CFS PHOENIX

GLUTATHIONE DEPLETION—METHYLATION CYCLE BLOCK:

A HYPOTHESIS FOR THE PATHOGENESIS OF CHRONIC FATIGUE SYNDROME

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by Richard A Van Konynenburg, Ph.D.

Independent Researcher and Consultant, (richvank@aol.com)

INTRODUCTION AND HYPOTHESIS

At the Seventh International Conference of the AACFS in 2004, the author proposed and defended the hypothesis that glutathione depletion is an important part of the pathogenesis of CFS (1).

In the conclusions of that paper it was noted that it seemed likely that there are vicious circle mechanisms involved in CFS that prevent glutathione repletion from being the complete answer for treating this disorder.

Recent autism research (2,3) suggests that in that disorder a vicious circle involving the methylation cycle apparently chronically holds down the level of glutathione.

The present author has recently proposed (4) that this same mechanism is active in many cases of CFS. This model for CFS will be referred to as the Glutathione Depletion—Methylation Cycle Block (GD-MCB) Hypothesis.

This mechanism appears to be capable of explaining and drawing together numerous features of CFS that have been reported in the peer-reviewed literature.

What is the methylation cycle and what does it do?

(See diagram)

The methylation cycle (also called the methionine cycle) (5) is a major part of the biochemistry of sulfur and of methyl (CH3) groups in the body. It is also tightly linked to folate metabolism and is one of the two biochemical processes in the human body that require vitamin B12 (the other being the methylmalonate pathway, which enables use of certain amino acids to provide energy to the cells).

This cycle supplies methyl groups for a large number of methylation reactions, including those that methylate (and thus silence) DNA (6), and those involved in the synthesis of a wide variety of substances, including creatine (7), choline (7), carnitine (8), coenzyme Q-10 (9), melatonin (10), and myelin basic protein (11). Methylation is also used to metabolize the catecholamines dopamine, norepinephrine and epinephrine (12), to inactivate histamine (13), and to methylate phospholipids (14), promoting transmission of signals through membranes.

The role of the methylation cycle in the sulfur metabolism is to supply sulfur-containing metabolites to form a variety of important substances, including cysteine, glutathione, taurine and sulfate, via

its connection with the transsulfuration pathway (5).

This cycle balances the demands for methylation and for control of oxidative stress (15)

How is the methylation cycle dysfunctional in autism, and how is this related to glutathione depletion?

In autism the methylation cycle was found by James et al. (2,3) to be blocked at methionine synthase, which is the step involving methylation of homocysteine to form methionine (see diagram).

Two effects of this block that they measured are a significant decrease in the level of plasma methionine and lowering of the ratio of S-adenosylmethionine to S-adenosylhomocysteine. The latter causes a decreased capacity for promoting methylation reactions (16).

In addition, they found (2,3) that the flow through the transsulfuration pathway (see diagram) was also decreased, resulting in lower plasma levels of cysteine and glutathione and a lowered ratio of reduced to oxidized glutathione, all of which they measured. This lowered ratio reflects a state of oxidative stress (17).

The block in the methylation cycle and the glutathione problem were found to be linked, since supplements used to restore the methylation cycle to normal operation (methylcobalamin, folinic acid and trimethylglycine) also restored the levels of reduced and oxidized glutathione (2).

Do genetic factors contribute to producing this methylation cycle dysfunction in autism?

It is known from studies of twins that genetics plays an important predisposing role in autism (18). The fact that the rate of incidence of autism has increased dramatically in recent years is evidence that there is also an important environmental component in the development of cases of autism (3), since the population's genetic inheritance is relatively constant over much longer periods.

James et al. (3) found that there are measurable genetic differences between children with autism and healthy controls. The differences they measured are associated with genes that encode enzymes and other proteins impacting the methylation cycle, the folate metabolism and the glutathione system.

In particular they found differences in allele frequency and/or significant gene-gene interactions for genes encoding the reduced folate carrier (RFC), transcobalamin II (TCN2), catechol-O-methyltransferase (COMT), methylenetetrahydrofolate reductase (MTHFR), and one of the glutathione transferases (GST M1).

These genetic results, combined with the biochemical observations of dysfunction in the methylation cycle, strongly suggest that variations in genes associated with this cycle and its related biochemistry are involved in the genetic predisposition to developing autism.

