Detoxification: The link to life

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Abstract

As the American population becomes sicker, detoxification is a missing link to improved health. Metabolic dysfunctions and environmental insults present a challenge to detoxification. The prevalent practice in medicine today is to offer medications that address symptoms but not root causes. Symptoms may give ambiguous information as to cause of disease. Without addressing root causes and imbalances, further imbalances may be caused by the measures given to address symptoms. Our present "medication-for-symptoms medical system" is no longer acceptable. As it has been said that genes only create possible predisposing issues, and that if the "right" stimuli is never encountered then the problem is only theoretical, then, too, we must apply this idea of environmental insult to the medical treatments we employ: Any detoxification protocol must be undertaken with due diligence, ensuring that the patient's body will be able to handle the possible consequential effects of a given detoxifying agent's action upon other physiological pathways and systems.

Keywords: detoxification, metabolism, enzymes, pathway

Detoxification is the missing link as the American population becomes sicker. As medicine stands today, all we think we can do is offer medication to reduce symptoms. We need to consider what is happening today in the environment, food and water sources, and air quality. Through these, we are exposed to heavy metals, pesticides, herbicides, fungicides, solvents, petrochemicals, and lipid peroxidases. Other sources of toxins include: fish with mercury; vaccines with thimerosal, aluminum and formaldehyde; fertilizers; chicken with arsenic; soy with aluminum; and margarine with nickel. Food sources contaminated with antibiotics and steroids add to the list of the toxins our body must clear. Our bodies begin to malfunction, leaky gut begins, and these toxins and undigested food constituents seep into our bloodstream. Our gastrointestinal tract becomes dysbiotic leading to the acceleration of bacterial endotoxins, which further aggravate malabsorption, maldigestion, and toxic overload.

The government agencies that are supposed to help are being influenced by forces not in the citizens' best interest. Insurance companies are paying attention to the immediate bottom line, unfortunately without regard for what really needs to be done or provided for the consumers. A higher premium plus less coverage exemplifies the present situation with insurance. It is in this perilous environment that we find ourselves stranded. It is our body, mind and spirit that must find a way to survive.

One of the major keys to survival is detoxification. This is a two-phased system that takes pollutants, waste, hormones, neurotransmitters, and medication and prepares them for excretion. The conversion for many products is from fat-soluble to water-soluble, which can then be excreted. This detoxification process handles all environmental exposures, as well as all in vitro preparations. As with any complicated process, there are ways for it to malfunction. This process may not be readily visible with immediate symptoms, which is what we are trained to look for. This malfunction process is slow and insidious, which proceeds from the molecular level gradually and methodically to the level of tissues and then organs.

The complexity, diversity and non-specificity of what is seen all contribute to the difficulty of diagnosis. On the surface, many symptoms present are usually treated without looking for the true cause of the problem. One of the great and far reaching malfunction side effects is free radical production and damage to multiple systems. The two-phase detoxification system must be capable of clearing all substrates and minimizing the complications. Complications in the conversion process will lead to damage, which over time leads to "disease". The "disease" will be foreshadowed by symptoms, which can give ambiguous clues to the cause; this in turn leads to multiple medications to alleviate the symptoms.

The present epidemic of autism is but a tip of the iceberg. The complex problems including ASD, chronic fatigue, fibromyalgia, asthma, and other, which are being set in motion, will confuse and baffle many. Treating symptoms will give temporary relief, but then symptoms will reappear or other symptoms will begin to surface. The addition of multiple medications will only lead to frustration and further deterioration. It is from this point that one must now ask, "Why?" Why did this originally start? Why did the new symptoms begin? Why didn't the prescribed medication have the expected affect? Why have more symptoms begun? It is through the asking of "why" that many will begin to realize it is a basic process and functioning of the body that is not coping.

The detoxification system is at the basis of many multisystem malfunctions. We will show how the intricate design of the body is manifested. Each reaction, cofactor, constituent and the intermediate compounds are an intricate part of the puzzle. Each new toxin is decomposed through the two-phased system. Problems can develop if phase one is faster than phase two. This discrepancy will lead to free radicals, which are more toxic than their precursors. This leads to increased oxidative stress,

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leading to cellular damage, which can affect mitochondrial, and eventually the cellular, DNA.

