By the early 21st century our society has increasingly been exposed to more and more toxic compounds in the air we breathe, the water we drink and in the foods we eat. For example, the Environmental Protection Agency (EPA) estimates that in 1994 alone, over 2.2 billion pounds of toxic chemicals were released into the environment in the United States. EPA estimates for 2002 had grown to 4.7 billion pounds. This includes the nearly 80,000 chemicals on the market in the US, many of which are used by millions of Americans in their daily lives and are unstudied and largely unregulated. Moreover, it is likely that at least 25% of the United States population suffers to some extent from heavy metal poisoning (e.g. mercury, arsenic, etc).

In addition, the body produces a steady stream of metabolic waste products (ammonia, bilirubin, urea, lactic acid, etc.) along with toxic byproducts called – exotoxins - as a result of microbial activity in the human intestine. Continual exposure to toxins originating from both within the body and outside the body gives credence to one of the body’s most vital functions – hepatic biotransformation. The sole purpose of the biotransformation process is to convert toxic compounds into non-toxic, water-soluble compounds which can easily be eliminated. The liver (hepatic) is the key player in this process.

Detoxification - for many years the term “detox” referred to breaking free of alcohol or drug addiction. Nowadays, detox means removing all toxins from the body; not just poisons from substance abuse, but also heavy metals, chemical additives, and other toxins found in our food, water and air. As well as the metabolic waste products produced by the body.

Biotransformation - is defined as a metabolic process whereby chemical modifications or alterations are made by the body on a specific chemical compound, usually by means of enzymatic activity. As mentioned these chemical compounds originate either from within the body, usually the result of metabolic waste and are called endogenous compounds or they come from outside the body and are called exogenous compounds.

Exogenous compounds enter the body through four primary routes – inhalation (nose/lungs), ingestion (mouth/intestines), transdermal (skin), or intravenous (veins). Exogenous compounds can either be compounds that the human body is familiar with (e.g. food, nutrients, water, oxygen) or they can be compounds which are foreign to the body (e.g. drugs, pesticides, solvents, industrial chemicals). Exogenous compounds which are foreign to the body are referred to as xenobiotics.

Hepatic biotransformation - is the chemical modification or alteration of compounds (endogenous or exogenous) via enzymatic activity, which takes place in the liver. Biotransformation can take place in most tissues anywhere in the body; however, the liver is the primary site for biotransformation to occur. This is due in part because of the large size of the liver and because it also contains the highest concentration of biotransformation enzymes.
Biotransformation enzymes exist in the smooth endoplasmic reticulum, cytosol (intracellular fluid) and to a lesser degree in the membranes of the mitochondria, nuclei and lysosomes (small spherical organelles) of the liver’s hepatocytes. The kidneys and lungs are the next major biotransformation sites, but only at 10 – 30% of the liver’s capacity. The skin, nasal mucosa and intestinal mucosa also have some biotransformation capacity.

Most xenobiotics entering the body are lipophilic (affinity for lipids). This property enables them to penetrate the lipid membranes of cells, to be transported by lipoproteins with blood, and to be rapidly absorbed by the target organ. However, the excretory mechanism of the body requires a certain degree of hydrophilicity (water loving) for efficient excretion. In other words, lipophilic compounds are more absorbable and retainable, while hydrophilic molecules are less able to cross cellular membranes and therefore are easily filtered out by the kidneys.

In the absence of efficient means of excretion constant exposure to lipophilic xenobiotics could result in accumulation of these compounds in human tissue potentially becoming toxic to the body. Therefore, the main function of biotransformational enzymatic activity is to make lipohilic compounds less toxic and harmful by converting or biotransforming them to hydrophilic compounds and preparing them for elimination.

Between the large variety of different metabolic waste products produced by the body and the larger amount of environmental toxins an individual is exposed to, the list of bio-chemical and chemical compounds that undergo biotransformation in the liver is practically endless.

Therefore, to properly protect the body, hepatic biotransformation takes place non-stop and is a high energy dependent enzymatic process. Meaning it requires and uses a tremendous amount of cellular energy. Along with needing large amounts of energy, hepatic biotransformation requires and uses a large variety of enzymes. Biotransformation enzymatic activity occurs in two sequential steps called - Phase I and Phase II. Also, Phase III occurs, however this phase requires the use of transporter proteins as opposed to enzymes.