What evidence suggests that this same dysfunction and similar genetic factors are also present in chronic fatigue syndrome?

1. Methionine concentrations are reported to be below normal in both plasma (19) and urine (20) in CFS patients. Low methionine can be caused by a methylation cycle block.

2. Four magnetic resonance spectroscopy studies in CFS (21-24) have found elevated choline-to-creatine ratios in various parts of the brain. Both choline and creatine arise partly from the diet and partly from synthesis in the body. Since the syntheses of these two substances are the main users of methylation (7), a methylation deficit would be expected to decrease the rate of synthesis of both of them, and hence to decrease their levels in the cells. When this occurred, it would be unlikely that their ratio would remain the same, since the fractions of each supplied by synthesis would not likely be the same, nor would the decrease in rates of synthesis of these two substances likely to be proportional to their levels in the cells. Since creatine synthesis is the

greater user of methylation (7), it might be expected that the choline-to-creatine ratio would increase, as is observed. It therefore appears that a methylation cycle block could explain this well-replicated observation in CFS.

What evidence suggests that this same dysfunction is also present in chronic fatigue syndrome? (continued)

3. Some substances that require methylation for their biosynthesis have been found to be at below-normal levels in CFS patients, and/or patients have been found to benefit by supplementing them. This has been reported in eleven of the studies in CFS of carnitine, beginning with the work of Kuratsune et al. (25-34), both the studies of coenzyme Q10 (35, 36), a study that included choline as phosphatidylcholine in a combination supplement (37), and one recent study of melatonin (38) (though it should be mentioned that earlier studies of melatonin in CFS found normal or elevated levels, and/or did not find benefit from supplementation (see review in ref. 39), suggesting that other issues in addition to the methylation deficit might be involved in the case of melatonin. See "Magnesium depletion" later in this paper).

4. Vitamin B12, which plays a key role in the methylation cycle and was one of the supplements used to restore this cycle in the autism work (2), has a long history (39,40) as one of the most helpful of the essential nutrients in CFS when given in high-dosage injections. Lapp and Cheney (41, 42) found that in urine organic acids testing of 100 CFS patients, 33% had elevated homocysteine, 38% had elevated methylmalonate, and 13% had both (29,30). The elevated homocysteine implicates the methylation cycle,

What evidence suggests that this same dysfunction is also present in chronic fatigue syndrome? (continued)

while the elevated methylmalonate indicates that the other pathway that requires vitamin B12 showed deficiency as well. Lapp and Cheney (42) found that 50 to 80% of over 2,000 patients reported benefit from high-dose vitamin B12 injections. Evengard et al. (43) reported that vitamin B12 levels in the cerebrospinal fluid of 10 of 16 CFS patients were below their detection limit of 3.7 pmol/L. Regland et al. (44) found both low vitamin B12 (in 10 out of 12 patients) and high homocysteine (in all 12 patients studied) in the cerebrospinal fluid of CFS patients. There were significant correlations between these parameters and symptoms.

Regland et al. (45) performed an open trial in which they gave 1,000 microgram weekly injections of hydroxocobalamin for at least 3 months to the 10 female patients from this study who had both low B12 and elevated homocysteine. They found that the treatment was significantly more beneficial if the patient did not have the thermolabile allele of the polymorphic gene for MTHFR. They concluded that vitamin B12 deficiency was probably contributing to the increased homocysteine levels. They also found that the effect of vitamin B12 supplementation was dependent on whether the available methyl groups were further deprived by the existence of thermolabile MTHFR. This work implicated the methylation cycle in

What evidence suggests that this same dysfunction is also present in chronic fatigue syndrome? (continued)

the pathogenesis of CFS, and it also pointed to the importance of a genetic component, involving one of the same genes that have been implicated in autism (3).

5. Folinic acid was recently found to produce subjective improvement in symptoms in 81% of 58 CFS patients tested (46). This was also one of the supplements used to restore the methylation cycle in the autism research (2).

6. Many studies have reported evidence for oxidative stress in CFS (47-61).

7. There have been several reports of depletion of reduced glutathione in at least a substantial

subset of CFS patients (49-51, 53,54,59,62). Reduced glutathione augmentation is now widely used by CFS clinicians, who have found that augmenting glutathione by various means has been helpful to many of their patients (49,50,63-65).

