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METHIONINE AND METHYLATION: CHICKEN OR THE EGG

BY NANCY MULLAN, MD, AND AMY YASKO, PHD, AMD, FAAIM

METHYLATION AS A GLOBAL REGULATORY PROCESS

You may be familiar with some version of the pathway in Figure 1. It is an extremely simplified representation of the intersection of three important biochemical pathways, the methionine cycle (A), the transsulfuration pathway (B) and the folate cycle (C). Many critical physiologic and neurologic functions are effected or facilitated by these conjoined pathways. As a result, in spite of their complexity, their proper functioning is intimately related to optimal health and wellbeing. Imbalances in the products of these pathways have been related to stroke, Down syndrome, neural tube defects, cancer, and even aggression and cognitive performance.

One of the end results of this pathway system is the production of methyl groups. They are generated in the methionine cycle. There are many more substrates that are necessary for its functioning than are represented in Figure 1. The term substrate is a general term for any initial substance involved in a chemical reaction. Essentially, it is a starting material, a material that needs to be present for the reaction to start and/or keep going. In an organic system, these materials can be amino acids, carbohydrates, lipids, vitamins, minerals, enzymes, cofactors, etc., in other words, any element or molecule that is involved in the reaction.

Methyl group production in the methionine cycle is intimately linked to other portions of the system, namely to folate and sulfate metabolism. These pathways cannot be isolated from one another. They supply each other with substrates and work together like gears, so if one cycle isn't moving in a progressive direction, the other two may not be either. Neither are these pathways isolated from other biochemical processes in the body. Hence, the idea is for all three of these cycles to have the starting materials they need and enough of their various substrates to optimally produce their final end products,

including methyl groups.

Methyl group attachment to another molecule is called *methylation*. A methyl group is the structure depicted in Figure 2. It is not that different from a water molecule. You are familiar with the idea of H₂O, two hydrogens with a single oxygen. The methyl group is another small structure. It is three hydrogens and a carbon, like the carbon in coal or diamonds. In spite of its size, when a methyl group attaches to or is detached from another molecule, it can produce significant changes in the character or function of that molecule.

DNA methylation, as depicted in Figure 3, is crucial for *epigenetic modification* of the genome. This is a critical concept as your genetic makeup cannot change, but epigenetics can change. Epigenetics involves the way the environment impacts the molecules in your body, and the subsequent impact that those molecules have on your genes. Epigenetics is like a second chance that allows the body to make changes to its genes. Epigenetics is involved in regulating

many cellular processes including embryonic development, genome transcription, and chromosome stability. The role of epigenetics is so crucial that it can make the difference between the presence or absence of disease in otherwise genetically identical twins.

Epigenetic modifications produce changes in gene expression patterns that are lasting and inheritable, but that do not change the sequence of the genes themselves. A growing number of human diseases have been found to be associated with aberrant DNA methylation. For example, DNA methylation patterns are globally disrupted in cancer with genome-wide hypomethylation and gene-specific hypermethylation events occurring simultaneously in the same cell. Pro-oxidant conditions in the cellular environment also have significant epigenetic consequence.

Examples of epigenetic modification are the methylation reactions that function to edit and repair DNA. Methylation reactions may also perform a silencing function. At any time, 80% of

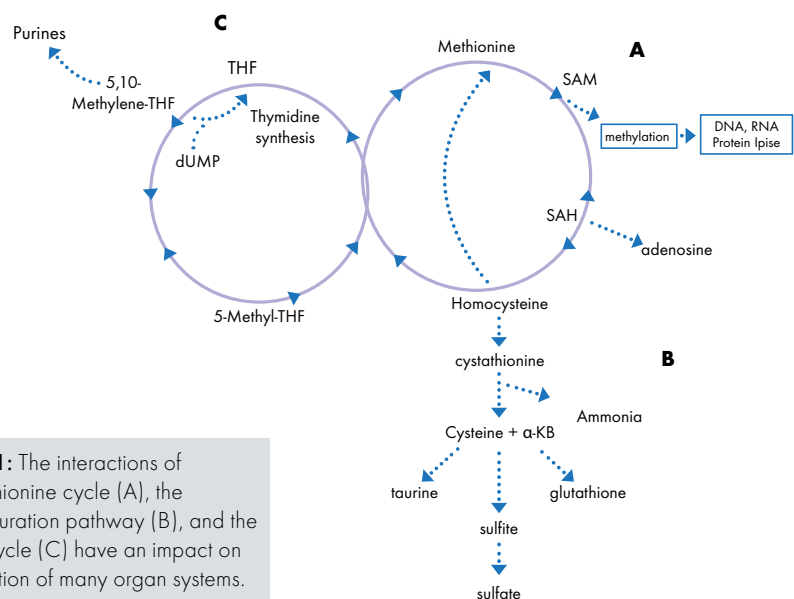


Figure 1: The interactions of the methionine cycle (A), the transsulfuration pathway (B), and the folate cycle (C) have an impact on the function of many organ systems.

