-MTHF and/or other forms of folate should be used; folic and folinic acid tested in published trials
D-ribose, RNA, inosine	All act to increase uric acid levels (peroxynitrite scavenger); all may act to help restore ATP pools	Published trial on D-ribose; trial currently in progress suggesting inosine can be helpful
IV high dose, buffered ascorbate	Lowers both ends of central couplet (see below); may be particularly helpful agent	Discussed in detail below
sauna therapy	Acts to increase BH4 availabiity (13); mechanism via increased synthesis of GTP cyclohydrolase I	Trials published on MCS, FM and CFS/ME; discussed further below

Fish oil	Established as anti-inflammatory agent	May also improve brain function

Most studies involved CFS/ME and/or FM; however studies with sauna therapy and IV ascorbate have been published with MCS patients.

It can be seen from Table 1, that agents lowering various aspects of the NO/ONOO- cycle are helpful in treatment of these three apparent NO/ONOO- cycle diseases. Specifically agents that lower oxidative stress, lower peroxynitrite, improve mitochondrial function, lower NMDA activity, increase BH4 availability or have anti-inflammatory activity all appear to be helpful in treatment. It is difficult to see how this could be the case unless the NO/ONOO- cycle or something very similar to it is the central cause of these multisystem diseases.

The evidence summarized in Table 1 also strongly suggests that the NO/ONOO- cycle makes very useful predictions in terms of therapy. Given the complexity of the cycle, as diagrammed in Fig. 1, it seems likely that multiple agents lowering various aspects of the cycle will be most effective in producing clinical improvements in apparent NO/ONOO- cycle disease.

Multiagent Protocols and the Allergy Research Group Nutritional Support Protocol

I described the responses to five multiagent protocols developed by different researchers, one of which I had a role in developing, in Chapter 15 in my book (1). Each of these five involved from 14 to 18 different agents or classes of agents that are predicted to down-regulate one or more aspects of the NO/ONOO- cycle. Each of these apparently produces substantial improvements in many patients suffering from these multisystem diseases, although four of the five have only been tested on one disease. In contrast, Teitelbaum's protocol has been tested on both CFS/ME patients and also on FM patients with apparent positive results (14).

More recently I have developed, in collaboration with the Allergy Research Group, an entirely over-the-counter protocol based on nutritional supplements chosen to down regulate the NO/ONOO- cycle. This nutritional support protocol is described elsewhere (2,3) as well as on a web page of my web site (thetenthparadigm.org/arg.htm). It includes 22 agents chosen for their ability to down-regulate various aspects of the NO/ONOO- cycle as well as other general nutrients.

The feedback I have gotten from clinical observations of physicians and others who have used it to treat their CFS/ME, FM and/or MCS patients is that roughly 80 to 85% of patients respond positively to it and that improvements are typically maintained if patients are able to avoid exposure to stressors that are predicted to up-regulate the cycle (2,3). Even patients who have been ill for two decades or more often respond positively. It should be noted, however, that the extent of improvement tends to vary considerably, varying from responses described as miraculous to modest improvements. Furthermore, about 15 to 20% of patients do not improve. Patients with high levels of mercury stored in their bodies may react negatively to the protocol, presumably because mercury can be mobilized by the alpha-lipoic acid found in the protocol. One group of patients who do not respond substantially, either positively or negatively are apparently the chronic Lyme disease patients. Note: I must point out to the reader that I do have a conflict of interest

here. I receive a small royalty from the Allergy Research Group for designing much of this nutritional support protocol.

Ingrid Franzon and her colleagues in Sweden have run a small pilot study on the Allergy Research Group protocol with a group of nine CFS/ME patients (personal communication). She finds, surprisingly for such a small group, statistically significant improvement in physical health measures within 4 weeks with a further, statistically significant improvement over another four weeks (personal communication; statistical analysis performed using a paired Student's t test).

In summarizing the last two pages of this article, there are four important points to be made: Firstly individual agents that down-regulate specific parts of the NO/ONOO- cycle have often been reported in clinical studies to produce improvements in these multisystem disease patients. Secondly multiple parts of the cycle are implicated from these clinical studies as well as from other studies, producing strong confirmation that a mechanism like the NO/ONOO- cycle is the central etiologic mechanism of these diseases. Thirdly and not surprisingly, treatment protocols using multiple agents predicted to down-regulate the cycle seem to be more effective than single agents. Fourthly, these several multiagent protocols are not effective with patients who are repeatedly or continuously exposed to stressors that otherwise up-regulate the NO/ONOO- cycle.

Clearly these four points are very important and exciting considering that the conventional wisdom has been that there is little that can be done to treat these illnesses.

My own view is that the Allergy Research Group nutritional support protocol is in many ways the most promising of these multiagent protocols, because it is relatively inexpensive, it is available over-the-counter in the U.S., Canada and much of Europe and because it apparently achieves good responses despite the limitations inherent in over-the-counter approaches.

The NO/ONOO- cycle etiology is best documented for CFS/ME, MCS, FM and PTSD (1-7) as well as for Gulf War syndrome/illness which appears to be a combination of the four (1,6). However, there is in addition, at least a superficial case to be made that 14 additional diseases, including the three classic neurodegenerative diseases, asthma, multiple sclerosis, tinnitus and autism, appear to also be caused by the NO/ONOO- cycle (1). The cases that have been made for each of these 14 are frankly relatively superficial (Chapters 14 and 7, ref. 1), except for tinnitus and post-radiation syndrome where more extensive cases have been made (15,16). Thus this approach to the treatment of chronic disease may not be limited to such diseases as CFS/ME, FM, MCS and PTSD but may have vastly broader implications.

How Can We Start Getting Substantial Numbers of Cures?

The use of multiple agent protocols where individual agents act to lower the NO/ONOO- cycle is an exciting and promising approach to therapy of these diseases. However, based on published evidence and to the extent I have access to it, unpublished evidence, none of these protocols produces any substantial numbers of cures. If we understand the NO/ONOO- cycle mechanism sufficiently and if we are effectively down-regulating it, we should start seeing substantial numbers of cures. Why has this not happened?

My own view, is that the part of the cycle that has been called the central couplet is insufficiently down-regulated in these protocols. The main argument that is being explored in this paper is that by more effectively lowering this central couplet mechanism, we may be able to extend these multiple agent protocols to obtain substantial numbers of cures. Let me remind you that the central couplet is the reciprocal relationship between peroxynitrite elevation on the one hand and BH4 depletion on the other. We need to focus, then on agents that lower peroxynitrite and its products at one end of the central couplet and also agents that raise BH4 availabliity on the other end of the central couplet.

There are at least ten available agents that are predicted to substantially lower the central couplet, summarized in Table 1 and we will explore each of them one at a time.

Table 1 Agents/Classes of Agents Predicted to Sustantially Lower the Central Couplet

Agent	Dosage	Presumed mechanism(s)
IV buffered ascorbate	7-50 g., repeated	1. Acts as peroxynitrite scavenger 2. Reduces BH3 back to BH4, helping restore BH4 levels 3. The very high levels obtained by IV treatment can lead to increased levels of hydrogen peroxide, leading to induction of GTP cyclohydrolase I, thus leading to increased *de novo* synthesis of BH4
oral ascorbate	circa 2-3g, repeated daily	Blood levels obtained are substantially lower than for IV treatment, above. However such levels may be adequate to trigger the first two mechanisms outlined immediately above.
sauna therapy	repeated	Induces GTP cyclohydrolase I, leading to increased *de novo* synthesis of BH4
reduced glutathione, liposomal, time release, nasal spray, IV or inhalant	150-500 mg, per day	Reduces BH2 back to BH4, thus helping restore normal BH4 levels and thus lowering the partial uncoupling of the nitric oxide synthases; some, particularly those with asthma-type symptoms may have some difficulty tolerating this treatment, depending on dosage regimen
Inosine, RNA or D-ribose	varies	Each of these has the capability of producing two responses: Restoration of adenine nucleotide pools and increased uric acid levels in blood. The latter will lead to lowered levels of peroxynitrite breakdown products, NO2 radical and carbonate radical. Each of these agents have drawbacks (see text).
5-methyl tetrahydro-folate (5-MTHF) or precursors folic or folinic acid	300 μg/day for 5-MTHF, higher doses for precursors	Acts as a potent peroxynitrite scavenger and will, therefore, help also restore BH4 pools; high dose folic or folinic acid will act to help raise 5-MTHF pools. 5-MTHF pools are depleted in CFS/ME presumably due to peroxynitrite mediated oxidation.
tetrahydro-biopterin (BH4) or precursors of BH4 biopterin or	circa 5 mg, or less, oral daily	Helps restore BH4 pools; also acts as peroxynitrite scavenger. This would be an off lable use of BH4

sepiapterin		
vasoactive intestinal peptide (VIP)	IV or inhalant	Induces GTP cyclohydrolase I, leading to increased de novo synthesis of BH4; this would be an off lable use
flavonoids, ellagic acid, other phenolic antioxidants	??, oral	Probably act to scavenge peroxynitrite and breakdown products and may also act more directly to help restore BH4; dosage and optimal sources are unclear
hydroxocobalamin	IM injection, nasal spray or inhalant	Acts in the reduced form (cobalt II) as a potent nitric oxide scavenger; this will indirectly lower peroxynitrite because of the role of nitric oxide as a peroxynitrite scavenger

Taken from the author's web site with permission.