8. Polymorphisms in the gene coding for the COMT enzyme were found by Goertzel et al. (66) to be some of the most important of those examined for distinguishing CFS cases from controls. As noted earlier, COMT is a methyltransferase, associated with the methylation cycle. In autism, the COMT 472G>A polymorphism showed significant difference between cases and controls (3).

If this same dysfunction is present in both autism and CFS, how can the obvious differences between these two disorders be explained?

Major differences are seen in the gender ratio and in the symptoms of these two disorders.

Autism is found primarily in boys, at a ratio of about 4 to1 (boys to girls) (67), while CFS occurs mainly in adult women at a ratio measured at 1.8 to 1 (women to men) by Jason et al. (68) in one large epidemiological study and 4.5 to 1 (women to men) by Reyes et al. (69) in another.

The most striking symptoms in autism involve the brain and are very characteristic of this disorder. They are described as follows by the Diagnostic and Statistical Manual of Mental Disorders (70):

1. Qualitative impairment in social interaction, as manifested by at least two of the following:
a. Marked impairment in the use of multiple nonverbal behaviors such as eye-to-eye gaze, facial expression, body postures, and gestures to regulate social interaction.
b. Failure to develop peer relationships appropriate to developmental level.
c. A lack of spontaneous seeking to share enjoyment, interests, or achievements with other people (e.g., by a lack of showing, bringing, or pointing out objects of interest).
d. Lack of social or emotional reciprocity.

2. Qualitative impairments in communication as manifested by at least one of the following:
a. Delay in, or total lack of, the development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication such as gestures or mime).
b. In individuals with adequate speech, marked impairments in the ability to initiate or sustain a conversation with others.
c. Stereotyped and repetitive use of language or idiosyncratic language.
d. Lack of varied, spontaneous make-believe play or social imitative play appropriate to developmental level.

If this same dysfunction is present in both autism and CFS, how can the obvious differences between these two disorders be explained? (continued)

3. Restricted repetitive and stereotyped patterns of behavior, interests, and activities, as manifested by at least one of the following:
a. Encompassing preoccupation with one or more stereotypic and restricted patterns of interest that is abnormal either in intensity or focus.
b. Apparently inflexible adherence to specific, nonfunctional routines or rituals.
c. Stereotypic and repetitive motor mannerisms (e.g., hand or finger flapping or twisting, or complex whole-body movements).
d. Persistent preoccupation with parts of objects.

CFS involves a large variety of symptoms (71,72), the chief ones being extreme fatigue, post-exertional malaise and/or fatigue, sleep dysfunction, muscle pain, and symptoms involving the brain that are significant but less profound than in autism (e.g. cognitive and memory difficulties).

The author proposes that these differences result at least in part from the different ages at onset. Autism develops early in life, before the brain is completely developed and before puberty, while the onset of CFS occurs after brain development is completed and (for the most part) after puberty.

Pangborn (73) has discussed five hypotheses that have been suggested to explain the higher

prevalence of autism in boys. Of these, the one that appears to be most consistent with the present author's hypothesis of a common pathogenesis between CFS and autism is the one put forward by Geier and Geier (74). Their hypothesis proposes

If this same dysfunction is present in both autism and CFS, how can the obvious differences between these two disorders be explained? (continued)

that the higher prevalence of autism in boys results from the potentiation of mercury toxicity by testosterone, while estrogen is protective. There is increasing evidence that mercury was a significant factor in the etiology of many cases of autism, because mercury-containing thimerosol was used as a preservative in vaccines given to them. Since thimerosol was removed from childhood vaccines, the number of new cases of neurodevelopmental disorders, including autism, has been found to be dropping (75).

The present author has proposed a hypothesis (76) to explain the higher prevalence of CFS in women, involving an additional bias toward oxidative stress due to redox cycling in the metabolism of estradiol when certain polymorphisms are present.

With regard to symptoms, it seems likely that the role of methylation in the formation of myelin basic protein (77) is at least part of the explanation for the major problems in brain development in autism and the symptoms that result from them.

Fatigue is not recognized to be a major feature of autism. However, it should be noted that the evaluation of fatigue is usually based on self-report, which is not possible in children who are unable to speak. Also, it seems possible

If this same dysfunction is present in both autism and CFS, how can the obvious differences between these two disorders be explained? (continued)

that fatigue may be manifested differently in very young children as compared with adults. Features such as hyperactivity and irritability may reflect fatigue in these patients.

