

# The Detoxification System

## Part III: Sulfoxidation and Sulfation

by  
Mark J Donohue

In two earlier reports – “**Sulfur and Sulfur Compounds in the Environment**” and “**Sulfur and Sulfur Compounds in the Human Body**” – it was reported that sulfur and sulfur compounds are ubiquitous. And regardless of the source, sulfur and sulfur compounds easily gain entry into the human body through the lungs, skin and intestines. Once in the body sulfur and sulfur compounds need to be metabolized and biotransformed for proper utilization by the body.

The metabolism and biotransformation of sulfur and sulfur compounds takes place as a two stage process – (1) **sulfoxidation** and (2) **sulfation**. In some individuals these processes are impaired, resulting in a wide range of physiological symptoms and diseases.

### Sulfoxidation

Sulfoxidation is the process by which sulfur-containing compounds are metabolized into **inorganic sulfite**. The inorganic sulfite is then used for the Phase II biotransformation sulfation pathway. As mentioned the sources of sulfur for the sulfoxidation process are many, however, the most common source comes from the sulfur amino acids (SAAs) - **cysteine** and **methionine** found in food proteins. The sulfoxidation process involves several steps, whereby cysteine is first converted to **cysteinesulfinic acid**, then cysteinesulfinic acid is converted to **sulfite** and finally, sulfite is converted to sulfate.

Problems can arise in individuals with a poorly functioning sulfoxidation system. This results in an increased ratio of either cysteine to sulfate or sulfite to sulfate in their serum and urine. In such situations cysteine and/or sulfite levels can build-up and become **cytotoxic** (toxic to cells), especially to the nervous system, becoming **neuro-toxic** and/or **excite-toxic**. Researchers have identified many poor sulfoxidizers with diminished sulfation and reported that these individuals are most susceptible to **multiple chemical sensitivities** and problems of the nervous system.

### Cysteine (C<sub>3</sub>H<sub>7</sub>NO<sub>2</sub>S)

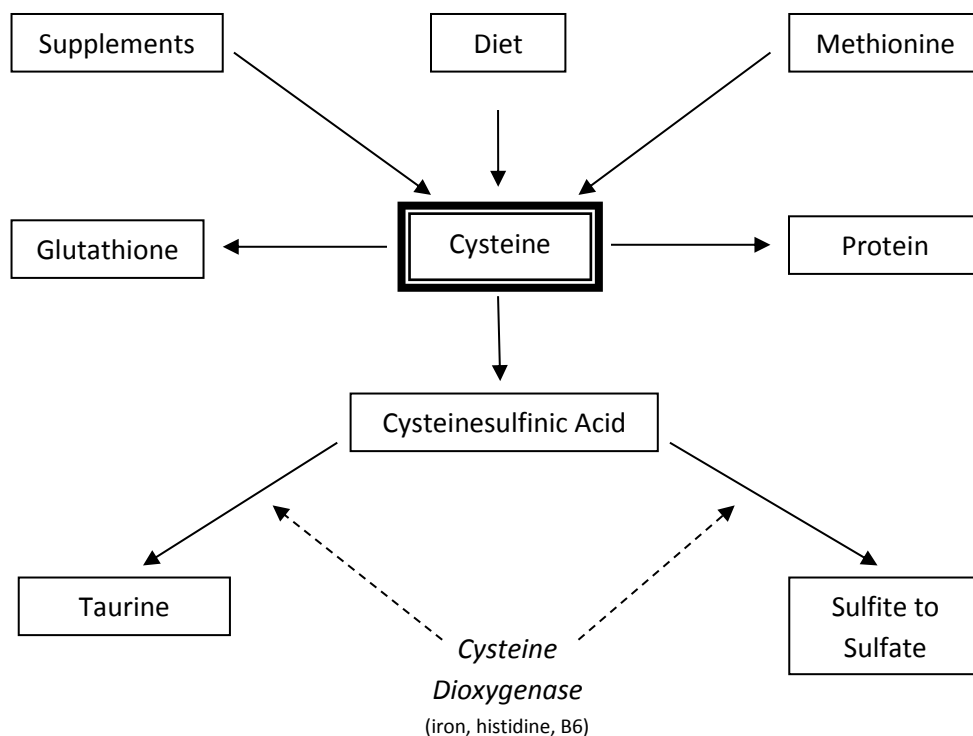
Cysteine is a non-essential thiol containing amino acid imperative to the growth and function of all living organisms. Cysteine is obtained from the diet through the breakdown of protein foods and/or is biosynthesized from the essential amino acid - methionine. Methionine and cysteine are metabolically linked via the unidirectional **transsulfuration pathway** that allows methionine to be converted to cysteine.

These sulfur amino acids enter the plasma and circulate as free amino acids until they are removed by tissues. Although cysteine is metabolized to some extent by many tissues, the liver clearly plays the dominant role in cysteine metabolism. Cysteine participates in a wide variety of reactions and pathways in the human body such as:

- ❖ Cysteine is involved in the biosynthesis of proteins.

- ❖ Cysteine is involved in the biosynthesis of **Coenzyme A** via the enzyme - **pantothenate kinase**.
- ❖ Cysteine is the rate-limiting precursor to the production of **glutathione** via the enzyme - **Glutamate-cysteine ligase (GCL)**, also known as -**glutamylcysteine synthetase**
- ❖ Cysteine is the rate-limiting precursor to the production of **taurine**.
- ❖ Cysteine's sulfhydryl group has a high affinity for heavy metals and will tightly bind with metals such as mercury, lead and cadmium - forestalling the entry of heavy metal ions into the brain (across the blood-brain barrier).
- ❖ Cysteine counteracts the poisonous effects of **acetaldehyde** by supporting the next step in metabolism, which turns acetaldehyde into the relatively harmless acetic acid.
- ❖ Cysteine also has the ability to breakdown proteins found in mucous that settles in the lungs. As a result it may be useful in the treatment of bronchitis and other respiratory problems.
- ❖ Cysteine is found in large amounts in **keratin** - the protein that makes up fingernails, skin and hair. Human hair is approximately 14% cysteine.
- ❖ Cysteine excites neurons.

However, one of cysteine's most important functions is in its role as the major precursor to **inorganic sulfate** via the sulfoxidation pathway. Inorganic sulfate is the rate limiting factor for the biotransformation of **xenobiotics** and the biosynthesis of many macromolecules, via the sulfation pathway. However, before cysteine can be converted to inorganic sulfate it must first be converted to cysteinesulfinic acid and sulfite. This conversion is catalyzed by the enzyme – **cysteine dioxygenase**.



## Cysteine Dioxygenase (CDO)

Cysteine dioxygenase tightly regulates the catabolism of cysteine levels so as to be sufficiently high enough to meet the needs of protein synthesis and the production of other essential molecules - glutathione, coenzyme A, taurine, and sulfite/sulfate. While at the same time making sure cysteine concentrations remain below the threshold of cytotoxicity.

Cysteine dioxygenase is an **iron-histidine- B6 enzyme** which is found at high levels in the liver, where it is uniformly distributed in hepatocytes. Cysteine dioxygenase is expressed in lower modest levels in the kidney, brain, lungs and thyroid. Cysteine dioxygenase catalyzes the addition of molecular oxygen (oxidizes) to the sulfhydryl group of cysteine, yielding cysteinesulfinic acid. Cysteinesulfinic acid lies at a branch-point in cysteine catabolism, where it can follow one of two pathways resulting in the formation of either taurine or sulfite. Thus, oxidation of cysteine via cysteine dioxygenase leads to both taurine and sulfite formation.

Cysteine dioxygenase activity shows diurnal (daytime) variation, being decreased at night. Hence, fasting measurements of plasma cysteine and inorganic sulfate in the early morning show higher levels of cysteine and lower levels of sulfate, than later in the day, where dietary amino acids will influence the result. In addition, up-regulation of hepatic cysteine dioxygenase concentration occurs in animals fed diets with increased levels of dietary protein or sulfur amino acids, and cysteine dioxygenase reaches a new steady state within a few hours of a change in diet.

Most interesting is the fact that CDO is known to be **polymorphic** (with different forms) in human populations and researchers have found that there are sub-sets with approximately 30% of the population having a lower CDO activity.

## Cysteine Toxicity/Excitotoxicity

When cysteine dioxygenase levels are reduced or functioning at a suboptimal level, cysteine oxidation, therefore, will also be reduced. This will in turn cause normal amounts of cysteine to become elevated and potentially becoming dangerous to organs in the body, especially the brain. Excess free cysteine is recognized as a potent excitotoxin which acts on the **NMDA glutamate receptors**, hence altering neuronal transmission and finally leading to cell death. Cysteine is comparable in its potency to other excitatory amino acids such as **glutamate** and **aspartate**. Cysteine toxicity/excitotoxicity due to poor or reduced activity of cysteine dioxygenase has been implicated in several degenerative diseases.

- Rheumatoid arthritis
- Systemic Lupus
- Alzheimer's disease
- Parkinson's disease
- Motor neuron disease
- Environmental illness (MCS)

**Box:** Diseases implicated in Cysteine Toxicity

In such disorders researchers have reported depressed levels of sulfate in plasma, elevated fasting plasma cysteine concentrations, elevated cysteine to sulfate ratios and lower sulfation, all of which are consistent with impaired cysteine oxidation. The etiology of these diseases could be linked to functional impairment of cysteine dioxygenase, leading to elevated levels of cysteine as well as deficiencies in taurine and sulfate.