General Strategy and Relationship to Lowering Hypertension

The general strategy is that an effort to lower the central couplet will be made, as a second phase to a treatment in which the first phase is a wide ranging protocol lowering other aspects of the cycle together with avoidance of stressors that will otherwise up-regulate the cycle. The Allergy Research Group nutritional support protocol for doing this is described on a page on my web site (thetenthpardigm.org/arg/htm) as well as elsewhere (2,3). The overall concept is that by lowering various aspects of the cycle and then focussing on lowering the central couplet, we should start seeing some cures of NO/ONOO- cycle diseases. Let me remind the reader, that I am a PhD, not an MD and nothing I say or write should be viewed as medical advice.

A second part of this general strategy is that by using relatively high doses of agents that act collectively to lower both ends of this central couplet, one may get responses that may go up as much as the square of the dose of these combinations of agents. Relatively high doses of agents that are acting at the same time to lower both sides of the couplet may be most effective. This may be expected because this central couplet is, just that, a couplet where lowering peroxynitrite will increase the availability of BH4 and independently, increasing the availability of BH4 will lower peroxynitrite and its products. Therefore, doing both of these simultaneously can have a major impact in lowering this central couplet.

The effectiveness of agents in accomplishing this task may be judged, to some extent by their ability to lower hypertension. Hypertension is thought to be caused, to a great extent by shifting the ratio of nitric oxide to peroxynitrite, towards excessive peroxynitrite, something that is produced by the action of the central couplet. This role in hypertension is a consequence of the following: Whereas nitric oxide is a vasodilator, peroxynitrite is a vasoconstrictor, acting in part by raising the levels of isoprostanes which are potent vasoconstrictors. For example, vasopressin II acts to produce hypertension by inducing higher levels of NADPH oxidase (17), an enzyme whose activity produces superoxide. The reaction of superoxide with nitric oxide will produce peroxynitrite and thus turn on the central couplet. Depletion of BH4 levels has been shown to have an important role in causing hypertension (18-20).

Treatments that lower hypertension may be suggested to be effective agents in lowering the central couplet. However, because hypertension occurs outside the central nervous system but some NO/ONOO- cycle diseases may be localized, to a

great extent in the brain, agents that fail to traverse the blood-brain barrier may act on hypertension but may not effectively lower central nervous system located NO/ONOO- cycle diseases. It is important, therefore, to keep this restriction in mind because it may limit the prediction that treatments that lower hypertension will work to produce improvements in NO/ONOO- cycle diseases.

Let's discuss the apparent mechanisms of action of the 10 agents discussed in Table 1.

IV Ascorbate

Intravenous (IV) ascorbate (vitamin C) can produce levels of ascorbate in the blood of 100 times or more the upper level of "normal" ascorbate (21-24). By doing so, it may be able to produce effects that are vastly higher than one will get from normal pools sizes of ascorbate. IV ascorbate, typically using 7 to 50 g. of buffered ascorbate, has been successfully used to treat MCS or CFS/ME patients (25-28) and, in addition, I am aware of a number of physicians who have reported successfully treating such patients with such doses of buffered IV ascorbate. Such IV ascorbate treatments appear to be well tolerated, even at doses roughly 4 times the highest doses suggested here (21-24,29), except possibly when two contraindications are present (see below).

There are three effects of ascorbate that may be expected to occur in response to such high levels of ascorbate:

1. Ascorbate is a scavenger of peroxynitrite and its breakdown products, but has only modest scavenging activity at normal ascorbate blood levels (30-32). It will be expected to have much greater scavenging activity with levels many times the normal upper level.
2. When peroxynitrite oxidizes BH4, the initial product is BH3, the one electron oxidation product. BH3 can be reduced back to BH4 by ascorbate (30,32), which is, of course, a reducing agent. However BH3 is itself unstable (9,10,30) and will probably, therefore, require high levels of ascorbate to efficiently produce such reduction.
3. The very high levels of ascorbate produced by such IV treatment produces hydrogen peroxide via ascobate oxidation and concomitant reduction of molecular oxygen (21-24,33,34). Hydrogen peroxide is known to be able to induce the enzyme GTP cyclohydrolase I (35-37), the first and rate-limiting enzyme in the *de novo* pathway to synthesize BH4. It follows that IV ascorbate may be expected to increase the availability of BH4 by this mechanism, as well as by the preceding one.

It follows that IV ascorbate may be able to favorably effect both sides of the central couplet, lowering peroxynitrite and its products and also, via two distinct mechanisms, increasing availability of BH4. This set of three mechanisms, collectively produces a rationale for the use of IV ascorbate in the treatment of these multisystem illnesses. To my knowledge, there has been no previous rationale for such treatment, despite the reported effectiveness of such treatment.

It will probably be important to determine that patients to be treated with such IV ascorbate do not have highly elevated levels of free iron, to avoid triggering extensive Fenton chemistry with the ascorbate treatment. Typically this means that

serum iron binding capacity should be no more than the upper limit of "normal", that is no more than 55% saturated.

In addition, those with a genetic glucose-6-phosphate dehydrogenase (G6PD) deficiency are susceptible to hemolysis caused by IV ascorbate because they are less able to detoxify the consequent hydrogen peroxide, so that treating such patients with IV ascorbate is contraindicated (38). Patients should be tested, therefore, for possible G6PD deficiency and for elevated free iron and only those lacking both of these contraindications should be treated with high dose IV ascorbate.

IV ascorbate used in such treatment should be buffered to the physiological pH of the blood (7.4) to avoid shifting the pH. Such buffering particularly important in those with kidney dysfunction who are less able to regulate the pH of the blood.

With the exception of cancer treatment, where IV ascorbate is thought to act mainly via increased production of hydrogen peroxide (22,24), there has been no widely applicable rationale for its reported effectiveness in the treatment of other diseases. The mechanisms described in this section are important, therefore, in providing such a rationale, one that makes important predictions on where IV ascorbate treatment may be useful and on what strategies should be used to maximize its efficacy.

Oral Ascorbate

Oral ascorbate can yield levels typically circa three times the upper range of normal with doses of 2 to 3 g. Such doses and somewhat lower doses are reported to produce lowered hypertension (18,39-41), suggesting that they may be able to act to lower the central couplet. Typically such high blood levels are only maintained for relatively short periods of time, on the order of 4 hours (22). Although 2-3 g. of oral ascorbate lead to absorption over 3- 4 hours, there is also rapid excretion of high blood levels of ascorbate, that is of levels well in excess of normal (22). The levels of ascorbate produced by 2 to 3 g. or higher doses of oral ascorbate may be expected to trigger substantial peroxynitrite scavenging (30-32), as well as some chemical reduction of BH3 to BH4 (30,32), but not any substantial hydrogen peroxide-induced increased levels of GTP cyclohydrolase I (see previous section).

It follows that such doses of oral ascorbate may be expected to act to lower the central couplet, although they will be less active in so doing than the much higher IV doses.

Sauna Therapy

Sauna therapy has been reported to be helpful in the treatment of MCS, FM, and CFS/ME (42-49), as well as with other diseases characterized by BH4 depletion (50). Sauna therapy is thought to act via two distinct mechanisms to induce higher levels of GTP cyclohydrolase I and thus increased availability of BH4 (50). Substantial increased availability probably only occurs after repeated sauna treatment.

In terms of strategy, therefore, it seems likely that sauna therapy could be useful in trying to cure these diseases, as follows: After several sauna treatments, subsequent sauna treatments should be accompanied by treatments with one or more agents that scavenge peroxynitrite and possibly also one or more agents that help reduce previously oxidized biopterin forms, such as BH3 and/or BH2, to BH4.

Reduced Glutathione

BH4 oxidation by peroxynitrite produces initially BH3 much of which is rapidly oxidized further to BH2, the two electron oxidation product. BH2 can be reduced back to BH4 by reduced glutathione and other thiol compounds (30). Therefore raising reduced glutathione levels may be useful in helping restore BH4 availability.

Oral glutathione typically gets degraded in the GI tract but a number of approaches can be used to try to provide increased glutathione. These include using oral liposomal or possibly time-release oral glutathione, or reduced glutathione via nasal spray, IV or as a nebulized inhalant. Reduced glutathione has, of course, several other antioxidant properties that should make it useful in the treatment of NO/ONOO- cycle diseases so clearly its actions are not specific to lowering of the central couplet.

There is one complication to using reduced glutathione in those who have asthma type responses. Such people report that reduced glutathione treatment may trigger asthma attacks. I think that this is probably due to the action of reduced thiols in activating some of the transfer receptor potential (TRP) receptors, including TRPA1. In any case it can cause problems. This problem is probably most substantial when using inhaled nebulized glutathione but other treatment modalities may occasionally cause such reactions. Nevertheless, I am aware of reports that reduced glutathione treatment can be very helpful in the treatment of NO/ONOO- cycle diseases, so it should be considered as a therapeutic agent.