Chronic pain may also be difficult to identify and characterize in children who do not have speech. A recent paper suggests that chronic pain may be the initial presenting symptom in cases of undiagnosed autism (78).

Many of the other phenomena found in CFS are also found in autism, but historically they have not received as much attention in autism as the brain-related symptoms, perhaps because the latter are so striking and profound. Some of the other phenomena that autism has in common with CFS in addition to those already mentioned are elevated proinflammatory cytokines (79), Th2 shift in the immune response (80), low natural killer cell activity (81), mitochondrial dysfunction (82, 83), carnitine deficiency (83), hypothalamus-pituitary-adrenal (HPA) axis dysfunction (84), gut problems (85), and sleep problems (86).

How does the Glutathione Depletion—Methylation Cycle Block (GD-MCB) Hypothesis explain other aspects of chronic fatigue syndrome?

Etiology: According to the GD-MCB Hypothesis, CFS is caused by a combination of two factors:

(1) a genetic predisposition (87), which is currently only partly known, and

(2) some combination of a variety of physical, chemical, biological and/or psychological/emotional stressors, the particular combination differing from one case to another (See Ref. 1 for a review.).

So far, polymorphisms in genes coding for the following proteins have been found to be associated with CFS in general or with a subset:

(1) Serotonin transporter (5-HTT) gene promoter (88)

(2) Corticosteroid binding globulin (CBG) (89)

(3) Tumor necrosis factor (TNF) (90)

(4) Interferon gamma (IFN-gamma) (90)

(4) Proopiomelanocortin (POMC) (91)

(5) Nuclear receptor subfamily 3, group C, member 1, glucocorticoid receptor (66,91)

(6) Monoamine oxidase A (MAO A) (91)

(7) Monoamine oxidase B (MAO B) (91)

(8) Tryptophan hydroxylase 2 (TPH2) (66,91)

(9) Catechol-O-methyltransferase (COMT) (66)

How does the GD-MCB Hypothesis explain other aspects of chronic fatigue syndrome?

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In addition, a COMT polymorphism has reported to be associated with fibromyalgia (92, 93), and polymorphisms in the genes for the detoxication enzymes CYP2D6 (cytochrome P450 2D6) and NAT2 (N-acetyl transferase 2) have been found to be associated with multiple chemical sensitivities (94). These may be relevant to CFS because of its high comorbidities with these two disorders.

All these proteins touch on the pathogenesis mechanism described in this paper, which is what would be expected if this Hypothesis is valid.

With regard to the stressors found to precede onset of CFS, they are known to raise cortisol secretion (prior to onset and early in the course of the illness), to raise epinephrine secretion and to place demands on glutathione, leading to oxidative stress (1).

According to this Hypothesis, when reduced glutathione is sufficiently depleted and the oxidative stress therefore becomes sufficiently severe in a person having the appropriate genetic predisposition, a block is established at methionine synthase in the methylation cycle (95,2,3). Because the methylation cycle is located upstream of cysteine and glutathione in the sulfur metabolism, these are further depleted, and a vicious circle is formed.

How does the GD-MCB Hypothesis explain other aspects of chronic fatigue syndrome?

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Note that infectious pathogens are included among the possible biological stressors that can contribute to the onset of CFS. In particular, Borrelia burgdorferi, the bacterium responsible for Lyme disease, has been found to deplete glutathione in its host (96). This may explain the very similar pathophysiologies of chronic Lyme disease and CFS. This may also explain the epidemic clusters of CFS, which seem to have been produced by a virulent infectious pathogen (or pathogens). Perhaps the genetic factors are less important in producing the onset if a very virulent pathogen is present.

Epidemiology: According to the GD-MCB Hypothesis, the prevalence of CFS is determined by the frequency in the population of the combined presence of certain genetic polymorphisms (yet to be completely identified) and of the above described stressors occurring coincidentally in those having the polymorphisms. As noted earlier, the author has proposed that the higher prevalence in women is a result of increased bias toward oxidative stress, resulting from redox cycling in the metabolism of estradiol when certain polymorphisms in detoxication enzymes are present (76).

Suppression of parts of the immune response: Elevation of cortisol due to long-term stressors causes

a suppression of the cell-mediated immune response and a shift to Th2 (97).

