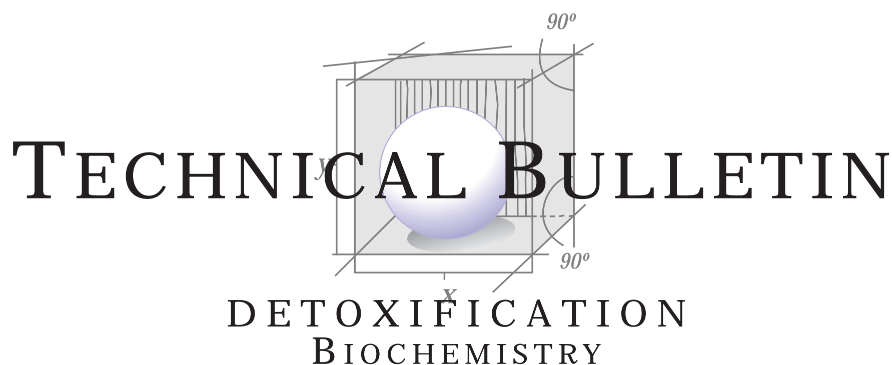
Technical Bulletin



Detoxification Biochemistry

Introduction

Our bodies are exposed to an increasing number of toxic compounds in the environment, as well as to a growing variety of drugs. Given these exposures, the individual's ability to detoxify is now recognized as a key factor to overall health. A significant body of literature suggests an association between the ability of the body to efficiently detoxify and the etiology of various puzzling chronic diseases such as fibromyalgia, multiple chemical sensitivities, and chronic fatigue syndrome.[1] Furthermore, recent research supports the link between chronic neurological diseases, such as Parkinson's,[2] as well as certain types of cancer, with the subject's ability to adequately detoxify.[3,4]

The body uses the process of detoxification - a complex series of reactions - to get rid of molecules (toxins) whose prolonged presence may have damaging effects on tissues or lead to undesirable imbalances. Toxins may be of external origin (also referred to as xenobiotics or exogenous toxins), such as chemicals in the air, water, food additives, or drugs. They may also be generated internally (also referred to as endogenous toxins) as the end-products from the metabolism of hormones, bacterial by-products, and other complex molecules.

In order to successfully integrate detoxification protocols into clinical practice, it is important to review the biochemistry of detoxification and realize the key role played by nutrition. This Technical Bulletin offers a brief review on the subject; more in-depth reviews, containing additional references, are recommended.[5,6] Please refer to the Technical Bulletin: *"Detoxification - Clinical Perspective"* for an overview of the clinical aspects of detoxification.

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DETOXIFICATION OVERVIEW

Detoxification is the conversion of non-polar (lipophilic) toxins into polar (hydrophilic), non-toxic metabolites, for their ready elimination by the excretory organs (kidneys, liver, lungs, and skin). The process serves to reduce the biological half-life of the toxins, to reduce the exposure of the organism to the toxins, and to avoid internal toxin accumulation.

The detoxification process occurs in two classical steps - named Phase I and Phase II - each involving a battery of enzymes of broad specificity. The products generated from Phase I reactions are often reactive intermediate metabolites and/or reactive oxygen species, which may cause tissue damage. The reactions in Phase II generally involve conversion of the intermediate metabolites of Phase I into the final products that are eliminated. In some cases, a toxin may be directly converted via Phase I or Phase II. Although both phases have different characteristics, it is essential that they function in balance with one another in order to minimize the presence of intermediate metabolites and carry through an effective detoxification. The detoxification process is depicted in Figure 1.