Although cysteine may not readily cross the blood-brain barrier, any damage to this area, whether by toxins, viruses or trauma would make the central nervous system more vulnerable. It has also been suggested that the lipophilic nature of cysteine allows it to penetrate the brain easily where it is oxidized to cysteinesulfinic acid which is also an excitatory amino acid and the actual toxic agent. Damage is typically restricted to **circumventricular regions** of the brain (hypothalamus, pineal gland, posterior pituitary) which lack blood-brain barrier protection.

Indications of cysteine toxicity are gas, bloating, fatigue and mental dullness in individuals after ingestion of sulfur containing foods. Individuals with these types of reactions, along with a high serum cysteine to sulfate ratio, may be identified as “*sulfur reactors*”.

### **The Culprits:**

Some possible theories as to why cysteine dioxygenase can function at a sub-optimal level are:

1. **Inflammation** - researchers have indicated that the combination of high cysteine and low sulfate levels may reflect the presence of inflammatory *cytokines* (immune signaling molecules), specifically - **tumor necrosis factor-  $\alpha$  (TNF-  $\alpha$ )** and **transforming growth factor-b (TGF-b)**. Both TNF-  $\alpha$  and TGF-b have been shown to inhibit the expression of cysteine dioxygenase in neuronal and hepatic human cell lines. Cytokine release may therefore modulate sulfate production and hence regulate formation of sulfated bio-components.

The finding that cytokines can affect sulfate production is of importance in furthering the understanding of chronic inflammatory disease processes. It has been shown that patients with rheumatoid arthritis (RA), where TNF-  $\alpha$  levels are usually high, have low plasma sulfate and reduced sulfation of **synovial fluid** (joint fluid) and synovial proteins.

The primary role of TNF-  $\alpha$  is in the regulation of immune cells. TNF is able to induce apoptotic cell death, to induce inflammation, and to inhibit tumor production and viral replication. TGF-b controls proliferation and cellular differentiation and plays a role in immunity, cancer, heart disease and diabetes.

2. **Measles vaccination** - has been found to increase the levels of cytokines - **interferon, interleukins** and TNF-  $\alpha$ . **Omega 3 fatty acids** can be used to reduce the level of cytokines, such as interleukin-1 (IL-1), IL-2, IL-6 and TNF-  $\alpha$ . This is a function of **prostaglandins** (lipid compound), formed from the omega 3 fatty **eicosapentaenoic acid (EPA)**. EPA is obtained from oily fish, and the body can also make it from **linolenic acid** in flaxseed oil, and a few other oils. Fish oil has been found to be helpful in treating rheumatoid arthritis possibly because this can reduce cytokine levels and so increase sulfate supply.
3. **Allergies** – individuals with allergies may also have high TNF-  $\alpha$  levels.
4. **DNA mutations** – in the coding for cysteine dioxygenase. Research indicates individuals with cysteine toxicity/excitotoxicity symptomatology, had a family history of chronic illness, suggesting that this could be a susceptibility factor.

Most interesting is the fact that CDO is known to be polymorphic, with different forms, in human populations. Researchers have found that there are sub-sets with approximately 30% of the population having a lower CDO activity.

5. **Adrenal dysfunction** - corticosteroids up-regulate cysteine dioxygenase. Corticosteroids are a class of steroid hormones that are produced in the adrenal cortex. Corticosteroids include both the glucocorticoids – cortisol, and mineralocorticoids – aldosterone.

Therefore, adrenal dysfunction would not only result in low levels of corticosteroids, but it would also result in the down regulation of cysteine dioxygenase.

6. **Low pantothenate (B-5) levels** - pantothenate is required to produce cortisol which up-regulates CDO. An inadequate level of pantothenate may cause poor conversion of cysteine to sulfate.

To further avoid cysteine toxicity in those with a poorly functioning cysteine dioxygenase, dietary changes are suggested to minimize the intake of foods containing the sulfur amino acids - methionine and cysteine. It is also suggested to avoid sulfur containing supplements such as N-acetyl-cysteine, lipoic acid, glutathione, etc. .

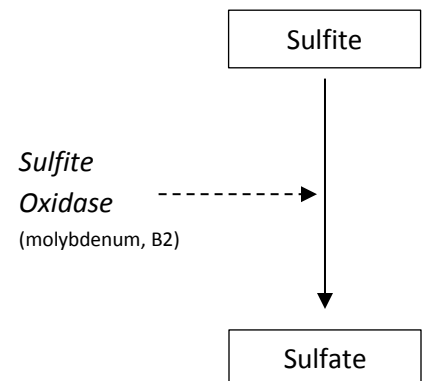
Cysteine and Methionine Containing Foods	
<b>High Levels:</b> ( > 100 mg/100g )	beef, pork, chicken, turkey, duck, fish, eggs, cheese, soy products nuts/seeds (including peanuts), wheat products
<b>Moderate Levels:</b> ( >20mg/100g )	red peppers, garlic, onions, broccoli, brussel sprouts, asparagus, corn cauliflower, avocado, peas, spinach, potatoes, oats, beans, rice, milk and yogurt

### Sulfite ( $\text{SO}_3^{2-}$ )

Sulfite, produced by the body as a cysteine metabolite, is also a powerful neurotoxin. Considerable quantities of sulfite are generated in the body by normal catabolic processing of sulfur-containing amino acids. The function of sulfite in the body is unknown other than as a toxic metabolite. Because of sulfite's potential damaging effect to cells it must be converted (oxidized) into the safer compound – sulfate ( $\text{SO}_4^{2-}$ ). This conversion takes place via the mitochondrial enzyme – **sulfite oxidase**.

### Sulfite Oxidase

Sulfite oxidase's sole function is to convert the cysteine metabolite - sulfite into the safer compound - inorganic sulfate. Sulfite oxidase is found in high concentrations in the liver and lungs and requires the co-factors - molybdenum and vitamin B2.



### Sulfite Toxicity and Sensitivity

Individuals with a deficiency of and/or a poorly functioning sulfite oxidase will have a build-up of sulfites in their body – **sulfite toxicity**. Individuals with such a build-up are usually sensitive to sulfites and are referred to as having – **sulfite sensitivities**.

Sulfites can react with a variety of cellular components - proteins, lipids, DNA, etc. Neuronal cells are highly susceptible to high levels of sulfites. Researchers have found sulfite accumulation is more likely to occur in neuronal cells compared with hepatic and renal cells whose high sulfite oxidase activity would facilitate its oxidation to sulfate more efficiently. This could possibly contribute to the more pronounced decrease in ATP production within neuronal cells, thereby potentially having neuro-toxic effects on brain tissue and causing neurological disorders. Sulfites can also inhibit 90% of lung ATP energy production, can impair liver cell ATP energy production, inhibit glutamate metabolism and can deplete glutathione.

Symptoms of sulfite toxicity/sensitivity occur after inhalation, ingestion, injection or dermal contact with sulfites. The symptomatology ranges in severity from mild discomfort to life-threatening episodes and death. There is currently no reliable blood or skin allergy test for sulfite toxicity/sensitivity. However, sulfite levels can be checked by dipping sulfite test strips in the urine. Normal test results should measure – zero sulfites in the urine; there should be no sulfites in the urine. Exogenous or endogenous sulfur and sulfur compounds are eliminated in the urine as the safer compound – sulfate. The measurement of serum levels or urinary sulfite is diagnostic.

### **The Culprits:**

Some possible theories as to why sulfite oxidase can function at a sub-optimal level are:

1. **DNA mutations** - though mutations to the genes that code for sulfite oxidase do occur, they however, are quite rare and are usually fatal.
2. **Molybdenum deficiency** - molybdenum is an essential trace element occurring as a cofactor in three important enzymatic reactions - **xanthine oxidase/dehydrogenase, aldehyde oxidase** and **sulfite oxidase**. Despite the body's need for molybdenum, the published minimum daily requirement for molybdenum is just 75 micrograms.

Researchers found that many patients presenting with sulfite toxicity/sensitivity symptoms had virtually no detectable blood molybdenum. Normal values are from 10-100 ppb. Studies also indicate that molybdenum may be a rate-limiting nutrient for the conversion of sulfite to sulfate and supplementation may be indicated.

In humans, supplemental molybdenum is well absorbed via oral and intravenous routes of administration. Molybdenum research in humans indicates absorption rates range from 70-90 percent. Once absorbed, molybdenum peaks in the plasma within 40-60 minutes, is rapidly cleared, and within three hours is excreted in the urine as molybdate. A portion of the absorbed molybdenum is deposited in "slow-turnover" tissue such as the liver, muscle, and bone, while another portion is deposited in "fast-turnover" tissue such as the adrenal

### **Symptoms of Sulfite Toxicity**

- Asthma
- Wheezing
- Shortness of breath
- Chest tightness
- Racing heart
- Hypotension
- Fatigue
- Nausea
- Stomach Cramps
- Diarrhea
- Coughing
- Itching
- Reddening of skin – hives
- Tingling sensation
- Flushing
- Headaches
- Nasal congestion
- Runny nose
- Swelling of eyes, hands and feet
- Seizures
- Anaphylactic shock

glands and gastrointestinal tract. The half-life for molybdenum in slow-turnover tissue has been reported to be 42-74 days, compared to 1.7-2.5 days for fast-turnover tissue.