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Methyl group – CH₃

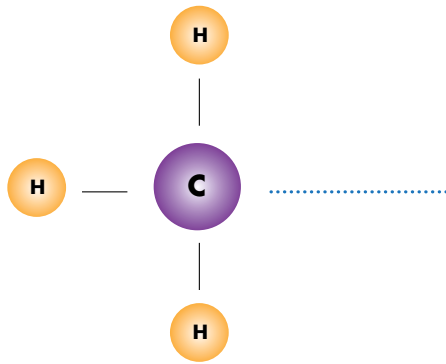


Figure 2: A representation of the one-carbon three-hydrogen methyl group CH₃.

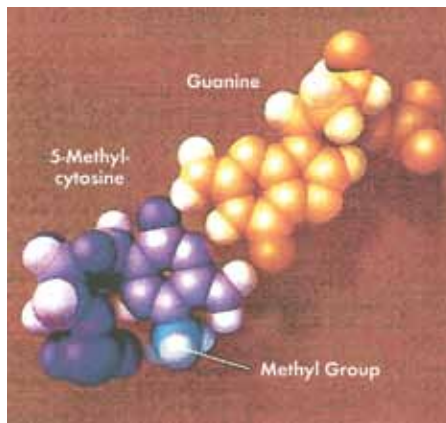


Figure 3: A methyl group bonding to cytosine, converting the molecule into 5-methyl-cytosine, may change the activity of the genome itself.

the DNA in your body should not be expressing. The attachment of methyl groups to certain molecules in the DNA inactivates it. Methylation also silences viral DNA, keeping the virus from replicating, expressing, and making you ill with syndromes that range from a cold or flu to cancer. Alternately, methylation can turn on genes that are normally inactive.

Aside from the importance of methyl groups in terms of epigenetics, they also play a direct role in a number of other functions in the body. The building blocks for DNA and RNA, purines and pyrimidines, are produced by balanced and progressive function of the methionine and folate cycles. Any reduced capacity for methylation and/or for purine and pyrimidine synthesis, reduces DNA and RNA production. This means that new cell synthesis may be impaired. For an organism to live it must create new cells as fast as cells die. This requires that the body make millions of cells every minute. Reduced capacity for

synthesis of DNA and RNA is a particular issue for cells such as bone marrow cells, lymphocytes, erythrocytes, and some brain cells, that may normally have difficulties meeting their needs for these substrates. Intestinal mucosal cells also cannot make all of the building blocks they need and must be supplied.

Stress and cell repair after injury also increases the need for DNA and RNA, as does immune system activation. Poorly regulated and ineffective immune cell responses may result from insufficient DNA and RNA production. Antibodies meant to deal with foreign antigens may cross react with your own cells. Alternately, your immune response may become hyperactive and react excessively to an exogenous antigen, like peanuts or shellfish. Your response to foreign bodies may also become exaggerated. Besides these factors, methylation deactivates histamine, an amino acid intimately involved with the allergic response. If methyl group production

is compromised and histamine cannot be deactivated, excessive allergic reactions result. Methylation also deactivates noradrenalin, a neurotransmitter associated with cortisol and the stress response.

When you are startled and alerted for danger, methylation activates adrenaline and increases the activity of your sympathetic nervous system. When the threat is over, methylation inactivates adrenaline and increases the activity of your parasympathetic nervous system producing relaxation. Because of its action on neurotransmitters, methylation impacts every important organ function and system including balance, movement, blood pressure regulation, pulse rate, respiratory rate, gastrointestinal and urinary tract function. Methylation is necessary for the proper myelination of nerves, recovery from anesthesia, and reducing blood levels of homocysteine, a molecule associated with cardiovascular disease. It is also necessary for metal detoxification, cell membrane function, and energy production.

The management of methylation has been an important focus in the alternative therapeutics of psychiatric disorders. Because of its action on dopamine, norepinephrine and serotonin, methylation impacts mood, memory, concentration and sleep. Methylation has also been carefully scrutinized by researchers and clinicians interested in autism and chronic fatigue syndrome.

HOW METHYL GROUPS ARE PRODUCED

The major source of methyl group production in the body is the methionine cycle (Figure 4, cycle A). Methionine is an amino acid that comes from protein in your diet. It cannot be synthesized in the body. It is the “first among equals” of essential amino acids, those that must be in the diet because they cannot be synthesized in the body. It has several important functions, and cells optimally will have an abundance of it. Any condition that impairs digestion and absorption of nutrients, including poor quality diet and disorders of digestion and absorption, may result in low levels of this critical amino acid. Methionine contains both a methyl and a sulfur group. The methyl group is important for methylation reactions and the sulfur for detoxification through the transsulfuration pathway (Figure 4, cycle B).