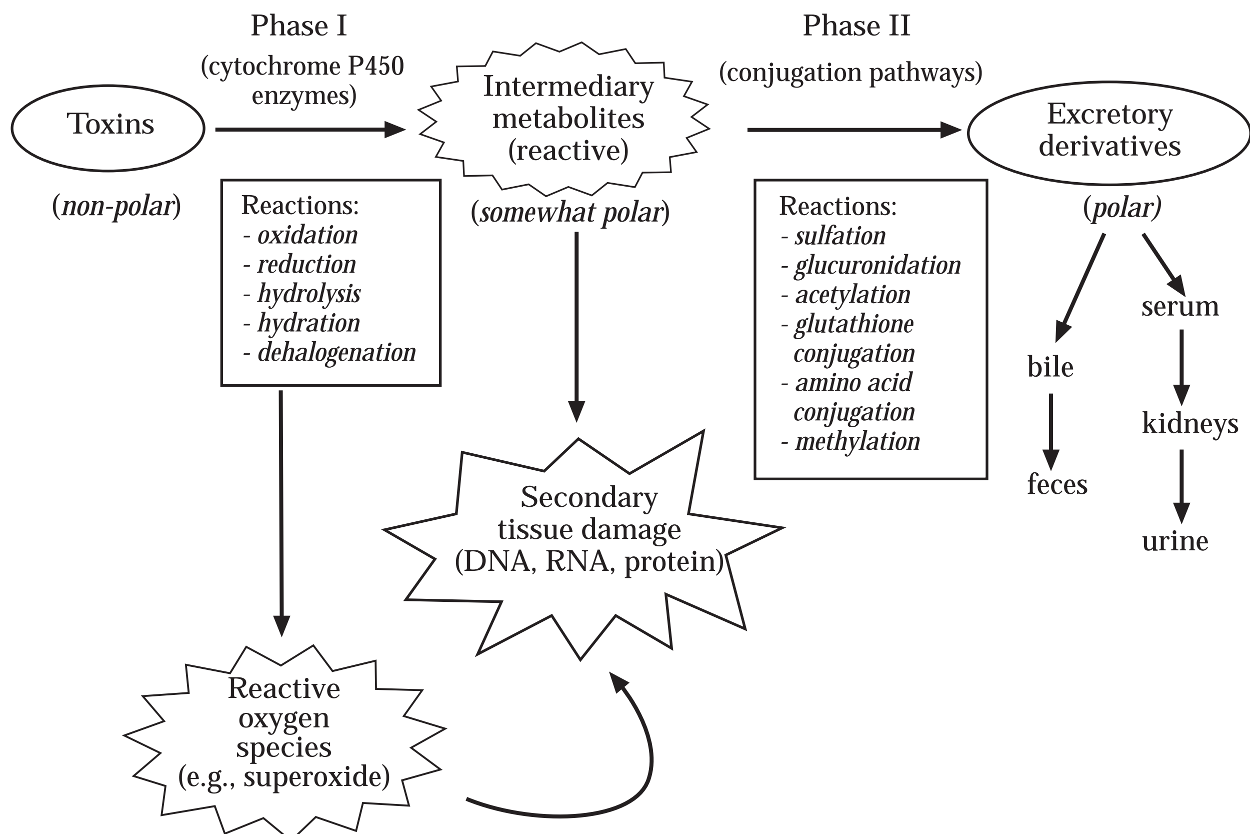

Figure 1. The Phases of Detoxification

While as much as seventy-five percent of detoxification activity occurs in the liver, much of the remainder takes place in the intestinal mucosa wall. Still an additional small percentage occurs in other tissues. Although we usually think of the liver as “the” detoxification site, it makes sense that the intestine also plays an important role in detoxification, since the gastrointestinal lining provides the initial physical barrier to the largest load of xenobiotics, including orally ingested drugs. The GI tract has indeed developed a complex physical and biochemical system to manage this load, as evidenced by the high concentration of detoxification enzymes present in the tip of the villi,[7,8] and by the “antiporter activity” as described later.[9] The GI tract also influences detoxification by hosting gut microflora capable of producing compounds that may either induce or inhibit detoxification.[10,11] Although the liver and intestinal mucosa contain the majority of the detoxification enzymes, the importance of detoxification to the integrity of cells and tissues explains the occurrence of some degree of detoxification activity in all cells.