- **Phase I Bioactivation** – is the process whereby enzymes act upon a compound to biotransform it; however during this phase the compound is only partially biotransformed. Thereby creating an intermediate or metabolite of the original chemical compound which has been bioactivated into a more chemically reactive, toxic compound. This reactive compound, if not fully biotransformed, will remain in the body potentially causing damage, especially to the liver where it was formed. And/or it will be stored in adipose (fat) tissue where it will be difficult to excrete.

- **Phase II Conjugation**– is the process whereby the bioactivated Phase I intermediate is further acted upon by a different set of enzymes and undergoes further biotransformation. The result is a safer, non-toxic water soluble compound. This process is sometimes referred to as – bioinactivation.

- **Phase III Efflux** – is the process of removing the water soluble Phase II conjugated compound from the cell. Phase III uses transporter proteins rather than enzymes to complete this process.

Once an unwanted compound has been completely biotransformed and removed from the cell, it will then be eliminated from the body via – kidneys, bowels, breath, sweat, saliva or hair - completing the detoxification process.

Though there are numerous biotransformation enzymes, each enzyme has an affinity for a certain molecular compound or substrate. A substrate is a specific compound (endogenous or exogenous) of which a specific enzyme acts upon and biotransforms it. Regulation of the biotransformation enzymatic process is highly complex. It is the widespread
variability in the regulation of biotransformation enzymes which determines the efficiency of the biotransformation process. And therefore a determining factor in whether an individual is highly sensitive or reactive to xenobiotic compounds or is more resilient and less sensitive.

Some studies have suggested an association between the ability of the body to adequately biotransform xenobiotics and metabolites, and the etiology (cause or origin) of various puzzling disease entities such as chronic fatigue syndrome, fibromyalgia, and multiple chemical sensitivity. Research has also begun to validate the hypothesis that chronic neurologic symptoms, such as those seen in Parkinson’s disease, may result from impairment of detoxification ability. And a link between compromised detoxification ability and certain types of cancer has also been reported. Therefore, it is highly suggestive that an individual’s ability to remove toxins from the body may play a role in the etiology or exacerbation of a range of chronic conditions and diseases.

**Biotransformation Regulation**

The regulation, expression and activity of biotransformation enzymes and whether or not a xenobiotic compound is completely detoxified or only bioactivated is determined in large part by two general factors: (1) environmental factors - the level/amount of exposure or ingestion of a toxic compound and (2) biochemical factors – an individual’s unique level of biochemistry; referred to as biochemical individuality.

Biochemical individuality is a simple concept that states all humans differ biochemically from others. And that biochemical individuality directly affects the degree to which a chemical compound is biotransformed from person to person. Some of the factors that determine a person’s level of biochemical individuality and therefore biotransformation capacity are:
Genetics factors - the structure, amount of or complete lack of a specific biotransformation enzyme may differ among individuals and this can give rise to differences in rates of biotransformation. These genetic variations are referred to as – **polymorphism**.

Non-genetic host factors – such as disease, stress, obesity, physical exercise and age. For example - In some disease states, detoxification activities appear to be up-regulated, while in other conditions these activities may be inhibited. Another example - elderly people are generally more “sensitive” due to a less active life style causing poor blood flow to the liver, along with the fact that they produce fewer biotransformation enzymes.

Co-factors - differences in the availability of co-factors and nutrients cause very different biotransformation abilities from person to person, or even within the same person due to a daily changing nutritional status.

A person’s unique biochemical individuality along with the wide variety of environmental factors regulates the interplay between **inducible** and **inhibitory** functions of the biotransformation process. In both phase I and phase II biotransformation enzyme families, some enzymes are continuously expressed and some are inducible. Various compounds such as – xenobiotics, natural toxins, plant compounds, etc. – can induce Phase I and Phase II processes. This is done by inducing production of their enzymes, thereby leading to faster detoxification or bioactivation. On the flip side these same types of compounds can also inhibit Phase I and Phase II processes.

### Inducers

Inducers can be either: **mono-functional** - affecting only one enzyme or one phase of biotransformation or they can be **multi-functional** – affecting multiple enzymes or phases. Mono-functional inducers that increase Phase I but not Phase II can result in:

A) Bioactivation - the increased formation of highly reactive metabolic intermediates which are associated with damage to proteins, RNA, DNA or increased inflammation, cell death or cancer.

B) The increased biotransformation of multiple compounds potentially clearing and reducing availability of a desirable substance – such as a helpful medication.

Multi-functional inducers tend to affect both phases by: increasing Phase II activity and to either slightly increase Phase I activity or to slow Phase I relative to Phase II – generally a good thing.