Inosine, RNA or D-Ribose

I have lumped these three agents together because each of them is expected to produce two specific favorable responses. One of these two responses acts to lower the central couplet. Let's consider the response unrelated to the central couplet first and then consider the one that lowers the couplet.

Each of these three agents will raise levels of purine nucleotides in the body, including the adenine nucleotides that include ATP and others (ADP and AMP) that can act as precursors for ATP. ATP is, of course the "energy currency" in the body and its levels will be depleted whenever there is mitochondrial dysfunction. Because mitochondrial dysfunction is part of the NO/ONOO- cycle this will occur in cycle diseases. When it is sufficiently severe, it will lead to accumulation of fairly large amounts of AMP which will be degraded further, lowering the levels of all of these adenine nucleotides (that is ATP+ADP+AMP). This causes a longer term problem because when and if there is an improvement in mitochondrial function, the lowered adenine nucleotide levels mean the cell has a problem is producing normal ATP pools, even when the mitochondria are otherwise capable of doing so. Each of these three agents will allow the production of increased adenine nucleotides potentially leading in turn to increased ATP. This is the interpretation that has been given to the improvements reported for D-ribose treatment of both CFS/ME and FM (51) and it may be partly responsible for that improvement.

However, there is a second response to all three of these agents that will directly lower the central couplet. By leading to increased purine nucleoside and nucleotide pools, they will subsequently produce increased purine degradation. The end product of such purine degradation is uric acid, an important scavenger of

peroxynitrite and its breakdown products in humans (31,52). Of course, by lowering peroxynitrite and its oxidant products, uric acid will lower the central couplet.

Uric acid levels in the blood are often circa 4 to 5 times those of ascorbate, although there is quite a bit of variation around those figures. However the effectiveness of uric acid in scavenging peroxynitrite and its products, per mole, is roughly similar to that of ascorbate (31). Consequently, even though it is possible to raise ascorbate levels by much higher percentages than uric acid levels *in vivo*, it seems likely that raising uric acid levels may be expected to produce a substantial effect on peroxynitrite-mediated oxidations *in vivo* and therefore should be considered well worth pursuing in lowering the central couplet.

Uric acid has a half life of something like 20 hours in humans (53), so it should not take very long for one to increase its levels by increasing the availability of purine containing compounds in the body, such that when an increase in purine degradation is obtained, and it will be sustained substantially longer than any high level ascorbate elevation. Consequently, it makes sense to consider each of these three supplements, inosine, RNA and D-ribose as possible agents to raise uric acid levels.

While each of these three supplements is expected to be helpful in two ways, one of which lowers the central couplet, each of these three agents has a possibly problematic feature:

D-ribose is a potent glycating agent, being approximately 50 times more active in glycation than is D-glucose (the normal sugar in the blood) (54-56) with substantial possible physiological effects of such D-ribose mediated glycation (54,56). Protein glycation is associated with aging and produces dysfunction of many glycated proteins.

The commercial source of RNA is yeast and some of the sufferers of these diseases have yeast allergies so some may have difficulty in tolerating RNA.

Inosine is in general a well tolerated supplement (57). However, it is capable of stimulating the activation of mast cells and people with these illnesses often have problems with mast cell excessive mast cell activation. Inosine is known to act to stimulate mast cell activation via the adenosine A(3) receptor (58).

Of these issues, the one that concerns me the most is the glycation via D-ribose, although I know that Dr. Jacob Teitelbaum, whom I have great respect for, disagrees with me on this.

People with these diseases tend to be low in uric acid presumably because of the oxidation of uric acid by peroxynitrite and its breakdown products. Because of the important role of uric acid in lowering peroxynitrite-mediated damage, it seems likely that raising uric acid levels may be an important approach towards lowering the central couplet. One does need to be careful not to raise uric acid levels too much because excessive levels can cause gout. In normal people, this is not a problem because uric acid excretion greatly increases as blood levels exceed normal levels, but this may be a concern in those who are susceptible to gout, where the excretion mechanism may not function properly.

A second, related issue is that very high uric acid levels may act to produce hypertension, and while direct measurements suggest that uric acid lowers nitric

oxide synthase uncoupling, rather than raising it, this also suggests we should limit the rise in uric acid levels in these treatments.

With these two caveats in mind, a substantial rise in uric acid levels into the mid to upper normal range may be very helpful to people suffering from NO/ONOO- cycle diseases.

5-Methyltetrahydrofolate (5-MTHF)

It has been known for a number of years now, that high dose folic acid supplements can produce lowered partial nitric oxide synthase uncoupling (59-62) (this has been most studied with the eNOS nitric oxide synthase form), with much of this effect being due to increased availability of BH4. This response is dependent on the reduction of the folic acid by the enzyme dihydrofolate reductase, showing that a reduced form of folate probably has a role here. What has been unclear, until recently, is what the mechanism of action of the reduced folate may be.

It has been shown, however, that 5-methyltetrahydrofolate (5-MTHF) is an extremely potent peroxynitrite scavenger (63,64), so the probable mechanism of action is the lowering of peroxynitrite and its breakdown products. In other words, this is another situation where the central couplet is involved, such that by lowering one end of the couplet (the peroxynitrite end) one also lowers the other end (increasing BH4). Another reduced folate, tetrahydrofolate also acted as a peroxynitrite scavenger (63), although it was less active than was 5-MTHF.

This action of 5-MTHF is also supported by the role of 5-MTHF as an extremely active scavenger *in vivo* and *in vitro* of singlet oxygen (65). Singlet oxygen is known to share chemical similarities to peroxynitrite because both molecules have very weak oxygen-oxygen bonds, so the similar scavenging of both molecules by 5-MTHF should not be surprising.

It has been shown that high dose oral folic acid can lead to major increases in 5-MTHF. For example, Doshi et al (66) in their figure 5, showed that a single 5 mg folic acid supplement, in humans, led to roughly seven times the initial blood levels of 5-MTHF in 3 to 4 hours. They also showed that repeated daily 5 mg doses produced still higher 5-MTHF levels , roughly 15 times the initial levels, an effect that was attributed in part by the authors to an induction of the dihydrofolate reductase enzyme.

Jacobson et al (67) showed that levels of 5-MTHF in the sera from CFS patients were very low compared with normals and that other reduced folate pools were also depressed. I am aware of extensive unpublished data on CFS/ME patients, confirming these results. Gerwin reported that folate deficiency was one of the three most common systemic factors in myofascial pain syndrome (68), a condition closely linked to fibromyalgia. These studies strongly suggests that elevated peroxynitrite levels in CFS/ME and possibly other multisystem illnesses may produce a substantial loss of 5-MTHF and that some of the products of 5-MTHF oxidation are lost to the folate pools, thus leading to an overall lowering of folates in the body. The lowering

of 5-MTHF pools, have also led in the unpublished data to a much more modest (circa 10 to 15%) lowering of S-adenosylmethionine levels.[&]

It can be inferred from the studies discussed in this section, that the reaction between 5-MTHF with peroxynitrite, can have substantial impacts on both 5-MTHF levels and peroxynitrite-mediated responses in real physiological situations. With regard to the main focus of this paper, raising the levels of 5-MTHF can have a substantial impact on the central couplet by lowering the levels of peroxynitrite and its breakdown products. The practical question that faces us is whether this can be best accomplished by using high folic acid doses which acts as a precursor for 5-MTHF, using 5-MTHF itself and/or other reduced folates that can serve as precursors of 5-MTHF, such as folinic acid? The answer to that question is uncertain at this point.

There are two important complications to this story. I have received information from two sources, to the effect that using doses of 5-MTHF in substantial excess of 300 micrograms leads to negative reactions in patients suffering from presumed NO/ONOO- cycle diseases. My guess is that this may be due to the toxicity of some of the oxidation products of peroxynitrite mediated oxidation of 5-MTHF. If this interpretation is correct, it may be possible to increase the well tolerated dose if one uses other agents that lower peroxynitrite at the same time.

The second complication is that there must be very rapid turnover of the methyl group on intracellular 5-MTHF. There are massive amounts of methylation going on in the body and even though the great majority of that does not go through 5-MTHF, still there must be rapid turnover of the methyl group on 5-MTHF. It follows that the half life of intracellular 5-MTHF is probably on the order of few seconds and while the 5-MTHF can be regenerated after it acts as a methyl donor, the efficiency of that process is uncertain. Consequently, the effectiveness of an oral supplement of 5-MTHF on the scavenging of peroxynitrite may be expected to be greater in the extracellular space than it is intracellularly.