The Detoxification Process

PHASE I Detoxification System

Initially, toxins are typically non-reactive compounds(e.g., petrochemical hydrocarbons, drugs, and steroid hormones). This means that they do not contain a reactive site that can bond to the water-soluble moieties of the Phase II reactions. In Phase I, these toxins are subjected to oxidation, reduction, or hydrolysis reactions – also referred to as functionalization – which transform them into substances ready for the Phase II process. Infrequently, the end product of Phase I is directly eliminated.[12]

Phase I reactions are catalyzed by a multitude of enzyme activities; the most significant one is the cytochrome P450 (CYP450) supergene family of isoenzymes (mixed function oxidases), which have very broad substrate specificity.[13] The CYP450 enzymes use oxygen and the reduced form of nicotinamide adenosine dinucleotide (NADH) as cofactor, to add a reactive group (i.e., hydroxyl radical) to the substrates. The result of this reaction is the generation of a reactive molecule, which is often more toxic than the parent compound. Unless this intermediate

metabolite is further metabolized by a well-functioning Phase II system, it may react with and cause damage to proteins, RNA, and DNA within the cell. Furthermore, Phase I reactions also generate damaging free radicals.

Over 10 families of CYP450 enzymes have been identified in humans. Each of these families may contain several subfamilies of enzymes, grouped according to the degree of amino acid similarity between the enzyme proteins. The individual CYP450 isoenzymes are thus categorized by family and subfamily. The human liver contains CYP450 activities such as CYP3A4/5, CYP1A1, CYP1A2, CYP2D6, and CYP2C; their relative amounts reflect their importance in the metabolism of drugs and exogenous toxins, as well as endogenous molecules such as steroids.[14,15] The percentage distribution of the CYP450 isoenzymes in the liver is graphically represented in Figure 2.[16]

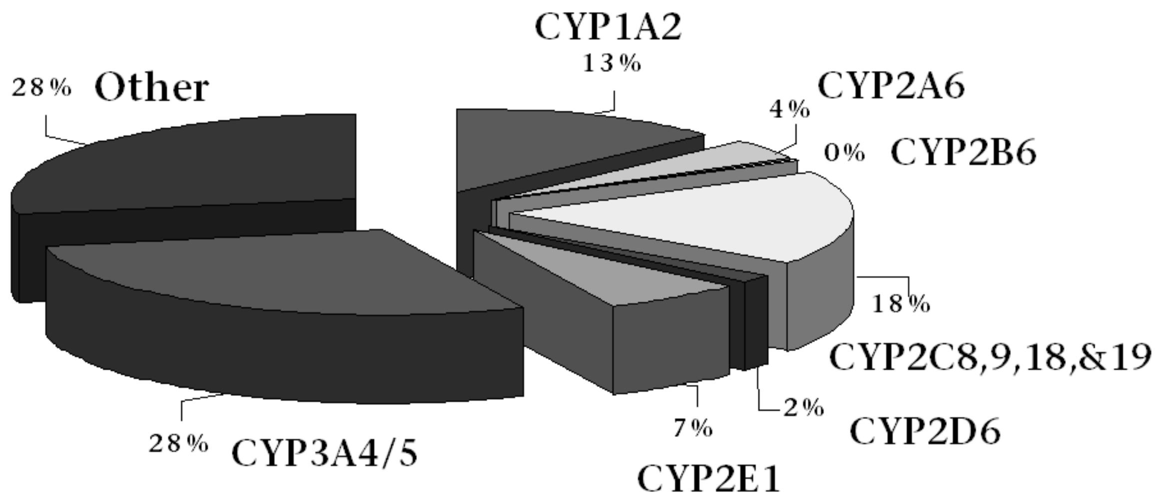

Figure 2. Distribution in the Liver of the Major Cytochrome P450 (CYP450) Enzymes

PHASE II Detoxification System

In this phase, the biotransformed molecules generated in Phase I are conjugated by the addition of a water-soluble group to the reactive site; this increases their solubility and thus facilitates excretion in the urine or bile.[13] Occasionally, in parent toxins with particular chemical configurations, Phase II proceeds directly without intermediary Phase I. The enzyme-mediated conjugation reactions of Phase II – glucuronidation, amino acid conjugation, sulfation, acetylation, glutathione conjugation, and methylation – require the presence of energy in the

form of adenosine triphosphate (ATP), and cofactors obtained through dietary sources.
The main types of enzymes catalyzing Phase II reactions are: glucuronyl transferases, glutathione transferases, sulfotransferases, N-acetyl transferases, N- and O- methyl transferases, amino acid transferases, and epoxide hydrolase.