Methionine acquires another simple molecule, an adenosyl group, which allows it to become S-adenosylmethionine (SAM). SAM is the body’s main methyl group donor. SAM gives up its methyl group and becomes S-adenosylhomocysteine (SAH). Optimally, the cycle is going in its progressive direction, so SAH gives up its adenosyl group and becomes homocysteine. Homocysteine then can use one of two pathways to acquire a methyl group again and become methionine. Methionine transits these cycles

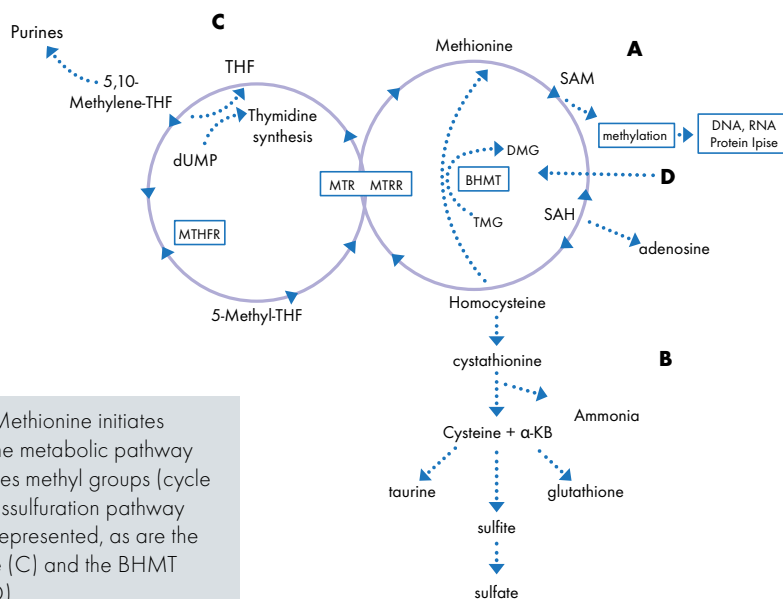


Figure 4: Methionine initiates activity in the metabolic pathway that produces methyl groups (cycle A). The transsulfuration pathway (B) is also represented, as are the folate cycle (C) and the BHMT pathway (D).

There are two opposing reactions, oxidation which involves the loss of electrons, and reduction which involves their gain. The balance of these two chemical processes is expressed in a ratio called the reduction/oxidation ratio, the redox ratio. Optimally, the redox ratio should be high. The option of gaining electrons should be available. If a molecule loses electrons, it needs to be able to regain them.

repeatedly to produce the optimal number of methyl groups for your body. This happens thousands of times a day in every functioning methionine cycle. There should be functioning methionine cycles in every cell in your body.

The folate cycle (Figure 4, cycle C) is the source for the methyl group that re-methylates homocysteine back into methionine. When the methionine cycle moves in a clockwise direction, the folate cycle can move counterclockwise. Then tetrahydrofolate (THF) becomes 5,10-methylenetetrahydrofolate (5,10-methylene-THF). Optimally, 5,10-methylene-THF is acted on by the enzyme methylenetetrahydrofolate reductase (MTHFR) and becomes 5-methyltetrahydrofolate (5-methyl-THF). It is 5-methyl-THF that passes its methyl group to hydroxycobalamin, vitamin B12. Hydroxy B12 then becomes methyl B12 which donates its methyl group to homocysteine. This reaction is catalyzed by the enzyme methionine synthase (MTR).

ENZYMES AND THEIR EXIGENCIES

Because the functions of methyl group production and methylation are central to organ systems' function, MTR has been extensively studied. This enzyme is deactivated by many factors. It is profoundly sensitive to mercury. Extremely low levels of mercury, nanogram levels, will completely inhibit MTR. How long MTR remains inactive depends on the amount of mercury present and how efficient your body is at excreting it. Lead, cadmium, arsenic and aluminum will do the same. Any condition that increases oxidative stress in the cell will inhibit MTR. If MTR becomes inactive, homocysteine may use a secondary pathway (Figure 4, pathway D) to go directly to methionine. Or it may transit through the transsulfuration pathway (Figure 4, pathway B), which then effects the production of an important antioxidant, glutathione.

When functioning optimally, the transsulfuration pathway generates glutathione (GSH). GSH is the body's main antioxidant and heavy metal detoxification agent. Thus, the inactivation of MTR by pro-oxidant conditions mediates increased activity in the production of a significant antioxidant defense molecule. However helpful oxidative inhibition of MTR may be for producing GSH, it reduces methyl group production and methylation reactions which, as noted earlier,

are critical. The function of the organism may be negatively impacted in many ways.

There are two opposing reactions, oxidation which involves the loss of electrons, and reduction which involves their gain. The balance of these two chemical processes is expressed in a ratio called the reduction/oxidation ratio, the redox ratio. Optimally, the redox ratio should be high. The option of gaining electrons should be available. If a molecule loses electrons, it needs to be able to regain them. The loss of electrons, oxidation, is burning. Fire is oxidation. Fire destroys its substrates. There must be reducing agents, antioxidants, in the cell to protect it from pro-oxidants. Pro-oxidants unopposed by antioxidants cause oxidative stress which threatens the cell, its function, and perhaps the organism. The cell and/or the organism may succumb and be destroyed.