Molybdenum supplements are generally available in one of several forms: sodium molybdate, ammonium molybdate, molybdenum picolinate, molybdenum citrate, and molybdenum aspartate. Although all forms of supplemental molybdenum appear to be well absorbed, research using oral dosages is limited. Therefore, an exact dosage for conditions such as sulfite toxicity/sensitivity has not been established. Supplementation with oral molybdenum covers a wide range of dosages from 50 mcg-6.0 mg daily in divided doses.

#### Case Report #1

A 53-year-old female asthmatic requiring numerous asthma medications to control her symptoms was found to have a urinary sulfite of 10-20 ppm (reference range = 0 ppm). Over a three-month period, the patient was treated with 250 mcg IV molybdenum twice weekly, gradually working up to 750 mcg for the last four injections. Urinary sulfite levels were reduced to 2-6 ppm, wheezing decreased significantly, and the patient was able to discontinue theophylline and prednisone; dosages of two inhaler medications were reduced by 50 percent.

#### Case Report #2

A 68-year-old man with a 30-year history of asthma was treated with IV molybdenum for 90 days. His urinary sulfite value decreased from 30-40 ppm to 3-7 ppm, while organic urinary sulfate increased from 14 percent to 30 percent, indicating improved sulfite metabolism

Along with molybdenum supplementation, the incorporation of molybdenum foods is also recommended. Because the molybdenum content of plants depends on the soil molybdenum content and other environmental conditions, the molybdenum content of foods can vary considerably. Some of the more common foods which contain molybdenum are:

Richest source - legumes (beans, lentils and peas), and dark leafy vegetables

Good source - grain products, and nuts

Low source - meat, eggs, milk

3. **Xanthine oxidase and aldehyde dehydrogenase** – are Phase I detoxification enzymes which also require molybdenum as a co-factor. Therefore, if the diet is high in their particular substrates - purines, caffeine or alcohol – this can lead to depletion of molybdenum.
4. **Elevated levels of boron** – boron binds to the sulfite oxidase co-factor - vitamin B2, causing it to be excreted and therefore no longer available for detoxification. Foods high in boron are tomatoes, peppers, apples, pears, peaches, plums, grapes, soya, parsnips, rosehips, hazelnuts, peanuts and almonds.

5. **Heavy metals** - heavy metals such as lead, mercury and tungsten interfere with molybdenum.
6. **Vitamin B-12 deficiency**
7. **Porphyria** – (needs further research)
8. **Excessive sulfite ingestion and inhalation** – if an individual has sulfite toxicity/sensitivities it is imperative that they lower the sulfite load on the body by avoiding excessive exposure to such compounds. Unfortunately, the act of avoiding exogenous sources of sulfites is easier said than done. Sulfites are ubiquitous in our environment. Sulfites can be found as naturally occurring compounds that nature uses to prevent microbial growth. They are found naturally on grapes, onions, garlic, and on many other plants. Sulfites are also found naturally occurring in some foods undergoing fermentation (i.e. beer and wine).

However, the main source of sulfites in the human body comes from the addition of sulfites and sulfur dioxide to many food products. Sulfites have antioxidant and preservative properties and are used as food additives by the food industry. For example sulfite and sulfur dioxide are used:

- In 'fresh' food products they are used to prevent discoloration and oxidation (browning) of the food.
- On grapes they are used as a fungicide.
- In wines they are used to kill certain unwanted bacteria and assist in the aging process.
- On shrimp and lobster they used to prevent black spots.
- Most pickled and vinegar-containing foods contain sulfites.
- In packaged (cans, bottles, boxes) processed food they are used as a preservative (e.g. - dried fruits, dried potato products, jams, canned vegetables, soup mixes, condiments, etc. etc. etc..)
- They are used as a bleaching agent for food starches.
- They are used as a strengthener in dough conditioners

Making the situation more difficult is that sulfites are also hidden in hundreds of ingredients like corn syrup and gelatin which find their way into thousands of foods. In addition, sulfites are used in pharmaceuticals to maintain the stability and potency of some medications. Sulfites are added as preservatives to a variety of injectable and inhalable drug preparations, infusion solutions (IVs), and dialysis solutions.

#### **Names of Sulfites used in Foods and Medicines which are Approved by the FDA for use as Additives**

Sulfite	Sulfur Dioxide	Sodium Sulfite
Sodium Bisulfite	Sodium Metabisulfite	Calcium Bisulfite
Calcium Metabisulfite	Potassium Bisulfite	Potassium Metabisulfite

Sulfite treatment levels in foods vary widely by application. Residual levels do not usually exceed several hundred parts per million (ppm) but may approach 1,000ppm in certain fruit and vegetable products. If a food contains more than 10ppm of sulfite, the FDA requires that the product label list the amount of sulfite. 10ppm is the same as 10mg (milligrams) per liter, or about 2mg per cup of a product liquid.



Though the primary route of exogenous sulfites is via the digestive system, the inhalation of sulfites, in the form of sulfur dioxide, is also of great concern. The inhalation of sulfur dioxide contaminated air is also difficult to avoid - levels are particularly high in polluted urban air and in the vicinity of coal or oil-burning plants. Patients with sulfite toxicity/sensitivity and asthma are particularly susceptible to insult in this environment eliciting a medical response called - ***sulfite-induced hypersensitivity response***.

Researchers have reported that the activity of sulfite oxidase in the normal human lung is 135 times less than that of the human liver. They concluded that the normal human lung has a diminished capacity to detoxify inhaled sulfites/sulfur dioxide.

#### **Environmental Sources of Sulfur Dioxide that can be Inhaled**

Geothermal activity – hot springs and volcanic gases  
Natural decay of vegetation on land, in wetlands and oceans  
Forest fires  
Power plants – burning of coal accounts for over 60% of human sulfur dioxide emissions  
Commercial-Institutional heating plants  
Industrial boilers  
Petroleum refining  
Metal smelters - smelting of sulfide ores  
Pulp mills - production of paper products  
Cement factories – heating of calcium sulfate  
Textile plants - bleaching  
Food processing plants - production of food preservatives  
Waste incineration plants  
Homes – burning fuel oil to heat homes  
Vehicle Exhaust (gasoline, diesel)

### **Sulfation**

Sulfation, also known as ***sulfate conjugation***, is the biochemical process whereby inorganic sulfate is conjugated with a specific substrate. This process is catalyzed by the super-family of enzymes called – ***sulfotransferases (SULTs)***. Depending on the specific substrate and the specific sulfotransferase enzyme involved in the process, sulfation will serve one of two primary functions:

1. **Phase II biotransformation/detoxification** – sulfation is important for biotransforming/detoxifying several drugs, food additives, and xenobiotics. In addition to environmental toxins, sulfation is also used to detoxify some normal body chemicals and is the main pathway for the elimination of steroid hormones, thyroid hormones, neurotransmitters and in the elimination of toxins from intestinal bacteria.
2. **Biosynthesis of macromolecules** – sulfation is important in the biosynthesis of a variety of macromolecules such as proteins, peptides, glycosaminoglycans (GAGs), and intestinal mucins.

Regardless of which functional process sulfation is involved in, sulfation will not take place without the rate limiting compound – **sulfate**.

### **Sulfate** ( $\text{SO}_4^{2-}$ )

Sulfate is essential for many biological processes in the human body. Sulfate is a heavily oxidized sulfur compound used in the sulfation pathways which can originate from one of several sources:

- ❖ **Sulfoxidation pathway** – this is the main source of sulfate in the human body. Approximately 64% of the body's total sulfate pool originates from the sulfoxidation of both exogenous and endogenous sulfur compounds into inorganic sulfate. This is because the dietary absorption of sulfate, from food sources, across the gastrointestinal tract is relatively inefficient.
- ❖ **Inorganic sulfate** – though the absorption of sulfate in the intestinal tract is inefficient, it still accounts for one-third of the sulfate pool in the human body. Approximately 19% of the body's total sulfate pool comes from the direct ingestion of inorganic sulfate from foods. Inorganic sulfate is found in many foods and water supplies and is absorbed from the stomach, small intestine and colon via a sodium-ion dependent transport system. Better absorption rates occur with soluble sulfate salts (e.g., potassium sulfate or sodium sulfate), as opposed to insoluble sulfate salts, such as barium sulfate, where no absorption occurs.

Much of the sulfate found in foods is added during processing. For example, ferrous, calcium, and ammonium sulfate are permitted additives in bread manufacturing (home baked bread using commercially available flour does not have a high sulfate content). Calcium sulfate is used to remove temporary hardness and lower pH in some beer recipes. Addition of sulfite to fruits, vegetables, beverages and other food products as a preservative is another source of dietary sulfate, because sulfate is a common impurity in sulfite - some sulfite is oxidized to sulfate during storage and cooking.

The inorganic sulfate contained in water and beverages, account for approximately 17% of the sulfate found in the body. Most public water supplies contain sulfate concentrations of less than 500 mg/L. Sulfate levels in water around 250 mg/L and above are detectable due to an off odor and taste, and this generally causes those exposed to water with higher concentrations of sulfate to switch to bottled water sources for drinking.