Folinic acid supplements were shown to produce major improvements in a group of CFS/ME patients (69). A number of other studies have reported major improvements in CFS/ME or FM patients with treatment protocols including high dose

---

[&] S-adenosylmethionine (SAMe) is the main direct methyl donor in living organisms, being produced by the methylation cycle and acting, in turn, to methylate many different substrates in the cell. There have been quite a number of claims that these illnesses are caused by lowered methylation cycle activity. I think that these claims not valid. There is a modest lowering of methylation activity caused by peroxynitrite-mediated 5-MTHF oxidation, but whether such modest lowering of methylation has any causal role is unclear. What should be clear is that such a modest methylation cycle lowering should be normalized by an effective down-regulation of the NO/ONOO- cycle, including especially the central couplet. That is the treatment approach explored in this paper is the approach that should be used to normalize various properties of these NO/ONOO- cycle diseases, including the modest lowering of methylation cycle activity.

folic acid or other folates, but it is difficult to determine the role of the folates themselves in such complex protocols.

Based on the compelling biochemistry, I think that folates, both folic acid and reduced folates, are among the most attractive agents in lowering the central couplet.

Tetrahydrobiopterin (BH4)

Perhaps the most obvious agent to use to lower the central couplet is BH4 itself, or alternatively sepiapterin or biopterin which are precursors of BH4. BH4 supplements have been reported to be helpful for the treatment of autism patients (70-72) and autism is one of the proposed NO/ONOO- cycle diseases (1). There are, however, some complications that need to be considered in using BH4 to lower the central couplet.

Firstly, it is known that BH4 when taken orally, is largely oxidized and must, therefore be reduced back to BH4 before it can function in target cells. Most of this reduction occurs occurs intracellularly through enzymatic reduction. However, the rapid peroxidation of the BH4 leads to questions of whether this oxidation may produce peroxidative damage. For example, although Parkinson's disease is thought to involved BH4 depletion, an animal model study on Parkinson's disease showed that high doses of BH4 produced Parkinson's-like symptoms and neuronal damage (73,74), providing some support for this view. In any case, it may be important to limit the dosage of BH4, if it is used directly to prevent any major consequences of BH4 peroxidation. It is possible that reducing agents such as high dose ascorbate may minimize this peroxidation and it is possible that using BH4 along with high dose ascorbate may be helpful in constructing therapeutic strategies.

An alternative approach is to use precursors of BH4, such as biopterin or sepiapterin as oral supplements to provide increased availability of BH4.

Vasoactive Intestinal Peptide (VIP)

VIP has been used by two physicians to treat CFS/ME patients or chemically sensitive patients (unpublished data), with apparently good responses in both. For example, Dr. William Rea has used VIP with his chemically sensitive patients with apparently good responses (personal communication). VIP is known to lower several parts of the NO/ONOO- cycle and the most likely mechanism for this, in my view, is the reported role of VIP in inducing GTP cyclohydrolase I activity and consequently raising BH4 levels (75). This view is supported by the well documented role of VIP in improving vasculature function. VIP is known to lower hypertension and vascular endothelial dysfunction, and both of these are caused, in part, by BH4 depletion.

Flavonoids, Ellagic Acid and Other Phenolic Antioxidants

A number of flavonoids have been shown to act as scavengers of peroxynitrite and also its precursor superoxide it has been suggested that they can be active *in vivo* in lowering peroxynitrite-mediated effects (76). Other phenolic antioxidants can also have important roles here and perhaps one of the most important may be ellagic acid which scavenges peroxynitrite (77). It is not clear to me which sources of these phenolics are the most likely to be useful here, but perhaps pomegranate extract, which contains substantial amounts of ellagic acid (78) and also several flavonoid-

containing extracts that are reported to lower hypertension and improve vascular endothelial dysfunction (79-82). Ghosh and Scheepens (80) list cocoa, wine, grape seed, berries, tea, tomatoes (polyphenolics and nonpolyphenolics), soy, hawthorn and pomegranate as attractive possibilities for phenolic antioxidants that may lower hypertension and improve vascular endothelial dysfunction. Schmitt and Dirsch (81) list cocoa, pomegranate, both green and black tea, olive oil and soy among food sources. They (81) also list ginkgo, hawthorn and ginseng among herbal sources. Extracts of each of these should be considered as agents for possibly lowering the central couplet.

Hydroxocobalamin Form of Vitamin B-12

Hydroxocobalamin has been used for over 70 years to decrease fatigue in people with chronic fatigue, long before CFS/ME was a well defined illness. It was shown in a clinical trial of patients with a CFS/ME-like illness, that 5 mg intramuscular (IM) injections twice a week produced statistically signficant improvements as compared with placebo(83). In this study (83), it was also shown that there was no correlation between initial B-12 levels and response to hydroxocobalamin therapy, suggesting that the hydroxocobalamin was not acting primarily to allay a B-12 deficiency. Lower doses of another form of B-12 that were adequate to allay a possible B-12 deficiency produce no clinical improvement (84,85) and other evidence also strongly suggests that high dose hydroxocobalamin is not acting here to allay a B-12 deficiency.

Other uncontrolled studies have suggested that the hydroxocobalamin form of vitamin B-12 produces clinical improvement in people with these multisystem diseases (86,87, Chapter 6 in ref. 1). It has been inferred that B-12 is acting as a potent nitric oxide scavenger and that this is the probable mode of action in the treatment of these multisystem diseases (87; Chapter 6 in ref. 1). People with these diseases report essentially across the board improvement in symptoms when treated with hydroxocobalamin, suggesting that hydroxocobalamin acts to lower the basic etiologic mechanism of these diseases, consistent with a nitric oxide scavenging mechanism.

In order to act as a nitric oxide scavenger, hydroxocobalamin and the chemically similar aquacobalamin must have the cobalt at the center of the molecule reduced from the cobalt III form to the cobalt II form (88). Such reduction is a process that occurs *in vivo* and is necessary for all cobalamins to have vitamin B-12 activity as well as for hydroxocobalamin to serve as a nitric oxide scavenger.

Nitric oxide does not have a direct role in the central couplet, but it does serve as a direct precursor of peroxynitrite, such that nitric oxide scavenging will inevitably lower peroxynitrite levels *in vivo*. It can be argued, therefore, that hydroxocobalamin will act to lower the peroxynitrite end of the central couplet by scavenging nitric oxide.

Summary and Overall Strategy

Of the 10 agents/classes of agents described above that are known or predicted to lower the central couplet, nine individually appear to produce substantial improvements in this group of diseases based on clinical trial studies, clinical observations or both. The only one of the nine where this is not true is with oral ascorbate. These observations make the central couplet an attractive part of the cycle to focus on in trying to obtain substantial numbers of cures for these diseases.

The question being raised here is whether combinations of these 10, especially combinations designed to effectively lower the central couplet, when added to the strategy I previously advocated for treatment of these diseases, will produce such cures?

That strategy suggested here is as follows: Avoid stressors that will otherwise up-regulate the NO/ONOO- cycle while using multiple agents that each lower one or more aspects of the cycle and collectively should lower several aspects of the cycle (1-3). There are multiple approaches each using such a multiple agent strategy, although the one that I have most worked on is the Allergy Research Group nutritional support protocol which appears to produce positive responses in roughly 80 to 85% of such patients. In general such multiple agent approaches seem to have been effective in producing clinical improvements in most such patients but have failed to give any substantial numbers of cures, based on published information (2,3, thetenthparadigm.org/arg.htm).

I think that the basic problem has been the failure to effectively down-regulate, the central couplet of the NO/ONOO- cycle. The proposal here is that we should add a second phase to these previous therapeutic approaches, one aimed at lowering that central couplet. More specifically, this means using agents that lower peroxynitrite and/or its breakdown products on the one hand. It also means using agents that increase BH4 availability on the other. Increased BH4 availability can be produced by using agents that reduce oxidized products of BH4 back to BH4. Such increased BH4 availability can also be produced by agents that induce the enzyme GTP cyclohydrolase I, the first and rate limiting enzyme in the *de novo* pathway for the synthesis of BH4. What I have provided, then is an overall strategy for getting some cures and a description of ten agents/classes of agents that should be useful in carrying out such a strategy. I have not, however, provided a detailed protocol for getting such cures.

I do think it is possible that IV buffered ascorbate alone, when added to one of these broad ranging protocols lowering the NO/ONOO- cycle and avoiding stressors that will raise the cycle, may be effective in obtaining some cures. I suspect that most of the other agents that lower the central couplet should be used as multiagent combinations, however. And, it is quite possible that even repeated IV ascorbate will be improved by using some of the other agents/classes of agents. The general strategy is to lower both ends of the couplet simultaneously, and probably repeatedly to progressively lower the cycle into insignificance. There is predicted to be synergistic interactions when using agents that work simultaneously to lower both ends of the central couplet.

I would be delighted to work with physicians and other health care providers who are interested in exploring this approach.

What I would say, is that if the view proposed in this paper can be shown to be correct, then we will be in a new era in medicine. That will be true even if the relevance of this approach is limited to such diseases as CFS/ME, MCS and FM. If other proposed NO/ONOO- cycle diseases can also be cured by this approach, diseases such as tinnitus, Parkinson's, Alzheimers, ALS, asthma, autism and MS, then the impact on medicine will be comparable with the previous biggest therapeutic breakthrough, the development of wide spectrum antibiotics.