There are a number of redox pairs which can indicate the amount of oxidative stress affecting the cell. The ratio of reduced glutathione (GSH) to oxidized glutathione (GSSH) is thought to reflect intracellular redox status. Extracellular redox status is measured by the ratio of cysteine to its oxidized form cystine. In addition to its function in the methionine cycle, methionine is an active scavenger of pro-oxidants. The ratio of methionine to methionine oxide is another gauge of intracellular redox status that also gives information about methyl group production capacity.

GENETIC INFLUENCES ON METHYL GROUP PRODUCTION

Besides the fact that oxidizing conditions inhibit MTR, there are other ways in which enzyme function in the methionine cycle can be disrupted and its ability to produce methyl groups impaired. Genes encode for the production of enzymes. Genes are made up of nucleotides, which code for amino acid bases arranged in a specific order. If the gene encodes a different amino acid sequence from the normal sequence, that may affect the function of the enzyme it produces. The enzyme may not be affected at all, it may have increased activity, it may have reduced activity, or it may be totally inhibited by the change. There are two copies of each kind of gene in the cell's genetic material, the genome.

The condition of having a different nucleotide

base from the one normally present at a particular location is called a SNP, a single nucleotide polymorphism. The SNP can be present on one or both of the genes. How much impact the SNP has on enzyme function depends upon where the SNP occurs in the strand of genetic material, and whether one or both genes have it. A SNP on the MTR gene(s) may occur at position A2756C, which affects its activity and which makes increased B12 supplementation important.

The methionine cycle is also impacted by SNPs in the genes encoding for MTHFR (Figure 4, cycle C). A SNP at position C677T will decrease the activity of the enzyme and reduce the amount of 5,10-methylene-THF that becomes 5-methyl-THF. 5-methyl-THF is critical for the remethylation of homocysteine, so this downregulating SNP is significant. Folinic acid, also called 5-formyltetrahydrofolate (5-formyl-THF), is the immediate precursor to 5,10-methylene-THF. Folinic acid is a form of folate found in many supplements. Giving folinic acid will not enhance methyl group production when this SNP is present. Supplementing 5-methyl-THF itself makes this substrate available to MTR. There are genetic profiles which contraindicate the supplementation of high levels of methyl group donating substrates. Optimal levels of methyl group supplementation based on a particular individual's SNP profile can help determine appropriate supplementation.

The MTHFR gene has another SNP location at A1298C that impacts folate cycle function (Figure 5). A SNP at this location makes the gene insensitive to regulation by SAM. Optimally, when the availability of SAM is sufficient or high, the A1298C location functions to increase MTHFR enzyme activity in the clockwise direction. This has the impact of inducing retrograde function in the methionine cycle reducing the amount of SAM produced. When the gene is insensitive to this regulatory input, clockwise function of the folate cycle is impaired.

One effect of this can be a reduction in the production of the neurotransmitters serotonin and dopamine. Tetrahydrobiopterin (BH4) is necessary for the synthesis of both dopamine and serotonin. The A1298C mutation is reported to reduce the amount of dihydrobiopterin (BH2) converting into tetrahydrobiopterin (BH4). The lack of BH4 in turn can impact neurotransmitter

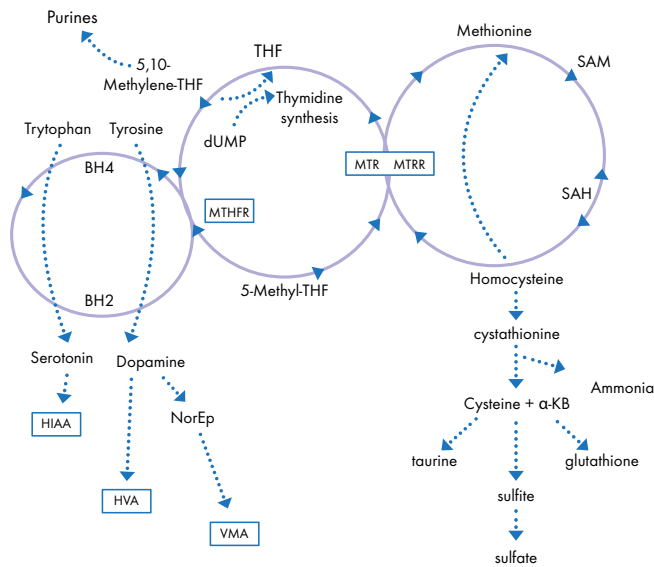


Figure 5: Sulfur is metabolized through transsulfuration, an important pathway for excretion of toxins.