The Antiporter System, PHASE III Detoxification?

Identification of the intestinal "antiporter activity" strongly suggests a mechanism supportive of detoxification which plays a particularly significant role in the metabolism of drugs and other xenobiotics, and is thus being called the Phase III detoxification system.[17] The antiporter is an energy-dependent efflux pump that moves xenobiotics from the inside to the outside of the cell to decrease their intra-cell concentration.[18] This is thought to be a mechanism allowing reabsorption, and therefore re-exposure of the non-metabolized xenobiotics to Phase I activity, thus increasing the efficiency of detoxification. The resistance to drugs such as chemotherapeutic agents, seen in some individuals, may be due to increased activity of the antiporter system.[18]

The Regulation of Detoxification

A number of factors modulate the activity of the enzymes involved in detoxification – either "inducing" or "inhibiting" them individually or in groups – with various effects on specific detoxification pathways. When the body is burdened with a high amount of a particular xenobiotic, the Phase I and Phase II enzymes involved with the detoxification of that compound can be induced in order to increase its biotransformation. Lifestyle habits such as smoking, xenobiotics from the environment, and medications can cause a selective induction of one enzyme or one phase of detoxification, which may have a negative end-effect on overall detoxification. On the other hand, dietary habits may enhance detoxification by providing nutrients that induce several Phase II enzyme activities. Age and gender, genetic polymorphism, and disease are also important factors that affect the detoxification processes. Below are some examples of regulatory effects by different factors.

Induction

The induction of enzymes involved in detoxification may be caused by substances that selectively upregulate a Phase I enzyme without co-induction of the corresponding Phase II enzyme. Since this may eventually lead to a higher level of the reactive (harmful) intermediate compounds that can cause damage to DNA, RNA, and proteins, it is considered an undesirable effect. Examples include the polycyclic hydrocarbons from cigarette smoke,[19] aryl amines from charbroiled meats,[20] and prolonged intake of the antiepileptic medication, phenobarbital.[21,22]

On the other hand, induction may be caused by substances with beneficial effects on the detoxification processes. Such is the case with many compounds of the flavonoid family found in specific fruits and vegetables, which are capable of inducing multiple Phase II enzyme activities. In general, two of the Phase II enzymes – glutathione S-transferase and glucuronyl transferases – are co-induced. This effect has been proposed as an explanation for the ability of some fruits and vegetables to protect against many types of cancer.[23, 24]

Diet and Nutrients that Support Detoxification

Nutrition plays a significant role in the support of detoxification. In general terms, the reactions involved in detoxification are driven by enzymes, which require cofactors, coenzymes, and other molecules provided through the diet. Specifically:

- Phase I reactions require general support nutrients, which provide cofactors for the CYP450 enzymes carrying out the functionalization reactions.
- Phase II conjugation reactions require the specific nutrients that are used as cofactors for each Phase II enzyme, as well as the specific molecules that are attached in the different conjugation pathways (i.e., sulfate, glucuronic acid, glutathione, specific amino acids). Additionally, since Phase II requires ATP, nutrients providing support for ATP production (energy) are also needed.
- Defense against oxidative stress requires the support of compounds with antioxidant properties, which are helpful to prevent the potential tissue damage from the highly reactive oxygen species often produced during Phase I activity. Antioxidants help by

"neutralizing" these reactive oxygen species.

Figure 3 summarizes some of the most important nutrients that support detoxification.

NUTRIENTS supporting PHASE I CYP450 enzymes

- Riboflavin (vit. B_2)[27]
- Niacin (vit. B_3)[28]
- Vitamin C[27]
- Thiamine (vit. B_1)[27]
- Folic Acid[28]
- Flavonoids[27]
- Phospholipids[27]
- Indoles (cruciferous)[27]
- Coumarins[27]
- Methylxanthines[27]
- Minerals:[28] Iron, Selenium, Zinc, Copper, Magnesium

ANTIOXIDANTS and NUTRIENTS supporting INTERMEDIATE PHASE

- β–carotene (vit. A)[30]
- Ascorbic Acid (vit. C)[30]
- Tocopherols (vit. E)[30]
- Minerals:[29] Selenium, Iron, Zinc, Copper, Manganese
- Flavonoids[30]
- Coenzyme Q_{10}[31]