<table>
<thead>
<tr>
<th><strong>Examples of Multi-Functional Inducers</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ellagic acid</strong> – induces Phase II while decreasing Phase I, found in raspberries, strawberries, cranberries, walnuts, pecans and pomegranates.</td>
</tr>
<tr>
<td><strong>d-limonene</strong> – is a strong inducer of both Phase I and Phase II and can be found in caraway, dill seed, and in citrus peel and juice of citrus fruit – but not grapefruit.</td>
</tr>
<tr>
<td><strong>Brassica vegetables</strong> – stimulates both Phase I and Phase II, found in broccoli, cauliflower, cabbage, kale, bok choy, and brussels sprouts which provide <strong>indole-3-carbimol</strong>.</td>
</tr>
</tbody>
</table>
Inhibitors

Inhibition of Phase I and Phase II processes can be caused by:

A) Two or more compounds competing for the same enzyme
B) Depletion of nutrients and co-factors
C) Selective inhibition of an enzyme by pharmaceuticals

It can be strategic to inhibit Phase I or Phase II systems, generally more true of Phase I. For example acute care in some poisoning includes administering the right Phase I inhibitor – this would be done if it is the bioactive intermediate that is the real poison and needs to be avoided. This can give the body time to cope with the slowed stream of toxic production. Also, it can be strategic to inhibit Phase I when availability of Phase II cofactors are limited - by poor nutrition or large toxic burden, this can help Phase II keep up.

Ideally, Phase I and Phase II detoxification mechanisms work synergistically. More specifically, as long as there is no deficiency of Phase II cofactors, Phase II reactions in general occur faster than Phase I reactions. This prevents the buildup of highly reactive bioactive Phase I intermediate compounds. However, if Phase I detoxification is highly active and Phase II detoxification is lethargic or if an individual is exposed to large amounts of xenobiotics along with a weakened biochemical individual response, imbalances between Phase I and Phase II can occur.

In such situations critical nutrients and co-factors can become depleted allowing unwanted compounds and bioactive intermediates to buildup in the body’s tissues. Individuals with such situations are referred to as a “pathological detoxifiers” (diseased detoxification) a condition which contributes significantly to free radical formation, oxidative stress and ultimately tissue damage.

Interestingly, the body will down regulate Phase I after about 24 hours of extreme calorie reduction (fasting or starvation). The fact is that fasting or starvation is the most potent of all the lipolytic, fat-soluble mobilizing factors. Non-detoxified lipophilic xenobiotics are stored in adipose tissue and during times of extreme calorie reduction are released into the blood stream. During this time concentration of xenobiotics in the urine can be ten times higher than normal. Therefore, the down regulation of Phase I during such times maybe the body’s wisdom in handling an increased amount of xenobiotics.

Additionally, during a fast or in times of starvation Phase II is deprived of its vital cofactors / nutrients and this too maybe another reason for the body to down regulate and slow the Phase I process. This will help Phase II keep up. Therefore, traditional approaches to fasting or detoxification involving minimal nutritional support for an extended period of time may have negative clinical consequences in chronically ill individuals. For excretion of xenobiotics to be effective, phase I activity requires antioxidant support and phase II activity requires specific nutritional support.

**Interesting note:** *chronic* exposure (≥ 3-4 days) to a compound that is a substrate for metabolism frequently causes up-regulation of enzyme synthesis, resulting in a net increase in enzymatic activity. In contrast, *acute* exposure may inhibit and/or destroy the enzyme, causing a net decrease in the rates of metabolism of other compounds that are metabolized by the same enzyme.
Phase I - Bioactivation

Hepatocytes are bathed in blood as the blood passes through the sinusoids - 70% of the hepatocytes surface membrane contacts the blood in the sinusoid. This provides for a tremendous surface area across which various compounds, especially xenobiotics, can gain entry into the hepatocytes. This can occur by one of several ways: a) compounds may passively diffuse across the sinusoidal membrane of the hepatocytes, b) they may be exchanged between blood transport proteins and the sinusoidal membranes, or c) their carrier proteins may bind to sinusoidal membrane receptors and then undergo endocytosis (cells absorb or engulf).

Once mobilized in the hepatocyte, unwanted compounds can contact and interact with biotransformation enzymes, the first being the Phase I enzymes. Phase I enzymes are lipid membrane bound proteins and are mostly found in the endoplasmic reticulum membrane. The primary function of Phase I enzymatic activity is to either:

A) Biotransform a toxic lipophilic compound directly to a more hydrophilic compound so it can be directly excreted in the kidneys (e.g. caffeine). Though, Phase I usually results in only a small amount of direct hydrophilicity and excretion.