Is this all delusional optimism? Clearly we won't know until we look. But what we do know is that all of these diseases are chronic diseases, with cases of each apparently initiated by stressors that should be able to initiate the cycle. And we have evidence with all of them for important roles of such cycle elements as oxidative stress, inflammatory biochemistry, mitochondrial dysfunction and excessive NMDA activity. Where they have been looked at, we also have evidence for BH4 depletion and NF-κB elevation. It is difficult to see how these cycle elements could be involved unless the NO/ONOO- cycle or something very similar to it is not central to the etiology of these diseases.

Mechanisms have consequences. It is time, in my view, for the sufferers of these diseases to fully benefit from the predictions of the NO/ONOO- cycle mechanism.

Correspondence:
Martin L. Pall
638 NE 41st Ave.
Portland, OR 97232-3312 USA
503-232-3883
martin_pall@wsu.edu

References

1. Pall ML. Explaining "Unexplained Illnesses": Disease Paradigm for Chronic Fatigue Syndrome, Multiple Chemical Sensitivity, Fibromyalgia, Post-Traumatic Stress Disorder, Gulf War Syndrome and Others. Harrington Park Press, 2007.
2. Pall ML. Multiple chemical sensitivity: Toxicological questions and mechanisms. In General and Applied Toxicology, 3rd Edition, Bryan Ballantyne, Timothy C. Marrs, Tore Syversen, Eds., pp. 2303-2352, John Wiley & Sons, London, 2009.
3. Pall ML. The NO/ONOO- cycle mechanism as the cause of chronic fatigue syndrome/myalgia encephalomyelitis. Chapter 2 in Chronic Fatigue Syndrome: Symptoms, Causes and Prevention, Edita Svoboda and Kristof Zelenjcik, Eds. Nova Publishers, 2009, in press.
4. Pall ML. The NO/ONOO- cycle as the cause of fibromyalgia and related illnesses: etiology, explanation and effective therapy. Chapter 2 in New Research in Fibromyalgia, John A. Pederson, Ed., Nova Science Publishers, pp 39-59, 2006.
5. Pall ML. Elevated, sustained peroxynitrite levels as the cause of chronic fatigue syndrome. Med Hypotheses 2000: 54:115-125.
6. Pall ML. Common etiology of posttraumatic stress disorder, fibromyalgia, chronic fatigue syndrome and multiple chemical sensitivity via elevated nitric oxide/peroxynitrite. Med Hypotheses 2001; 57:139-145.
7. Pall ML, Satterlee JD. Elevated nitric oxide/peroxynitrite mechanism for the common etiology of multiple chemical sensitivity, chronic fatigue syndrome and posttraumatic stress disorder. Ann NY Acad Sci 2001; 933:323-329.
8. Hinoi E, Takarada T, Ueshima T, Tsuchihashi Y, Yoneda Y. Glutamate signaling in peripheral tissues. Eur J Biochem 2004: 271:1-13.
9. Milstien S, Katusic Z. Oxidation of tetrahydrobiopterin by peroxynitrite: Implications for vascular endothelial function. Biochem Biophys Res Commun 1999; 263:681-684.
10. Laursen JB, Somers M, Kurz S, et al. Endothelial regulation of vasomotion in apoE-deficient mice: implications for interactions between peroxynitrite and tetrahydrobiopterin. Circulation 2001; 103: 1282-1288.

11. Pall ML. Nitric oxide synthase partial uncoupling as a key switching mechanism for the NO/ONOO- cycle. Med Hypotheses 2007;69:821-825.
12. Foxton RH, Land JM, Heales SJ. Tetrahydrobiopterin availability in Parkinson's and Alzheimer's disease; potential pathogenic mechanisms. Neurochem Res 2007;32:751-756.
13. Pall ML. Do sauna therapy and exercise act by raising the availability of tetrahydrobiopterin? Med Hypotheses 2009; 73:610-613.
14. Teitelbaum JE, Bird B, Greenfield, et al. Effective treatment of chronic fatigue syndrome and fibromyalgia: arandomized, double-blind, placebo-controlled, intent to treat study. J Chronic Fatigue Syndr 2002; 8(2):3-28.
15. Pall ML, Bedient SA. The NO/ONOO- cycle as the etiological mechanism of tinnitus. Int Tinnitus J 2007;13:99-104.
16. Pall ML. Post-radiation syndrome as a NO/ONOO- cycle, chronic fatigue syndrome-like disease. Med Hypotheses 2008;71:537-541.
17. Griendling KK, Ushio-Fukai. Reactive oxygen species as mediators of angiotensin II signalling. Regul Pept 2000; 91:21-27.
18. Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, Mitch WE, Harrison DG. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. J Clin Invest. 2003 Apr;111(8):1201-9.
19. Schulz E, Jansen T, Wenzel P, Daiber A, Münzel T. Nitric oxide, tetrahydrobiopterin, oxidative stress, and endothelial dysfunction in hypertension. Antioxid Redox Signal 2008;10:1115-1126.
20. Moens AL, Kass DA. Tetrahydrobiopterin and cardiovascular disease. Arterioscler Thromb Vasc Biol 2006;26:2439-2244.
21. Padayatty SJ, Levine M. New insights into the physiology and pharmacology of vitamin C. CMAJ 2001;164:353-355.
22. Padayatty SJ, Sun H, Wang Y, et al. Vitamin C pharmacokinetics: implications for oral and intravenous use. Ann Intern Med. 2004;140:533-537.
23. Levine M, Rumsey SC, Daruwala R, Park JB, Wang Y. Criteria and recommendations for vitamin C intake. JAMA 1999;281:1415-1423.
24. Riordan HD, Hunninghake RB, Riordan NH, et al. Intravenous ascorbic acid: protocol for its application and use. P R Health Sci J 2003;22:287-290.
25. Heuser G, Vojdani A. Enhancement of natural killer cell activity and T and B cell function by buffered vitamin C in patients exposed to toxic chemicals: the role of protein kinase C. Immunopharmacol Immunotoxicol 1997; 19:291-312.
26. Kodama M, Kodama T, Murakami M. The value of the dehydroepiandrosterone-annexed vitamin C infusion treatment in the clinical control of chronic fatigue syndrome (CFS). I. A Pilot study of the new vitamin C infusion treatment with a volunteer CFS patient. In Vivo 1996;10:575-584.
27. Kodama M, Kodama T, Murakami M. The value of the dehydroepiandrosterone-annexed vitamin C infusion treatment in the clinical control of chronic fatigue syndrome (CFS). II. Characterization of CFS patients with special reference to their response to a new vitamin C infusion treatment. In Vivo 1996; 10:585-596.
28. Kodama M, Kodama T. Four problems with the clinical control of interstitial pneumonia, or chronic fatigue syndrome, using the megadose vitamin C infusion system with dehydroepiandrosterone-cortisol annex. In Vivo 2006; 20:285-291.
29. Levy, TE. Curing the Incurable: Vitamin C, Infectious Disease and Toxins 3rd Edition. LivOn Books, Henderson, NV, 2009.

30. Kuzkaya N, Weissmann N, Harrison DG, Dikalov S. Interactions of peroxynitrite, tetrahydrobiopterin, ascorbic acid and thiols: implications for uncoupling of endothelial nitric oxide synthase. J Biol Chem 2003; 278:22546-22554.
31. Whiteman M, Ketsawatsakul U, Halliwell B. A reassessment of the peroxynitrite scavenging activity of uric acid. Ann N Y Acad Sci 2002; 962:242-259.
BH4 oxidation by carbonate and NO2 radical (cite also for ascorbate reduction)
32. Patel KB, Stratford MRL, Wardman P, Everett SA. Oxidation of tetrahydrobiopterin by biological radicals and scavenging of trihydrobiopterin radical by ascorbate. Free Rad Biol Med 2002; 32: 203-211.
Hydrogen peroxide (see Padayatty et al, Levine et al, Riordan et al) and:
33. Chen Q., Espey M. G., Krishna M. C., Mitchell J. B., Corpe C. P., Buettner G. R., Shacter E., Levine M. (2005) Pharmacologic ascorbic acid concentrations selectively kill cancer cells: action as a pro-drug to deliver hydrogen peroxide to tissues. *Proc Natl Acad Sci U S A* 102,13604-13609.
34. Clément M. V., Ramalingam J., Long L. H., Halliwell B. (2001) The in vitro cytotoxicity of ascorbate depends on the culture medium used to perform the assay and involves hydrogen peroxide. *Antioxid Redox Signal* 3,157-163.
35. Shimizu S., Shiota K., Yamamoto S., Miyasaka Y., Ishii M., et al. (2003) Hydrogen peroxide stimulates tetrahydrobiopterin synthesis through the induction of GTP-cyclohydrolase I and increases nitric oxide synthase activity in vascular endothelial cells. *Free Radic Biol Med* 34,1343-1352.
36. Ishii M., Shimizu S., Wajima T., Hagiwara T., Negoro T., Miyazaki A., Tobe T., Kiuchi Y. (2005) Reduction of GTP cyclohydrolase I feedback regulating protein expression by hydrogen peroxide in vascular endothelial cells. *J Pharmacol Sci* 97,299-302.
37. Kalivendi S., Hatakeyama K., Whitsett J., Konorev E., Kalyanaraman B., Vásquez-Vivar J. (2005) Changes in tetrahydrobiopterin levels in endothelial cells and adult cardiomyocytes induced by LPS and hydrogen peroxide--a role for GFRP? *Free Radic Biol Med* 38,481-491.
38. Rees DC, Kelsey H, Richards JD. Acute haemolysis induced by high dose ascorbic acid in glucose-6-phosphate dehydrogenase deficiency. BMJ 1993; 306:841-842.
39. May JM. How does ascorbic acid prevent endothelial dysfunction? Free Radic Biol Med 2000; 28:1421-1429.
40. Fotherby MD, Williams JC, Forster LA, Craner P, Ferns GA. Effect of vitamin C on ambulatory blood pressure and plasma lipids in older persons. J Hypertens 2000; 18:411-415.
41. Bates CJ, Walmsley CM, Prentice A, Finch S. Does vitamin C reduce blood pressure? Results of a large study of people aged 65 or older. J Hypertens 1998;16:925-932.
42. Gibson PR, Elms AN-M, Ruding LA. Perceived treatment efficacy for conventional and alternative therapies reported by persons with multiple chemical sensitivity. Environ Health Perspect 2003;111:1498–1504.
43. Rea WJ. Chemical sensitivity. Boca Raton, FL: Lewis Publishers; 1992.
44. Crinnion W. Components of practical clinical detox programs – sauna as a therapeutic tool. Altern Ther Health Med 2007;13:S154–S156.