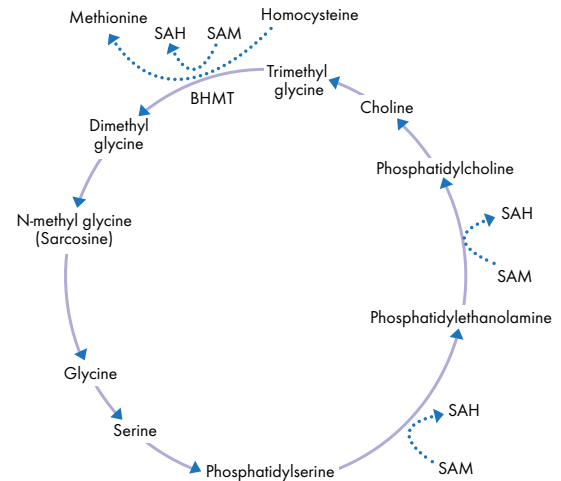


Figure 6: Homocysteine can be methylated by the enzyme betaine homocysteine methyl transferase in the presence of SAM and any one of the following: phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine or TMG.

levels. One solution is again to add 5-methyl-THF. The addition of this substrate may drive the clockwise portion of the reaction. In both of the above cases, 5-methyl-THF is added but for different reasons.

5-methyl-THF is one of several substrates which have a specific binding site on MTR as part of the remethylation process of homocysteine. Homocysteine binds to one, 5-methyl-THF to another and hydroxycobalamin to a third. The hydroxyl group is released from hydroxycobalamin, and the methyl group from 5-methyl-THF replaces it at the same location. The newly created methylcobalamin then donates the methyl group to homocysteine turning it back into methionine. There is an active site on the cobalamin molecule that first houses the hydroxyl radical. This site releases the hydroxyl radical, acquires a methyl group, and then releases the methyl group. This site can very rapidly become oxidized and needs to be reduced again before it can be used. This occurrence slows down the production of methyl groups. This is a problem particularly under pro-oxidant conditions. Therefore, antioxidant molecules are a particularly important component of the cell's environment.

It is possible for this important site on the cobalamin molecule to be reduced again, but this necessity slows down the reaction considerably. The enzyme methionine synthase reductase (MTRR) functions to keep cobalamin reduced, and it expedites methylation. There are at least six locations on the genes that encode for MTRR that impact enzyme activity. One seems related to increased methylation cycle function. Another produces a mild downregulation of

enzyme activity if only one gene is affected, but a significant downregulation if both of the two genes have SNPs. The other four locations produce profound downregulations if even only one gene is affected. The solution in all cases is increased B12 of the appropriate kind for the individual involved. Profound downregulations require surprising amounts of B12 of the appropriate type for the particular individual's genetics. There are clinical signs and biochemical testing that indicate when an optimal level of B12 supplementation has been reached.

A MORE EXPEDITIOUS WAY TO METHYLATE HOMOCYSTEINE

The clinical improvement that occurs when a patient who has not been methylating well starts to do so can be dramatic. Therefore, judicious support for methyl group production should start early. There are a number of factors that must be clarified and/or addressed before clinically effective inducement of the methionine and folate cycles' function can take place.

The second pathway by which homocysteine can be remethylated is represented in Figure 4, pathway D. The enzyme betaine homocysteine methyltransferase (BHMT) catalyses the transfer of a methyl group from trimethylglycine (TMG), which is also called betaine, to homocysteine. TMG becomes dimethylglycine (DMG), and homocysteine is converted to methionine. This pathway is less problematic to activate more quickly. A number of its substrates can be supplemented without much incident, and possible genetic interference is less. The use of phospholipids, phosphatidylserine, phosphatidylethanolamine

and phosphatidylcholine feeds directly into this portion of the pathway. In addition, phospholipids are key components of cell membranes. Figure 6 is an elaborated but still extremely simplified version of pathway D.

The gene that encodes for the BHMT enzyme has at least four locations that alter enzyme function. Three are downregulations, and one is an upregulation. The three downregulating SNPs are associated with increased gastrointestinal symptoms. Not pictured in Figure 6, but necessary to the process of these reactions, are B6, folic acid, B12, magnesium, manganese and riboflavin. SAM is important also. Many substrates in this pathway are critical for neurologic function and contribute to cell membrane integrity.

Before a clinician attempts to induce any particular metabolic pathway to function, appropriate broad spectrum nutritional supplementation should be in place. Appropriate supplementation implies neither too much nor too little of any substrate. Excessive amounts of a substrate unbalance pathway function as badly as too little. Clinicians may give unbalancing amounts of a substrate in an attempt to drive a reaction or remove a symptom. This trades long-term recovery for putative short-term gain. The treatment may end up without convincing direction. Supplementation needs to be reasonably precise and address the extremely intertwined and complex biochemical interactions of the whole organism. For didactic purposes, certain critical pathways are highlighted, but recovery is a whole body phenomenon. Biochemical testing optimally would show pathways that are in balance, and the patient's progress should reflect this circumstance.