B) The bulk of Phase I enzymatic activity takes place in the form of altering unwanted compounds in a way as to either expose or introduce a functional group. Functional groups such as: Carboxyl group (−COOH), hydroxyl group (−OH), amino group (−NH2), or sulfhydryl group/thiol (−SH).

This Phase I enzymatic alteration results in the unwanted compound now becoming a bioactivated intermediate. As mentioned, this gives rise to a more reactive and potentially more toxic and harmful substance than the original compound. Therefore, it must be acted upon rapidly by antioxidants and/or Phase II enzymes.

Complicating matters, often a single compound goes through a series of two to three Phase I reactions before it is ready for Phase II, which may occur in different parts of the cell (e.g. ER - lysosomes - mitochondria). This gives rise to increased opportunity for a toxic intermediate to encounter a target molecule and have a toxic effect during the transition.

Phase I enzymatic activity occurs by means of one of three possible chemical reactions: hydrolysis, reduction or oxidation. The type of reaction depends on the chemistry of the original compound and whether or not the compound is a substrate for one of the various Phase I enzymes.

Hydrolysis

Hydrolysis is a chemical process in which a molecule is cleaved into two parts by the addition of a molecule of water. One fragment of the parent molecule gains a hydrogen ion (H+) from the additional water molecule. The other fragment collects the remaining hydroxyl group (−OH), which is a functional group and thereby prepares the compound for Phase II. The major enzymes associated with the actions of hydrolysis are: esterases, peptidase and epoxide hydrolase.
Reduction

The process of moving electrons from one element to another element during chemical reactions is called reduction and oxidation or redox. Reduction is the opposite of oxidation, whereby oxygen is removed or in the absence of oxygen at least one electron is added when compounds come into contact with each other. Compounds that have the ability to reduce (take oxygen away from) other compounds are said to be reductive and are known as reducing agents. The major reduction enzymes associated with Phase I are:

- Azo reduction
- Carboxyl reduction
- Disulfide reduction
- Quinone reduction
- Sulfoxide reduction
- Nitro reduction
- Reductive dehalogenation

Oxidation

Though hydration and reduction enzymatic reactions do occur, the overwhelming majority of Phase I reactions occur as oxidative enzymatic reactions. Oxidation is commonly defined as the addition of an oxygen molecule to any compound it comes in contact with to form an oxide. Compounds that have the ability to oxidize (add oxygen to) other compounds are said to be oxidative and are known as oxidizing agents, oxidants or oxidizers. However, it is not necessary for oxygen to be present for oxidation to occur and therefore oxidation is also defined as the loss of at least one electron when two or more compounds interact.

In Phase I, oxidation is carried out by a large family of enzymes called - mixed function oxidases or monooxygenases. These enzymes introduce oxygen into the chemical structure of unwanted compounds, creating bioactivated intermediates such as reactive oxygen species (ROS). These reactive oxygen species, also known as free radicals, can be extremely toxic, far more so than the original compound.

Examples of Phase I Bioactivated Intermediates

- Alcohol
- Aldehydes (formaldehyde – embalming agent)
- Chloral hydate (identical to the knock-out drug - “Mickey Finn”)
- Epoxide
- Endogenous benzodiazepines (similar to valium and other tranquillizers and sleeping pills)

Viewing this short list makes it easier to understand how chronic fatigue, for instance, can develop when a toxic overload is present. The harmful effects of these intermediates are primarily controlled by antioxidants and being acted upon by Phase II enzymes.

There are well over one hundred mixed function oxidase or monooxygenase enzymes used in the Phase I hepatic biotransformation process. Listing each one of these enzymes with their substrates is beyond the scope of this report. However, below is a description of the most common Phase I biotransformation enzyme – Cytochrome p450 (CYP450).
Cytochrome p450 Monooxygenases (CYP450)

The most extensively studied mechanism responsible for the metabolism of xenobiotics is the action of the cytochrome p450 enzymes (CYP450). The CYP450 enzymes constitute a super family of proteins (containing a heme cofactor) that are not only responsible for metabolism of xenobiotics and metabolic waste, but are also involved in the metabolism of nutrients, fatty acids, cholesterol and steroid hormones. These enzymes are widely distributed throughout the body with the greatest concentrations in the liver and in tissues exposed to the external environment (e.g. skin, intestines, lung, eyes), as well as the kidneys, adrenals, testes, and brain. This huge system processes more compounds than all the rest of Phase I enzymes combined.