Oxidative stress results in multisystem stress and dysfunction [1-3]. The following are diseases or conditions partly caused by oxidative stress:

1) Inflammation and immune injury: glomerulonephritis, vasculitis, autoimmune diseases, rheumatoid arthritis, hepatitis
2) Ischemia: stroke, myocardial infarction, arrhythmias, angina, organ transplantation, inflamed rheumatoid joint, frost bite, Dupuytren's contracture, cocaine induced fetal damage
3) Drug and toxin induced reactions: heavy metal reactions, encephalopathy, antibiotic reactions, nitro radicals, azo compounds, radiation damage
4) Iron overload: idiopathic hemochromatosis, dietary iron overload (Bantu), thalassemia, other chronic anemias, nutritional deficiencies, alcoholism, cardiopulmonary bypass, fulminant hepatic failure, prematurity, alcohol related iron overload, cancer chemotherapy/radio therapy
5) Radiation injury: consequences of nuclear explosions, accidental exposures, radon gas, cataracts
6) Aging: premature aging, age related diseases, aging itself
7) Red blood cells: phenyl hydrazine, primaquine, lead poisoning, protoprophyrin, malaria, sickle cell anemia, favism, Fanconi's anemia, hemolytic anemia of prematurity, chemotherapy
8) Respiratory tract: effects of cigarette smoke, snuff inhalation, other smoke inhalations, emphysema, chronic obstructive pulmonary disease, hyperoxia, bronchial pulmonary dysphasia, exposure to air pollutants (0_3, NO_2, SO_2, diesel exhaust), acute respiratory distress syndrome, mineral dust pneumoconiosis, asbestosis, carcinogenicity, bleomycin toxicity, paraquat toxicity, skatole toxicity, asthma, cystic fibrosis
9) Heart and cardio vascular system: alcohol cardiomyopathy, Keshan disease (selenium deficiency), atherosclerosis, athracycline cardiotoxicity, cardiac iron overload
10) Kidney: autoimmune nephritic syndromes, aminoglycoside nephrotoxicity, heavy metal nephrotoxicity (Pb, Cd, Hg), myoglobin/hemoglobin damage, hemodialysis, transplant storage/rejection
11) Gastrointestinal tract: Betel nut related oral cancer, liver damage from endotoxins, liver damage from hydrocarbons, exposure to diabetogenic agents, NASAID-induced gastrointestinal lesions, oral iron poisoning
12) Brain/nervous system/neuromuscular disorders: Hyperbaric oxygen, Vitamin E deficiency, exposure to neurotoxins (e.g., heavy metail), Alzheimer's disease, Parkinson disease, Huntington's chorea, stroke, neuronal ceroid lipofuscinoses, allergic encephalomyelitis, aluminiun overload, sequale of traumatic injury, muscular dystrophy, multiple sclerosis, amyotrophic lateral sclerosis, Guam dementia
13) Eye: Cataract, ocular hemorrhage, degenerative retinal disease/macular degeneration, retrolental fibroplasias, photic retinopathy, penetration of metal objects
14) Skin: UV radiation, thermal injury, porphyria, hypericin, exposure to other photosensitizers, contact dermatitis, baldness

Full list plus comments available in *Free radicals in disease process: a complilation of cause and consequence* by JMC Gutterridge (Free Rad. Res. Commun. 1993,19:141) and reference [3].

As we can see from the list of diseases, this covers all systems and all specialties. It is from this platform that we must ask "why" and then proceed from the symptomatic approach and begin to examine system malfunction. It is at this juncture that we must examine the detoxification system. This system is made up of two phases: substrates proceed through Phase I and then into Phase II.

Phase I is made up of several super families of enzymes. The types of reactions that are carried out by the cytochrome P450 enzymes are as follows: aromatic hydroxylations, aromatic epoxidations, aliphatic hydroxylations, alkene epoxidation, N-dealkylation, O-dealkylation, S-dealkylation, N-hydroxylation, S-oxidation, aldehyde oxidation, androgen aromatization, halothane oxidation, halothane reduction, arginine oxidation, cholesterol side-chain cleavage, dehydrogenation, dehalogenation, azoreduction, deamination, desulphation, amide hyudrolysis, ester hydrolysis, peroxidation and denitration [4:77]. This group of enzymes is the first to begin the processing of xenobiotics in the conversion from fat-soluble to water-soluble for excretion. These enzymes have a broad specificity so that they can process a wide variety of substrates, and at the same time are able to adapt to new substrates that they encounter. The processes carried out in Phase I detoxification have the potential to create more toxic intermediates; these intermediates must be quickly processed through Phase II. If this is not the case, then these reactive species are released into the cell [4-6]. This toxic process can be exemplified by the epoxidation of polycyclic aromatic hydrocarbons, this process yields electrophiles and several of these are highly chemically reactive [5;6:384 (Table 10.4) and 401 (Table 10.8)]. If the system is working well then these compounds will be conjugated quickly into neutrophiles. However if Phase II is not able to keep up with the rate of Phase I then the release of these potential carcinogens will attack proteins and DNA. These detrimental reactions can be controlled if the activity of the epoxide hydrolases and glutathione-S-transferase are in sufficient quantities and quality to metabolize the toxic intermediates; this is but one of the Phase II reactions.

