

Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome

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Topics to be discussed in these talks

- What is the history of ME and CFS?
- What is the definition of myalgic encephalomyelitis/ chronic fatigue syndrome (ME/CFS)?
- What are the symptoms of ME/CFS?
- Who has CFS (epidemiology)?
- Is there a genetic predisposition to ME/CFS?
- What are the likely causes (etiology) of ME/CFS?

- What is glutathione, and what does it do ?
- What is the methylation cycle, and what does it do?
- What is included in the sulfur metabolism?

- What is the Glutathione Depletion—Methylation Cycle Block (GD-MCB) hypothesis for ME/CFS?

- How does the GD-MCB hypothesis explain the various aspects of ME/CFS?

- What testing is available to find out if the GD-MBC hypothesis applies to a given case?

- What treatment is suggested by the GD-MCB hypothesis?

- What clinical study of this treatment has been done, and what were the results?

Disclaimers

1. A person undergoing treatment for ME/CFS as discussed in these talks must be under the care of a **licensed physician**.
- Much of what will be discussed in this talk is **hypothesis** (unproven theory). I will try to distinguish between what is hypothesis and what is well established and considered to be scientifically true at this time. **References** for most of the work of others cited in these talks can be found in my IACFS/ME poster papers, at

<http://forums.phoenixrising.me/forumdisplay.php?91-General-Wiki-Pages>
3. **I have no financial interest** in the tests or supplements discussed in these talks.

What is the history of ME?

- The name was assigned in **1956** by **Dr. A.Melvin Ramsay** to describe an epidemic at the Royal Free Hospital in the UK in 1955.
- The name means “**pain in the muscles and inflammation in the brain and the spinal cord.**” Muscle pain is indeed frequently reported by patients. Recent autopsies on the bodies of ME patients have indeed found signs of inflammation in the spinal cord.
- There had been **previous epidemics**, such as at the Los Angeles County General Hospital in California, USA, in 1934.
- Dr. Byron Hyde of Canada has traced the history of ME-like disease back to **as early as the ancient Egyptians in 1900 B.C.**

What is the history of CFS?

- It was first defined in the U.S. by the **Centers for Disease Control and Prevention (CDC) in 1989**, after clusters of cases appeared at a few locations, the best-known being near Incline Village on Lake Tahoe in Nevada.
- It has since been recognized that CFS **had probably existed for much longer**, but under different names.
- It **seems that the prevalence has been much higher starting in the 1980s**, but it is not possible to prove this because of lack of early epidemiological data.
- The **cause or causes have not been established and agreed upon**. The most recent suspect is the **XMRV retrovirus**.
- **In the UK** and some other countries, “chronic fatigue syndrome” has largely been brought into the province of **psychiatry**.
- There has been **controversy as to whether CFS is the same as ME**, but the trend in recent years among those who recognize a physiological basis for it has been to **combine them into “ME/CFS,”** and most recently, to **abolish** the term “chronic fatigue syndrome” completely.

Definitions of ME/CFS

- Since there is no agreed-upon diagnostic test, ME/CFS is defined in terms of a **set of symptoms**, and is diagnosed when other possible causes for them have been ruled out.
- Several definitions have been developed by various committees. These definitions have attempted to identify a **symptom complex that is specific to ME, CFS, or ME/CFS** for use in diagnosis or for selecting patients for research studies, or both.

(The development of definitions has been **very controversial**.)

Ramsay (1986) ME definition

Holmes et al.(1988) CFS working case definition (U.S. CDC)

Lloyd et al.(1990) Australian CFS case definition

Sharpe et al.(1991) “Oxford criteria” for CFS in the UK

Fukuda et al.(1994): U.S. CDC-sponsored international research definition for CFS

Carruthers et al.(2003): Canadian ME/CFS consensus clinical working definition

Reeves et al.(2003) “Empirical” CFS case definition (U.S. CDC)

Jason et al.(2007) IACFS ME/CFS pediatric case definition

Carruthers et al.(2011) ME International Consensus Criteria

ME International Consensus Criteria

(Carruthers, B.M. et al., J Intern Med. 2011 Jul 20)

These are the **most recently developed** criteria.

They were developed by an international panel of 25 people, representing **12 countries**.

These criteria **do not use the term “chronic fatigue syndrome.”**
They require that “a patient will meet the criteria for—

- (1) **post-exertional neuroimmune exhaustion**,
- (2) at least one symptom from three **neurological** categories,
- (3) at least one symptom from three **immune/gastrointestinal/genitourinary** impairment categories, and
- (4) at least one symptom from **energy metabolism/transport impairments**.”

ME International Consensus Criteria (summary)

Post-exertional neuroimmune exhaustion (PENE): “a pathological inability to produce sufficient energy on demand, with prominent symptoms primarily in the neuroimmune regions.”

Neurological impairments: neurocognitive impairments, pain, sleep disturbance, and neurosensory, perceptual and motor disturbances.

Immune, gastro-intestinal & genitourinary impairments: flu-like symptoms, susceptibility to viral infections, nausea, abdominal pain, bloating, urinary urgency or frequency, nocturia, sensitivities to food, medications, odors or chemicals.

Energy production/transportation impairments: cardiovascular, respiratory, loss of thermostatic stability, intolerance of extremes of temperature.

(The full text of the criteria document describes these in greater detail.)

Other aspects of ME/CFS

- How is **fibromyalgia** related to ME/CFS?

The original diagnostic definition for fibromyalgia from the American College of Rheumatology focused on **pain and pain sensitivity**. Many people satisfied the definitions for both CFS and fibromyalgia, but others did not. The proposed new clinical case definition moves fibromyalgia closer to ME/CFS, by including a **widespread pain index and categorical scales for cognitive symptoms, unrefreshed sleep, fatigue, and number of somatic symptoms**. The cause of fibromyalgia is likewise not known.

- Does ME/CFS have **a sudden or a gradual onset**? There are some cases of each.
- Does ME/CFS occur in **epidemics or clusters**, or does it occur **sporadically**? Some of each.
- What's the **severity** of ME/CFS? Some people with ME/CFS are able to continue working full time, some carry on a few normal activities, some are housebound, some are bedridden, and a few even die from it, though the cause of death may be assigned to something more immediate. Most people who develop CFS are chronically ill for many years.

What are some of the symptoms of ME/CFS?

The most characteristic symptoms are included in the case definitions and criteria.

However, there are **many others** that have been reported by patients. Beginning with those most often reported, here is a partial list of symptoms (Hilgers and Frank, 1996):

- | | |
|---------------------------|---------------------------|
| 1. fatigue/exhaustion | 16. dryness of eyes/mouth |
| 2. lack of concentration | 17. visual disorders |
| 3. memory disorders | 18. allergy |
| 4. sleep disorders | 19. nausea |
| 5. myalgia | 20. paresthesia |
| 6. swings of mood | 21. hair loss |
| 7. headache | 22. lymphadenopathy |
| 8. respiratory infections | 23. skin alterations |
| 9. depression | 24. dyscoordination |
| 10. palpitations | 25. chest pain |
| 11. dizziness | 26. personality changes |
| 12. pharyngitis | 27. skin rashes |
| 13. dyspepsia | 28. general infections |
| 14. nocturnal sweating | 29. muscle twitching |
| 15. arthralgia | 30. urinary infections |

What can account for such a variety of symptoms, involving so many different organs and body systems?

After considering this question for a while, I suspected that there must be a problem in the basic biochemistry that can affect a large number of different cells, tissues and organs.

What are the important parameters that control the biochemistry?

Temperature, pH, concentrations of substances, and redox environment.

Which of these is most abnormal in ME/CFS?

Redox environment, as evidenced by oxidative stress.

In 1993, **Dr. Majid Ali** published a paper entitled “**Hypothesis: chronic fatigue is a state of accelerated oxidative molecular injury.**”

There are now at least 20 published studies showing evidence for oxidative stress in ME/CFS.

What maintains the redox environment of the cells?

Mainly, Glutathione.

Is there a genetic predisposition to developing ME/CFS?

Yes, at least for the sporadic cases.

Evidence for this comes from:

- 1. Family studies (Walsh, 2001; Albright, 2011)**
- 2. Twin studies (Buchwald, 2001; Hickie, 2001; Sullivan, 2005; Schur, 2007)**
- 3. Genomic polymorphism frequency studies (Smith, 2006; Narita, 2007; Carlo-Stella, 2009; Fukuda, 2010; Sommerfeldt, 2011).**

Genes that have polymorphisms (SNPs) in ME/CFS (as reported in the published literature)

Immune system:

- Tumor necrosis factor (TNF)
- Interferon gamma (IFN-gamma)
- Human leukocyte antigen (HLA-DRB1)
- Killer cell immunoglobulin-like receptors (KIR's)
- Interleukin-17F (IL17F)

Others:

- Receptor for Advanced Glycation End-Product (RAGE)
- Disrupted in Schizophrenia-1 (DISC 1)

Neurotransmitter systems:

- Tryptophan hydroxylase 2 (TPH2)
- Serotonin transporter (5-HTT) gene promoter
- Serotonin receptor subtype HTR2A
- Monoamine oxidase A (MAO A)
- Monoamine oxidase B (MAO B)
- Catechol-O-methyltransferase (COMT)
- Beta-2 adrenergic receptor (ADRB2)

HPA Axis:

- Angiotensin converting enzyme (ACE)
- Proopiomelanocortin (POMC)
- Corticosteroid binding globulin (CBG)
- Glucocorticoid receptor--nuclear receptor subfamily3, group C, member 1 (NR3C1)

Are there other genetic polymorphisms involved in ME/CFS?

- Yes.
- Dr. Amy Yasko has found that there are polymorphisms in the genes for several enzymes associated with the **methylation cycle and related biochemical pathways**, in patients with autism as well as patients with ME/CFS.
- This work **has not been documented or replicated in peer-reviewed publications**, but many patients are following her genetically-guided treatment program.
- **There is a need for good statistical analysis** of the available data on polymorphisms in methylation-related genes, both to help with understanding the pathogenesis and pathophysiology, and for guiding treatment.
- It would also be helpful to know if there are higher frequencies of polymorphisms in genes coding for enzymes in **the glutathione system and the antioxidant system in general**.

Who has ME/CFS?

- Estimates vary from about **2.4 to 25.4 per 1,000 people in U.S.** studies, depending on the definition used (Jason, 1999; Reyes, 2003; Reeves, 2007)
- Evengard et al. (2005) reported that the **6-month prevalence of “CFS-like illness”** in the Swedish twin registry was **23.6 per 1,000 people**.
- A recent study of **3 regions in England** (Nacul, 2011) reported between **0.3 and 2 per 1,000 people**, depending on the definition used.
- Hamaguchi et al. (2011) found a prevalence of **10 per 1,000 people** in a community study in Japan.
- Prevalence is **higher in women than in men** in the U.S.: Ratio 1.8 (Jason, 1999) or 4.5 (Reyes, 2003) or metropolitan: 11.2, urban: 1.7, rural: 0.8 (Reeves, 2007)
- Prevalence is **higher in adults than in children** in the U.S.
- **Minorities have higher prevalences** than the non-minority population in the U.S.
- It was estimated that **only about 15%** of those in the U.S. who have ME/CFS **have been diagnosed**.

What are the likely causes of ME/CFS?

Risk factor studies indicate that **a variety of stressors** may contribute to the onset of ME/CFS:

- **Physical**: Aerobic exercise (especially of long duration), physical trauma (especially motor vehicle accidents) and surgery.
- **Chemical**: Exposure to toxins such as organophosphate pesticides, solvents and ciguatoxin.
- **Biological**: Infections, immunizations, blood transfusions, insect bites, allergic reactions, and eating or sleeping less.
- **Emotional/Psychological**: (see next slide) The body's **nonspecific stress response system** deals with this type of stressor in the same way it responds to physical, chemical and biological stressors.

The involvement of emotional/psychological stressors **does not mean that ME/CFS is a psychiatric disorder**. The **nonspecific parts** of the body's stress response systems produce the **same physiological response for all types of stressors**.

What are the likely causes of ME/CFS (continued)

Emotional/Psychological:

Stressful life events, including death of a spouse, close family member or close friend; recent marriage; troubled or failing marriage, separation, or divorce; serious illness in immediate family; job loss, starting new job, or increased responsibility at work; and residential move.

Difficulties, including ongoing problems with relationships, persistent work problems or financial problems, mental or physical violence, overwork, extreme sustained activity, or "busyness."

Dilemmas *"A dilemma is a situation in which a person is challenged to choose between two equally undesirable alternatives."*[45] Choosing inaction in response to a dilemma leads to further negative consequences.

Problems in childhood, including significant depression or anxiety, alcohol or other drug abuse, and/or physical violence in parents or other close family members; physical, sexual or verbal abuse, low self-esteem and chronic tension or fighting in the family.

What aspect of the biochemistry do all these potential causes impact?

These stressors all tend initially to increase the secretion of cortisol, epinephrine and norepinephrine.