Each human CYP450 enzyme appears to be expressed by a particular gene and most compounds are largely metabolized by a single CYP450 enzyme. Though, there is a lot of overlap and redundancy in this system, for example: one CYP450 enzyme maybe involved in the metabolism of one or more substrates while a single substrate may be acted upon by multiple CYP450 enzymes. Interestingly, it is known that the gene for the cytochrome p450 has existed for more than 1.5 billion years. This indicates xenobiotic (e.g. pharmaceuticals) metabolism by the CYP450 system is a new and secondary role for these enzymes.

Genes encoding CYP450 enzymes are designated with the abbreviation "CYP", followed by a numeral indicating the gene family. Below is a list of the cytochrome p450 enzyme families found in the human body.

<table>
<thead>
<tr>
<th>CYP1</th>
<th>CYP2</th>
<th>CYP3</th>
<th>CYP4</th>
<th>CYP5</th>
<th>CYP7</th>
<th>CYP8</th>
<th>CYP11</th>
<th>CYP17</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP19</td>
<td>CYP20</td>
<td>CYP21</td>
<td>CYP24</td>
<td>CYP26</td>
<td>CYP27</td>
<td>CYP39</td>
<td>CYP46</td>
<td>CYP51</td>
</tr>
</tbody>
</table>

There are eighteen CYP450 families in the human body which can be subdivided into subfamilies. There are approximately forty-four subfamilies which are indentified by a capitol letter (following the family numeral). These subfamilies can be further divided by individual genes of which there are approximately fifty and are identified by another numeral (following the subfamily letter). For example, some of the cytochrome p450 “non-detoxification” functions with their associated – family/subfamily/gene - are:

- **Bile acid metabolism**: CYP7A
- **Vitamin D metabolism**: CYP24A1
- **Cholesterol metabolism**: CYP51A1
- **Fatty acid metabolism**: CYP2J2, CYP4, CYP5, CYP8A1
- **Steroid metabolism**: CYP2G1, CYP7, CYP8B1, CYP11, CYP17, CYP19, CYP21, CYP27A1, CYP46, CYP51

Of the many CYP genes within the human genome, the three families expressing CYP1, 2, and 3 constitute the majority of the exogenous and endogenous Phase I biotransformation detoxification enzymes. The primary cytochrome p450 enzymes involved in the Phase I hepatic biotransformation process are:
CYP1: CYP1A1, CYP1A2

CYP2: CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2E1

CYP2D6 – is the most thoroughly studied CYP enzyme and exhibits a number of phenotypes (biochemical characteristics). 5-10% of Caucasians and <2% of Asians and African Americans are poor metabolizers

CYP3: CYP3A4 – The most abundant P450 enzyme in liver.

Chart: Proportion of drugs metabolized by different CYPs

CYP450 Substrates:

Although CYP450 enzymes detoxify both endogenous and exogenous compounds, most of the available information is derived from pharmaceutical companies and their studies on drug metabolism. However, since environmental xenobiotics are detoxified by the same enzymes as drugs, much can be learned from this research about how to detect the effects of environmental xenobiotics on detoxification. Because listing a specific CYP450 enzyme with its many drug substrates, inducers and inhibitors is beyond the scope of this report, I have instead provided links to websites where such information can be found.

CYP450 substrate, inducer, inhibitor chart #1
CYP450 substrate, inducer, inhibitor chart #2
CYP450 substrate, inducer, inhibitor chart #3
CYP450 Inducers:

The cytochrome p450 family of biotransformation enzymes (CYP1, 2, 3) can be induced by numerous compounds; except for CYP2D6, which cannot be induced. Below is a short list of several compounds known to induce CYP450 enzymes.

- Chemicals – air pollution, petroleum derivatives, solvents, organochlorines, herbicides, pesticides, acetate, paint fumes, dioxin, carbon tetrachloride (fire extinguisher, refrigerants, cleaning agents), etc.
- Drugs – acetaminophen, diazepam, sleeping pills, contraceptive pills, steroids, barbiturates, sulfonamides/sulfa drugs, nicotine, ethanol/alcohol, caffeine, phenobarbital, etc. A more detailed list of pharmaceutical compounds known to induce cytochrome p450 enzymes can be seen in linked charts above.
- Foods – charcoal-broiled fats, saturated fats, high protein diets, brassica vegetables, d-limonene, sassafras.
- Supplements – thiamin (B1), riboflavin (B2), niacin (B3), ascorbic acid (C), protein powders, St. John’s wort.