<u>Food</u> (mg/g)	<u>Sulfate Content</u>
Almonds	0.9
Bread (brown)	1.5
Bread (white)	1.3
Broccoli	0.9
Brussel sprouts	0.9
Cabbage	0.8
Cauliflower	0.5
Dates	1.1
Dried apples	4.9
Dried apricots	3.0
Pasta, durum wheat	0.3
Peanuts	0.7
Prunes	1.0
Raisins	1.3
Sunflower seeds	0.6

**Table:** Sulfate Content of Foods

<u>Beverage</u> (mg/L)	<u>Sulfate Content</u>
Beer	260
Cider	270
Coconut milk	500
Cola	80
Juice, apple	70
Juice, grape	200
Juice, tomato	250
Milk, cow	100
Milk, human	5
Wine, red	380
Wine, white	300

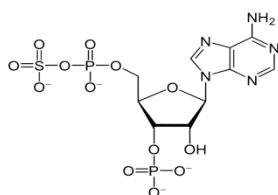
**Table:** Sulfate Content of Beverages

Sulfate ingestion from drinking water is highly variable and depends on the area of the country from which the water is obtained. Some well water in rural areas of the United States have been known to contain upwards of 500 mg/L and some of the “mineral” waters sold with health claims have been reported to exceed this level. Distilled water contains very little, if any, sulfate, and de-ionized water contains no sulfate.

- ❖ **Kidneys** - sulfate is usually eliminated in the urine along with a conjugated substrate. However, when serum sulfate levels fall below a certain threshold re-absorption of sulfate occurs in the kidneys. Chemicals can have a direct effect on renal tubular sulfate reabsorption. For example - salicylic acid (found in aspirin and many foods) increases the renal clearance of inorganic sulfate, while Probenecid (gout med.) inhibits the renal re-absorption of inorganic sulfate. In both examples serum sulfate levels will further decrease.
- ❖ **Macromolecules** (endogenous) - under conditions of low serum sulfate levels due to – fasting /starvation, high sulfate renal clearance, and/or a dysfunctional sulfoxidation process - sulfate can be supplied by catabolism of proteins and other macromolecules, especially glycosaminoglycans (GAGS).

The consumption of high protein foods containing organosulfur compounds (methionine and cysteine), along with the consumption of inorganic sulfated compounds in both food and beverages, is usually sufficient enough to meet the body's requirement for sulfate. However, in humans, the serum sulfate level varies dramatically over 24 hours, and is decreased in individuals who are fasting or ingesting high levels of substances that are metabolized by sulfation (such as acetaminophen). Humans excrete approximately 20 to 25 millimoles of sulfate in 24 hours; therefore, sulfate reserves must be maintained through dietary intake of sulfur-containing amino acids and inorganic sulfate.

The body's primary use for inorganic sulfate is as a co-substrate for the sulfation pathways. Sulfate serves these functions in its active form called - **3'-phosphoadenosine-5'-phosphosulfate (PAPS)**.



### **3'-phosphoadenosine-5'-phosphosulfate (PAPS)**

PAPS - also known as **active sulfate** – is dependent on the availability of inorganic sulfate and **adenosine-5'-triphosphate (ATP)**. The highest concentration of PAPS is found in the liver, followed by the kidney, then the lungs, intestines and brain.

PAPS's primary function is to serve as the universal sulfate donor for all sulfation pathways which are catalysed via sulfotransferase reactions. PAPS is synthesized in a two-step, coupled reaction. The first step combines ATP and inorganic sulfate to form **APS** and **pyrophosphate (PPi)**. The reaction is catalysed by **ATP-sulfurylase** in the presence of Mg<sup>2+</sup> (magnesium). The subsequent step combines the APS formed in the first step with additional ATP to form PAPS and **ADP**. This reaction is catalyzed by **APS-kinase** in the presence of Mg<sup>2+</sup>. PAPS synthesis takes place in the cytosol, after which it is transported to the golgi apparatus by PAPS transporters, where it is used for sulfation of various molecules ([see flow chart](#))

Interestingly, the ability to synthesize PAPS from inorganic sulfate is a more important factor in sulfation capacity than is PAPS steady-state concentrations. For example, infusion of sodium sulfate, cysteine and methionine increases serum and tissue sulfate concentrations. However, tissue PAPS levels are not increased markedly. Therefore, it appears that steady-state PAPS concentrations in various tissues cannot be increased by increasing tissue sulfate concentrations.

Instead, the availability of PAPS for sulfation is dependent on its synthesis. PAPS tissue concentrations are low compared with the amount of PAPS required to sustain high rates of sulfation, indicating high rates of PAPS biosynthesis. This is important because it has been demonstrated that sulfation is a high-affinity, low-capacity enzymatic process in which the entire liver content of available PAPS can be consumed in less than 2 minutes. Therefore, rapid biosynthesis of PAPS is required for sulfation.

### **Sulfotransferases (SULTs)**

With the formation of PAPS as a co-substrate, the process of sulfation can now take place with assistance from a super-family of enzymes called – sulfotransferases (SULTs). SULTs catalyse the transfer of the sulfate group from PAPS onto the target molecule – substrate. In humans there are three SULT families - SULT1, SULT2, and SULT4, which contain at least thirteen distinct members and have a wide tissue distribution in the body.

SULTs can also be divided into two broad functions or classes which relate directly to the two primary functions of sulfation, which are:

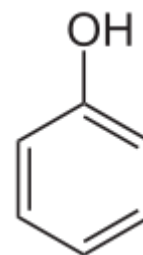
1. **Phenolsulfotransferase (PST) or Cytosolic SULTs** - are employed for use in the Phase II biotransformation sulfation pathway. This pathway is responsible for the biotransformation of xenobiotics and the inactivation of endogenous substrates such as steroids, bile acids, thyroid hormones and neurotransmitters. There are three major isoforms of phenolsulfotransferase:
  - **SULT1A1** - is selective for **phenol** substrates and can be found throughout the body with the liver, intestine, brain and platelets containing particularly high levels.
  - **SULT1A2** – detected in the liver
  - **SULT1A3** - is selective for **amine** and **phenol** substrates and is mainly found in the intestine.
2. **Membrane-bound SULTs** – (located in the Golgi apparatus) are employed for use in the biosynthesis of macromolecules via the sulfation pathway. Some examples of macromolecules produced via sulfation and membrane bound SULTs are: mucins, gastrin, CCK and glycosaminoglycans (GAGS).

### **Phase II Biotransformation - Substrates**

One of the primary functions of sulfation is in the Phase II biotransformation/detoxification of xenobiotics and other compounds. The sulfation pathway is vital for the biotransformation of two large classes of chemical compounds – **phenols** and **amines**.

#### **Phenols**

Phenols are a class or family of chemical compounds consisting of a **hydroxyl group (-OH)** bonded directly to an **aromatic ring**. **Phenol (C<sub>6</sub>H<sub>5</sub>OH)** is the simplest member of the class of phenols and the structure upon which the entire group is based. The term “aromatic” was assigned due to the fact that many of the compounds have a sweet scent. The aromatic ring in this case is – **benzene (C<sub>6</sub>H<sub>6</sub>)**.



Phenols are ubiquitous, occurring both naturally in nature as well as being synthesized as manufactured chemicals. Naturally, phenol is a constituent of coal tar and creosote, decomposing organic material, human and animal wastes, and as a common compound found in many foods as well as non-foods. Phenol is also formed during forest fires, and by atmospheric degradation of benzene in the presence of light.

Synthetically produced phenols are commonly used both in industry and in consumer products. Phenol is a high volume chemical with production exceeding 3-billion pounds annually in the U.S. It ranks in the top 50 in production volumes for chemicals produced in the U.S. with the housing and construction industries accounting for about half of the phenol used. Manufacture of phenol resins is the largest single use of phenol and is usually sold commercially as a thick liquid.

Phenol is also used as an antiseptic, a general disinfectant, and a slimicide (kill bacteria and fungi in slimes), in medical preparations including lotions, ointments, mouthwashes, salves. Phenol is also the active ingredient in some over-the-counter oral anesthetics sprays used as a treatment for sore throats. Minor uses of phenol include – manufacture of paint and varnish removers, lacquers, paints, rubber, ink, illuminating gases, tanning dyes, perfumes, soaps and toys.

In addition, very small amounts of phenol is produced endogenously as a breakdown product of protein metabolism by the action of bacteria on normal constituents of the diet in the gut and excreted independent of external exposure to the compound. Some of this internally produced phenol may be eliminated in the feces and some may pass to the blood. Phenols are readily absorbed following inhalation, ingestion and skin contact.

**Tables (below):** list sources of phenols, which are substrates for the sulfation pathway

Exogenous Phenols - Xenobiotics	Examples
Bisphenol A (BPA)	Polycarbonate plastic & containers, liner of food & drink cans, liner of water pipes, plastic cling wraps, specialty resins (lacquers, varnishes, inks, adhesives, epoxies), flame retardants, dental sealants & composite materials, sunglasses, CDs, DVDs, electronic equipment, water coolers, sports equipment, auto parts, etc.
Benxophenone-3 (BP3)	Sunscreen, photostabilizer, cosmetic and personal care products (nail polish & enamels, bath, makeup, hair, skin care), etc.
2,4-Di & Tri-chlorophenols	Herbicides, fungicide, insecticide, antiseptic, defoliant, etc.
2,5-Dichlorophenol (25DCP)	Mothballs, toilet deodorizer blocks
ortho-Phenylphenol (o-PP)	Fungicide & germicide (post-harvest for “waxing” citrus fruit and nuts), disinfectant, etc.
4-tert-Octylphenol (4-t-OP)	Surfactant (wetting agent that lower the surface tension of a liquid, allowing easier spreading) used in industrial & domestic detergents, cleaners and pesticides. Also used in cosmetics, adhesives, carbonless paper, plastics, etc.
Nonylphenol	Surfactant found primarily in industrial detergents, but is also used in pesticides, paint, rubber, steel manufacturing, and power generation. Nonylphenols can be found in many commercially sold products, such as household cleaners and detergents, in wax used for coating fruits and vegetables, plastic food packaging, and cosmetics.