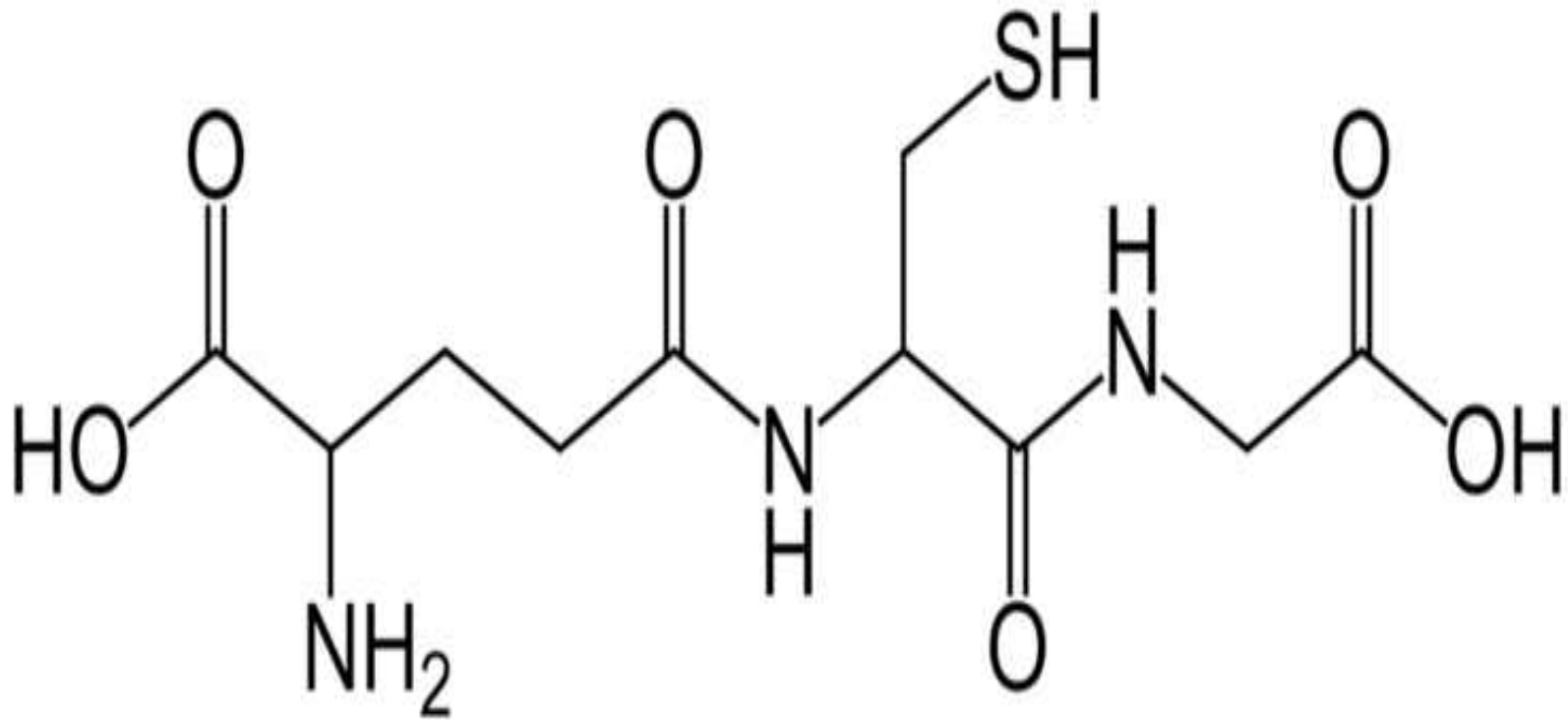
Ultimately, directly or indirectly, they all place demands on. . .

glutathione.

(Note that Droge and Holm first suggested in 1997 that glutathione is depleted in ME/CFS, and in 1998, Dr. Paul Cheney reported that nearly all his ME/CFS patients were depleted in glutathione.)

Structure of Glutathione

(from GlutathionePill.com)



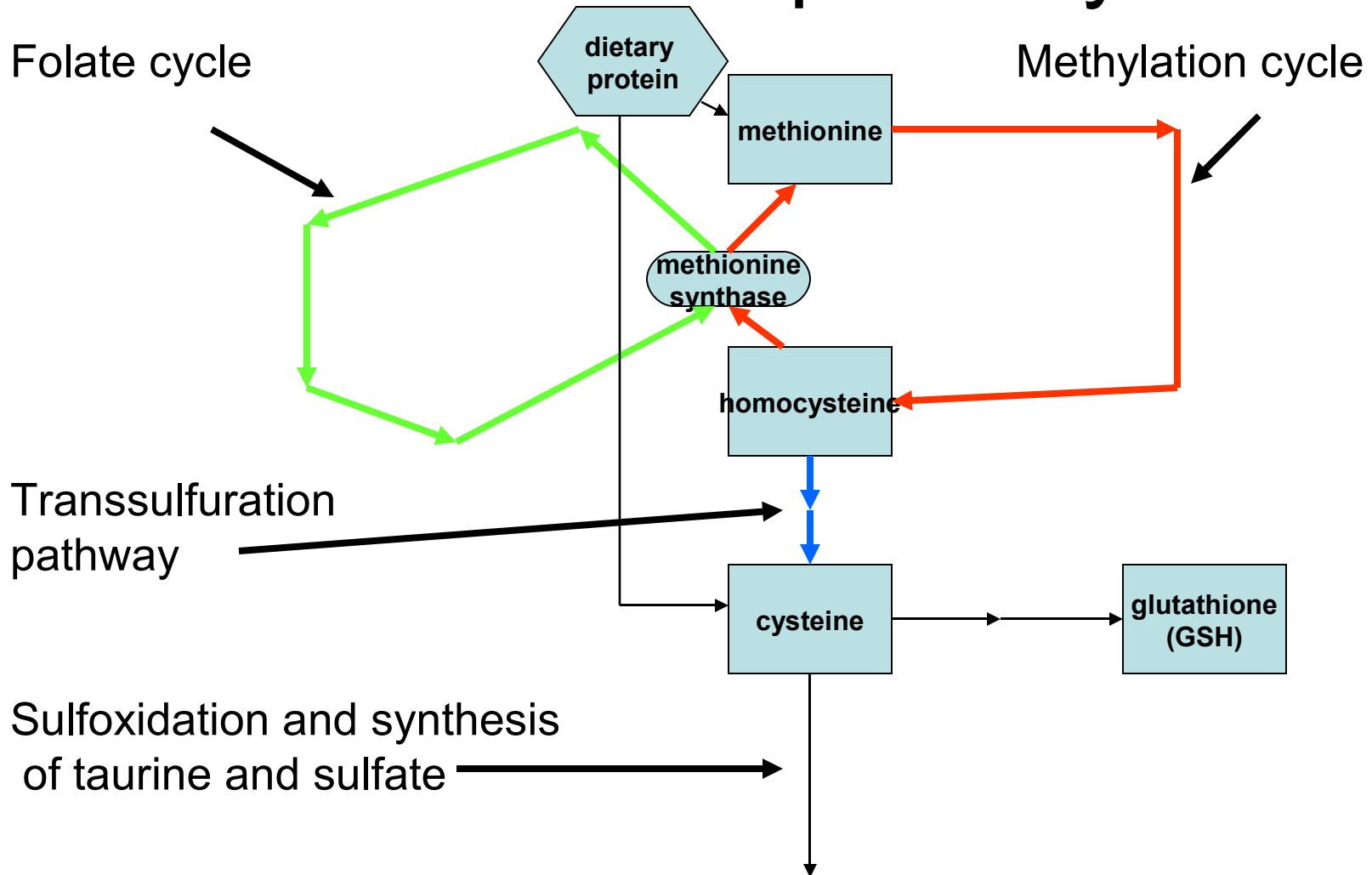
Glutathione—What is it and what does it do?

- A **tripeptide**, composed of glutamate, cysteine and glycine
- **Found in all cells**, blood, bile and epithelial lining fluid of the lung
- Synthesized particularly by **liver and red blood cells**
- The **most abundant thiol** (sulfhydryl)-containing substance in cells
- Has **reduced and oxidized forms**, GSH and GSSG
- Ratio of GSH to GSSG **controls the redox potential** in cells
- Serves as basis for the **antioxidant system**, quenching reactive oxygen species

Glutathione (continued)

- **Conjugates** several classes of toxins for removal from the body in Phase II detox, and quenches free radicals generated in Phase I detox in general
- **Supports immune system**, especially cell-mediated immunity
- Plays important role in **synthesis of proteins** that contain cysteine
- Participates in **bile production**
- **Protects vitamin B12** inside the cells and assists in its metabolism
- Has **many other functions**

Methylation cycle and associated biochemical pathways



What does the methylation cycle do?

- **Distributes methyl (CH₃) groups** to a large number of biochemical reactions in the body.
- **Controls the overall sulfur metabolism**, balancing the needs for methyl groups, for GSH to control oxidative stress, and for other sulfur metabolites, including cysteine, taurine and sulfate.
- Together with the folate metabolism, it **coordinates the production of new DNA with the supply of methyl groups**, which are used to methylate DNA, among many other roles.

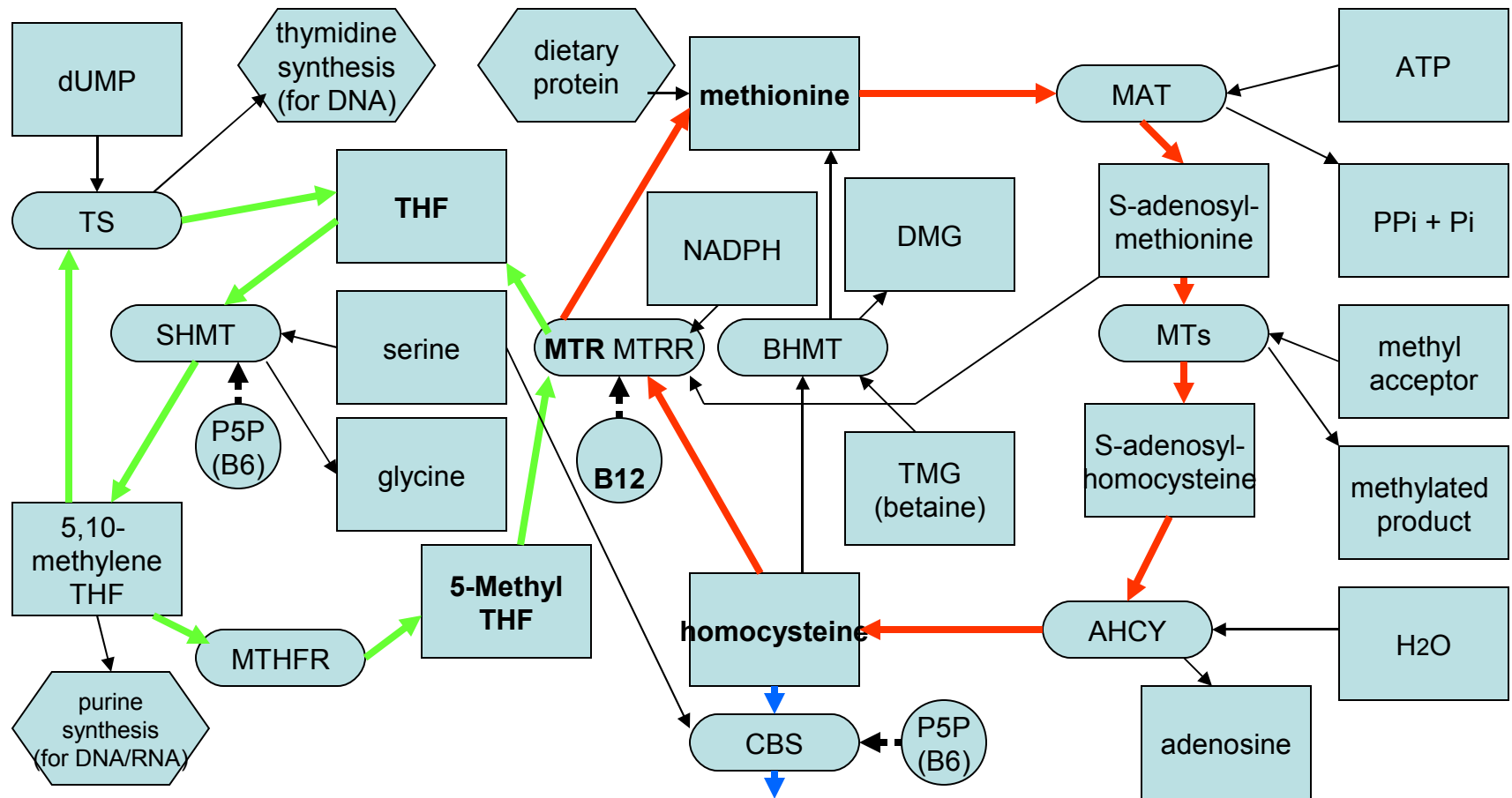
What are the functions of vitamin B12 (cobalamin)?

- In the form of **methylcobalamin** it act as a **coenzyme for the enzyme methionine synthase**, in the methylation cycle. This enzyme converts homocysteine to methionine.
- 2. In the form of **adenosylcobalamin** it acts as a **coenzyme for the enzyme methylmalonate CoA mutase**, which helps to feed certain fuels into the Krebs cycle to help in making ATP, the energy source for many of the biochemical reactions.