CYSTATHIONINE BETA-SYNTASE: A CRITICAL ENZYME FOR METHIONINE CYCLE FUNCTION

The information that is essential before attempting induction of methionine and folate cycle function is the status of cystathionine beta-synthase (CBS) enzyme activity. CBS is the enzyme which catalyses the reaction which converts homocysteine into cystathionine (Figure 7). CBS enzyme activity can be increased by metabolic influences or by SNPs in genes that encode for CBS enzyme. There are several locations on the CBS gene where SNPs can increase enzyme activity. The stronger of these two upregulations can increase enzyme activity ten-fold.

Excess sulfur may increase CBS enzyme activity. This can result from improper supplementation, a diet containing too many sulfur foods, BHMT downregulating SNPs, other causes of BHMT pathway overactivity or sulfur-based chelation. Excess sulfur generates excess sulfur breakdown products like hydrogen sulfide, sulfite and other toxic molecules. It can also result in reduced glutathione production because of unbalanced and non-optimal function of the transsulfuration pathway. Sulfur is able to directly activate the stress/cortisol response that can lead to elevations in adrenaline and depletion of dopamine and norepinephrine. A constant state of fight or flight produces sympathetic versus parasympathetic overload and a wide range of secondary effects in the body, including changes in the magnesium/calcium ratio, decreased levels of serotonin and dopamine, effects on the methionine cycle via BHMT pathway substrate levels, changes in GABA/glutamate balance, as well as potentially depleting important glucose metabolizing enzymes and causing blood sugar fluctuations.

Overenthusiastic vitamin B6 supplementation can increase CBS enzyme activity, as will elevated glucose, excess cortisol or excess protein in the diet. CBS upregulation frees up nitrogen molecules that were complexed in protein in the methionine cycle, wasting them from the body and increasing the production of the neurotoxin ammonia. Immune system activation and/or bacterial infection increase the inflammatory cytokine TNF-alpha, which also increases CBS activity, as will any oxidizing condition or cause of inflammation.

Increased flux through the BHMT pathway is both caused by and results from increased CBS activity. Other causes of increased BHMT activity, and hence CBS overactivity, are stress, inappropriate supplementation of BHMT pathway substrates, BHMT genetic upregulations, impaired MTR activity or excess sulfur. Excess sulfur is also both caused by and results from BHMT enzyme overactivity. The methionine and folate cycles are readily depleted of their substrates by increased CBS activity. Normal genetic expression moves this conversion slowly and leaves enough homocysteine to convert back to methionine.

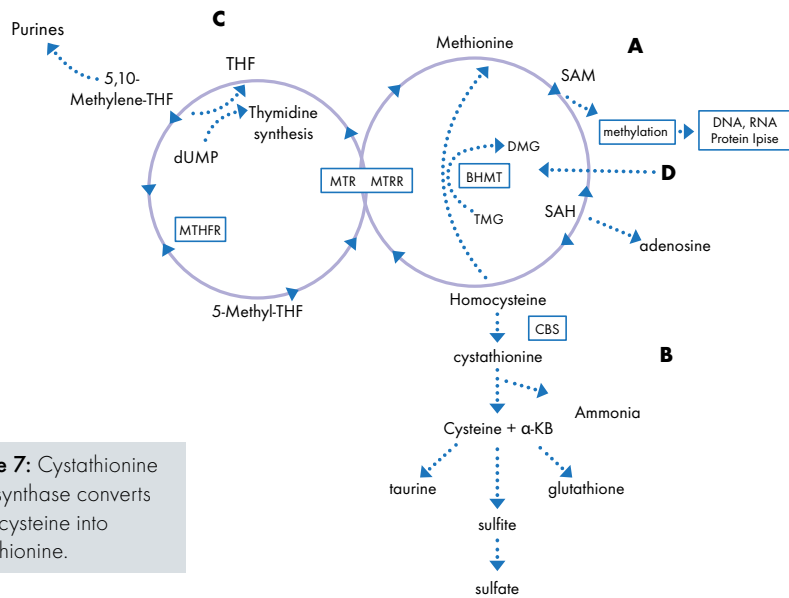


Figure 7: Cystathionine beta-synthase converts homocysteine into cystathionine.

It is critical that the methionine and folate cycles have adequate material with which to start and continue functioning. The restraint of CBS activity is necessary in order for the cycles to fill in. This also conserves sulfur and nitrogen stores in the body. SAM helps to stabilize and modify CBS activity, as do specifically formulated nutritional supplements. Accumulation of homocysteine favors its conversion to methionine, which helps maintain substrate levels in the cycles. The function of these cycles is possible only when adequate substrate levels are present.

SULFITE OXIDASE AND TRANS-SULFURATION PATHWAY FUNCTION

One of the products resulting from CBS activity is the amino acid cysteine. Recall above that the ratio of cysteine to cystine is used as a measure of the redox status of the fluid surrounding the cell. Intracellular hepatic cysteine occupies a pivotal position for determining the amount of both glutathione and taurine that are produced in the liver. Cysteine is acknowledged to be the rate-limiting substrate for GSH production. However, the level of cysteine in the liver cell appears to determine whether or not taurine or GSH is produced. Cysteine sits at a junction, a fork in the road, with regard to the production of these substrates (Figure 8).