The conversion process from lipophilic to hydrophilic can proceed in different directions when one considers the functional groups (i.e., O_2, OH, SH, NH, etc.) that are to be converted. Phase I is considered as functionalization, while Phase II is called conjugation due to the addition to the compounds. Functional groups can be considered electrophilic if they have alpha, beta unsaturated carbonyl groups, and nucleophilic if they have alcoholic, phenolic hydroxyl groups, amino or sulfhydryl groups. Electrophiles have a significantly greater possibility of being cytotoxic and/or mutagenic due to their increased affinity for proteins, RNA and DNA, which is electron rich [5:83-104]. It is the Phase II terminal conjugations to the functional groups that extinguish the electrophilic threat, by making them inactive or unable to react with proteins, RNA, DNA or further interact with receptors. Nucleophilic compounds do not

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bond covalently and are less likely to bind to biologically active macromolecules.

The Phase I major enzymes are the oxidoreductases and hydroxylases; these two major groups are further divided into super families. The major super family is the cytochrome P450 dependant monooxygenases (CYP), flavin-containing monooxygenases (FAAO), monoamine oxidase (MAO) and cyclooxygenases (COX) [5:85]. Most of these enzymes act by adding oxygen to a substrate or removing electrons from it. Hydrogen can be removed or added to substrates by dehydrogenases, e.g., alcohol and aldehyde and/or by reductases, e.g., Carbonyl which assists with xenobiotic metabolism. The other major activity is carried out by the hydroxylases, which hydrolyze esters, amides, epoxides and glucuronides. In our consideration of Phase I, the major enzyme super family we will discuss will be the Cytochrome P450-dependant monooxygenases, which are the oxidoreductase enzymes. These enzymes tend to be fixed in the endoplasmic reticulum and are predominately identified by their absorbance maximum at 450 nm [5:86]. However the complexity of isolation, induction and isoenzymes has lead to the expanding numbers of enzymes. At present 38 different cyp genes have been identified in humans with groupings by familiarity; 14 different families have been identified with 40% of their amino acid sequences identical. They are further divided into subfamilies, which consist of 55% identical amino acid sequences [5:86]. These enzymes transfer one atom of molecular oxygen into a substrate; the remaining oxygen is reduced to water, with the hydrogen being provided by NADPH (this process is far more complicated, and for a step by step reaction discussion see: Ortiz de Montellano, PR, Cytochrome P450-Structure, Mechanism and Biochemistry). This process -- whether epoxidation, hydroxylation, or desaturation -- proceeds through a number of sequential steps. There is some evidence that NADPH-P450 reductase and several CYP450 come together and function as a complex [5:88 (Fig. 2)]. In the center of the complex is an iron in the Fe^{3+} state; an enzymatic pocket is formed where substrates are engaged and the conversion/reduction from Fe^{3+} to Fe^{2+} via NADPH-450 reductase produces 1 electron transfer and O^2 is bound to the Fe^{2+}. This is then further reduced via NADH-dependent cytochrome b5 reductase resulting in a highly reactive $Fe^{2+}O^{2-}$ that will transfer oxygen to a substrate or release active oxygen species like hydrogen peroxide. These steps require adequate cofactors (NADH/NADPH) and have the potential to release reactive oxygen species if that reacive groups is blocked [6:1-34].