What are the roles of the folates?

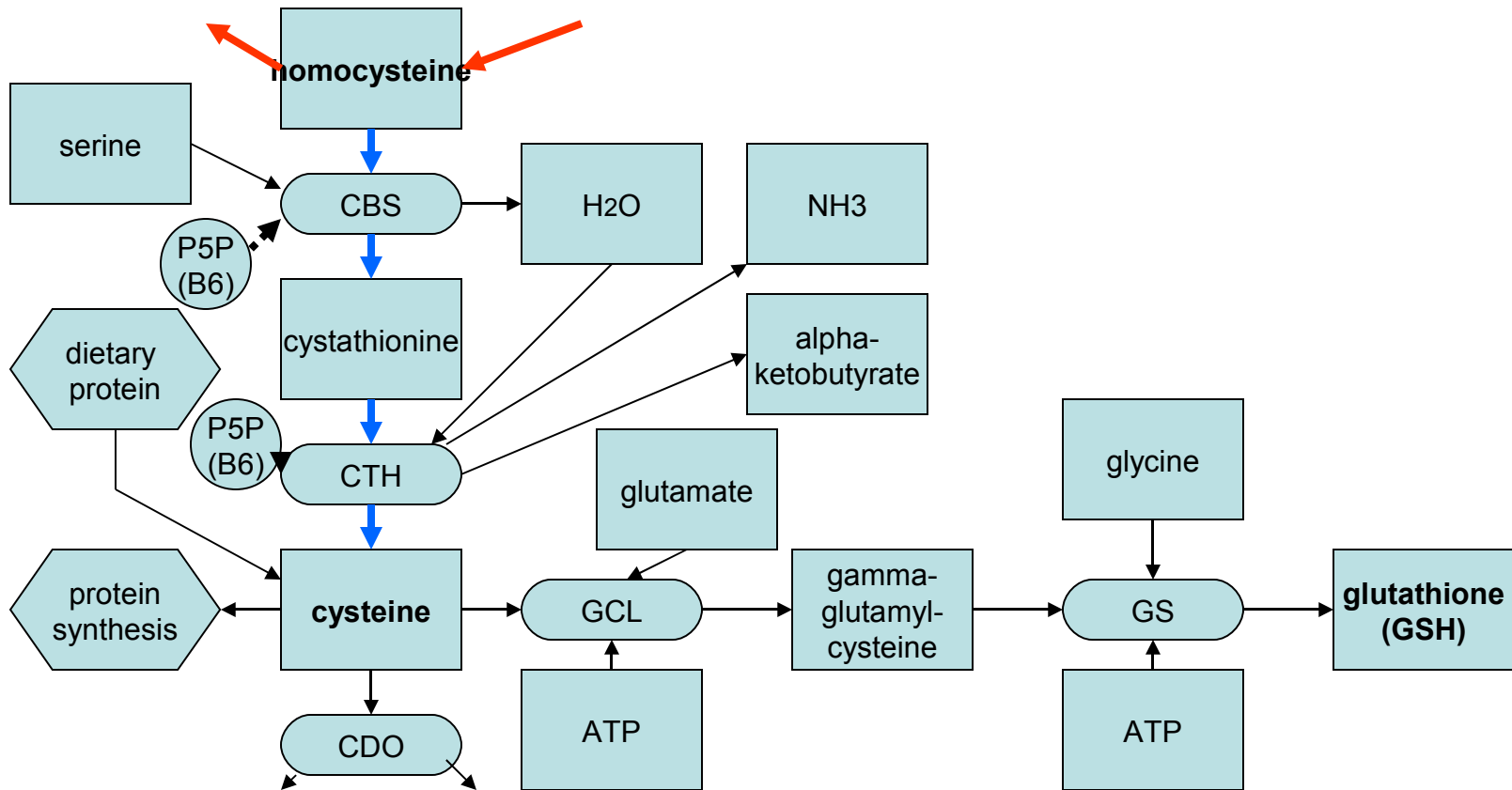
- Acts as the source of **methyl groups** for the methylation cycle.
- Participates in the **metabolism of the amino acid histidine**.
- Participates in the **synthesis of thymidine**, which is needed for DNA.
- Participates in the **synthesis of purines**, needed for DNA, RNA, and ATP.

(combined) **Methylation** and **Folate** cycles (showing link to **transsulfuration** pathway via CBS)

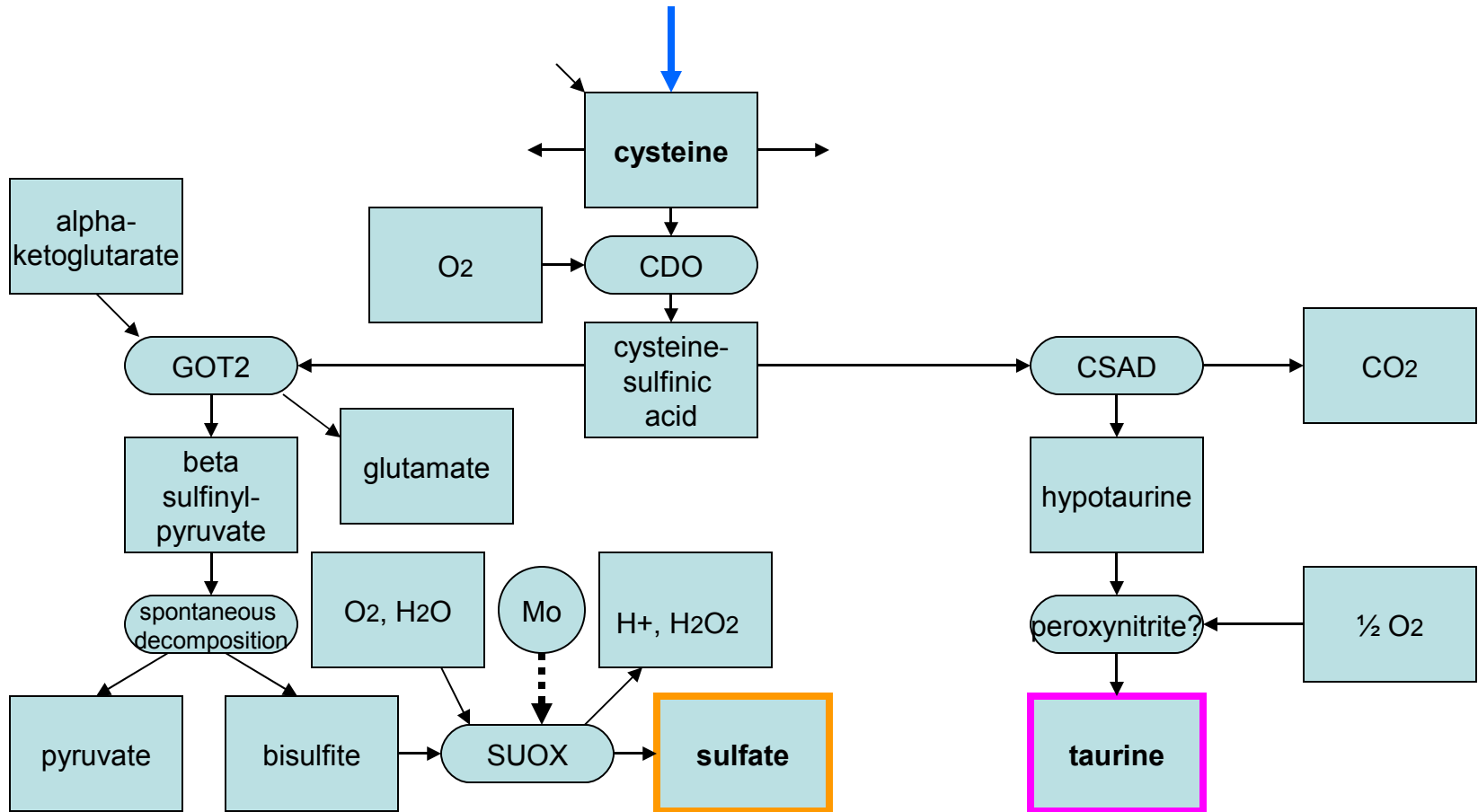


Transsulfuration pathway

[Note that a complete transsulfuration pathway is found only in cells of the liver, kidneys, pancreas, intestine, lens of the eye, and (at much lower capacity) the brain.]



Sulfoxidation and synthesis of sulfate and taurine



The Glutathione—Methylation Cycle Block (GD-MCB) Hypothesis

What causes ME/CFS, according to this hypothesis?

Genetic predisposition (only partially characterized at this time, and important for the sporadic cases, but less so for the epidemic cases)

together with:

Stressors

(Some combination of a variety of physical, chemical, biological and or psychological/emotional stressors, the particular combination differing from one case to another).

The stressors initially raise cortisol and epinephrine, and they deplete glutathione by various direct and indirect mechanisms in a person who has the genetic predisposition.

(Note that the cortisol levels usually decrease later in the illness as the HHPA axis becomes dysfunctional.)

Everyone experiences stressors, so why doesn't everyone get ME/CFS?

- A major reason is likely to be differences in the combinations of **inherited genetic polymorphisms**.
- There has **not yet been a complete genome study** of the polymorphisms that are more frequent in CFS than in the general population.
- However, as I noted earlier, **there is evidence** from family and twin studies as well as from limited polymorphism studies **that there is a genetic component** in the development of CFS.

Pathogenesis (disease development) of most cases of ME/CFS, according to this hypothesis

1. **Stressors deplete glutathione** (GSH), which produces oxidative stress, allows toxins to accumulate, and interferes with the protection and intracellular metabolism of vitamin B12.
2. A **functional deficiency of vitamin B12** develops, and accumulated toxins react with much of the vitamin B12.
3. Lack of sufficient methyl-B12 establishes a **partial block of methionine synthase**, and the sulfur metabolism therefore becomes dysregulated.
4. **Sulfur metabolites drain** excessively into the transsulfuration pathway and are eventually excreted, depleting methionine in many cases. This stabilizes the depletion of glutathione.

(continued on next slide)

Pathogenesis (continued)

5. **Folate drains from the cells** into the blood via the “methyl trap” mechanism.
6. Glutathione depletion and the partial block in methionine synthase form a **vicious circle**, and this vicious circle becomes chronic. **This vicious circle is the reason ME/CFS is a chronic condition.**
7. **Most of the various biochemical abnormalities and symptoms of CFS result from glutathione depletion, the functional deficiency of vitamin B12, the methylation deficit, and low intracellular folate.**

Elaborating the GD-MCB Hypothesis

I have used two approaches to elaborate this hypothesis:

- **Identify the normal functions** of glutathione, B12, methylation and folates, **consider what would happen if these functions were not performed**, and **compare the results** to the abnormalities found in ME/CFS.
This is the forward or “**gedanken experiment**” approach.

2. **Identify the abnormalities found in ME/CFS, determine their causes** based on known physiology and biochemistry, and **determine whether they can be explained by the aspects of the vicious circle mechanism** proposed by the GD-MCB hypothesis.
This is the **retrospective approach**.

What are some things that might be expected if glutathione were depleted? Are they observed in ME/CFS?

- Oxidative stress—**observed**.
- Mitochondrial dysfunction and low ATP output—**observed**; and diastolic dysfunction in the heart, leading to low cardiac output—**observed**.
- Buildup of toxins, including heavy metals—**observed**.
- Immune dysfunction—**observed**.
- Reactivation of herpes family viral infections—**observed**.
- Thyroid problems—**observed**.
- Low secretion and dysregulation of certain cysteine-containing secretory proteins, including ACTH, antidiuretic hormone, and perforin:

Low and dysregulated ACTH leads to blunting of the HHPA axis—**observed**. Low antidiuretic hormone leads to high daily urine volumes and—**observed**. Low perforin leads to low cytotoxic activity of the natural killer cells and the CD8 (“killer”) T cells—**observed**.

What are some things that would be expected if there were a functional deficiency in vitamin B12?

- High methylmalonate—observed.
- Partial methylation cycle block—observed.
- Energy deficit—observed.
- Neuropathy—observed in some cases.
- Macrocytic anemia—not usually observed, but high mean corpuscular volume is found in some cases.

What are some things that might be expected if the methylation capacity were diminished?

- Overexpression of many genes because of lack of gene silencing by methylation—**observed**.
- Lowered synthesis of choline and creatine—**abnormal ratio of choline to creatine is observed in the brain**.
-
- Lowered synthesis of carnitine—**deficit is observed**.
- Lowered synthesis of coenzyme Q-10—**deficit is observed**.
- Lowered synthesis of three components of myelin— **slow brain processing speed is observed**.