Triclosan (TRCS)	Anti-microbial (anti-bacteria, anti-fungal, anti-viral) - used in domestic cleaning and personal care products (hand soaps, dish soaps, cleaners, detergents, cosmetics, lotions, creams, toothpastes, mouthwashes, deodorants, etc.). Also used in kitchen ware, computer equipment, clothes, children's toy, etc.
Butylated Hydroxytoluene (BHT)	Antioxidant food additive/preservative (butter, meats, cereals, chewing gum, baked goods, snack food, dehydrated potatoes, beer). It is also used as an antioxidant additive in packaging, animal feed, cosmetics, pharmaceuticals, jet fuel, rubber, petroleum products, electrical transformer, embalming fluid, etc.
Picric Acid	One of the most acidic phenols and is used primarily as an explosive.
Xylenol	Resins, laminates, construction materials, fire resistant hydraulic fluids in aircraft, lubricants in power plants, transformers, electric motors (cars, home appliances, power tools, etc)
Pentachlorophenol (PCP)	Used as an herbicide, insecticide, fungicide, algacide, and disinfectant. It is also used in leather, masonry, and wood preservation. <b>Banned since 1980's</b>
Cresols	Used to dissolve other chemicals, as disinfectants (Lysol), deodorizers and to make pesticides. Cresols are also found in photographic developers, creosote, cresolene and cresylic acids (wood preservatives). Cresols are found in the atmosphere as pollutants from the burning of wood, coal (power plants), gasoline (car exhaust), oil refineries, and cigarette smoke  In addition cresols are found naturally occurring in foods (see below)

Exogenous Phenols - Naturally Occurring	Examples
Cresols	Cresols are found naturally occurring in foods - tomatoes, asparagus, cheese, butter, bacon and smoked foods. Cresols are also found naturally in crude oil & coal tar.
Tyrosine	Amino acid found in high protein foods
Raspberry Ketone	The primary aroma compound of red raspberries. It is used in perfumery, cosmetics and as a food additive to impart a fruity odor.
Eugenol	A clear, yellow oily liquid extracted from certain essential oils – clove oil, nutmeg, cinnamon, basil and bay leaf. Used in perfumeries, flavorings, essential oils and medicine as a local antiseptic (antimicrobial) and anesthetic.
Capsaicinoids	Capsaicin & dihydrocapsaicin are the major active components that create the sensation of heat in chili peppers. Minor active components are – nordihydrocapsaicin, homodihydrocapsaicin and homocapsaicin. Capsaicin is also used as topical ointments to relieve shingle pain, minor aches and arthritic pain.

## Flavonoids

Flavonoids are the polyphenol plant pigments found in most plants (flowers, vegetables, fruits). Flavonoids are the largest group of naturally occurring phenol compounds. These pigments exhibit various biological activities in humans, the most important one being the antioxidant activity. Other flavonoid biological activities include – antimicrobial, antiulcer, anti-arthritis, estrogenic, estrogen receptor binding, anti-cancer, etc.

There are thousands of flavonoids that are classified into sub-categories such as:

- a. Flavones – found in celery, thyme, parsley. The major dietary flavones are:
  - Luteolin
  - Apigenin
- b. Flavonols – found in onion, scallions, kale, broccoli, lettuce, tomato, apple, grape, berries, tea, red wine. The major dietary flavonols are:
  - Quercetin
  - Kaempferol
  - Myricetin
  - Isorhamnetin
- c. Flavanones – found in citrus fruits/juices. The major dietary flavanones are:
  - Hesperetin
  - Naringenin
  - Eriodictyol
- d. Flavanols/Catechins/Proanthocyanidins – found in tea, chocolate, red wine, apples, apricot, grapes, berries, peach, cherry. The major dietary flavanols are:
  - Catechin
  - Gallocatechin
  - Epicatechin
  - Epigallocatechin
  - Epicatechin 3-gallate
  - Epigallocatechin 3-gallate
- e. Isoflavones – found in soy beans, soy products and legumes. The major dietary isoflavones are:
  - Daidzein
  - Genistein
  - Glycitein
- f. Anthocyanins – found in violet, blue and purple colourants in fruits, and berries such as black currant, red currant, red cabbage, plum, cranberry, blueberry, strawberry, cherry, black grape, etc. The major dietary anthocyanins are:
  - Cyanidin
  - Delphinidin
  - Malvidin
  - Pelargonidin
  - Peonidin
  - Petunidin

Salicylic Acid	<p>Is a phenol phytohormone which is found in many plants and foods. It is also synthesized and used in acne medicines, aspirin, in many common health and beauty products. Foods which contain high levels of salicylic acid or its salt – salicylates are:</p> <p>Fruit – apples, blueberries, blackberries, dates, grapes, cherries, mandarin, apricots, cantaloupe, raisins</p> <p>Vegetables – avocados, alfalfa, cucumbers, mushrooms, radishes, spinach, broccoli, sweet potato, green pepper, olive, tomato,</p> <p>Seasonings – cumin, curry powder, dill, oregano, paprika, rosemary, thyme, turmeric, mustard</p> <p>Nuts – pine, peanuts, pistachios, almonds, macadamia</p>
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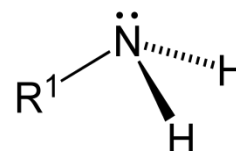
Endogenous Phenol Compounds	Examples
Steroids	<p>Steroid hormones - are a general class of chemical compounds that are structurally related to one another and share the same chemical skeleton - contain a specific arrangement of four rings that are joined to each other. Steroids differ from each other by the functional groups attached to these rings. Steroids can be classified as:</p> <ul style="list-style-type: none"> <li>➤ Sex Steroids – hormones that produce sex differences or support reproduction <ul style="list-style-type: none"> <li>a. Androgens (anabolic steroids) – hormones which develop and maintain male characteristics <ul style="list-style-type: none"> <li>○ testosterone – primary androgen</li> <li>○ dehydroepiandrosterone (DHEA) – a prohormone</li> </ul> </li> <li>b. Estrogens – hormones which develop and maintain female characteristics <ul style="list-style-type: none"> <li>○ estradiol – predominant form in non-pregnant females</li> </ul> </li> <li>c. Progestagens – hormones named for their function in maintaining pregnancy. The progestagen class of steroids serve as precursors to all other steroids including – estrogens, androgens, mineralocorticoids and glucocorticoids. Thus, all tissues producing steroids, such as the adrenals, ovaries, and testes must be capable of producing progestagens. <ul style="list-style-type: none"> <li>○ pregnenolone – the first step in the steroidogenic pathway</li> <li>○ progesterone – involved in female menstrual cycle and pregnancy.</li> </ul> </li> </ul> </li> <li>➤ Corticosteroids – produced in the adrenal cortex and involved in a wide range of physiologic systems. <ul style="list-style-type: none"> <li>a. Glucocorticoids – hormones involved in carbohydrate, fat and protein metabolism with anti-inflammatory properties. <ul style="list-style-type: none"> <li>○ cortisol – the most important glucocorticoid.</li> </ul> </li> </ul> </li> </ul>



	<p>b. Mineralocorticoids – hormones involved in salt-water balance.</p> <ul style="list-style-type: none"> <li>o aldosterone – the primary mineralocorticoid</li> </ul>
Catecholamines	<p>Catecholamine hormones –are produced in the adrenal glands and are involved in the “fight or flight” response to stress by increasing heart rate, blood pressure, breathing rate, muscle strength and mental alertness. Catecholamines are also produced in the brain and function as neurotransmitters</p> <ul style="list-style-type: none"> <li>o Epinephrine (adrenaline)</li> <li>o Norepinephrine (noradrenalin)</li> <li>o Dopamine</li> </ul>
Iodothyronines	<p>Iodothyronines - are any of the iodinated (attached with iodine) derivatives of thyronine (a phenol amino acid).</p> <ul style="list-style-type: none"> <li>o Triiodothyronine (T3) - a hormone made by the thyroid gland. It has three iodine molecules attached to its molecular structure. It is the most powerful thyroid hormone, and affects almost every process in the body, including body temperature, growth, and heart rate.</li> <li>o Tetraiodothyronine (thyroxine – T4) – a hormone made by the thyroid gland. It has four iodine molecules attached to its molecular structure. It is deiodinated to form T3.</li> </ul>
Neurotransmitters	<p>Catecholamine neurotransmitters – produced and released in nervous tissue where they exhibit both excitatory and inhibitory effects in the nervous system. These compounds are also produced in the adrenal gland and function as hormones.</p> <ul style="list-style-type: none"> <li>o Epinephrine (adrenaline)</li> <li>o Norepinephrine (noradrenalin)</li> <li>o Dopamine</li> </ul> <p>Serotonin – 80% of the body’s total serotonin is located in the gut where it is used to regulate intestinal movements. The remaining 20% is synthesized in the central nervous system and functions to regulate mood, appetite, sleep, muscle contraction, and cognitive functions.</p>
Bile Acids	<p>Are steroid carboxylic acids produced in the liver to break down fats. The primary bile acids are cholic and chenodeoxycholic acids. They are conjugated with glycine, taurine and sulfate before they are secreted into the bile.</p>

## Amines

Amines are a class or family of chemical compounds which contain nitrogen. Amines are derivatives of ammonia (NH<sub>3</sub>), where one or more of the hydrogen atoms have been replaced with a substitute atom. (the word amine was derived from the word “ammonia”). Amines have strong odors and is often described as “fishy” since the odor of raw fish comes from the amines contained.