44. Baird DN, Rea WJ. The temperomandibular joint implant controversy. Part II: its clinical implications. J Nutr Environ Med 1999;9:209–222.
45. Rogers SA. Detoxify or die. Syracuse, New York: Prestige Publishers; 2002.
46. Krop J. Chemical sensitivity after intoxication at work with solvents: response to sauna therapy. J Altern Complement Med 1998;4:77–86.
47. Masuda A, Munemoto T, Tei C. A new treatment: thermal therapy for chronic fatigue syndrome. Nippon Rinsho 2007;65:1093–1098.
48. Brockow T, Wagner A, Franke A, Offenbacher M, Resch KL. A randomized controlled trial on the effectiveness of mild water-filtered near infrared whole-body hyperthemia as an adjunct to standard multimodal rehabilitation in the treatment of fibromyalgia. Clin J Pain 2007;23:67–75.
49. Matsushita K, Masuda A, Tei C. Efficacy of Waon therapy for fibromyalgia. Intern Med 2008;47:1473–1476.
50. Pall ML. Do sauna therapy and exercise act by raising the availability of tetrahydrobiopterin? Med Hypotheses 2009;73:610-613.
51. Teitelbaum JE, Johnson C, St Cyr J. The use of D-ribose in chronic fatigue syndrome and fibromyalgia: a pilot study. J Altern Complement Med 2006;12:857-862.
52. Ducrocq C, Blanchard B, Pignatelli B, Ohshima H. Peroxynitrite: an endogenous oxidizing and nitrating agent. Cell Mol Life Sci 1999; 55:1068-1077.
53. Geren W, Bendich A, Bodansky O, Brown GB. The fate of uric acid in man. J Biol Chem 1950; 183:21-31.
54. Wei Y, Chen L, Chen J, Ge L, He RQ. Rapid glycation with D-ribose induces globular amyloid-like aggregations of BSA with high cytotoxicity to SH-SY5Y cells. BMC Cell Biol. 2009 Feb 13;10:10.
55. Bailey AJ, Sims TJ, Avery NC, Halligan EP. Non-enzymic glycation of fibrous collagen: reaction products of glucose and ribose. Biochem J 1995; 305(Pt 2):385-390.
56. Yan H, Harding JJ. Glycation-induced inactivation and loss of antigenicity of catalase and superoxide dismutase. Biochem J 1997; 328(Pt 2):599-605.
57. Haskó G, Sitkovsky MV, Szabó C. Immunomodulatory and neuroprotective effects of inosine. Trends Pharmacol Sci 2004; 25:152-157.
58. Tilley SL, Wagoner VA, Salvatore CA, Jacobson MA, Koller BH. Adenosine and inosine increase cutaneous vasopermeability by activating A(3) receptors on mast cells. J Clin Invest 2000; 105:361-367.
59. Hyndman ME, Verma S, Rosenfeld RJ, Anderson TJ, Parsons HG. Interaction of 5-methyltetrahydrofolate and tetrahydrobiopterin on endothelial function. Am J Physiol Heart Circ Physiol. 2002 Jun;282(6):H2167-72.
60. Moat SJ, Clarke ZL, Madhavan AK, Lewis MJ, Lang D. Folic acid reverses endothelial dysfunction induced by inhibition of tetrahydrobiopterin biosynthesis. Eur J Pharmacol. 2006 Jan 20;530(3):250-8.
61. Verhaar MC, Wever RM, Kastelein JJ, van Dam T, Koomans HA, Rabelink TJ. 5-methyltetrahydrofolate, the active form of folic acid, restores endothelial function in familial hypercholesterolemia. Circulation. 1998 Jan 27;97(3):237-41.
62. Gori T, Burstein JM, Ahmed S, Miner SE, Al-Hesayen A, Kelly S, Parker JD. Folic acid prevents nitroglycerin-induced nitric oxide synthase dysfunction and nitrate tolerance: a human in vivo study. Circulation. 2001 Sep 4;104(10):1119-23.

63. Antoniades C, Shirodaria C, Warrick N, Cai S, de Bono J, Lee J, Leeson P, Neubauer S, Ratnatunga C, Pillai R, Refsum H, Channon KM. 5-methyltetrahydrofolate rapidly improves endothelial function and decreases superoxide production in human vessels: effects on vascular tetrahydrobiopterin availability and endothelial nitric oxide synthase coupling. Circulation 2006; 114:1193-1201.
64. Rezk BM, Haenen GRMM, van der Vijgh WJF, Bast A. Tetrahydrofolate and 5-methyltetrahydrofolate are folates with high antioxidant activity. Identification of the antioxidant pharmacophore. FEBS Lett 2003; 555: 601-605
65. Offer T, Ames BN, Bailey SW, Sabens EA, Nozawa M, Ayling JE. 5-Methyltetrahydrofolate inhibits photosensitization reactions and strand breaks in DNA. FASEB J. 2007; 21:2101-2107.
66. Doshi SN, McDowell IF, Moat SJ, Payne N, Durrant HJ, Lewis MJ, Goodfellow J. Folic acid improves endothelial function in coronary artery disease via mechanisms largely independent of homocysteine lowering. Circulation 2002; 105:22-26.
67. Jacobson W, Saich T, Borysiewicz LK, Behan WM, Behan PO, Wreghitt TG. Serum folate and chronic fatigue syndrome. Neurology 1993;43:2645-2647.
68. Gerwin RD. The management of myofascial pain sydromes. J Musculoskeletal Pain 1993; 1(3/4): 83-94.
69. Lundell K, Qazi S, Eddy L, Uckun FM. Clinical activity of folinic acid in patients with chronic fatigue syndrome. Arzneimittelforschung 2006; 56:399-404.
70. Danfors T, von Knorring AL, Hartvig P, et al. Tetrahydrobiopterin in the treatment of children with autistic disorder: a double-blind placebo-controlled crossover study. J Clin Psychopharmacol 2005; 25:485-489.
71. Fernell E, Watanabe Y, Adolfsson I, et al. Possible effects of tetrahydrobiopterin treatment in six children with autism--clinical and positron emission tomography data: a pilot study. Dev Med Child Neurol 1997; 39:313-318.
72. Naruse H, Hayashi T, Takesada M, Nakane A, Yamazaki K. [Metabolic changes in aromatic amino acids and monoamines in infantile autism and development of new treatment related to the finding] No To Hattatsu 1989 Mar;21:181-189.
73. Choi HJ, Lee SY, Cho Y, No H, Kim SW, Hwang O. Tetrahydrobiopterin causes mitochondrial dysfunction in dopaminergic cells: implications for Parkinson's disease. Neurochem Int 2006;48: 255-262.
74. Lee SY, Moon Y, Hee Choi D, Jin Choi H, Hwang O. Particular vulnerability of rat mesencephalic dopaminergic neurons to tetrahydrobiopterin: Relevance to Parkinson's disease. Neurobiol Dis 2007; 25:112-120.
75. Anastasiadis PZ, Bezin L, Gordon LJ, Imerman B, Blitz J, Kuhn DM, Levine RA. Vasoactive intestinal peptide induces both tyrosine hydroxylase activity and tetrahydrobiopterin biosynthesis in PC12 cells. Neuroscience 1998; 86:179-189.
76. McCarty MF. Scavenging of peroxynitrite-derived radicals by flavonoids may support endothelial NO synthase activity, contributing to the vascular protection associated with high fruit and vegetable intakes. Med Hypoth 2008; 70:170-181.
77. Ippoushi K, Takeuchi A, Azuma K. Prevention of peroxynitrite-induced oxidation and nitration reactions by ellagic acid. Food Chemistry 2008; 112:185-188.
78. Basu A, Penugonda K. Pomegranate juice: a heart-healthy fruit juice. Nutr Rev. 2009; 67:49-56.
79. Perez-Vizcaino F, Duarte J, Andriantsitohaina R. Endothelial function and cardiovascular disease: effects of quercetin and wine polyphenols. Free Radic Res 2006; 40:1054-1065.
80. Ghosh D, Scheepens A. Vascular action of polyphenols. Mol Nutr Food Res 2009; 53:322-331.
81. Schmitt CA, Dirsch VM. Modulation of endothelial nitric oxide by plant-derived products. Nitric Oxide 2009; 21:77-91.