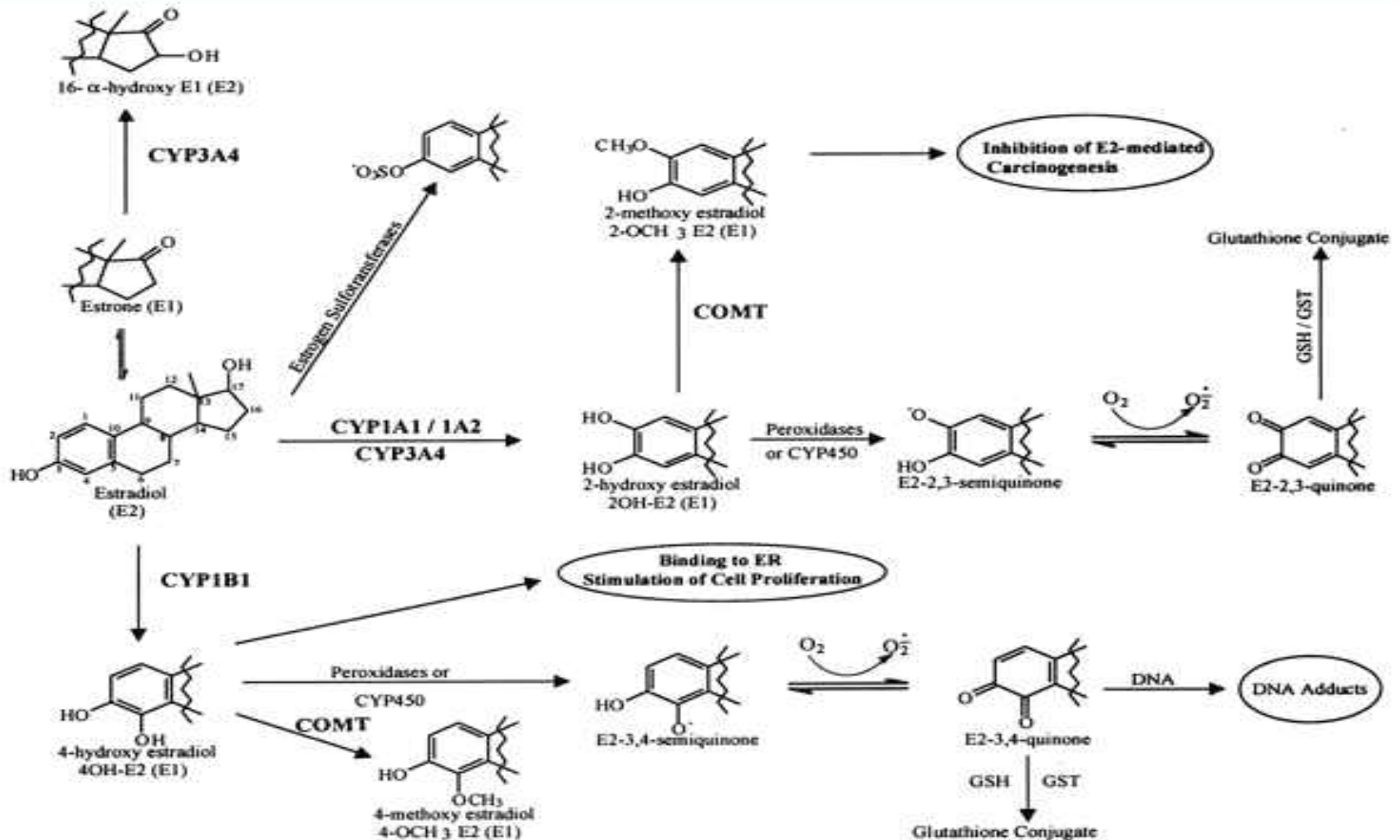
What are some things that would be expected if folate drained from the cells?

- Rise in formiminoglutamate (“Figlu”) in urine—**observed**.
- Low red and white blood cell counts—**low white cell counts observed in some cases**.
- Elevated red blood cell volume (MCV)—**observed in some cases**
- Hair loss— **observed in some cases**
- Poor digestion and absorption—**observed**

How does this hypothesis account for the higher prevalence of ME/CFS in women than in men?

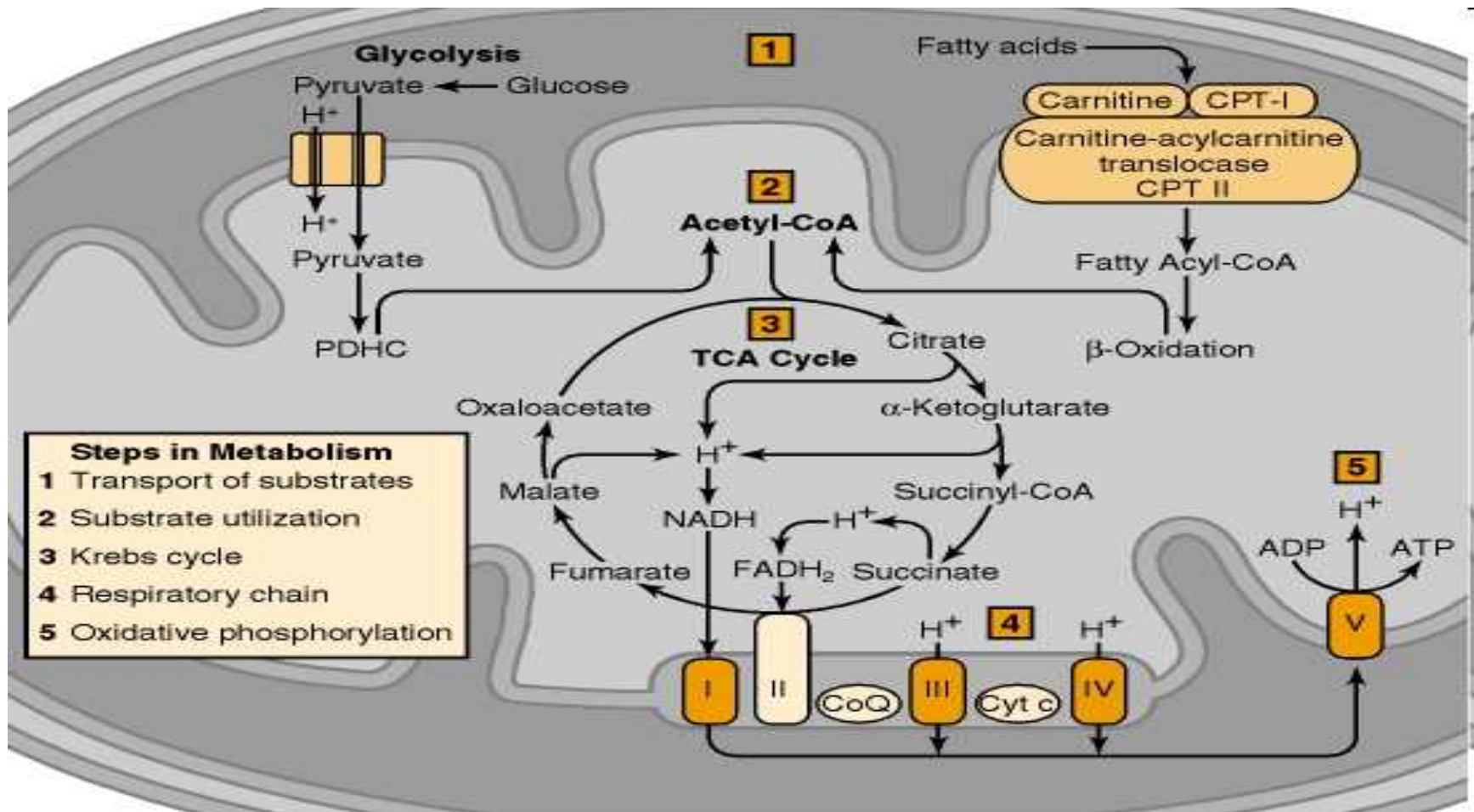
- During their potentially reproductive years, **estrogens** are produced in larger amounts in women, and **must be metabolized**.
- Some people (both men and women) inherit certain **polymorphisms** in the genes that code for some of the **detox enzymes involved in the metabolism of the estrogens (CYP1B1, COMT and GST enzymes)**. I have found this combination of polymorphisms in several women who have ME/CFS.
- In women, these polymorphisms can lead to **redox cycling** when metabolizing estrogens. This adds an additional bias toward development of **oxidative stress**.
- **Oxidative stress depletes glutathione** and initiates the pathogenesis of CFS.

Estradiol Metabolism



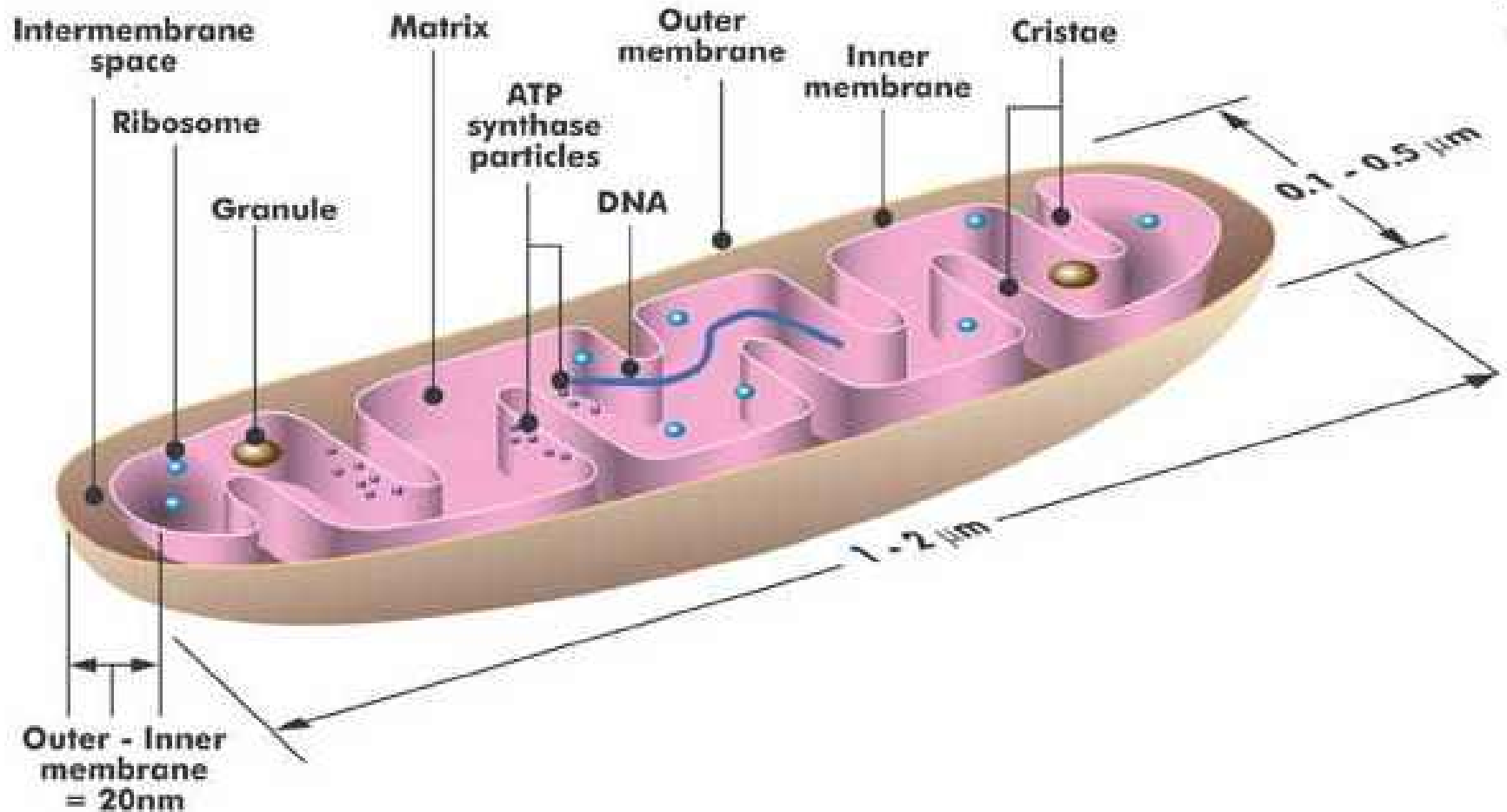
Energy Metabolism

(Diagram from Siegel et al., 1999)



Structure of the Mitochondrion

(Diagram from Canadian Academy)



What causes the fatigue in ME/CFS, according to the GD-MCB hypothesis?

Mitochondrial dysfunction.

Initially, the depletion of glutathione and the increase in oxidative stress inhibit enzymes in the Krebs (citric acid) cycle and the respiratory (electron transfer) chain, lowering ATP production.

The partial methylation cycle block causes depletion of carnitine, coenzyme Q10, phosphatidylcholine and creatine, all of which require methylation for their synthesis, and all of which are needed by the mitochondria.

Over time, toxins build up in the mitochondria because of the lack of glutathione to take them out, and they block enzymes.