How does the GD-MCB hypothesis explain other aspects of chronic fatigue syndrome?

(continued)

Depletion of reduced glutathione likewise causes a shift to Th2 (98, 99).

The elevation of cortisol prior to onset and in the early course of the illness also (temporarily) suppresses inflammation (100).

The cytotoxicity of natural killer (NK) cells and CD8 T cells in CFS has been found to be low, and Maher et al. found this to be associated with a deficiency of perforin secretion (101). According to the GD-MCB Hypothesis, in CFS perforin secretion is inhibited by depletion of reduced glutathione because glutathione is needed to form the disulfide bonds in their proper configurations in secretory proteins (102). Depletion of glutathione therefore causes misfolding and recycle of perforin molecules, which have twenty cysteine residues and thus ten disulfide bonds (103). This misfolding mechanism would affect other secretory proteins in CFS that are synthesized in cells having glutathione depletion as well, which may account for the observation of misfolded proteins in the spinal fluid of CFS patients by Baraniuk et al. (104).

Proliferation of T lymphocytes is inhibited by the block in the folate cycle, which inhibits production of new RNA and DNA (105).

How does the GD-MCB Hypothesis explain other aspects of chronic fatigue syndrome?

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Viral and intracellular bacterial reactivation: According to the GD-MCB Hypothesis, depletion of reduced glutathione is the trigger for the reactivation of latent viral and intracellular bacteria in CFS. The infections found initially in a case of CFS are usually due to those pathogens that are capable of residing in the body in the latent state, suggesting that these infections arise by reactivation (106). In general, intracellular glutathione depletion is associated with the activation of several types of viruses (1, 107-111) as well as Chlamydia (112), and it may account for reactivation of other latent intracellular bacteria as well. In herpes simplex type 1 viral infection, raising the glutathione concentration inhibits viral replication by blocking the formation of disulfide bonds in glycoprotein B (111). Since glycoprotein B appears to be present in all herpes virus types (113), it is likely that glutathione depletion is responsible for reactivation of Epstein-Barr virus, cytomegalovirus and HHV-6 in CFS.

The Coxsackie B3 virus genome is known to code for glutathione peroxidase, a selenium-containing enzyme (114). Taylor has suggested (115) that such viruses suppress the immune system of the host by depleting its selenium, thus inhibiting the host's use of glutathione peroxidase. Since glutathione peroxidase makes use of

How does the GD-MCB Hypothesis explain other aspects of chronic fatigue syndrome?

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glutathione, depletion of reduced glutathione itself would therefore assist this virus in its mechanism of infection.

Populations more deficient in selenium would be expected to be more vulnerable to Coxsackie B3 infection. It is interesting to note that nearly all the studies of Coxsackie virus in CFS have come from the UK. The population there has become more deficient in selenium since the 1970s, when major sources of grain in the diet were changed to areas with selenium-deficient soils (116).

Immune activation: This occurs when the immune system detects the reactivation of pathogens

(117).

Activation of 2-5A, RNase-L pathway (118): This pathway is activated by interferon and double stranded RNA as part of the cellular response to viral reactivation. According to the GD-MCB Hypothesis, RNase-L remains activated in CFS because of the suppression of the cell-mediated immune response and the consequent failure to defeat the viral infection (See "Suppression of parts of the immune response," above.)

Mitochondrial dysfunction and the onset of physical fatigue: As hypothesized by Bounous and Molson (119), competition between the oxidative skeletal muscle cells and

How does the GD-MCB Hypothesis explain other aspects of chronic fatigue syndrome? (continued)

the immune system for the decreased supply of glutathione and cysteine causes depletion of reduced glutathione in the skeletal muscles. According to the GD-MCB Hypothesis, this inhibits the glutathione peroxidase reaction and allows hydrogen peroxide to build up. This in turn probably exerts product inhibition on the superoxide dismutase reaction, which allows superoxide, produced as part of normal oxidative metabolism, to rise in the mitochondria of the oxidative skeletal muscle cells. Superoxide reacts with nitric oxide to produce peroxynitrite, as Pall (120) has pointed out. Superoxide also interacts with aconitase in the Krebs cycle to inhibit it (121), and peroxynitrite can cause partial blockades in the Krebs cycle and also the respiratory chain (120, 122). These reactions lower the rate of production of ATP, and this constitutes mitochondrial dysfunction. Since ATP is needed to power muscle contraction, lack of it produces physical fatigue.