CYP450 Inhibitors:

Many substances inhibit cytochrome p450 enzymes. This situation can cause substantial problems as it makes toxins potentially more damaging because they remain in the body longer before detoxification. Such blocking results in a build-up of more toxic compounds in tissue. This can in turn lead to a spreading phenomenon, with increasing sensitivity to more chemicals such as perfumes, colognes, cleaners, detergents and many other environmental chemicals. Also, the spreading phenomenon can eventually cause an individual to become sensitive and reactive to even natural chemicals occurring in foods, pollen and mold.

Dr William Rae of the Environmental Health Centre in Dallas says that the most severely ill chemically sensitive patients not only have abnormally low anti-pollutant enzymes, in addition to toxic suppression and nutrient depletion, but in some instances antibodies are produced against cytochrome p450 enzymes and may inhibit or decrease their effectiveness. Some examples of substances that inhibit CYP450 enzymes are:

- Chemicals – Carbon monoxide, etc.
- Drugs – H2-blockers (for acid reflux), benzodiazepines (for anxiety, insomnia), anti-histamines (for allergies), anti-fungals, barbiturates (sedatives, anticonvulsants), etc. A more detailed list of pharmaceutical compounds known to inhibit cytochrome p450 enzymes can be seen in linked charts above.
- Foods – naringenin (flavonoid in grapefruit), curcumin (turmeric), capsaicin/cayenne (chili peppers), eugenol (clove oil), quercetin (onions), etc.
- Supplements – quercetin (flavonoid), calendula (calendula officianalis), N-acetylcysteine, etc.
- Endogeneous – gut bacteria endotoxins/exotoxins
- Other – aging, loss of oxygen
Other Phase I Oxidative Enzymes:

As mentioned there are well over one hundred mixed function oxidase or monooxygenase enzymes used in the Phase I hepatic biotransformation process. And again, it is beyond the scope of this report to list and describe these enzymes. However, other than the cytochrome p450 monooxygenases described above, some of the other more common phase I biotransformation enzymes are:

- Flavin-Containing Monooxygenases (FMO)
- Alcohol Dehydrogenase
- Aldehyde dehydrogenase
- Aldehyde Oxidase
- Xanthine oxidase
- Diamine oxidase
- Monomine oxidase
- Molybdenum hydroxylase

Phase II - Conjugation

Whether endogenous or exogenous, some compounds by-pass Phase I and enter Phase II directly. However, most compounds enter Phase II as Phase I bioactivated intermediates. The primary function of Phase II is to further biotransform compounds to a less toxic, more hydrophilic compound.

To do this, Phase II incorporates the use of another type of enzyme called a transferase enzyme. Transferase enzymes are a family of enzymes whose function is to catalyze the transfer of various chemical groups from one compound to another. In hepatic biotransformation, the transferase enzyme transfers and attaches a co-factor to the exposed functional group of the entered Phase I intermediate. This process is referred to as conjugation.

<table>
<thead>
<tr>
<th>Phase II Pathway</th>
<th>Transferase Enzyme</th>
<th>Co-Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylation</td>
<td>N-Acetyltransferase</td>
<td>Acetyl-CoA</td>
</tr>
<tr>
<td>Amino Acid Conjugation (Glycination)</td>
<td>Glucuronosyltransferase</td>
<td>Glucuronic Acid</td>
</tr>
<tr>
<td>Amino Acid Conjugation</td>
<td>Glutathione-S-transferase</td>
<td>Glutathione</td>
</tr>
<tr>
<td>Glucuronidation</td>
<td>Methyltransferases</td>
<td>Methyl group</td>
</tr>
<tr>
<td>Glutathione Conjugation</td>
<td>Sulfurtransferase</td>
<td>PAPS</td>
</tr>
</tbody>
</table>

In Phase II when a specific transferase enzyme works with a specific co-factor it is referred to as a Phase II pathway. Individual compounds that enter Phase II usually follow one or two distinct pathways. Once a compound has become conjugated, via one of the Phase II pathways, it has now completed the biotransformation process of becoming hydrophilic and is ready for transport out of the cell. The Phase II biotransformation detoxification pathways are:
**Acetylation**

**Primary enzyme:** N-acetyltransferase  
**Co-factor:** acetyl-Coenzyme A  
**Nutrients needed:** pantothenic acid (B5), vitamin C  
**Inducers:**  
**Inhibitors:** deficiency of pantethenic acid (B5), or ascorbic acid (C)  
**Substrates:** amines  
**Notes:** Incidence of slow acetylators – 70% in Middle Eastern populations, 50% in Caucasians, 25% in Asians. This rises to as high a level as 80% among the chemically sensitive population. Their N-acetyltransferase activity is thought to be reduced and this prolongs the action of drugs and other toxic chemicals, thus enhancing their toxicity.