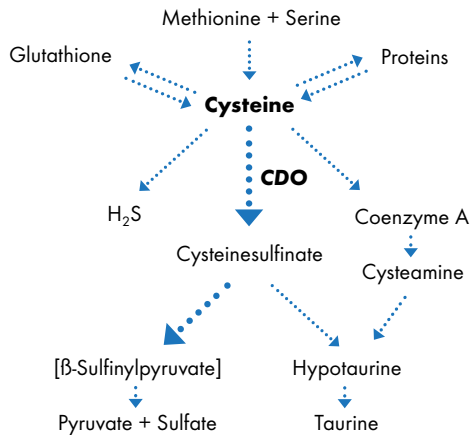
When cysteine levels in the liver are closer to low normal, the enzyme cysteine dioxygenase (CDO) is broken down and less taurine is produced. Conversely, when cysteine levels are in the higher range of normal, CDO is not degraded, which mediates increased taurine synthesis. The level of cysteine is in part determined by CBS which regulates the flow of substrates from the methionine cycle.

Stipanuk and colleagues (2006) describe at length the role of cysteine as follows:

"The mammalian liver tightly regulates its free cysteine pool, and intracellular cysteine in rat liver is maintained between 20 and 100 nmol/g even when sulfur amino acid intakes are deficient or excessive. By keeping cysteine levels within a narrow range and by regulating the synthesis of glutathione, which serves as a reservoir of cysteine, the liver addresses both the need to have adequate cysteine to support normal metabolism and the need to keep cysteine levels below the threshold of toxicity. ...Short-term regulation of GSH...occurs mainly via the availability of cysteine, the limiting substrate, and perhaps by feedback inhibition.... The rate of GSH synthesis is extremely sensitive to changes in the cellular cysteine level. Not only is GSH synthesis highly dependent on the cellular cysteine concentration, but normal GSH turnover plays a crucial role in maintenance of cellular cysteine levels....

Elevated tissue cysteine levels should be avoided [emphasis added] because they may lead to auto-oxidation of cysteine to form cystine and ROS, oxidation of protein thiol groups, neurotoxicity mediated by NMDA-type glutamate receptors or membrane cystine/glutamate exchanger activity, or excess production of H₂S via desulfhydration reactions.... Cysteine catabolism is tightly regulated via regulation of CDO levels in the liver, with the turnover of CDO being dramatically decreased when intracellular cysteine levels increase. This occurs in response to changes in the intracellular cysteine concentration via changes in the rate of CDO ubiquitination [the addition of ubiquinone or CoQ10] and, hence, degradation. ...Under conditions where intracellular cysteine levels were low, CDO was rapidly ubiquitinated and degraded....

Figure 8: This metabolic flow chart illustrates the position of CDO within the many pathways of cysteine metabolism. CDO catalyzes the first step in the major cysteine catabolic pathway and shunts cysteine toward the production of cysteinesulfonic acid, pyruvate, sulfate, hypotaurine and taurine.



In contrast, when intracellular cysteine levels were high, the ubiquitination of CDO was markedly attenuated, and the half-life of the protein was significantly prolonged.... The body uses...CDO as a means of disposing of excess cysteine obtained through the diet and in the process conveniently generates cysteinesulfinate, the biosynthetic precursor of the essential metabolites sulfate, hypotaurine, and taurine. These final end products of cysteine sulfoxidation, from a toxicity standpoint, are far more benign than cysteine."

Both GSH and taurine play important roles in the body and, as with the rest of this complex pathway, balance is key. In an effort to induce GSH production, supplementation of high levels of cysteine or cysteine containing compounds can result in less GSH because cysteine increases CDO levels. Overactivity of CBS may also result in higher levels of taurine and lower levels of GSH by virtue of the increase in cysteine levels subsequent to increased CBS activity. Taurine can then be degraded to form sulfites that are cumulative with the sulfite already produced via the transsulfuration pathway.

The effects of excess sulfite are familiar to many. Sulfites in wine and other preserved foods are known to cause severe asthma, headache, gastroesophageal reflux disease and a list of additional symptoms. The FDA has tight regulations regarding the amount of sulfites contained in foods and strict labeling requirements regarding them. Sulfites need to be converted into sulfates, less toxic molecules. This reaction uses the enzyme sulfite oxidase (SUOX) as seen in Figure 9. SUOX requires adequate B12 and molybdenum to function properly. Boron and manganese may become depleted through the activity of this enzyme. Their levels should be monitored to ensure optimal enzyme function.