RNase-L cleavage, leading to formation of the low molecular weight version (123): Depletion of reduced glutathione removes inhibition of the activity of calpain (124), which is located in the cytosol with RNase-L, and calpain cleaves RNase-L (125). (Elastase, the other enzyme found by Englebienne et al. (125) to be able to cleave RNase-L in the laboratory, is confined to granules and vesicles inside living cells (126), and thus is not in contact with RNase-L.)

How does the GD-MCB Hypothesis explain other aspects of chronic fatigue syndrome? (continued)

Failure to defeat viral and intracellular bacterial infections and continuing immune activation: According to the GD-MCB Hypothesis, these occur because of depletion of reduced glutathione (127) and also because the folate metabolism block prevents production of new DNA and RNA for proliferation of T lymphocytes (105).

Depletion of magnesium: There is a long history showing depletion of magnesium in CFS and benefits of supplementation, both orally and by injection (See review in Ref. 39). Magnesium depletion may be responsible for a variety of symptoms that are found in CFS (128), including mitochondrial dysfunction, muscle twitching, muscle pain, sleep problems and cardiac arrhythmia. In connection with sleep problems, Durlach et al. have found that magnesium depletion is associated with abnormalities in the level of melatonin and dysregulation of biorhythms (129). Manuel y Keenoy et al. (54) found that the subset of CFS patients that was resistant to repletion of magnesium in their clinical study also showed glutathione depletion. It has also been found that glutathione depletion causes magnesium depletion in red blood cells (130). According to the GD-MCB Hypothesis, the depletion of intracellular magnesium in CFS is another result of depletion of reduced glutathione.

Buildup of toxins: Glutathione depletion allows toxins, including heavy metals, to build up, because there is not

How does the GD-MCB Hypothesis explain other aspects of chronic fatigue syndrome? (continued)

enough glutathione to conjugate these toxins as rapidly as they enter the body. Mercury is of particular concern, because the population in general has considerable exposure to it from dental

amalgams, fish consumption, and environmental sources such as nearby coal-fired power plants. There is considerable clinical experience of mercury buildup in CFS patients (1). Immune testing has also shown evidence that the immune system has responded to elevated mercury in CFS patients (131-133).

Solidification of the vicious circle: After the vicious circle has developed involving the methylation cycle block and the depletion of glutathione, another factor must come into play to lock in this situation chronically. It seems likely that buildup of toxins is the factor responsible for this, by blocking the formation of methylcobalamin and thus the activity of methionine synthase. It has been shown that one of the important roles of glutathione normally is to protect the very much smaller (by six orders of magnitude) concentrations of cobalamins from reaction with toxins by forming glutathionylcobalamin (134). Without this protection, cobalamins are vulnerable to reaction with a variety of toxins. An example is mercury. It has been found that very small concentrations of mercury are required to block the methionine synthase reaction (135). Because of this additional factor, attempts simply to correct the glutathione depletion and the oxidative stress after the

How does the GD-MCB Hypothesis explain other aspects of chronic fatigue syndrome? (continued)

cobalamins have reacted with toxins in most cases will not restore normal function of the methylation cycle (1).

Neurotransmitter dysfunction: The production of melatonin from serotonin as well as the metabolism of the catecholamines require methylation, as noted earlier, and according to the GD-MCB Hypothesis, they are inhibited because of the decreased methylation capacity. Also, genetic polymorphisms involving enzymes in the neurotransmitter system have been found to be more frequent in at least some subsets of CFS patients, as noted earlier. These factors cause dysfunction of the neurotransmitters.

Further development of mitochondrial dysfunction: As the course of the illness progresses, it is likely that other factors that result from glutathione depletion and the methylation cycle block come into play and further suppress the operation of the mitochondria. These include the buildup of toxins and infections, depletion of magnesium, and damage to the phospholipid membranes of the mitochondria by oxidizing free radicals (136). Because the essential fatty acids in these membranes are polyunsaturated, they are the most vulnerable to oxidation (137), and they become depleted, at least in some CFS patients (See review in Ref. 39).