The cytochromes are divided on the basis of function. In 1977, Rendic and DiCarlo broke the cytochromes by the types of plant chemicals and drug metabolism through each family: 1A—flavones, psoralens, phytoalexins, aflotoxin B1, tannis; 2A—coumarin, xanthotoxin; 2B—cocaine, nicotine; 2C—betulinic acid; 2D—quinine, quinidine, yohimbine, sparteine; 2E: ethanol, ethylene glycol; 3A—quinine, quinidine, pyrrolizidine alkaloids, aflotoxin B1 [7]. The CYP1 family is broken down into the A and B families: CYP 1A1, CYP1A2 and CYP1B1. The CYP1A1 is located and coder for at the genetic location 15q22-q24 [4:40 (Table 2.6)] with nuclear receptor control through AhR and ARNT [4]. CYP 1A1 metabolizes the polycyclic aromatic hydrocarbons (PAH); these are potentially carcinogenic pollutants, which are activated by the detoxification system. In the substrate form they are inert, but through the oxidative metabolism they become mutagenic compounds. The CYP 1A1 substrates include: acetaminophen, amitryptyline, chlorinated benzenes, caffeine, coumarin activation, clomipramine, clozapine, cyclobenzene, estridol (2-hydroxylation), flexeril, fluvoxamine (luvox), haloperidol, imipramine N-DeMe, mexiletine, naproxen (in part), ondansetron (in part), planacetin, propanolol, riluzole, ropivacaine, tacrine, testosterone, theophylline, verapamil®, warfarin, zileuton, and zolmitriptan [8]. Aside from the substrates, the cytochromes can be affected by other compounds. Inhibitors may or may not be substrates but they will reduce the enzyme activity when they are present, these include: amiodarone, cimetidine, fluroquinolones, fluvoxamine, furafylline, interferon (possibly), methoxansalen, mibefradil, N_2O, propolol, ticlopidine, apigenin and benzoflavone [8]. Another group of compounds will induce the enzymes when they are present; these may or may not be substrates: these include: polycyclic aromatic hydrocarbons, cigarette smoke, charbroiled foods, beta-naphthoflavone, insulin, methyl cholanthrene, modafinil, nafcillin, omeprazole and cruciferous vegetables [8]. The polycyclic aromatic hydrocarbons will induce the transcription of the CYP1A1 gene by activating the aryl hydrocarbon receptor. Therefore, with exposure, the induction of CYP1A1 will increase and transform these into toxic intermediates. When there is no exposure, there is no CYP1A1 in the liver, but may be found in the lymphocytes and the lungs [5:83-109].

CYP 1A2 is principally doing the 2-hydroxylation, its location is 15q22-q24 [4:1-210]. This enzyme is always found in the liver and it also handles polycyclic aromatic hydrocarbons, also activating procarcinogens. This usually accounts for a smaller amount of the CYP1A constituents but this may vary with each individual. Aside from activation of procarcinogens it also activates hepatotoxic mycotoxin aflotoxin B1 [5:83-109]. Substrates include: polycyclic aromatic hydrocarbons, aromatic amines, phenacetin, caffeine, warfarin, aflotoxin B1, estradiol, aryl amines [5:Table 2]. Inhibitors include: furafylline and induction can be seen with polycyclic aromatic hydrocarbons and 2,3,7,8-tetrachlorodibenzene [4:87 (Table 4.8)].

CYP1B1 also participates in the metabolism of polycyclic aromatic hydrocarbons; one of its major roles is the 4-hydroxylation of beta-estradiol: the estradiols affect the Ah receptors and thereby stimulate their own toxic intermediates. The ligand binds to the receptor then is released and transported to the cell nucleus and the targeted genes are promoted [4:14,89]. **Substrates include:** amitryiptyline, clomipramine, imipramine, acetaminophen, caffeine, clozapine, coumarin activation, estradiol, estrone (4-hydroxylation), heterocyclic amines, naproxen, propnolol, tacrine, testosterone, theophylline inhibitors: cimetidine, ciprofloxacin, erythromycin, fluvoxamine, pyrene, ticlopidine, grapefruit juice, ginseng. **Inducers:** omperazole, phenyltoin, Phenobarbital, rifampin, polycyclic aromatic hydrocarbons, cigarette smoke, charbroiled foods [8].

The CYP1 subfamily is susceptible to stimulation by estrogens; these can affect both the AhR receptors. These receptors can be stimulated by endogenous, phytoestrogens and xenoestrogens. This interplay can have an effect on hormone homeostasis, which can lead to increased cancer in susceptible people. The other complication that may occur is blocking of receptors

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by false transmitters/hormones—this leads to unbalanced activity hormonal and neural activity. These actions can be traced to nuclear receptor activity shown by Lake and Lewis in 1996 [9].