82. Romero M, Jimenez R, Sanchez M, et al. Quercetin inhibits vascular superoxide production induced by indothelin-1: Role of NADPH oxidase, uncoupled eNOS and PKC. Atherosclerosis 2009; 202: 58-67.
83. Ellis FR, Nasser S. A pilot study of vitamin B12 in the treatment of tiredness. Br J Nutr 1973; 30:277-283.
83. Lawhorne L, Ringdahl D. Cyanocobalamin injections for patients without documented deficiency. JAMA 1989; 261:1920-1923.
84. Kaslow JE, Rucker L, Onishi R. Liver extract-folic acid-cyanocobalamin vs placebo for chronic fatigue syndrome. Arch Intern Med 1989; 149: 2501-2503.
85. Lapp CW. Using vitamin B-12 for management of CFS. The CFIDS Chronocle 1999; 12(6): 14-16.
86. Hoh D. Treatment of the Cheney clinic. The CFIDS Chronicle 1998; 1(4): 13-14.
87. Pall ML. Cobalamin used in chronic fatigue syndrome therapy is a nitric oxide scavenger. J Chronic Fatigue Syndr 2001; 8(2): 39-44.
88. Wolak M, Zahl A, Schneppensieper T, Stochel G, van Eldik R. Kinetics and mechanism of the reversible binding of nitric oxide to reduced cobalamin B(12r) (Cob(II)alamin). J Am Chem Soc 2001; 123:9780-8791.

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

Author: Martin L. Pall, PhD[1]

[1]Professor Emeritus of Biochemistry and Basic Medical Sciences, Washington State University and Research Director, The Tenth Paradigm Research Group. 638 NE 41st Ave. Portland, OR 97232-3312 USA. email: martin_pall@wsu.edu

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.

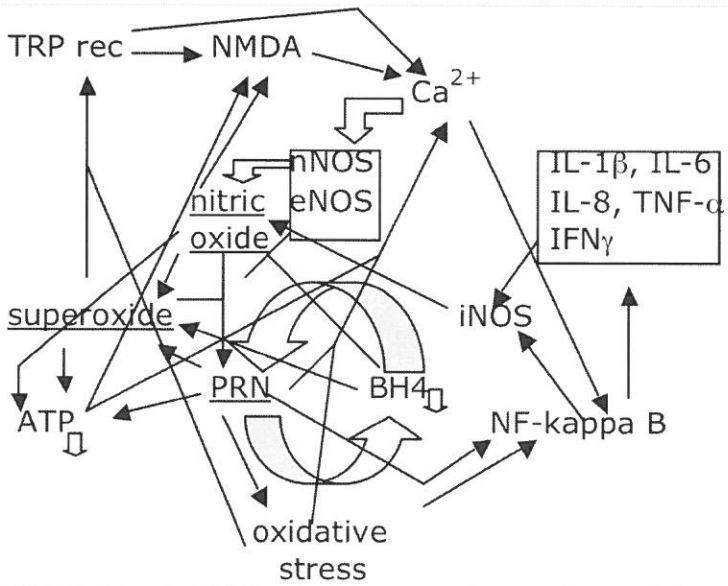
The reason for this is because the three small compounds in the cycle, NO, ONOO- and superoxide all have short half lives in biological tissues and the mechanisms of the cycle, the various arrows shown in Figure 1, all act at the levels of individual cells. Because of its primarily local nature, the 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 beta 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.

References

1. Pauling L: Orthomolecular psychiatry: varying the concentration of substances normally present in the human body may control human disease. *Science,* 1968; 160: 265-271.
2. Pauling L: Orthomolecular psychiatry: varying the concentration of substances normally present in the human body may control human disease. *J Nutr Environ Med,* 1995; 5: 187-198.
3. Hoffer A., Osmond H, Callbeck MJ, et al: Treatment of schizophrenia with nicotinic acid and nicotinamide. *J Clin Exp Psychopathol,* 1957; 18: 131-158.
4. Hoffer A, Osmond H: The adrenochrome hypothesis and psychiatry. *J Orthomol Med,* 1999; 14: 49-62.
5. Hoffer A: *Niacin Therapy in Psychiatry.* Charles C. Thomas, publishers, Springfield, IL, 1962.
6. Ames BN, Elson-Schwab I, Silver EA: High-dose vitamin therapy stimulates variant enzymes with decreased coenzyme binding affinity (increased Km): relevance to genetic disease polymorphisms. *Am J Clin Nutr,* 2002; 75: 616-658.
7. Hoffer A, Prousky J: Successful treatment of schizophrenia requires optimum dose of vitamin B_3. *Altern Med Rev,* 2008; 13: 287-291.
8. Chen Q, Espey MG, Krishna MC, et al: Pharmacologic ascorbic acid concentrations selectively kill cancer cells: action as a pro-drug to deliver hydrogen peroxide to tissues. *Proc Natl Acad Sci,* USA, 2005; 102: 13604-13609.
9. Padayatty SJ, Sun H, Wang Y, et al: Vitamin C pharmacokinetics: implications for oral and intravenous use. *Ann Intern Med,* 2004; 140: 533-537.
10. Duconge J, Miranda-Massari JR, Gonzalez MJ, et al: Pharmacokinetics of vitamin C: insights into the oral and intravenous administration of ascorbate. *P R Health Sci J,* 2008; 27,7-19.
11. Pall ML: Multiple chemical sensitivity: toxicological questions and mechanisms. In. eds. Ballantyne B, Marrs TC, Syversen T. *General and Applied Toxicology. 3rd ed.* New York, John Wiley & Sons. 2009; 2303-2352.
12. Pall ML: The NO/ONOO- Vicious Cycle Mechanism as the Cause of Chronic Fatigue Syndrome/ Myalgic Encephalomyelitis. In. eds. Svoboda E, Zelenjcik K. *Chronic Fatigue Syndrome: Symptoms, Causes and Prevention.* New York, Nova Science