Despite this foul reputation, the amines are essential to life as constituents of amino acids. They occur in drugs and vitamins and are essential starting materials for many synthetic processes.

**Tables (below):** list sources of amines, which are substrates for the sulfation pathway

Exogenous Amines – Xenobiotics	Examples
Aniline	Is an aromatic amine which is used in the production of dyes (indigo – the blue in blue jeans), drugs (acetaminophen), explosives, plastics, photographic chemicals
Ethanolamines	In industry they are used as absorbents for acidic compounds. They are used emulsifiers in numerous household products. Also used as a corrosion inhibitor for automobile antifreeze solutions and engine coolants.

Exogenous Amines – Naturally Occurring	Examples
Alkaloids	Are found primarily in plants and more than 3,000 different types have been identified. Certain plant families are particularly rich in alkaloids such as the poppy family. Some well known alkaloids are: <ul style="list-style-type: none"><li>○ Morphine</li><li>○ Quinine</li><li>○ Ephedrine</li><li>○ Nicotine</li><li>○ Strychnine</li></ul>
Tyramine	Acts as a catecholamine releasing agent and helps control blood pressure. Tyramine is produced in foods from the natural breakdown of the amino acid tyrosine. Tyramine is not added to foods. Tyramine levels increase in foods when they are: aged, fermented, stored for long periods of time, or are not fresh. Below is a list of foods which contain high levels of tyramine. <ul style="list-style-type: none"><li>○ Cheese – aged cheese: blue, brick, brie, cheddar, swiss, roquefort, mozzarella, provolone, emmentaler, colby, american, parmesan</li></ul>

- Fruits – figs, grapes, oranges, pineapples, plums, prunes, raisins, overripe fruit and dried fruit
- Meat & Fish – aged, dried, fermented, salted, smoked or pickled – pepperoni, salami, liverwurst, bologna, bacon, frankfurters, ham.
- Vegetables – snow peas, fava or broad beans, sauerkraut, pickles, olives, avocados, eggplant, tomatoes
- Fruit – bananas, raisins, plums, raspberries
- Soy – fermented: miso, soy sauce, teriyaki sauce, tofu, tempeh
- Nuts and Seeds – all nuts
- Beverages – all alcoholic beverages, all non-alcoholic fermented beverages
- Other – yeast, brewers extracts, chocolate, caffeine, coke

Endogenous Compounds	Examples
Neurotransmitters	<p>Catecholamine neurotransmitters – dopamine, epinephrine, and norepinephrine contain both phenol compounds as well as amine compounds are referred to as – <b><i>phenol amines</i></b>. Serotonin is another phenol amine.</p> <p>Histamine derived from the amino acid histidine acts as a neurotransmitter mediating arousal and attention, as well as a pro-inflammatory signal released from mast cells in allergic responses.</p>
Melatonin	<p>Melatonin is a hormone produced in the brain by the pineal gland from the amino acid tryptophan. The synthesis and release of melatonin are stimulated by darkness and suppressed by light, suggesting the involvement of melatonin in circadian rhythms. Levels of melatonin in the blood are highest prior to bedtime.</p>
Amino Acids	<p>The building blocks of protein. There are 20 such amino acids.</p> <p>Essential amino acids - Isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine</p> <p>Non-essential amino acids – alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, tyrosine, arginine, histidine, ornithine, taurine</p>

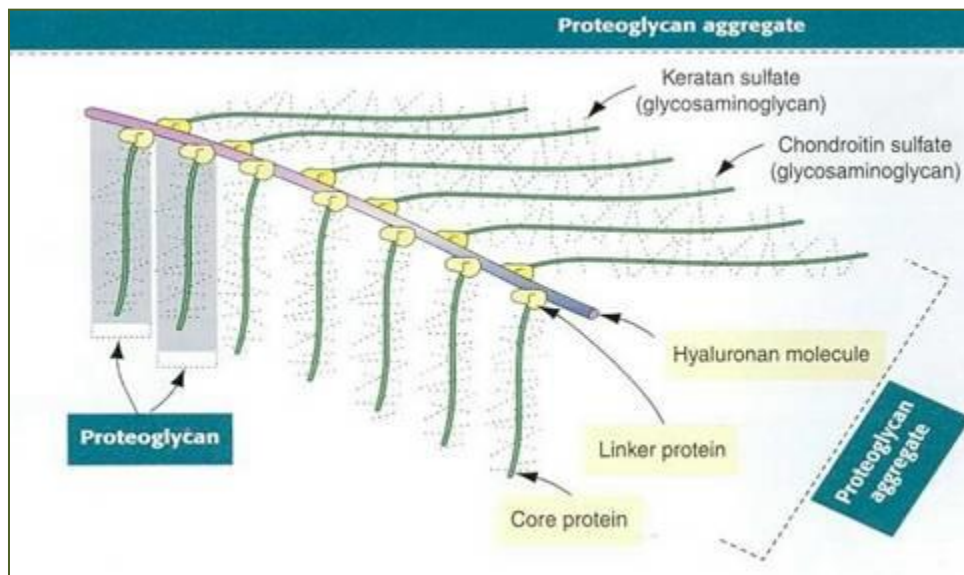
## Macromolecule Biosynthesis

Another primary function of sulfation is in the biosynthesis of macromolecules such as glycosaminoglycans (GAGs), mucin, gastrin and cholecystokinin (CCK).

### Glycosaminoglycans (GAGs)

In general terms, glycosaminoglycans (GAGs) are large complex carbohydrate molecules that maintain a negative charge on the membrane of cells and serve also as hydration sites which maintain the properties of mucous membranes.

More specifically, GAGs are membrane-associated macromolecules which are large complexes of negatively charged **polysaccharide** chains (long molecules composed of repeating carbohydrate/sugar structures). Many of these chains attach to a protein core and are collectively referred to as a **proteoglycan** (also called **mucopolysaccharides**). The GAGs extend perpendicularly from the protein core in a brush-like structure similar to a bottlebrush with the bristles representing GAGs. These long polysaccharide chains are composed of repeating **disaccharide** units (acidic sugar – amino sugar). The amino sugar is either **D-glucosamine** or **D-galactosamine**. The acidic sugar is either **D-glucuronic acid** or **L-iduronic acid** and together with sulfate give GAGs their strongly negative charge allowing for the attraction and binding of water – viscous.



**Figure:** Proteoglycan with Glycosaminoglycans (GAGs)

Along with the high viscosity of GAGs comes low compressibility, which makes these molecules ideal as a lubricating fluid in the joints. At the same time, their rigidity provides structural integrity to cells and provides passageways between cells, allowing for cell migration. Proteoglycans and GAGs are continuously but slowly turned over. Sulfation of the carbohydrate chain occurs after the **monosaccharide** to be sulfated had been incorporated into the growing carbohydrate chain. The source of the sulfate is 3'-phosphoadenosyl-5'-phosphosulfate (PAPS) and is catalysed by one of the sulfotransferase enzymes.

GAG synthesis occurs, in the endoplasmic reticulum and the goigi, rather than the cytosol (as in the biotransformation pathway). GAGs are then transferred to and located on the surface of cells and in the **extracellular matrix (ECM)**. The extracellular matrix is the non-cellular component of connective tissues surrounding all cells. Cartilage is the primary example of extracellular matrix in joints.

There are several major classes of GAGs:

- **Chondroitin sulfate** – a very large molecule, is the most abundant GAG in the body. Found in cartilage, tendons, ligaments, bone, skin, arteries and the cornea. It is also located inside certain neurons and may provide an endoskeletal structure, helping to maintain their shape.
- **Keratin sulfate** – found in loose connective tissue, cartilage, intervertebral discs, and the cornea.
- **Dermatan sulfate** – a relatively small GAG is widely distributed in the body found in skin, blood vessels, heart valves and the cornea.
- **Heparan sulfate** – found in lungs, blood vessels, **basement membrane** (a thin, delicate layer of connective tissue underlying the epithelium of many organs) and as a ubiquitous component of cell membranes.
- **Heparin** – unlike other GAGs that are extracellular compounds, heparin is an intracellular component of mast cells. Heparin is synthesized by and stored exclusively in mast cells that line arteries, liver, lungs and skin. When secreted into the blood it acts as an anti-coagulant.
- **Hyaluronic acid** – is different from other GAGs as it is unsulfated, not covalently attached to protein and the only GAG not limited to animal tissue – also found in bacteria. Hyaluronic acid is found in synovial fluid of joints (acts as a lubricant, space filler wetting agent), eyes, skin, loose connective tissue and cartilage.

With the exception of keratin sulfate, which has a half-life of greater than 120 days, the GAGs have a relatively short half-life, ranging from about three days for hyaluronic acid to ten days for chondroitin and dermatan sulfate.

The two main characteristics of sulfated GAGs is in their ability to maintain a negative charge on cell membranes and serve also as hydration sites which maintain the properties of mucous membranes. Meaning, GAGs have the special ability to bind large amounts of water, thereby producing the gel-like matrix that forms the basis of the body's **ground substance** or extracellular matrix.