How does the GD-MCB Hypothesis explain other aspects of chronic fatigue syndrome? (continued)

HPA axis blunting (138): According to this Hypothesis, glutathione depletion in the pituitary gland inhibits production of proopiomelanocortin (POMC) (which has

two disulfide bonds in its N-terminal fragment (139)), and hence secretion of ACTH (which is part of POMC), by the same mechanism as inhibition of perforin synthesis (102) (See "Suppression of parts of the immune response," above.). This results in the lowering of cortisol secretion by the adrenal glands, which is a late finding in the course of the illness (140). As noted earlier, genetic polymorphisms in POMC may also be involved in a subset of CFS patients (91).

Diabetes insipidus (excessive urination, thirst, decrease in blood volume): According to this Hypothesis, glutathione depletion inhibits production of arginine vasopressin (141), which has one disulfide bond (142), by the same biochemical mechanism by which it inhibits perforin and ACTH synthesis (102). It is likely that the secretion of oxytocin, which also has one disulfide bond and is also synthesized in the hypothalamus, is also inhibited. Measurements of oxytocin in CFS have not been reported, but there is evidence that it is low in some fibromyalgia patients (143), which may be relevant because of the high comorbidity of CFS and fibromyalgia. A clinician has reported benefit from oxytocin injections in fibromyalgia patients (144).

How does the GD-MCB Hypothesis explain other aspects of chronic fatigue syndrome? (continued)

Low cardiac output (145): According to this Hypothesis, this occurs because depletion of reduced glutathione in the heart muscle cells lowers the rate of production of ATP, as in the skeletal muscle cells. This produces diastolic dysfunction as observed by Cheney (146, 147). Both low blood volume (see Diabetes insipidus, above), which produces low venous return, and diastolic dysfunction, which decreases filling of the left ventricle, produce low cardiac output. In addition, in some cases, as observed by Lerner et al., viral infections produce cardiomyopathy (148). According to the GD-MCB Hypothesis, this is a result of depletion of reduced glutathione and suppression of cell-mediated immunity. This is another factor that can decrease cardiac output in CFS.

Orthostatic hypotension and orthostatic tachycardia (149): According to this Hypothesis, these occur because of low blood volume, low cardiac output and HPA axis blunting (See Diabetes insipidus, Low cardiac output, and HPA axis blunting, above.).

Loss of temperature regulation: As pointed out by Cheney (146), this occurs because of low cardiac output (see Low cardiac output, above), which causes the autonomic nervous system to decrease blood flow to the skin. This removes the ability to regulate the rate of heat loss from the skin.

How does the GD-MCB Hypothesis explain other aspects of chronic fatigue syndrome?

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Hashimoto's thyroiditis (150) and elevated incidence of thyroid cancer (151): According to this Hypothesis, Hashimoto's thyroiditis occurs in CFS because depletion of reduced glutathione in the thyroid gland allows damage to thyroglobulin by hydrogen peroxide, as proposed by Duthoit et al. (152). In addition, hydrogen peroxide damage to DNA in the thyroid gland may be responsible for the elevated incidence of cancer there. Hydrogen peroxide is produced normally by the thyroid to oxidize iodide in the process of making thyroid hormones (153).

Increasing variety of infections (154) and inflammation (155): According to this Hypothesis, viral, intracellular bacterial and fungal infections accumulate over time because the cell-mediated immune response is dysfunctional (See "Suppression of parts of the immune response," above.). Inflammation becomes more severe because of the decreased secretion of cortisol later in the course of the illness (See "HPA axis blunting," above), and because of the rise in histamine as a result of lack of sufficient methylation capacity to deactivate it (156).

Slow gastric emptying (157) and gastroesophageal reflux: According to this Hypothesis, in CFS these result from mitochondrial dysfunction in the parietal cells of the

How does the GD-MCB Hypothesis explain other aspects of chronic fatigue syndrome?

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stomach, due to depletion of reduced glutathione, which results in low production of stomach acid. (Anecdotally, many CFS patients have reported absence of eructation after ingestion of sodium bicarbonate solution on an empty stomach, suggesting low stomach acid status.) A slower rate of gastric emptying was found to be associated with higher pH, i.e. lower acid status (158).

Gut problems: According to this Hypothesis, several of the above factors converge to produce problems in the gut in CFS, often referred to as irritable bowel syndrome (IBS). These factors include glutathione depletion, low cardiac output, immune suppression, low stomach acid production, neurotransmitter dysfunction (note that serotonin plays a major role in gut motility), and increasing variety of infections and inflammation.