NUTRIENTS supporting PHASE II conjugation

- Flavonoids (cruciferous vegs.)[32]
- Indole-3-carbinols (cruciferous vegs.)[32]
- Carnosic Acid (rosemary)[33]
- Diallyl sulfides (garlic)[34]
- Isoflavones (soy)[35]
- Ellagic Acid (red grape skin)[36]
- Curcumin (turmeric)[37]
- Monoterpenoids [Mormilin, Limonene] (citrus)[38]

Figure 3. Nutrients that Support Detoxification

Inhibition

The Phase I and Phase II detoxification enzyme activities can also be inhibited through several mechanisms. In examples where the same detoxifying enzyme is used by two or more compounds, a dramatic increase of one will inhibit the detoxification of the other – a process called competitive inhibition.

Inhibition by Drugs

A particularly significant mechanism of inhibition is the binding of some drugs. For example, certain H2-blockers (cimetidine), macrolide antibiotics, and Selective Serotonin Reuptake Inhibitors (SSRIs) can bind to the reactive site of one or more of the Phase I detoxification enzymes and competitively inhibit their activity.[37,38]

This enzyme inactivation, and the resulting defective pathways, may have widely different manifestations such as overdose of a medication or a lack of efficacy due to increased elimination of the drug.[14,39]

Inhibition by Depletion of Nutrients

The depletion or insufficiency of any cofactor needed in the detoxification process is another significant inhibition mechanism, particularly for Phase II activities. One of the most sensitive activities to cofactor depletion is sulfation, which depends on the availability of sulfur-containing amino acids or inorganic sulfate from dietary intake. Fasting or consumption of high amounts of acetaminophen which is metabolized by sulfation, may deplete sulfate reserves and thus inhibit detoxification.[40]

Effects of AGE and GENDER

Age can play an important role in detoxification activity. Newborns and very young children have detoxification reaction rates that are much slower than adults. Equally important is the influence of gender in some detoxification pathways. For example, the effect of progesterone in inducing one of the families of Phase I enzymes (CYP3A4) seems to explain the fact that many women require higher doses of an antiepileptic drug (phenytoin) when pregnant.[17] Hormonal effects on Phase I may also explain the differences in drug doses needed by premenopausal women as compared to men and postmenopausal women.[41]

Effects of GENETIC POLYMORPHISM and DISEASE

Genetic individuality has a place in the detoxification picture. Genetic differences in the ability to metabolize xenobiotics are related to the presence of different "versions" of the gene encoding that activity, or genetic polymorphism. Poor metabolizers (PM) of many drugs have a gene that encodes a slower acting version of a CYP450 enzyme, which is involved in the detoxification of the respective drug. Many antiarrhythmics, antidepressants, and antipsychotics are metabolized by the Phase I CYP2D6 activity. Approximately seven to ten percent of Caucasians are poor metabolizers, that is, they carry a genetic polymorphism of the CYP2D6 gene that encodes a slower activity, and therefore metabolize these drugs at a slower rate. Lowering the dose of these drugs in PM subjects may avoid some of the adverse side effects associated with them.[42] Likewise, genetic polymorphism may explain the correlation between a slow Phase II N-acetyl transferase activity and the incidence of Parkinson's disease, and the higher risk for some types of cancer.[43]

An individual's health condition may also be an important factor in determining the level of detoxification at any particular time. Although this is an area of continuing study and not fully understood, there is evidence that many conditions (e.g., insulin-dependent diabetes and obesity) can lead to changes in the detoxification patterns.[44] Clearly, impairment of normal liver function caused by a chronic or acute disease will affect detoxification activity. Due to the separate localization of the Phase I and the Phase II activity within the liver cells (Phase I is primarily in the membrane, whereas Phase II is primarily in the cytosol), and the compartmentalization of the activities of both phases within the organ, diseases that affect selective areas of the liver will also selectively affect detoxification activities.[45]

Summarizing, research over the past decade has increased our understanding of detoxification. Key to this understanding is the importance of identifying the individual's "detoxification personality" and the relationship between proper detoxification and adequate nutrition.

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