Because of sulfated GAGs large number of negative charges they tend to be extended in solution. They repel each other and are surrounded by a shell of water molecules. When brought together, they “slip” past each other. This produces a “slippery” consistency of mucous secretions and synovial fluid. When a solution of sulfated GAGs is compressed, the water is “squeezed out” and the sulfated GAGs are forced to occupy a smaller volume. When the compression is released, the GAGs, spring back to their original hydrated volume. These characteristics of sulfated GAGs allow them to be involved in numerous functions in the body.

### Glycosaminoglycans (GAGS) Functions in the Human Body

- Act as structural components of the extracellular membrane.
- Act as a sieve in the extracellular membrane.
- In cartilage, they bind collagen and hold fibres in a tight, strong network.
- Are used to synthesize the chondroitin matrix of cartilage.
- Have role in compressibility of cartilage by acting as lubricants and shock absorbers.
- Contribute to the turgor (hydration) of various tissues.
- In the eye they play a role in corneal transparency and have a structural role in the sclera (white part).
- Act as anticoagulant.
- Facilitate cell migration - tissue formation during embryonic development, wound healing and immune responses all require the orchestrated movement of cells in particular directions to specific locations.
- Are essential components of cell surfaces and play an important role in mediating cell- cell signalling and adhesion.
- Are components of synaptic vesicles.
- Are involved in cell proliferation because they act as co-receptors for growth factors of the fibroblast growth factor family.
- Are widely distributed within the intestine where they maintain the integrity of the gut wall by surrounding cell surfaces with sulfated GAGs.

### Mucin

Mucins which line the gut are sulfated glycoproteins. Sulfate is essential in forming the mucin proteins which have two main functions: 1) protection – mucin production forms a barrier and blocks transport of toxins from the gut into the blood stream, 2) lubrication - mucins prevent gut contents from 'sticking' to the intestinal wall.

These two functions work together to increase resistance to colonization by pathogenic bacteria and viruses. It is interesting to note that *Helicobacter pylori* (cause ulcers) which can colonize the stomach, only does so when it has produced a **sulfatase** enzyme to de-sulfate the gastric mucins.

Sulfation has been shown to be essential in mucin formation and is controlled by another member of the sulfotransferase family, **tyrosyl protein sulfotransferase (TPST)**.

This reduced sulfation of mucin proteins may underlie the relatively common finding of *Candida* infections in the gut, since the slight negative charges on *Candida* cells would lead to their repulsion by the negatively charged sulfate groups on normal mucins. The reduction in mucin sulfation is probably an indirect cause of the alterations in gut flora which are often found in gastrointestinal inflammation.

It has been shown that low plasma sulfate levels and reduced sulfation activity of gut mucin is linked with several gastrointestinal problems such as:

- Gastrointestinal inflammation
- Irritable bowel disease
- Pathogenic microflora overgrowth (e.g. candida)
- Intestinal permeability (Leaky Gut)

The intestinal mucosa is the primary barrier to permeation of toxic compounds and macromolecules. Abnormalities of the intestinal barrier system may lead to enhanced uptake of substances that are normally excluded - macromolecules, endotoxins and xenobiotics. These foreign chemicals are presented to the liver's biotransformation system for processing and elimination. They can stress the detoxification capability of the liver or be partially processed and accumulate in the liver and adipose tissue. This can lead to increased tissue stores of toxic compounds and depressed immune status.

### **Gastrin, Cholecystokinin (CCK)**

Peptides can also be sulfated by the enzyme tyrosyl protein sulfotransferase (TPST); the gastric hormones gastrin and cholecystokinin (CCK) are good examples of this pathway. Both are involved in the digestive process and both are activated by sulfation. In a complex cascade, gastrin is sulfated and with hydrochloric acid from the stomach, causes release of CCK, which also requires sulfation. Together with peptide fragments released from gastric proteolysis (mediated by hydrochloric acid), this acts with the peptide hormone ***secretin*** on pancreatic tissue to induce the secretion of a range of ***proteolytic*** enzymes and also ***amylase*** and ***lipases***.

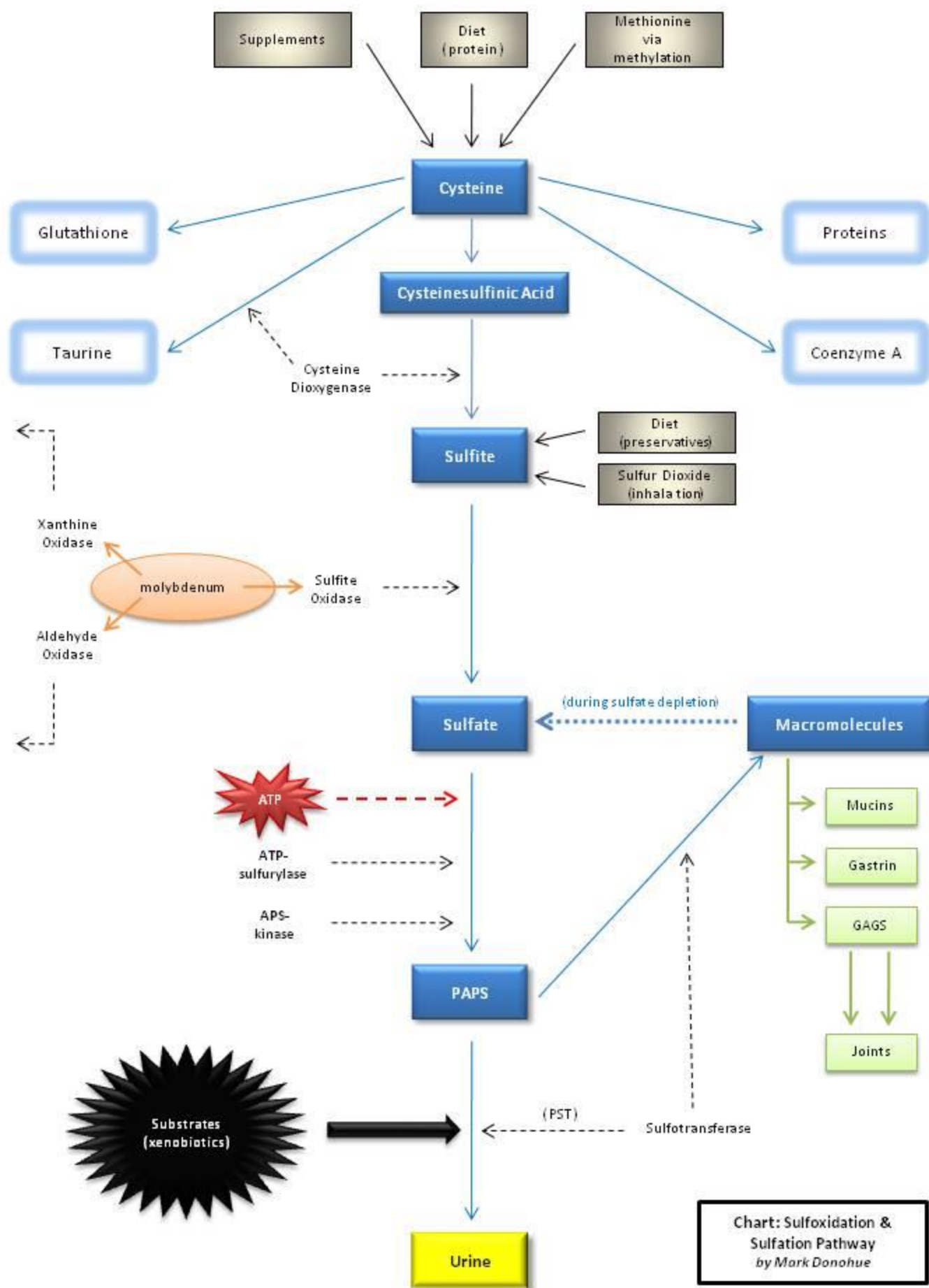
Lower levels of pancreatic amylase alter the digestibility of starch-based foods and allow increased fermentation of pathogenic bacteria while the decreased pancreatic lipase activity promotes formation of foul-smelling fatty stools which contain undigested triglycerides. Without the sulfation process to trigger the release of pancreatic proteases such as ***trypsin*** and ***chymotrypsin***, the complete digestion of proteins to their amino acid building blocks (proteolysis) cannot take place, so that peptides, rather than amino acids, are found in the gastrointestinal tract. As reduced sulfation of mucins may have made the gut more permeable, the stage is set to allow peptides to penetrate into the blood stream.

Some peptides, particularly those derived from casein and gluten, have been found to be neuro-active with effects on the brain where they can act at opioid receptors, affecting behavior, mood and responses to physical stimuli such as pain. This 'leaky gut' hypothesis therefore links with the opioid theory to explain why peptides from casein and gluten in particular seem to cause problems for some people. Although the blood-brain barrier is usually seen as being non-permeable to many compounds it may, like the gut, be 'leaky' in individuals with previous head injuries or who are living in stressful conditions.

### **Sulfoxidation and Sulfation Flow Chart**



[Back to PAPS](#)



**Chart: Sulfoxidation & Sulfation Pathway**  
by Mark Donohue



## PAPS Deficiency and SULTs Enzyme Dysfunction

The ability to conjugate with PAPS (active sulfate) is variable and requires both active SULTs (sulfotransferase) enzymes and adequate supplies of inorganic sulfate. Because sulfate is involved in numerous body functions, poor ability to conjugate with PAPS appears to be implicated in a variety of medical conditions. It is beyond the scope of this report to list and describe in detail these medical conditions, however, a short list with brief descriptions are provided below:

### ❖ Autism

- Researchers have demonstrated that autistic children have a compromised capacity for sulfation and therefore, are unable to effectively metabolize numerous compounds particularly phenolic amines such as dopamine, tyramine, and serotonin (present in many foodstuffs), which function as neurotransmitters. It was also found that plasma sulfate levels were greatly reduced in autism.