**Amino Acid Conjugation**

Several amino acids, primarily glycine and taurine are used to conjugate with and neutralize toxins. Glycine is the most commonly utilized and is referred to as – *glycination*.

**Primary enzyme:**  
**Co-factor:** glycine, taurine  
**Nutrients needed:** Bile acids are conjugated with glycine and taurine. Taurine deficiency leads to liver congestion. Environmental medicine specialist - Dr. Rae recommends 2 grams/day of taurine during detox.  
**Inducers:** glycine, taurine  
**Inhibitors:** low protein diet  
**Substrates:** - carboxylic acids  
**Notes:** Both glycine and taurine dependent reactions require an alkaline pH: 7.8 to 8.0. Environmental medicine specialists may alkalinize over-acidic patients by administering sodium and potassium bicarbonate in order to facilitate these reactions.

**Glucuronidation**

**Primary Enzyme:** UDP-glucuronosyltransferase (six forms)  
**Co-factor:** UDP-Glucuronic acid  
**Nutrients Needed:** Beta-glucuronidase, regarded as a dangerous enzyme, interferes with the glucuronidation process. A phyto-extract, D-glucarate, has been shown to support the glucuronidation pathway by inhibiting the activity of beta-glucuronidase. D-glucarate may be obtained naturally by emphasizing apples, grapefruit, broccoli, and brussels sprouts in the diet and by supplementing with calcium-D-glucarate. Research has shown D-glucarate inhibited beta-glucuronidase by 57% in the blood, 44% in the liver, 39% in the intestines, and 37% in the lungs.  
**Inducers:** fish oils, cigarette smoke, d-limonene, phenobarbital,  
**Inhibitors:** aspirin  
**Substrates:** carboxylic acids  
**Notes:** Glucuronidation is considered an important detoxification mechanism when sulfation or glycination is diminished or saturated. For most individuals, glucuronidation is a supplemental pathway.
**Glutathione Conjugation**

Glutathione is a tripeptide of glycine, cysteine and glutamic acid.

**Primary Enzyme:** Glutathione-S-transferase  
**Co-factor:** Glutathione  
**Nutrients Needed:** Vitamin C increase glutathione stores by stimulating the rate of glutathione synthesis  
**Inducers:** ellagic acid, brassicia vegetables, d-limonene  
**Inhibitors:** deficiency of selenium, B12, zinc  
**Substrates:** phenols, amines, thiols  
**Notes:** Glutathione is a “hot” topic in the alternative medicine community.

**Methylation**

A common but minor pathway, methylation involves conjugating methyl groups to toxins. The methyl group is attached by various methyl-transferase enzymes to an N, O, or S imbedded in a 6-carbon ring respectively called N, O and S-methylation.

**Primary Enzyme:** methyltransferases (four types – Pheno O-, Catechol O-, N-, S-)  
**Co-factor:** methyl groups – S-adenosylmethionine (SAM)  
**Nutrients Needed:**

- Most of the methyl groups used for detoxification comes from S-adenosylmethionine (SAM). SAM is synthesized from the amino acid methionine, a process which requires the nutrients choline, the active form of B12 – methyl cobalamin, and the active form of folic acid – 5-methyltertahydrofolate.

- The activity of the methyltransferase enzyme is dependent on magnesium and due to the frequency of magnesium deficiency; supplementation with this nutrient will often stabilize chemically sensitive individuals.

**Inducers:** choline, methionine, betain, folic acid, cobalamin  
**Inhibitors:** deficiency of folic acid, B12  
**Substrates:** phenols, amines  
**Notes:** According to environmental medicine specialist Dr. William Rae, the process most often disturbed in chemically sensitive people involves methylation reactions.

**Sulfoxidation & Sulfation** – the next report is devoted to this topic

**Phase III - Efflux**

This report has focused primarily on the liver’s role in the biotransformation process. Though this process also occurs in other tissues - eyes, lungs, intestines, etc – they have received little attention in the medical literature. For example, the small intestine functions predominantly as an absorptive organ extracting nutrients from luminal contents. Yet due to
the significance of its primary role in nutrition; neglected is the importance of intestinal metabolism of non-nutritive dietary constituents - xenobiotics. This is in spite of the fact that the small intestine is the first site of xenobiotic exposure. As a matter of fact, over the course of a lifetime, the gastrointestinal tract processes more than 25 tons of food, which represents the largest load of antigens and xenobiotics confronting the human body.