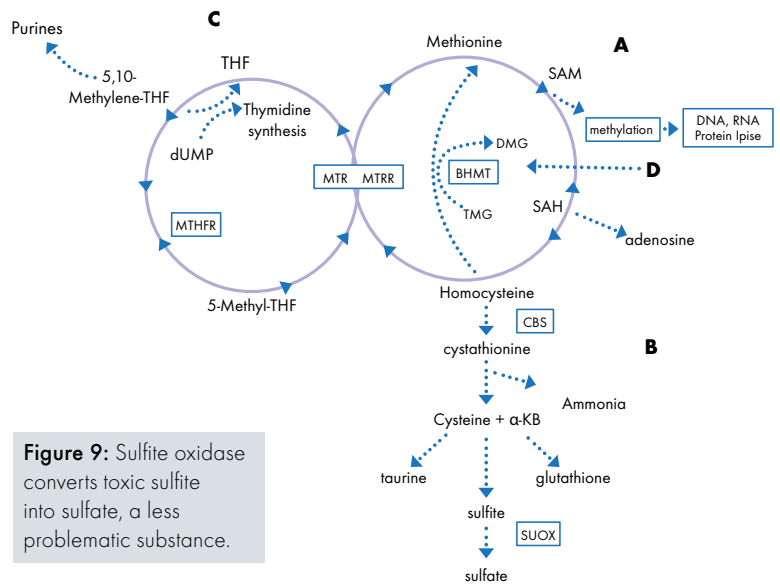


Figure 9: Sulfite oxidase converts toxic sulfite into sulfate, a less problematic substance.

There are at least three possibilities for SNPs in the genes that encode for SUOX enzyme production and hence function. One has no impact, another mediates increased enzyme activity, and a third significantly inhibits enzyme function. Those individuals with enzyme inhibition may be an extremely symptomatic population. They get significant symptoms from at least one toxin. They may have trouble with any perfume or scent. They may not be able to go into a dry cleaner or car wash without developing symptoms.

The problem is compounded when the patient has an upregulation at CBS. An upregulation at CBS and inhibited enzyme activity at SUOX is distinctly non-optimal. The pathway can produce multiple problematic sulfur compounds. The patient needs informed supplementation to avoid having symptoms get worse. Their diet may need modification because of the almost inevitable gastrointestinal problems that accompany this genetic profile. CBS enzyme activity needs to be restrained by removing whatever provoking conditions might be present and using the supplements developed for this purpose. Inducing more efficient sulfite to sulfate conversion at SUOX is possible. But while B12 is needed to do this, its administration—even in miniscule amounts—may induce extreme symptoms. Sufficient nutritional groundwork needs to be in place, and the gastrointestinal problems effectively treated, before B12 supplementation can be increased and the activity of SUOX optimized.

BALANCE IS THE OPERATIVE WORD

Overall, the conjoined action of these biochemical pathways can be envisioned as an intersecting series of gears. Each starting material and each product interact in a manner to drive these gears so that they move smoothly for optimal function of the pathway and balance in the body. Excessive amounts of any of the substrates or end

products can produce disease, oxidative stress, neurotransmitter imbalance and suboptimal levels of substrate for other critical reactions in the body. The key word for this complex process is balance.

Perhaps the centrality of this pathway grouping can be inferred from its role in DNA, RNA and methionine production. DNA is recognized as the material of life itself, the blueprint that constructs and runs your body and defines each of us as a unique entity. DNA is the first information to begin new life, the essence of each of us contained in the egg and sperm of our parents. This pathway grouping produces substrates for new DNA and RNA as well as methionine.

The blueprint determined by DNA is transferred to the RNA which then constructs the new protein. The "start codon," the three nucleotide sequence that begins every new DNA and RNA molecule, is the sequence for methionine. When the RNA starts to construct the new protein, it also begins with the three-nucleotide sequence for methionine. Even if the methionine is removed later, as the protein is being made, it starts with a methionine. (See *The Textbook of Biochemistry with Clinical Correlations* noted in the bibliography for further elaboration of this concept.)

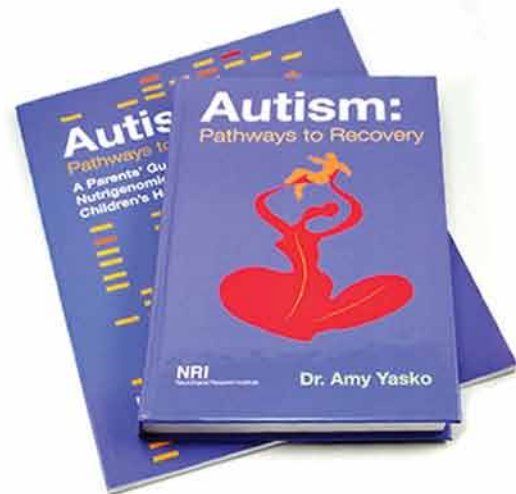
Methionine acts like the capital letter at the start of every sentence. Without this initial capital letter, the sentence cannot be written properly, and without this first amino acid, the protein cannot be made. Methionine is needed to start the production of every protein in your body. In addition, the methionine and folate cycles need to function properly to make the methionine and the nucleotide bases that allow all of this to happen. Clearly this is a critical pathway coupling. It is no surprise that it is so tightly regulated and intertwined. This is another "How high is the sky?" moment. Methionine/methylation function in the body may be the ultimate case of which came first, the chicken or the egg.

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