Publishers, Inc. 2010;chapter 2.
13. Pall ML: *Explaining "Unexplained Illnesses": Disease Paradigm for Chronic Fatigue Syndrome, Multiple Chemical Sensitivity, Fibromyalgia, Post-Traumatic Stress Disorder, Gulf War Syndrome and Others.* Bighamton, NY. Harrington Park (Haworth) Press. 2007.
14. Pall ML: The NO/ONOO- cycle as the cause of fibromyalgia and related illnesses: Etiology, explanation and effective therapy. In. eds. Pederson JA. *New Research in Fibromyalgia.* New York, Nova Science Publishers, Inc. 2006; 39-59.
15. Pall ML: NMDA sensitization and stimulation by peroxynitrite, nitric oxide and organic solvents at the mechanism of chemical sensitivity in multiple chemical sensitivity. *FASEB J,* 2002; 16:1407-1417.
16. Pall ML: Elevated nitric oxide/peroxynitrite theory of multiple chemical sensitivity: central role of N-methyl-D-aspartate receptors in the sensitivity mechanism. *Environ Health Perspect,* 2003; 111:1461-1464.
17. Pall ML, Satterlee JD: Elevated nitric oxide/peroxynitrite mechanism for the common etiology of multiple chemical sensitivity, chronic fatigue syndrome, and posttraumatic stress disorder. *Ann NY Acad Sci,* 2001; 933: 323-329.
18. Smirnova IV, Pall ML: Elevated levels of protein carbonyls in sera of chronic fatigue syndrome patients. *Mol Cell Biochem,* 2003; 248: 93-95.
19. Pall ML: Nitric oxide synthase partial uncoupling as a key switching mechanism for the NO/ONOO- cycle. *Med Hypotheses,* 2007; 69: 821-825.
20. Pall ML: Elevated, sustained peroxynitrite levels as the cause of chronic fatigue syndrome. *Med Hypotheses,* 2000; 54: 115-125.
21. Pall ML, Bedient SA: The NO/ONOO- cycle as the etiological mechanism of tinnitus. *Int Tinnitus J,* 2007; 13: 99-104.
22. Pall ML: Common etiology of posttraumatic stress disorder, fibromyalgia, chronic fatigue syndrome and multiple chemical sensitivity via elevated nitric oxide/peroxynitrite. *Med Hypotheses,* 2001; 57: 139-145.
23. Pall ML: Post-radiation syndrome as a NO/ONOO(-) cycle, chronic fatigue syndrome-like disease. *Med Hypotheses,* 2008; 71:537-541.
24. Hinoi E, Takarada T, Ueshima T, et al: Glutamate signaling in peripheral tissues. *Eur J Biochem,* 2004; 271:1-13.
25. Kalariti N, Pissimissis N, Koutsilieris M: The glutamatergic system outside the CNS and in cancer biology. *Expert Opin Investig Drugs,* 2005; 14:1487-1496.
26. Kodama M, Kodama T, Muramaki M: The value of the dehydroepiandrosterone-annexed vitamin C treatment in the clinical control of chronic fatigue syndrome. I. A pilot study of the new vitamin C infusion treatment with volunteer CFS patients. *In Vivo,* 1996; 10:575-584.
27. Kodama M, Kodama T, Muramaki M: The value of the dehydroepiandrosterone-annexed vitamin C treatment in the clinical control of chronic fatigue syndrome. II. Characterization of CFS patients with special reference to their reponse to a new vitamin C infusion treatment. *In Vivo,* 1996; 10:585-596.
28. Kodama M, Kodama T: The clinical course of interstitial pneumonia alias chronic fatigue syndrome under the control of megadose vitamin C infusion system with dehydroepiandrosterone-cortisol annex. *Int J Mol Med,* 2005;15:109-16.
29. Lundell K, Qazi S, Eddy L, et al: Clinical activity of folinic acid in patients with chronic fatigue syndrome. *Arzneimittelforschung,* 2006; 56:399-404.
30. Botez MI, Fontaine F, Botez T, et al: Folate-responsive neurological and mental disorders: report of 16 cases. Neuropsychological correlates of computerized transaxial tomography and radionuclide cisternography in folic acid deficiencies. *Eur Neurol,* 1977; 16: 230-246.
31. Ellis FR, Nasser S: A pilot study of vitamin B_{12} in the treatment of tiredness. *Br J Nutr,* 1973 ;30:277-283.
32. Pall ML: Cobalamin used in chronic fatigue syndrome therapy is a nitric oxide scavenger. *J Chronic Fatigue Syndr,* 2001; 8:39-44.
33. Heuser G, Vojdani A: Enhancement of natural killer cell activity and T- and B-cell function by buffered vitamin C in patients exposed to toxic chemicals: the role of protein kinase C. *Immunopharmacol Immunotoxicol,* 1997; 19: 291-312.
34. Pall ML: How Can We Cure NO/ONOO- Cycle Diseases? Approaches to Curing Chronic Fatigue Syndrome/Myalgic Encephalomyelitis, Fibromyalgia, Multiple Chemical Sensitivity, Gulf War Syndrome, and Possibly Many Others. *Townsend Lett Doctors Patients,* 2010; 319/320: 75-84.
35. Kuzkaya N, Weissmann N, Harrison DG, et al: Interactions of peroxynitrite, tetrahydrobiopterin, ascorbic acid and thiols: implications for uncoupling of endothelial nitric oxide synthase. *J Biol Chem,* 2003; 278: 22546-22554.
36. Whiteman M, Ketsawatsakul U, Halliwell B: A reassessment of the peroxynitrite scavenging activity of uric acid. *Ann NY Acad Sci,* 2002; 962:242-259.
37. Patel KB, Stratford MRL, Wardman P, et al: Oxidation of tetrahydrobiopterin by biological radicals and scavenging of trihydrobiopterin radical by ascorbate. *Free Rad Biol Med,* 2002; 32: 203-211.
38. Shimizu S, Shiota K, Yamamoto S, et al: Hydrogen peroxide stimulates tetrahydrobiopterin synthesis through the induction of GTP-cyclohydrolase I and increases nitric oxide synthase activity in vascular endothelial cells. *Free Radic Biol Med,* 2003; 34: 1343-1352.

39. Ishii M, Shimizu S, Wajima T, et al: Reduction of GTP cyclohydrolase I feedback regulating protein expression by hydrogen peroxide in vascular endothelial cells. *J Pharmacol Sci,* 2005; 97:299-302.
40. Kalivendi S, Hatakeyama K, Whitsett J, et al: Changes in tetrahydrobiopterin levels in endothelial cells and adult cardiomyocytes induced by LPS and hydrogen peroxide-a role for GFRP? *Free Radic Biol Med,* 2005; 38: 481-491.
41. Szabo C, Dawson VL: Role of poly (ADP-ribose) synthetase in inflammation and ischaemia-reperfusion. *Trends in Pharmacol Sci,* 1998; 19:287-298.
42. Pieper AA, Verma A, Zhang J, et al: Poly (ADP-ribose) polymerase, nitric oxide and cell death. *Trends in Pharmacol Sci,* 1999; 20:171-181.
43. Szabó C: Poly(ADP-ribose) polymerase activation by reactive nitrogen species--relevance for the pathogenesis of inflammation. *Nitric Oxide,* 2006; 14: 169-179.
44. Elliott RB, Pilcher CC, Stewart A, et al: The use of nicotinamide in the prevention of type 1 diabetes. *Ann N Y Acad Sci.,* 1993; 696:333-341.
45. Liu D, Gharavi R, Pitta M, et al: Nicotinamide prevents NAD+ depletion and protects neurons against excitotoxicity and cerebral ischemia: NAD+ consumption by SIRT1 may endanger energetically compromised neurons. *Neuromolecular Med,* 2009; 11:28-42.
46. Ungerstedt JS, Blömback M, Söderström T: Nicotinamide is a potent inhibitor of proinflammatory cytokines. *Clin Exp Immunol,* 2003;131:48-52.
47. Antoniades C, Shirodaria C, Warrick N, et al: 5-methyltetrahydrofolate rapidly improves endothelial function and decreases superoxide production in human vessels: effects on vascular tetrahydrobiopterin availability and endothelial nitric oxide synthase coupling. *Circulation,* 2006; 114: 1193-1201.
48. Rezk BM, Haenen GRMM, van der Vijgh WJF, et al: Tetrahydrofolate and 5-methyltetrahydrofolate are folates with high antioxidant activity. Identification of the antioxidant pharmacophore. *FEBS Lett,* 2003; 555: 601-605
49. Offer T, Ames BN, Bailey SW, et al: 5-Methyltetrahydrofolate inhibits photosensitization reactions and strand breaks in DNA. *FASEB J,* 2007; 21: 2101-2107.
50. Hyndman ME, Verma S, Rosenfeld RJ, et al: Interaction of 5-methyltetrahydrofolate and tetrahydrobiopterin on endothelial function. *Am J Physiol Heart Circ Physiol,* 2002; 282: H2167-H2172.
51. Moat SJ, Clarke ZL, Madhavan AK, et al: Folic acid reverses endothelial dysfunction induced by inhibition of tetrahydrobiopterin biosynthesis. *Eur J Pharmacol,* 2006; 530: 250-258.
52. Verhaar MC, Wever RM, Kastelein JJ, et al: 5-methyltetrahydrofolate, the active form of folic acid, restores endothelial function in familial hypercholesterolemia *Circulation,* 1998; 97: 237-241.
53. Gori T, Burstein JM, Ahmed S, et al: Folic acid prevents nitroglycerin-induced nitric oxide synthase dysfunction and nitrate tolerance: a human in vivo study. *Circulation,* 2001;104:1119-1123.
54. Hawkins D, Pauling L: *Orthomolecular Psychiatry: Treatment of Schizophrenia.* San Francisco, CA, W. H. Freeman.1973.
55. Osmond H, Hoffer H: Massive niacin treatment in schizophrenia. Review of a nine-year study. *Lancet,* 1962; 1: 316-319.
56. Hoffer A, Osmond H: Treatment of schizophrenia with nicotinic acid. A ten year follow-up. *Acta Psychiatr Scand,* 1964; 40: 171-189.
57. Godfrey PS, Toone BK, Carney MW, et al: Enhancement of recovery from psychiatric illness by methylfolate. *Lancet,* 1990; 336: 392-395.
58. Godfrey PS, Toone BK, Carney MW, et al: Enhancement of recovery from psychiatric illness by methylfolate. *Lancet,* 1990; 336: 392-395.
59. Passeri M, Cucinotta D, Abate G, et al: Oral 5'-methyltetrahydrofolic acid in senile organic mental disorders with depression: results of a double-blind multicenter study. *Aging* (Milano), 1993; 5:63-71.
60. Guaraldi GP, Fava M, Mazzi F, et al: An open trial of methyltetrahydrofolate in elderly depressed patients. *Ann Clin Psychiatry,* 1993; 5: 101-105.
61. Behzadi AH, Omrani Z, Chalian M, et al: Folic acid efficacy as an alternative drug added to sodium valproate in the treatment of acute phase of mania in bipolar disorder: a double-blind randomized controlled trial. *Acta Psychiatr Scand,* 2009; 120: 441-445.
62. Newbold HL: Vitamin B_{12}: placebo or neglected therapeutic tool? *Med Hypotheses,* 1989; 28:155-164.
63. Misra HP, Fridovich I: The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem,* 1972; 247: 3170-3175.
64. Babior BM, Kipnes RS: Oxidation of epinephrine by a cell-free system from human granulocytes. *Blood,* 1976;47: 461-471.