The CYP2A subfamily is complexly involved in the detoxification of testosterone [10] and they also metabolize drugs. CYP2A6 has the following profile. **Substrates:** nicotine, nitrosamines, halothane, methoxyflurane, valproic acid, disullfram, aflotoxin B1, coumarin, losigamone, SM-12502, fadrozole, cotinine, 534U87 and butadiene. **Inhibitors include:** grapefruit juice, pilocarpine and hepatitis A [9].

The CYP2B subfamily enzymes preferentially metabolize: 4-trifluoromethyl-7-ethoxycoumarin, bupropion, cyclophosphamide, ifosfamide, 7-pentoxyresorufin, antipyrine, benzphetamine, cocaine, nicotine, phenobarb, allylisopropamide, chloramphenicol, DDT, heptachlor, methoxychlor, phenyltoin, phenylbutazone Induced by phenobarbital Inhibitors include: octylamine, 2-phenylimidazole, orphenadrine and SKF-525A [4:91 (Table 4.2)]. Honkakoski and Negishi in 2000 showed that the induction is through an orphan receptor-constitutive androstane receptor [11].

The CYP2C subfamily has been shown to have a clear demarcation as to the preferred substrates [12]. This subfamily constitutes approximately 20% of the liver CYP content [5:89]. The collection of CYP2C pathways tends to have similar substrates; however there are some alterations seen in CYP2C19, which are specific to it [13]. This subfamily is divided into CYP2C8, CYP2C9 and CYP2C19; all of these are found in the liver, but only CYP2C9 is also found in the kidneys [5:89]. Rendic and DiCarlo in 1997 showed that CYP2C8 handled the total 6-alpha-hydroxylation [7]. Below, we review the enzymes of CYP2C9 and CYP2C19 as to substrates, inhibitors and inducer.

CYP2C9 **substrates:** ibuprofen, diclofenac, S-naproxen, Piroxicam, meloxicam, suprofen, amitriptyline, angiotensin, carvedilol, celecoxib, chloramphenicol, clomipramine, coumadin, diazepam, diclofenacdronabinol, fluoxetine, flurbiprofen, formoterol, glipizide, glyburide, hexobarbital, hyzaar, imipramine, indomethacine, irbesartan, isoniazid, losartan, Phenobarbital, phenyltoin, piroxicam, retinoids, rosiglitazone, sildenafil, sulfa drugs, suprofen, tamoxifen, THC, tolbutamide, torsemide and warfarin **Inhibitors:** fluvoxamine, paroxetine, sertraline, fluoxetine, Itraconazole, ketoconazole, fluconazol, amiodarone, cimetidine, chloramphenicol, disulfram, efavirenz, fenofibrate, flurouracil, fluvastatin, gemfibrozil, imatinib, isoniazid, lovastatin, metronidazole, omperazole, phenylbutazone, probenicid, retonavir, sulfamethoxazole-trimethoprim, sulfaphenazole, teniposide, ticlopidine, zafirlikast, teniposide, and possibly garlic and St. John's wart **Inducers:** aprepitant, carbamazepine, ethanol, Phenobarbital, phenytoin, primidone, rifampin, rifapentine and secobarbital [8].

CYP2C19 **substrates:** phenyltoin, S-mephenytoin, phenobarbitone, esomperazole, omperazole, lansoprazole, pantoprazole, rabeprazole, amitryptyline, clomipramine, desipramine, imipramine, carisoprodol, citalopram, cyclophosphamide, diazepam, felbamate, formoterol, hexobarbital, indomethacine, R-mephobarbital, moclobemide, nelfinavir, niliutamide, pentamidine, propanalol, premarin, primodone, progesterone, proquanil, teniposide, thioridazine, tolbutamide, R-warfin, voriconazole **Inhibitors:** fluoxetine, sertaline, paroxetine, fluvoxamine, citalopram, omeprazole, lansoprazole,cimetidine, fluconazole, ketoconazole, voriconazole, delavirdine, efavirdine, felbamate, fluvastatin, indomethacin, isoniazid, letozole, modafinil, oxcarbazepine, probenicid, retonavir, telmisartan, ticlopidine, topiramate, ginko biloba, kava kava, and possibly garlic, St. Johns wart and epigallacatechin **Inducers:** Carbamazepine, norethindrone, prednisone, Phenobarbital, phenyltoin and rifampin [8].

The CYP2D subfamily is usually associated with some basic drugs and other xenobiotics with nitrogen groups.