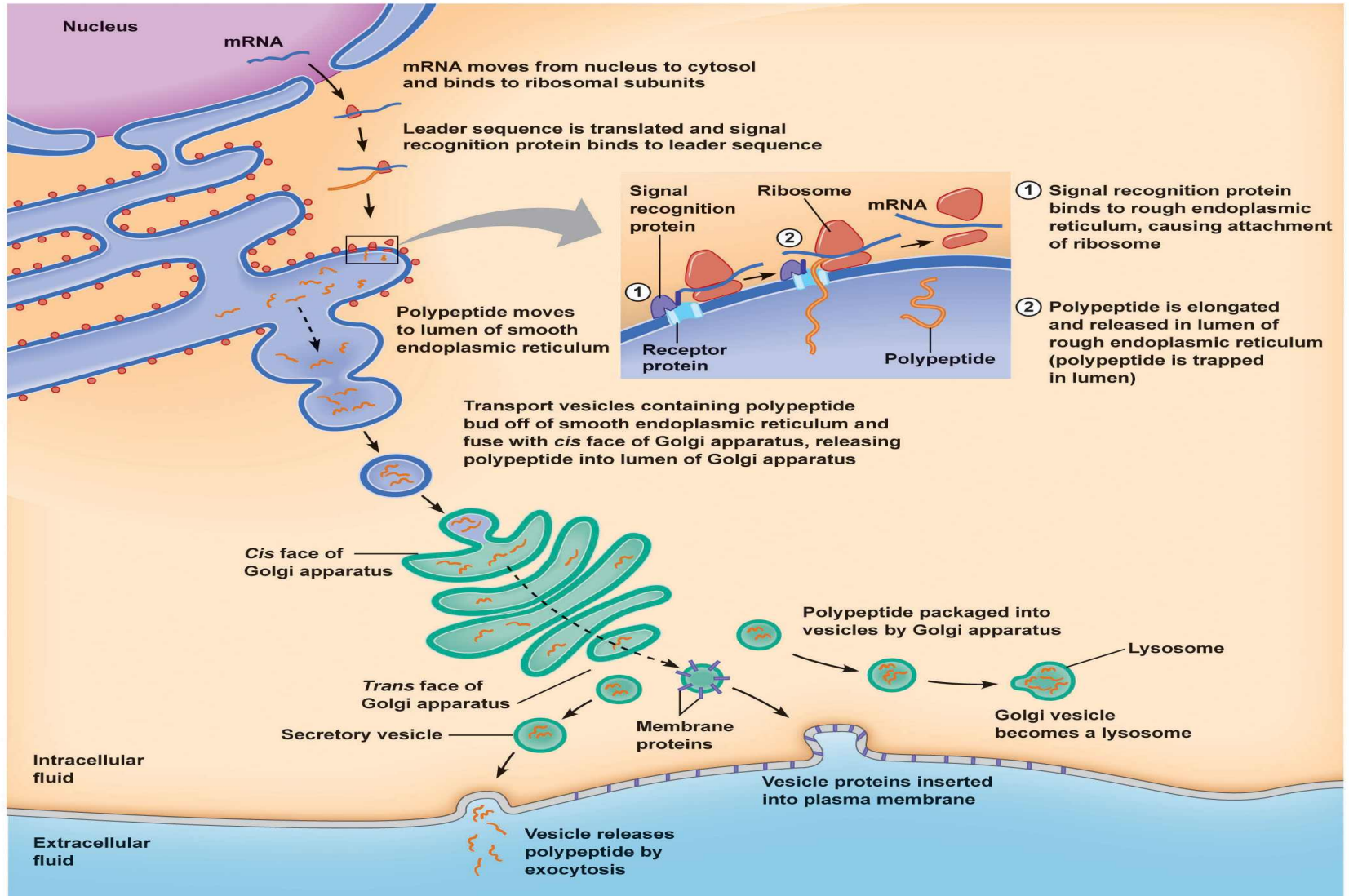
The lowered ATP production hinders the ability of the membrane ion pumps to control the concentrations of essential minerals in the mitochondria, and this also interferes with mitochondrial function.

All these factors add together to cause mitochondrial dysfunction and hence, fatigue.

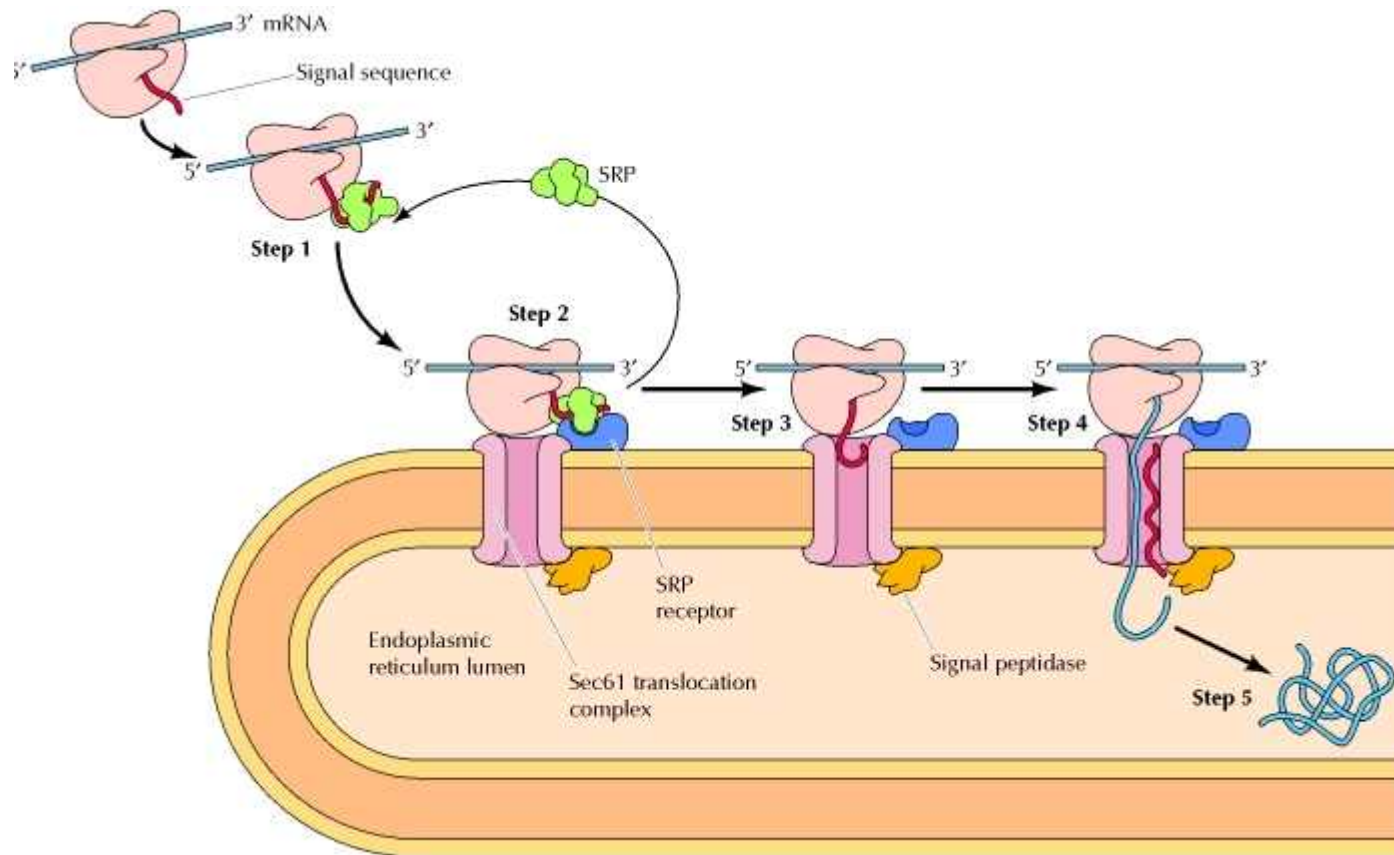
What causes the post-exertional fatigue in ME/CFS, according to the GD-MCB hypothesis?

- Exercise causes a higher rate of production of reactive oxygen species (ROS) by the mitochondria.
- The antioxidant system is dysfunctional because of the depletion of glutathione.
- The additional ROS damage the lipid membranes of the mitochondria.
- This lowers the ability of the mitochondria to produce ATP, which is needed to power the muscles.
- Some of the mitochondria may be damaged so much that they need to be destroyed by autophagy and replaced by binary fission of other mitochondria.
- It takes time to repair the mitochondrial membranes and to replace mitochondria, and this accounts for the period of post-exertional fatigue.
- This process explains why too much exercise can be damaging to people who have ME/CFS.

Secretory Protein Synthesis



Secretory Protein Synthesis



How does glutathione depletion cause problems in secretory protein synthesis?

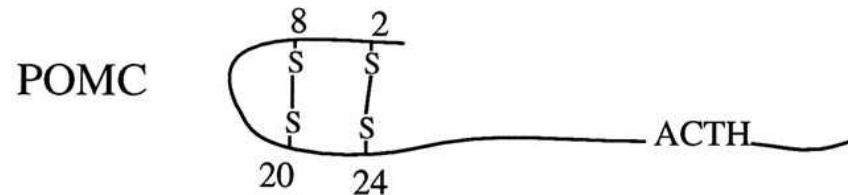
- Secretory proteins are proteins that are made inside cells, as are other proteins, and are then exported to do their jobs elsewhere. The peptide hormones are classified as secretory proteins.
- Secretory proteins are assembled initially as a chain of amino acids in the cytosol of cells. Then they are passed into the endoplasmic reticulum.
- Many secretory proteins contain cysteine in their chains, and disulfide bonds between the correct partner cysteine residues in the chains are normally formed in the endoplasmic reticulum to establish the proper tertiary structure of the protein molecules.
- If conditions become too oxidizing in the cytosol, which occurs when glutathione becomes depleted, cysteine molecules will react with each other there to form cystine, forming disulfide bonds (Chakravarthi and Bulleid, 2004).
- If this occurs too early, it will interfere with formation of their proper tertiary structures, and either the quality control system will recycle the proteins, or they will be routed to the unregulated secretory pathway or will be released in a misfolded configuration.
- I suggest that this causes lowered net synthesis of several secretory proteins in ME/CFS, and also that it causes dysregulation of ACTH secretion, in particular. I suggest that glutathione depletion is thus responsible for several of the endocrine abnormalities in ME/CFS.

A, alignment of the NH2-terminal regions showing homology between POMC, pro-vasopressin, pro-oxytocin, pro-dynorphin, pro-enkephalin, and chromogranin B.

A

Human POMC	W	C	L	E	S	S	Q	C	Q	D	L	T	T	E	S	N	L	L	E	C	I	R	A	C	K	P	D	L
Pig-tailed macaque POMC	W	C	L	E	S	S	Q	C	Q	D	L	T	T	E	S	N	L	L	A	C	I	R	A	C	K	P	D	L
Mouse POMC	W	C	L	E	S	S	Q	C	Q	D	L	T	T	E	S	N	L	L	A	C	I	R	A	C	K	L	D	L
Rat POMC	W	C	L	E	S	S	Q	C	Q	D	L	T	T	E	S	N	L	L	A	C	I	R	A	C	R	L	D	L
Guinea pig POMC	W	C	L	E	S	S	Q	C	Q	D	L	T	T	E	R	H	L	L	E	C	L	R	A	C	K	P	D	L
Bovine POMC	W	C	L	E	S	S	Q	C	Q	D	L	T	T	E	S	N	L	L	A	C	I	R	A	C	K	P	D	L
Porcine POMC	W	C	L	E	S	S	Q	C	Q	D	L	S	T	E	S	N	L	L	A	C	I	R	A	C	K	P	D	L
African clawed frog POMC	Q	C	W	E	S	S	R	C	A	D	L	S	S	E	D	G	V	L	E	C	I	K	A	C	K	T	D	L
Bullfrog POMC	Q	C	W	E	S	N	R	C	T	D	L	S	S	E	D	G	I	L	E	C	I	K	A	C	K	M	D	L
Frog POMC	Q	C	W	E	S	N	K	C	T	D	L	S	S	E	D	G	I	L	E	C	I	K	A	C	K	M	D	L
Ostrich POMC	P	C	W	E	S	G	K	C	Q	D	L	T	T	E	A	G	V	L	A	C	A	A	A	C	R	S	D	L
Salmon I POMC	E	C	W	E	N	P	R	Q	C	Q	D	L	N	S	E	N	S	I	L	E	C	I	Q	L	C	R	S	D
Salmon II POMC	Q	C	W	D	S	S	H	C	K	D	L	P	S	E	D	K	I	L	E	C	T	H	L	F	R	S	G	L
Trout A POMC	Q	C	W	E	N	P	R	C	H	D	L	S	S	E	N	N	L	L	E	C	I	Q	L	C	R	S	D	L
Trout B POMC	Q	C	W	D	S	S	H	C	K	D	L	P	S	E	D	K	I	L	E	C	I	H	L	F	R	S	G	L
Human vasopressin	A	C	Y	F	Q	N	-	C	P	R	G	G	K	R	A	M	S	D	L	E	L	R	Q	C	L	P	C	G
Human oxytocin	-	C	F	G	P	N	I	C	C	A	E	E	L	G	C	F	V	G	T	A	E	A	L	R	C	Q	E	E
Rat dynorphin	I	C	S	L	E	-	-	C	Q	D	L	V	P	P	S	E	E	W	E	T	C	R	G	F	W	S	F	L
Rat proenkephalin	D	C	S	Q	D	C	A	K	C	S	Y	R	L	V	R	P	G	D	L	N	F	L	A	C	T	L	E	C
Human chromogranin B	R	C	I	I	E	V	L	S	N	A	L	S	K	S	S	A	P	P	I	T	P	E	C	R	Q	V	L	K