### ❖ Asthma

### ❖ Chemical, Drug & Food Sensitivities

- Researchers have found defective sulfoxidation (low sulfate) in nearly all ***“universal reactors”*** (MCS).
- Researchers have found defective sulfoxidation in a large majority (over 85%) of those highly allergic to foods, inhalants and chemicals.

### ❖ Chronic Fatigue Syndrome/Myalgic Encephalomyelitis

### ❖ Degenerative Joint Disorders

- Sulfate is needed for the formation of proteins in joints. Proteoglycans are, with collagen type II, the major structural components of cartilage. While collagen type II is responsible for the tensile strength, the proteoglycans are responsible for the pressure-resistant properties of cartilage. The highly sulfated GAGs, which are covalently bound to the core protein of the cartilage proteoglycans, are accountable for these pressure-resistant traits. The negatively charged sulfate esters on the GAGs are crucial for the optimal functioning of cartilage.
- Sulfate is an essential co-substrate in the synthesis of cartilage GAGs. Meaning sulfate availability is a rate-limiting factor in the synthesis of GAGs. Low sulfate availability will inhibit normal cartilage GAG and other GAG synthesis. Therefore, sulfate deficiency results in the synthesis of proteoglycan cores with a reduced quantity of normal GAG chains.
- Small variations in serum sulfate concentrations in humans will result in an altered rate of GAG synthesis in cartilage. Human cartilage – especially GAG – reproduction suffers significant deterioration with an even slight decrease in serum sulfate. A reduction in the sulfate concentration from 0.3 mM to 0.2 mM resulted in a 33% reduction in human GAG synthesis.
- One of the first events in the pathogenesis of osteoarthritis is a reduced content of GAGs in the affected cartilage. Individuals with a low serum sulfate concentration might have a lower capacity of GAG synthesis and as a result a more limited potential of matrix repair than individuals with a high serum sulfate concentration. As a consequence, individuals with a low serum sulfate concentration might be more vulnerable to the development of osteoarthritis than those with a higher serum sulfate level.

- GAG synthesis in human cartilage will be very sensitive to the potential sulfate-depleting effects of drugs used in the treatment of rheumatoid arthritis and osteoarthritis.

#### ❖ Intestinal Disorders

- Researchers have found that sulfated GAGs are widely distributed within normal intestine. However, patients with **inflammatory bowel disease (IBS)** showed substantial loss of GAGs within the intestine, and those with **Crohn's disease** also had disruption of both vascular and interstitial GAGs within the sub mucosa.
- Reduced supplies of sulfate alter the synthesis of biocomponents such as the **mucin** proteins which line the gastrointestinal tract.
- Sulfate is needed to start the cascade of digestive enzymes released from the pancreas. Without proteases, lipases and amylases food is not digested efficiently.

#### ❖ Lupus

#### ❖ Neurodegenerative Diseases

- Neurodegenerative diseases are characterized by deterioration of neurons or their myelin sheath, disrupting transmission of sensory information, movement control, and more. Examples of neurodegenerative diseases are - Parkinson's disease, Alzheimer's disease, Multiple Sclerosis, and Huntington's disease

#### ❖ Neurotransmitter Imbalances

- Failure to inactivate neurotransmitters via the sulfation pathway leads to neurotransmitter imbalances which may have clinical consequences. Principally, sulfation is a major inactivation pathway for catecholamines such as the neurotransmitter dopamine, about 80% of which is sulfated in man. Usually, when chemical neurotransmitters are released in the central nervous system, they act at receptor proteins and are then inactivated by sulfation or by FAD-linked mono-oxygenases or alternatively are carried by transporter proteins back into the initiating neuron. Failure of a major pathway such as sulfation will lead to a neurotransmitter imbalance and this can have effects on behavior, mood and function and the development of depression, hyperactivity, anxiety, etc.

#### ❖ Migraines

- Patients with migraine usually have reduced activity of SULT 1A1 and sometimes SULT 1A3.
- Migraine patients often have dietary 'triggers', the main ones provoking the intense headaches typical of the condition being cheese, chocolate, and bananas which contain tyramine, phenylethylamine and serotonin, respectively. All these amines are CNS-active and any reduction in sulfation potential could presumably increase levels of the active parent compounds. The SULT 1A1 isoform is also inhibited by flavonoids from fruit and vegetables. This may underlie the reported sensitivity of many migraine patients to orange juice and red wine; as can be seen from orange juice contains substances, probably flavonoids, such as tangeretin, which are strongly inhibitory.

- The increased blood levels of compounds with neurotransmitter activity are thought to 'trigger' migraine headaches in those who are already susceptible. It has been shown that individuals who are susceptible to migraine are metabolically unstable (with raised excitotoxic amino acid levels) so that very small changes in blood and brain catecholamine levels are sufficient to provoke a migraine attack.

### **The Culprits:**

In cases of sulfate deficiency the sulfate pool must be replenished, however this can be tricky. Before treatment is attempted it should be determined why there is a sulfate deficiency. Low serum sulfate levels can be due to several factors:

- **Low protein diet** – protein foods contain the sulfur amino acids – methionine and cysteine which are converted via sulfoxidation into inorganic sulfate. Almost two-thirds of the sulfate pool is supplied from cysteine.
- **Dysfunctional sulfoxidation** – the primary source of inorganic sulfate in the body comes from the sulfoxidation of dietary organosulfur compounds from protein rich foods. A dysfunctional sulfoxidation process will result in the slowing and/or blockage of cysteine being converted into inorganic sulfate. A dysfunctional sulfoxidation process therefore results in the elevation of cysteine and/or sulfite and a depletion of inorganic sulfate.

Though most people with deficient sulfate levels and high xenobiotic loads will benefit from the supplementation of cysteine, methionine, glutathione and other organosulfur compounds, in individuals with a dysfunctional sulfoxidation pathway this treatment protocol is **contraindicate** (advise against due to possible dangers). Individuals with elevated plasma cysteine to sulfate ratios may worsen with cysteine, methionine and glutathione supplementation. This is because supplementation with these and other organosulfur compounds will potentially add to the cysteine load. A high cysteine load may then become neurotoxic to the nervous system. Also, adding such supplements may raise sulfite levels in individual which can also become neurotoxic.

Therefore, individuals with a dysfunctional sulfoxidation pathway should consider supplementation with inorganic sulfate to bypass this process. Several sources of inorganic sulfate are available:

- ✓ **Chondroitin sulfate**
- ✓ **Glucosamine sulfate**
- ✓ **Sodium sulfate**
- ✓ **Magnesium sulfate** (IVs or injections)
- ✓ **Magnesium sulfate** (Epsom salts) - best to use a dose of around 600mg (1/3 teaspoonful) in water once or twice a day with food. Compared to sodium sulfate, magnesium sulfate appears to be absorbed less completely and more erratically, and produces more adverse effects (upset stomach, diarrhea). Absorption of sulfate across the gut occurs via a sodium/sulfate protein transporter, which are very effective but easily saturated so that many tiny doses are more effective than one large dose.

Sulfate that is not absorbed in the upper gastrointestinal tract passes to the large intestine and colon, where it is either excreted in the feces, reabsorbed, or converted by **sulfate reducing bacteria** (*Desulfovibrio*, *Desulfomonas* and *Desulfobacter*) to hydrogen sulfide. Hydrogen sulfide may be pathogenic to the bowel mucosa, thereby, implicating sulfate and sulfite in the etiology of ulcerative colitis and other bowel diseases.

Alternatively, Epsom salts can be placed in the bath water where it is absorbed through the skin and thereby eliminating the complications of taking it orally.

- **Low dietary inorganic sulfate intake** – intake of inorganic sulfate from foods and beverages contributes to approximately one-quarter of the sulfate pool.
- **High molybdenum intake** – In attempts to optimize the sulfoxidation pathway caution must be taken when supplementing with molybdenum. Molybdenum, although an essential element is an analog (similar properties) of sulfate and competes with inorganic sulfate for ATP-sulfurylase, which catalyzes the first step in PAPS synthesis and can thus lower sulfate levels and impair sulfation ability.

*Environmental medicine* experts warn that molybdenum supplementation may be contraindicated in individuals with poor sulfation ability. Other compounds which also compete in this first step are - selenate, tungstate, chromate and arsenate.

- **Low ATP** - PAPS formation can also be inhibited by low level of available ATP. Active sulfate (PAPS) formation requires adequate amounts of ATP. It has been demonstrated that the rate of PAPS synthesis in isolated hepatocytes is directly related to ATP concentrations.
- **Heavy substrate exposure** – excessive exposure to sulfation substrates (phenol & amine compounds) will quickly deplete the body's inorganic sulfate pool.
- **High flavonoid intake** - the SULTs are potentially modulated by dietary components. Flavonoids and polyphenolic compounds found in fruits and vegetables, inhibit the SULT 1A1 isoform and partially inhibit SULT 1A3. Studies with fruit and vegetable showed that radishes, spinach, broccoli, bananas, elderberries, red cabbage, grapes, and red wine inhibit SULT 1A1 and also SULT 1A3.
- **Low magnesium levels** – active sulfate (PAPS) formation requires adequate amounts of magnesium.
- **Age**

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(same as Biotransformation)

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