The degree of abnormality of a lactulose breath test (indicating small intestinal bacterial

overgrowth) in fibromyalgia patients was found by Pimentel et al. to be greater than in IBS patients without fibromyalgia (159). In addition, they found that the abnormality was correlated with somatic pain (159). (This may be relevant because of the high comorbidity of CFS with fibromyalgia.)

How does the GD-MCB Hypothesis explain other aspects of chronic fatigue syndrome?

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Brain-related problems: According to this Hypothesis, several of the above factors also converge to produce problems in the brain. These include glutathione (and cysteine) depletion, low cardiac output, failure to defeat infections and continued immune activation, neurotransmitter dysfunction, decreased methylation capacity to maintain myelin, and increasing variety of infections and inflammation.

Relapsing (Crashing) (160): Many CFS patients have chronically low glutathione levels. According to this Hypothesis, when the level of stressors is temporarily increased, the levels of reduced glutathione become more severely depleted, and this produces the so-called crashing phenomenon. After a period of rest, reduced glutathione levels are increased to the chronically low levels that existed prior to the increased stressors.

Alcohol intolerance (161): According to this Hypothesis, because of mitochondrial dysfunction, the skeletal muscles of CFS patients depend more than normal on glycolysis for ATP production. Increased use of glycolysis requires increased use of gluconeogenesis by the liver to convert lactate and pyruvate back to glucose (Cori cycle). In CFS, this is hampered by low cortisol levels. The metabolism of ethanol by the liver further inhibits gluconeogenesis,

How does the GD-MCB Hypothesis explain other aspects of chronic fatigue syndrome?

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producing hypoglycemia and lactic acidosis. This accounts for the alcohol intolerance reported by many CFS patients.

Weight gain: According to this Hypothesis, the weight gain often seen in CFS results from the inability to metabolize carbohydrates and fats at normal rates, because of partial

blockades in the Krebs cycle produced by depletion of reduced glutathione. Excess carbohydrates are cycled back to glucose by gluconeogenesis, and ultimately are converted to stored fat.

Low serum amino acid levels (19): According to this Hypothesis, these result from the burning of amino acids as fuel at higher rates than normal. Amino acids are able to enter the Krebs cycle by anaplerosis, downstream of the partial blockades, so they can be used as fuel in place of carbohydrates and fats.

The pathogenesis of CFS becomes increasingly complex as it proceeds, because of the interactions and feedback loops that develop. For this reason, determining the cause-effect relationships for all the aspects of the resulting pathophysiology is a problem that is exceedingly difficult. Nevertheless, understanding the etiology and early pathogenesis provides a basis for developing a more effective treatment approach.

CONCLUSIONS

There is abundant and compelling evidence that the glutathione depletion—methylation cycle block mechanism is an important part of the pathogenesis for at least a substantial subset of chronic fatigue syndrome patients.

A pathogenesis hypothesis based on this mechanism is capable of explaining and unifying many

of the published observations regarding chronic fatigue syndrome, and it provides a basis for developing a more effective treatment approach.

KEY TO DIAGRAM

The diagram shows the methylation cycle at the top right, the folate cycle at the top left, and the transsulfuration pathway at the bottom right.

The enzymes that catalyze the reactions are shown in boxes:

BHMT Betaine homocysteine methyltransferase

CBS Cystathionine beta synthase

CDO Cysteine dioxygenase

CGL Cystathionine gamma lyase

GCL Glutamate cysteine ligase

GS Glutathione synthase

MAT Methionine adenosyltransferase

MS Methionine synthase

MSR Methionine synthase reductase

MTase Methyltransferase (a class of enzymes)

MTHFR Methylene tetrahydrofolate reductase

SHT Serine hydroxymethyltransferase

TS Thymidylate synthase

Most of the metabolites are spelled out. The ones that are abbreviated are as follows:

DMG Dimethylglycine

SAH S-Adenosylhomocysteine

SAM S-Adenosylmethionine

THF Tetrahydrofolate

TMG Trimethylglycine (betaine)

The cofactor and coenzyme are as follows:

P5P Pyridoxal phosphate, the active form of

Vitamin B6

B12 Methylcobalamin, one of the active forms of

Vitamin B12

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