B



Cool D R et al. J. Biol. Chem. 1995;270:8723-8729

Hippocampus-Hypothalamus-Pituitary-Adrenal (HHPA) Axis

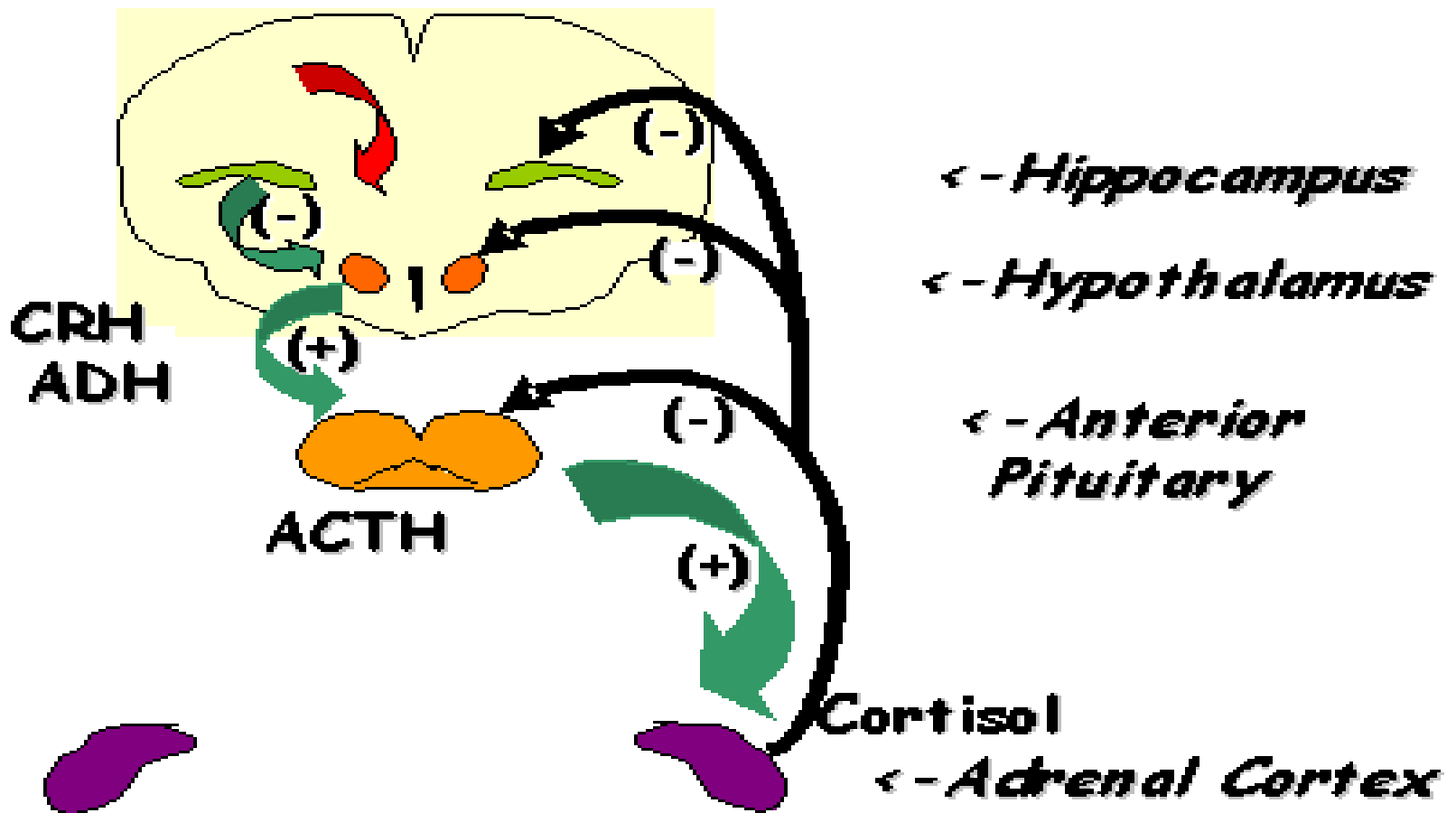


Figure 2: The hypothalamic-pituitary-adrenal (HPA) axis in www.onwellness.info
"Basic Primary Prevention" 2006 Review by Dr. Quentin Reilly, Australia

How does the GD-MCB hypothesis account for “adrenal fatigue” in ME/CFS?

- The cause of “adrenal fatigue” in ME/CFS is usually not in the adrenal glands themselves, but higher up in the HPA axis.
- The **secretion of ACTH is decreased and disregulated** by glutathione depletion in the pituitary, by the mechanism involving dysfunction of secretory protein synthesis described earlier.
- This occurs because the depletion of glutathione removes redox protection from cysteine, part of which becomes oxidized to cystine.
- This causes improper formation of proopiomelanocortin (POMC), which is the precursor to ACTH. Loss of the loop on the POMC molecule causes it to be recycled or to be routed to the constitutive, rather than the regulated, secretory pathway. This explains why cortisol is low and the diurnal cortisol variation is abnormal in ME/CFS.
- Also, the hippocampus is damaged by sustained high levels of cortisol during the high-stress initial period of ME/CFS, and the hippocampus is then not able to control the HPA axis normally.

How does the GD-MCB hypothesis explain the “mild” diabetes insipidus in ME/CFS?

- Many people with ME/CFS experience **high daily urine volume** and constant thirst.
- This is **diabetes insipidus** (not to be confused with diabetes mellitus).
- It is caused by **low secretion of antidiuretic hormone**, also called vasopressin. This hormone contains two cysteine residues.
- I suggest that the diabetes insipidus in ME/CFS results from **glutathione depletion in the hypothalamus**, which inhibits production of antidiuretic hormone.

What causes the brain-related problems in ME/CFS, according to the GD-MCB hypothesis?

- Glutathione depletion causes **excitotoxicity** by lowering the ATP production in the astrocytes in the brain. They are therefore not able to control the glutamate levels in the synapses, and this causes overstimulation of the NMDA receptors, producing excitotoxicity (anxiety, nervousness, insomnia). (Yasko)
- The methylation deficit leads to **dysfunction of the dopamine D4 receptors**, which require methylation of phospholipids for their operation, and thus problems with attention (Deth).
- The methylation deficit also causes **disrepair of myelin**, which leads to slow mental processing speed and long reaction time. The myelin damage causes “white spots” in MRI examination of the brain.
- **Mitochondrial dysfunction** in the cells in the brain accounts for the elevated lactate that has been observed in magnetic resonance spectroscopy of the brain.

Brain problems (continued)

- **Neurotransmitter synthesis and metabolism are defective** because of the methylation deficit.
- **Blood flow to the brain is lowered** because of lowered total blood volume (due to diabetes insipidus), diastolic dysfunction of the heart (due to mitochondrial dysfunction of the heart muscle cells), and defective control of blood circulation (due to problems with the sympathetic nervous system).
- **Infections** can occur in the brain due to suppression of immune system by glutathione depletion and folate draining.

What causes the immune dysfunction in ME/CFS, according to the GD-MCB hypothesis?

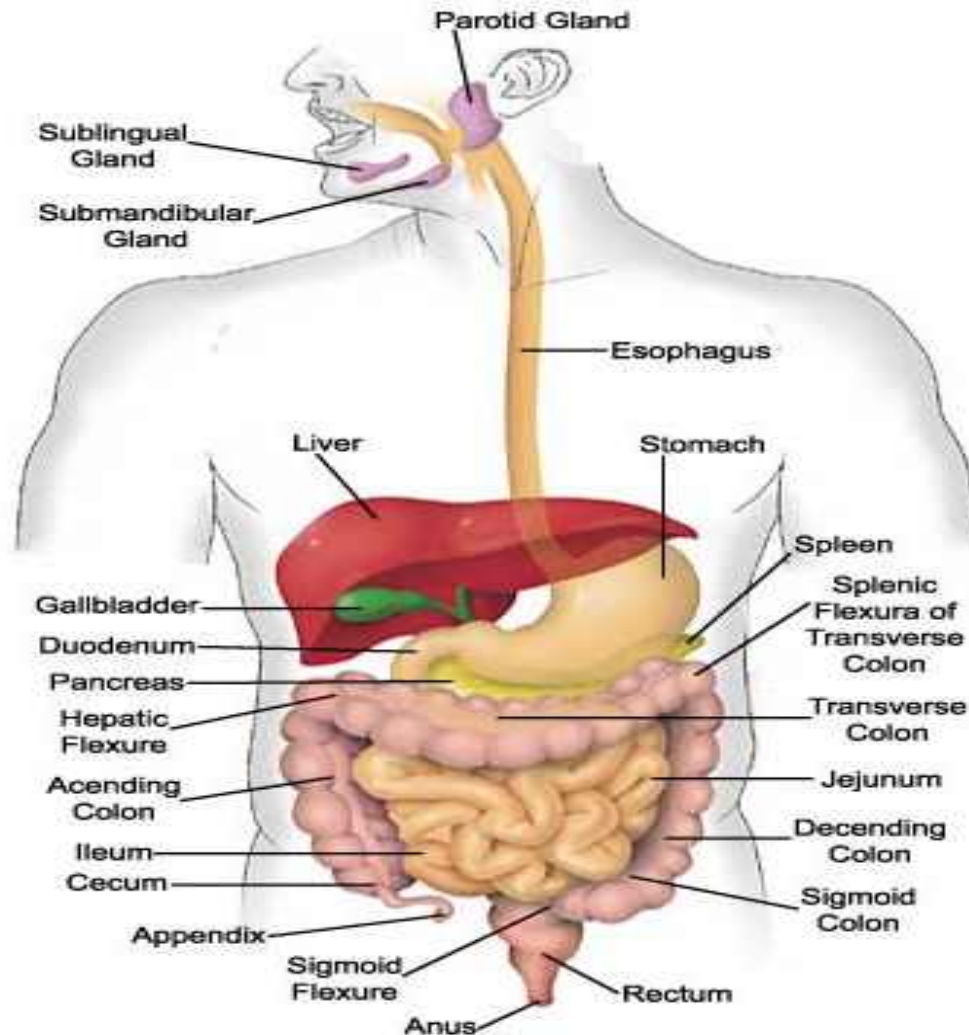
- **Low NK cell and CD8 cell cytotoxicity** is caused by low perforin secretion, which in turn is due to glutathione depletion in these cells, causing the problem in secretory protein synthesis described earlier.
- The **inhibition of the cell-mediated immune response and shift to Th2 immune response** is caused initially by the rise in cortisol, and later, after the HHPA axis becomes blunted, to glutathione depletion in the CD4 T cells.
- The **persistent interferon-driven RNase-L activation** is due to lack of a normal cell-mediated immune response, which normally takes over control of infections by viruses.
- The **cleaving of RNase-L and formation of the LMW RNase-L** is due to calpain activation in response to glutathione depletion.

Immune dysfunction (continued)

- The **low proliferative response of B and T cells to mitogens** is due to folate deficiency, which hinders production of DNA for new cells.
- The **rise in proinflammatory cytokines** reflects the ongoing activation of the dysfunctional immune system in response to pathogens, and the lack of control of inflammation by low cortisol.
- **Viruses that normally remain latent in most of the population reactivate** because of the lack of effective NK cell cytotoxicity and lack of an effective cell-mediated immune response.

Digestive System

(Diagram from mydigestivehealth.com)



What causes the problems in the digestive system in ME/CFS according to the GD-MCB hypothesis?

Glutathione depletion causes low ATP production in the mitochondria of the parietal cells. This results in low stomach acid production (high ATP supply is needed because of the large concentration gradient of the hydrogen ions).

The low stomach acid has several consequences:

Gastroesophageal reflux

Survival of yeasts and bacteria that come in with food, which promote dysbiosis.

Poor absorption of vitamin B12 and some essential minerals

Lack of conversion of pepsinogen to pepsin, so that protein digestion is inhibited.

Poor signaling to pancreas and gall bladder for release of digestive enzymes and bile, inhibiting digestion of food and absorption of nutrients in general.

Low methylation capacity and low glutathione inhibit production of digestive enzymes by the pancreas.

Digestive problems (continued)

Glutathione depletion causes **lowered bile volume**.

Poor digestion caused by the above factors leaves **food for the dysbiotic bacteria**.

The methylation cycle partial block **lowers the production of acetylcholine and serotonin**, which regulate flow through the intestine, causing dysmotility.

Glutathione depletion **removes protection of the gut from toxins and oxidative stress**.

Abnormality in cortisol level causes a **deficit in secretory IgA level**, which removes protection from the gut.

Lack of folate inhibits the production of new enterocytes (cells that line the small intestine).

The above factors lead to **leaky gut** (intestinal permeability), which in turn causes immune response and **multiple food sensitivities**.

How is mercury involved in ME/CFS, according to the GD-MCB hypothesis?