This outlook is perpetuated by the fact that CYP450 activity declines along the length of the intestine and from the tip of the microvilli to its base. Thus, as the absorptive function of the enterocyte (intestinal absorptive cell) and intestine decline, so does the expression of CYP450. Therefore, levels of intestinal cytochrome p450 are reported to be much less than those found in the liver. This difference between intestine and liver CYP450 activity is often used as the basis for the idea that the intestine is of little importance in the detoxification process.

However, this attitude is changing and the intestines are now being recognized as having a major role in the biotransformation process. This is in part because of the Phase I and Phase II activities found in enterocytes, along with a biochemical mechanism referred to as – antiporter. The antiporter is an energy dependent efflux pump (transport protein), contained within the enterocytes, whose function is to export unwanted compounds immediately back into the intestines. Once the exported unwanted compound arrives back into the intestine one of several things can take place:

1) It can be eliminated in the stool
2) It can re-enter the enterocyte only to be exported back out into the intestine and start the cycle over again
3) It can re-enter the enterocyte and pass into the blood stream and travel to the liver
4) It can re-enter the enterocyte and be acted on by the Phase I CYP3A4 enzymes thereby starting the biotransformation process before it is passed into the blood stream to travel to the liver.

Researchers have found that the antiporter activity in the intestines appears to be co-regulated with the Phase I CYP3A4 enzyme. It has been speculated that this allows more opportunities for Phase I activity to metabolize unwanted chemicals before they are taken into circulation. This makes antiporter activity an important factor in the first pass metabolism of pharmaceuticals and other xenobiotics. First pass effect is the biotransformation of unwanted
compounds by intestinal enzymes and the liver before the compound reaches systemic circulation. This results in lower systemic bioavailability of a parent compound, which is an important effect when pharmaceuticals are involved.

The question then must be asked: if the antiporter mechanism is part of the first pass metabolism and occurs pre-biotransformation or pre-Phase I and Phase II then why is it referred to as Phase III? The answer... after the completion of Phase II conjugation, unwanted compounds are now hydrophilic and ready for excretion from the cell. However, these compounds still remain in the cell and must be transported out of the cell in order for them to be eliminated from the body. This is obviously a vital function in the detoxification process and is accomplished by none other than the efflux transporters or efflux pumps - the same efflux pumps involved in the antiporter mechanism. It is this process, of transporting Phase II conjugates out of the cell, which is now being referred to as Phase III.

Therefore, efflux transporters / pumps serve a dual function with the Phase III terminology used to refer to both functions of:

A. Phase III metabolism – transporters act by eliminating both endogenous and exogenous Phase II conjugated metabolites from hepatocytes and other tissues.

B. Antiporter action – transporters acting as nature’s gatekeeper for cellular entry by eliminating unwanted compounds pre-biotransformation.

More than 350 unique human transporters have been identified. The best known and most studied transporter is the P-glycoprotein. These efflux transporters, like Phase I and Phase II enzymes, work on specific substrates. Efflux transporters can also be induced – increasing the transporters activity, and can be inhibited – causing substrate levels to become higher.

De-Conjugation

Once an unwanted compound completes the biotransformation process and is eliminated from the cell it finds its way into either the urine or the bile. Conjugated compounds released into the bile, may be subjected to the action of hydrolytic enzymes. Hydrolytic enzymes originate from some types of intestinal microflora (bacterial microorganisms), which varies from person to person. One of the functions of these intestinal microflora enzymes seems to throw a wrench into the whole detoxification process by de-conjugating some of the biotransformed compounds.

De-conjugation results in an increase in lipophilic compounds and renders them once again subject to passive uptake. Re-absorbed compounds enter the circulation via the hepatic portal vein, which shunts the compound back to the liver where the compound can, once again, go through the biotransformation process. This process is called entero-hepatic circulation (EHC). A compound may undergo several cycles of EHC resulting in a significant increase in the retention time of a compound in the body and increased toxicity. An example of de-conjugation is:

- Glucuronidation can be reversed (de-conjugated) by an enzyme called beta-glucuronidase. Beta-glucuronidase is found in the intestines and is produced by pathological bacteria. Calcium d-glucurate, a natural substance found in certain vegetables and fruits can inhibit beta-glucuronidase activity resulting in the proper elimination of conjugated toxins.
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