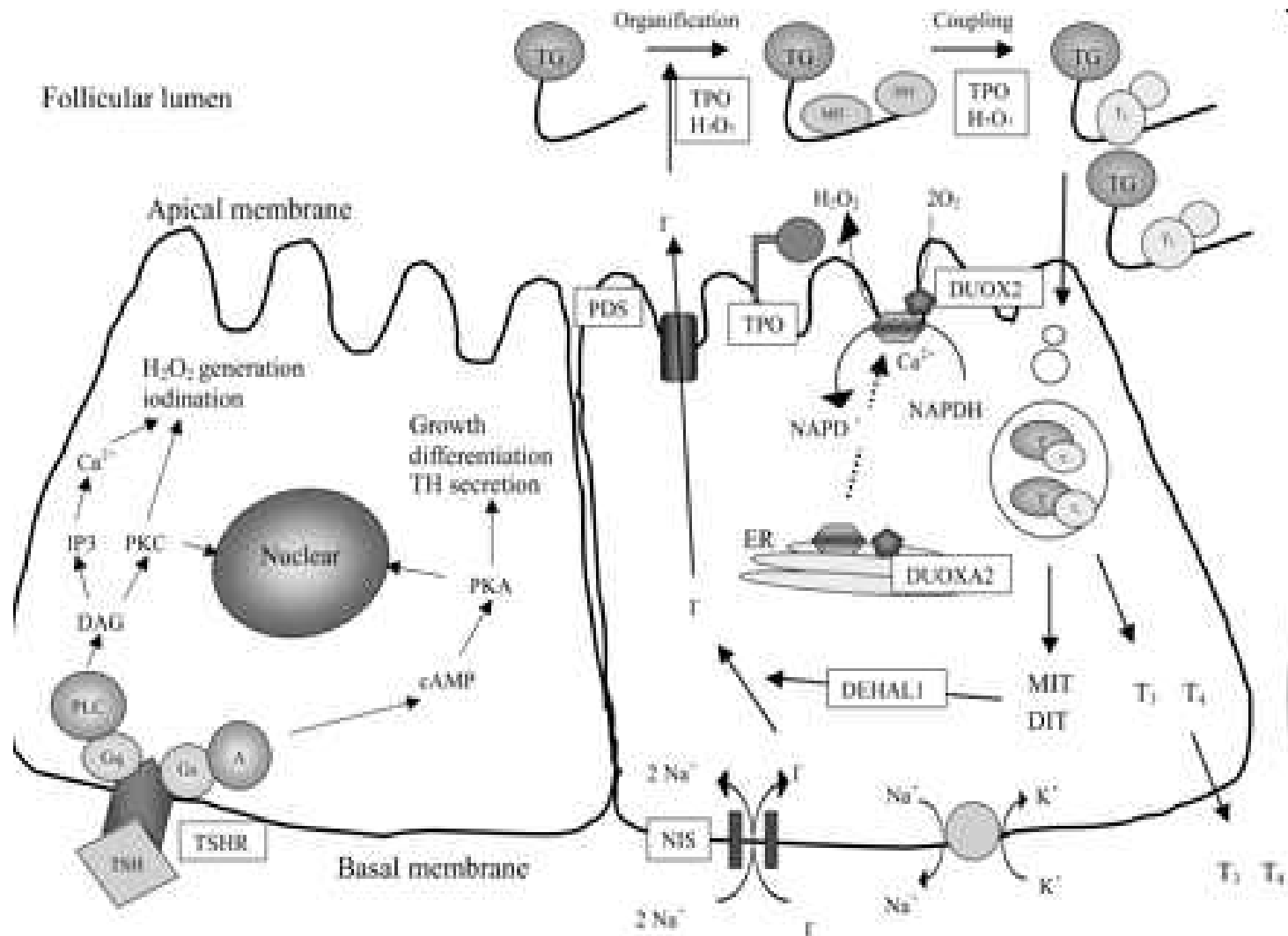
- Mercury can contribute to the onset of ME/CFS by helping to deplete glutathione, because glutathione is normally used to take it out of the body.
- Mercury toxicity can build up after the onset of ME/CFS, because not enough glutathione is available to take it out of the body. Mercury can inhibit several of the enzymes in the methylation cycle and related pathways, including the pathway for glutathione synthesis.

How does the GD-MCB hypothesis explain Hashimoto's thyroiditis in ME/CFS?

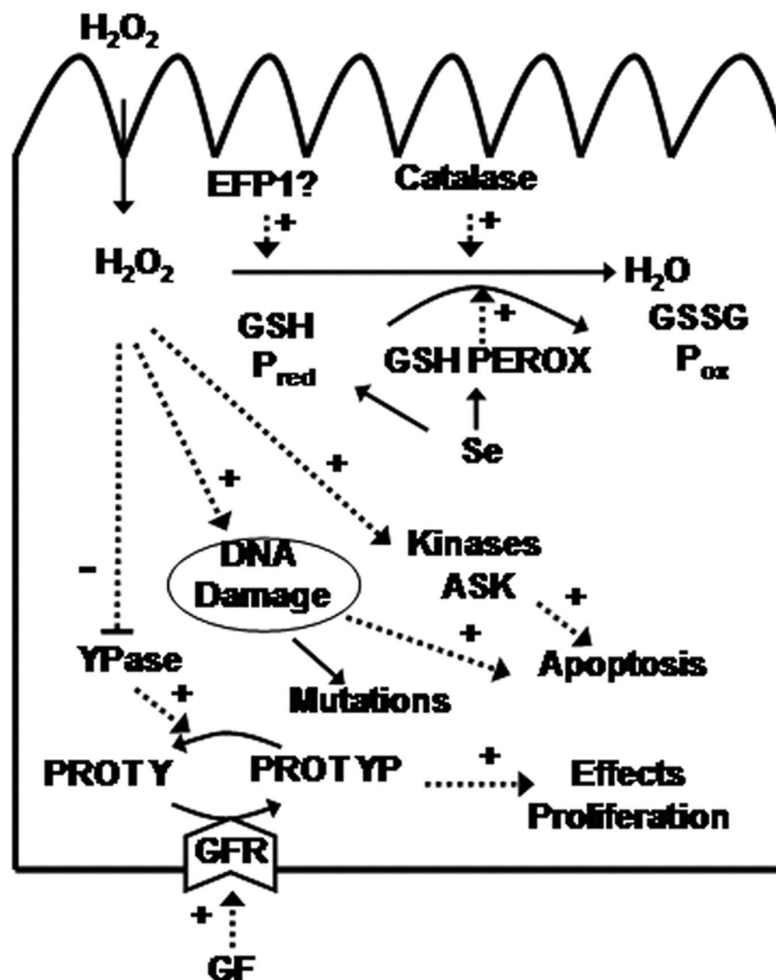
- Wikland et al.(2001) found that the prevalence of Hashimoto's thyroiditis is high in ME/CFS.
- Hyde has confirmed the high prevalence.
- The thyroid cells make hydrogen peroxide as part of the synthesis of thyroid hormones.
- Normally, glutathione protects the thyroid from its own hydrogen sulfide.
- When glutathione becomes depleted, hydrogen peroxide damages proteins in the thyroid.
- The immune system mounts an autoimmune reaction against these “foreign” proteins.
- This proposed mechanism is based on Duthoit et al. (2001)

Hydrogen peroxide and thyroid gland

(Diagram from Ohye and Sugawara, 2010)



Fate of H_2O_2 leaking back in the thyroid cell: catabolism by GSH peroxidase (GSH PEROX), peroxiredoxin reduced or oxidized (Pred or Pox), and catalase; proposed effects on protein tyrosine phosphatase (YPase) and kinases such as apoptosis signal kinase (ASK...
Lumen



Song Y et al. JCEM 2007;92:3764-3773

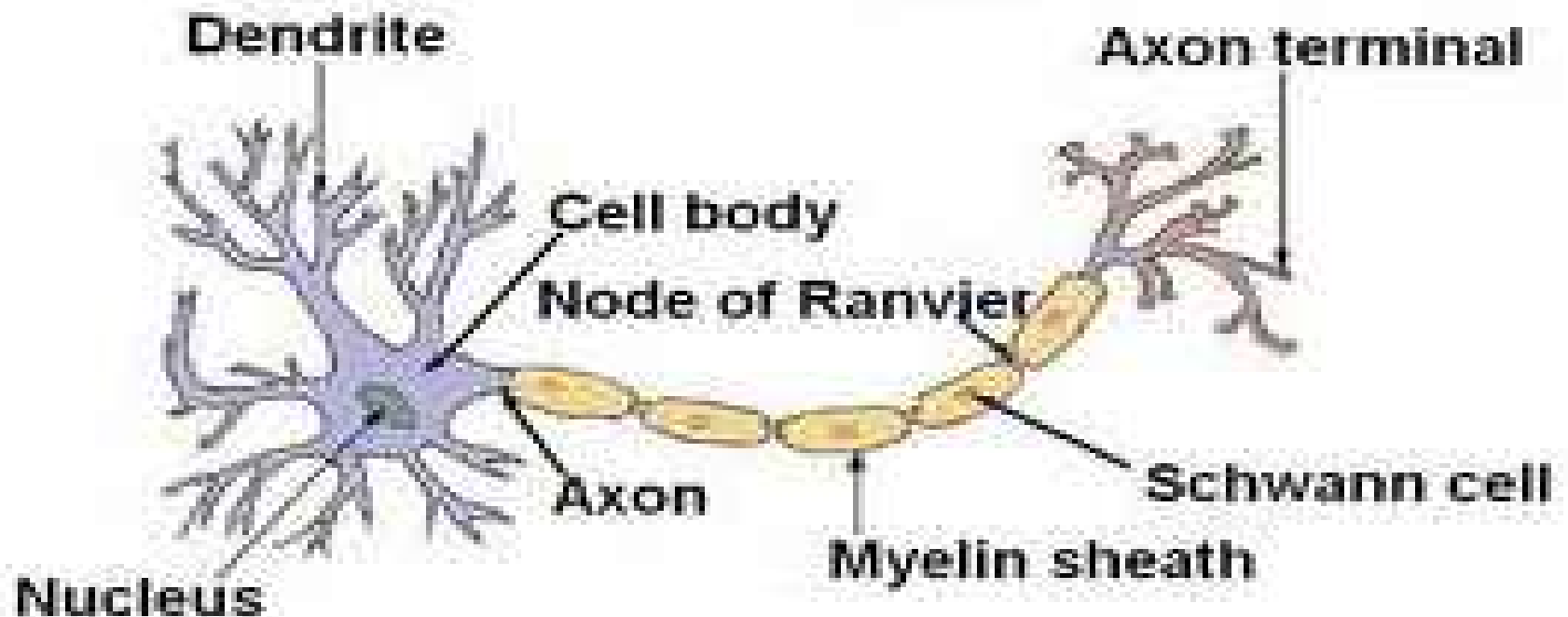
How does the GD-MCB hypothesis explain electromagnetic hypersensitivity in ME/CFS?

- Myelin normally serves as the electrical insulation on nerve axons.
- Over time, myelin becomes damaged and is normally repaired.
- At least three substances needed to repair myelin require methylation for their synthesis (myelin basic protein, phosphatidylcholine, and choline plasmalogen).
- The partial methylation cycle block lowers the methylation capacity, so that the synthesis of these substances is hindered.
- The myelin falls into disrepair and no longer provides effective insulation for the nerve axons.
- Electromagnetic radiation induces currents in the body, and they are able to penetrate into the nerves because of the lack of effective insulation.

Myelin

(Diagram from Multiple Sclerosis Resource Centre)

Structure of a Typical Neuron



What direct testing is available to see if this hypothesis applies to a given case?

Methylation Pathways Panel (offered by European Laboratory of Nutrients in the Netherlands and Health Diagnostics and Research Institute in the U.S.)

This panel can be ordered through Scandlab in Sweden.

Metabolites measured:

- Reduced glutathione (GSH) (plasma)
- Oxidized glutathione (GSSG) (plasma)
- S-adenosylmethionine (SAME) (red blood cells)
- S-adenosylhomocysteine (red blood cells)
- Adenosine (plasma)
- 5-methyl tetrahydrofolate (plasma)
- 10-formyl tetrahydrofolate (plasma)
- 5-formyl tetrahydrofolate (folinic acid) (plasma)
- Tetrahydrofolate (plasma)
- Folic acid (plasma)
- Folinic acid (whole blood)
- Folic acid (red blood cells)

What Alternative (Indirect) Testing Can be Used?

Urine organic acids testing for methylmalonic acid (MMA), formiminoglutamic acid (figlu) and pyroglutamic acid are also very helpful.

When MMA and figlu are elevated, they indicate low adenosylcobalamin and low tetrahydrofolate, respectively. If both are elevated, it is very likely that methionine synthase is partially blocked and folates have drained from the cells.

If pyroglutamic acid is high or low, it is likely that glutathione is depleted.

Additional alternative testing: Genomic polymorphisms on the Yasko nutrigenomic panel

Dr. Yasko characterizes polymorphisms in the following genes:

COMT	MTRR
VDR	BHMT
MAO A	ACHY
ACAT	CBS
ACE	SUOX
MTHFR	SHMT
MTR	NOS

It has not been shown that all these polymorphisms are more frequent in ME/CFS than in the general population, but Dr. Yasko has found that **their presence shows a tendency** toward problems with the methylation cycle and related pathways, **and influences choices about optimum treatment.**

What treatment is suggested by the GD-MCB hypothesis?

1. Building up glutathione directly—

This has been done by Drs. Salvato, Cheney, Enlander and others, but has been found to produce only temporary benefit in ME/CFS cases.

2. Supporting methionine synthase with B12, together with active folate to lift the methylation cycle block—

This was found to be effective in treating autism, which involves similar biochemical abnormalities (James et al., 2004).

Cofactor vitamins and minerals as well as amino acids may need to be supplied if they are deficient.

Previously-tested treatments of ME/CFS with B12 or folate or both

B12 alone:

Simpson (1991): Effect on RBC shape—half of patients improved.

Lapp and Cheney (1993): Found threshold for symptoms improvement at 2 to 2.5 milligrams IM.

Regland et al. (1998): 1.0 mg weekly injected for at least 3 months— significantly more beneficial **if patient did not have MTHFR C677T SNP** [This was an important observation, showing that folate was also involved, and thus pointing toward an inhibited methionine synthase reaction.]

Lapp (1999): 3.0 mg. IM q 2-3 days—50 to 80% had some improvement.

Folate alone:

Lundell et al. (2006): Folinic acid, oral, 15 milligrams—81% of patients improved.

Combined B12 and folate:

Kaslow et al. (1989): 220 mcg cyanoB12 and 800 mcg folic acid equivalent—significant improvement, but no better than placebo [We now know that the B12 dosage was not high enough, and other forms of B12 and folate are superior.]

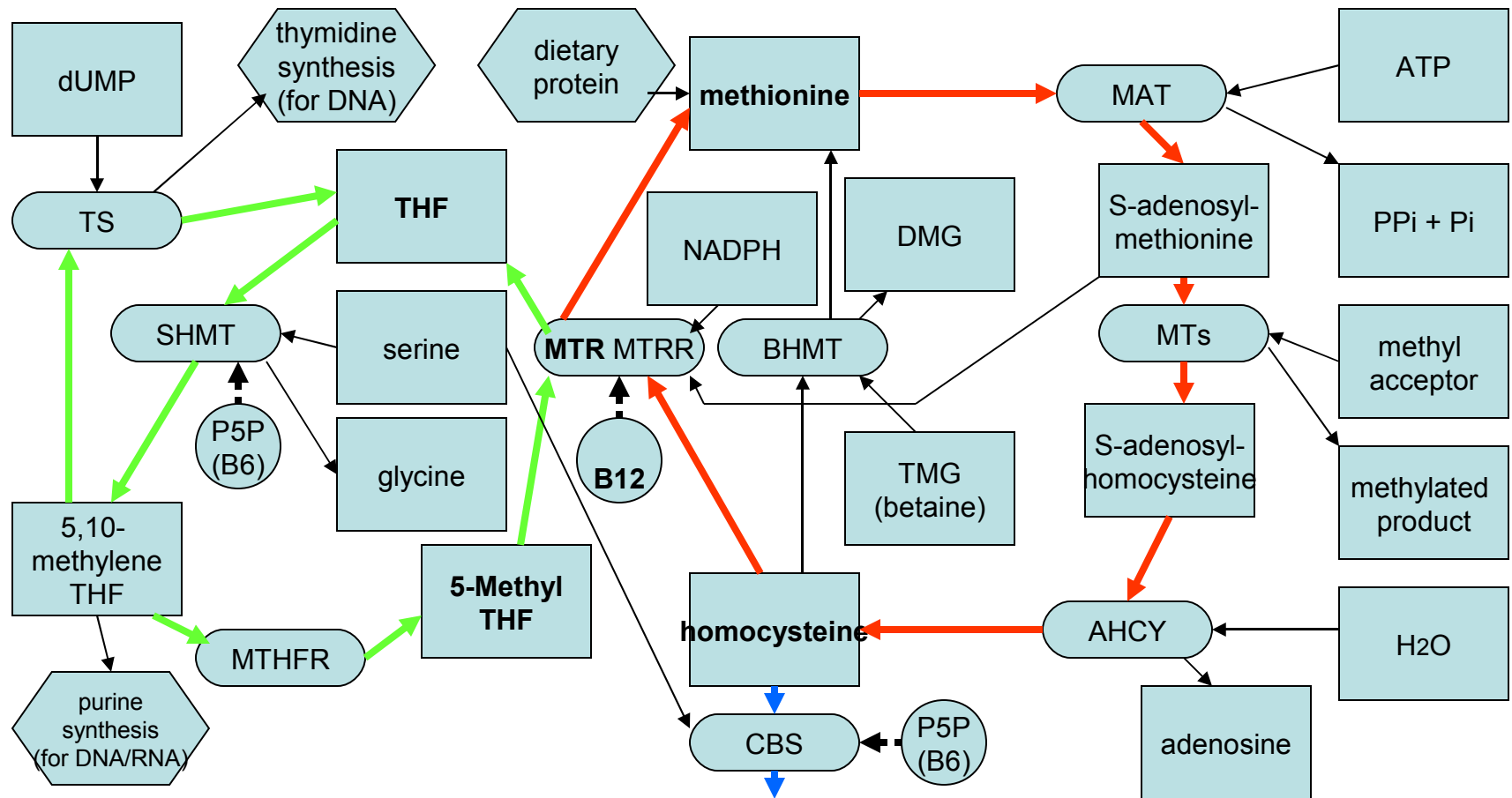
Pall (2001): Recommended hydroxocobalamin to scavenge nitric oxide. Later, recommended methylfolate to scavenge peroxynitrite. No results reported so far.

What protocols are in use now to lift the methylation cycle block?

1. “Defeat Autism Now!” (DAN!) protocols.
2. Yasko treatment program
3. Dr. Alan Vinitzky’s protocol
4. Fred Davis’s protocol
5. PamLab “medical foods” (Cerefolin-NAC, Metanx, Deplin)
6. “Simplified Treatment Approach”

All include forms of B12 (at dosages large compared to the RDA) and forms of folate.

(combined) Methylation and Folate cycles (showing link to transsulfuration pathway via CBS)



Components of the Simplified Treatment Approach Protocol (current version)

Daily (dosages can be adjusted):

- Hydroxocobalamin (sublingual drops, 2,000 mcg.)
- 5L-Methyltetrahydrofolate (sublingual drops, 210 mcg.)
- Folinic acid (oral, 200 mcg.)
- Yasko's General Vitamin Neurological Health Formula (multivitamin, multimineral) (oral, 2 tablets)
- Phosphatidylserine complex (oral, 500 mg.) or lecithin (oral, 1200 mg.)

Clinical Study of Simplified Yasko Protocol for CFS/fibromyalgia

- Type: **Open-label** clinical study
- Setting: The **private practice** of Dr. Neil Nathan in Springfield, Missouri, U.S.A.
- **Informed consent**: Patients signed forms after explanation of the study and its possible risks.
- Duration of treatment: **Six months** (However, note that after the 6-month study period, individualized treatments were added to the basic protocol for **an additional 3 months**.)
- Outcome measures: Objective testing using **methylation pathways panel** and **self-rating of symptoms**.
- **Restrictions on medications and additional supplements: None**, except that they and their dosages were not to be changed during the study without the knowledge and agreement of Dr. Nathan.



Neil Nathan

Richard Van
Kornenborg

Patients in the Clinical Study

- **Total number--30.** All had suffered from fatigue chronically.
- **Twenty-one met our strict criteria for ME/CFS** (Fukuda plus postexertional fatigue and malaise), and a statistical analysis was performed on their results from six months of treatment.
- Of these 21 patients, **18 also met the ACR criteria for fibromyalgia.**
- Sex: **All female**
- Ethnicity: **All Caucasian**
- Ages: **33 to 84** (mean—52) years
- Durations of illness: **1 to 20+ years**
- Histories of **previous treatment**: These patients had exhibited partial response to treatment ranging from **one to twelve years in duration** with a protocol that included evaluation and treatment of **adrenal, thyroid and sex hormones; food allergies; intestinal dysbiosis; heavy metal toxicity; infections (EBV, Lyme disease, mycoplasma); mold exposure; magnesium deficiency; and other nutritional imbalances.**

Patients in the Clinical Study (continued)

Additional diagnoses:

- Migraine headaches—15 patients
- Irritable bowel syndrome—13
- Chronic sinus infections—11
- Endometriosis—6
- Restless leg syndrome—5
- Mononucleosis (Epstein—Barr virus)—5
- Mold exposure and/or toxicity—5
- Multiple chemical sensitivity—4
- Lyme disease (previously treated)—2
- Interstitial cystitis—1
- Mycoplasma infections—1
- Chronic vulvitis—0

Supplement Protocol Used in Clinical Study

The treatment protocol used in this study was extracted from the full treatment program developed by Dr. Amy Yasko for the treatment of autism and adult neurological diseases. It was **an earlier version of the simplified treatment approach**. It consisted of five supplements:

- **Activated B12 Guard** (hydroxocobalamin): 1 sublingual lozenge (2,000 micrograms) daily
- **FolaPro** (5-methyl tetrahydrofolate): ¼ tablet (200mcg) daily
- **Intrinsi B12/folate**: ¼ tablet daily (Combination of [folic acid, 5-methyl tetrahydrofolate, and folinic acid] (200 mcg), cyanocobalamin (125 mcg), calcium (22.5 mg), phosphorus (17.25 mg), and intrinsic factor (5 mg))
[Note: Composition of this supplement has since been changed by Metagenics.]
- **General Vitamin Neurological Health Formula** (a multivitamin, multimineral supplement including antioxidants, trimethylglycine, nucleotides, supplements to support the sulfur metabolism, a high ratio of magnesium to calcium, and no iron or copper): starting with ¼ tablet and increasing the dosage as tolerated, to 2 tablets daily
- **Phosphatidyl Serine Complex** (phospholipids and fatty acids): 1 softgel capsule daily

Composition of General Vitamin Neurological Health Formula

- Serving Size: 6 Tablets (note that up to 2 tablets per day are used in the treatment)
- **Amount per serving:** Vitamin A (as palmitate)5000 IU,Vitamin C (ascorbic acid)500 mg,Vitamin D (as cholecalciferol)400 IU,Vitamin E (as d-alpha tocopheryl succinate)400 IU,Vitamin K (as phytonadione)40 mcg,Vitamin B-1 (as benfotiamine)25 mg,Vitamin B-2 (as riboflavin)12.5 mg,Niacin (as niacinamide)37.5 mg,Vitamin B-6 (as pyridoxal-5-phosphate)12.5 mg,Folic Acid100 mcg,Vitamin B-12 (cyanocobalamin B12)250 mcg,Biotin150 mcg,Pantothenic Acid (as d-calcium pantothenate)50 mg,Calcium (as calcium d-glucarate)25 mg,Magnesium (as citrate, oxide)100 mg,Zinc (as monomethionine)5 mg,Selenium (as L-selenomethionine)100 mcg,Manganese (as arginate)1 mg,Chromium (as polynicotinate)100 mcg,Molybdenum (as amino acid chelate)75 mcg,Potassium (as citrate)5 mg,Broccoli florets powder160 mg,Citrus bioflavonoids50 mg,Choline (as bitartrate)25 mg,Inositol25 mg,PABA (para-amino benzoic acid)5 mg,Garlic (*Allium sativum*) bulb powder200 mg,L-methionine150 mg,Milk thistle (*Silybum marianum*) seed extract100 mg,N-acetyl-cysteine75 mg,Pine (*Pinus maritimus*) bark extract25 mg,Taurine250 mg,Turmeric (*Curcuma longa*) root extract50 mg,Intrinsic Factor5 mg,Trimethylglycine (TMG)50 mg, Free Form Nucleotide Complex100 mg,Boron1 mg,L-Carnitine (Tartrate)100 mg.
- (Ref.: <http://www.holisticheal.com>)

Enumeration of Symptoms and Self-Rating of Outcome Measures

- The patients were asked to mark their symptoms on a checklist (initially and at 6 months) that included 38 symptoms.
- The patients were also asked to rate five outcome measures initially and at 3 and 6 months on visual analog scales ranging from 1 to 10. These measures consisted of energy, sleep, mental clarity, freedom from pain, and overall feeling of wellbeing.
- In addition, at 3 and 6 months they were asked to estimate their percentage of improvement.

Symptoms Checklist

- Confusion, disorientation
- Difficulty in word finding
- Impairment of concentration, difficulty assimilating new information
- Reduced task completion
- Hypersensitivity to bright light
- Night blindness
- Tearing, redness of eyes
- Blurred vision
- Chronic aching muscles
- Joint pain, morning joint stiffness
- Pain in weight bearing joints
- Nausea
- Loss of appetite
- Weight gain (How much, and over what period of time?)
- Abdominal pain
- Chronic sinus congestion
- Chronic cough that mimics asthma

Symptoms Checklist (continued)

- Shortness of breath
- Ice-pick like pain, or electrical pain that shoots into a muscle
- Nosebleeds
- Metallic taste or other unusual taste
- Vertigo, dizziness
- Ringing in the ears (tinnitus)
- Rage or inappropriate anger
- Panic attacks or anxiety
- Depression
- Tingling, “needles and pins” sensation
- Increased sensitivity to touch
- Difficulty with sleep
 - Difficulty with getting to sleep
 - Difficulty with staying asleep
- Mood swings
- Excessive thirst or frequent urination
- Impotence
- Irregular vaginal bleeding
- Low body temperature
- Chronic yeast infections
- Onset of menopause (if appropriate)