CYP2D6 is found in the liver and the gene local is 22q13.1 [5]. **The substrates:** amytriptiline, clomipramine, desipramine, doxepin, fluoxetine, fluvoxamine, imipramine, mirtazapine, nortriptyline, olanzapine, paroxetine, trazodone, venlafaxine, haloperidol, perphenazine, quetiapine, risperidone, thioridazine, alprenolol, bisoprolol, bufarolol, carvedilol, metoprolol, penbutolol, pindolol, propanolol, timolol, propafenonme, amphetamine, atomoxetine, cevimeline, chlorphenaramine, chlorpromazine, cimetadine, clozapine, codeine, cyclobenzaprine, debrisoquine, dexfenfluramine, dextromethorphan, dolasetron, donepezil, doxepin, encainide, fenfluramine, fentanyl, fexofenadine, flecainide, fluphenazine, formoterol, galantamine, hydrocodone, lidocaine, loratidine, maprotiline, meperidine, methadone, methamphetamine, methoxyamphetamine, metoclopramide, mexiletine, minaprine, morphine, ondansetron, perhexiline, phenacetin, phenformin, propoxyphene, oxycodone, quanoxan, risperidone, simvastatin, sparteine, tamoxifen, tolterodine, tramadol and venlafaxine [8:chart 1, m-5]. **Inhibitors:** citalopram, clomipramine, desipramine, fluoxetine, fluvoxamine, nefazodone, paroxetine, sertaline, venlafaxine, halperidole, perphenazine, risperidone, thioridazine, amiodarone, bupropion, celecoxib, chloroquine, chloropromazine, chlorpheniramine, cimetidine, cocaine, diphenhydramine, doxorubicin, fluphenazine, halofantrine, haloperidol, hydrochloroquine, imatinib, levomeproamide, methadone, mibefradil, moclobemide, propafenone, propoxyphene, ranitidine, ritonavir, prolixin, thioridazine, quinacrine, quinidine, quinine, terbinafine inducers: carbamazaapine, dexamethasone, ethanol, Phenobarbital, phenyltoin, primidone, rifampin ritonavir St. Johns wart [8:Chart 1].

There are some reports that certain alleles are linked to the formation of Parkinson's Disease. The polymorphisms, which appear in the CYP2D6, are associated with drug toxicities. This occurs by prolongation of the drug half-life due to an inactive allele. There is some evidence by Hohkakoski and Negishi that the nuclear receptors may be estrogen and possibly hepatocyte nuclear factor (HNF-4) [11].

The CYP2E subfamily of enzymes is hydrophilic and metabolizes a wide range of substrates. They also activate some procarcinogen; if activated by ethanol, there is an increased risk of activating the procarcinogens, and it can also generate reactive oxygen species from the metabolism of acetaminophen. Along with these medications, the CYP2E also metabolizes industrial solvents and petrochemicals.

CYP2E1 substrates: enflurane, halothane, isoflurane, methoxyflurane, sevoflurane, acetaldehyde, acetaminophen, aniline, benzene, chlorzoxazone, dapsone, dichloromethane, N, N-dimethylformamide, disuluram, ethanol, isoniazid, ketones, nitrosamines, styrene, theophylline, trichloroethylene, vinylbromide inhibitors: disulofuram, dithiocarbamate, chlormethiazole, black tea, broccoli (isothiocyanates), epigallocatechin, ellagic acid, garlic, watercress and possibly ginseng inducers: acetone, alcohol, isoniazid, pyrazole and obesity [8:Chart 1]

Lewis described other compounds: azoxymethane, 4-nitrophenol, carbon tetrachloride, dimethylnitrosamine, nitrosopyrrolidine, dimethyl sulphoxide, p-xylene, pyridine, pyrazole, imidazole, benzene, butan-2-ol, acetonitrilea and diehtylnitrosamine [14]

With this wide array of substrates, if these accumulate then this may affect half-life. When this occurs the heme iron is in

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the high-spin state which can produce reactive oxygen species. This may be part of the ethanol-induced damage. This may be part of the oxidative damage produced by obesity and possibly halothane induced hepatitis type II and I [3:554–5,573–6,729].

The final CYP subfamily to be discussed is CYP3A; of all of the subfamilies this has the greatest diversity in the base of substrates and molecular weight of substrates [15]. Gonzalez and Gelboin in 1994 showed that some procarcinogens are activated by this subfamily, specifically aflotoxin B1 [16]. Guengerich in 1999 showed that CYP3A4 genes were regulated by glucocorticoid receptor [17]. This is the largest percentage of cytochromes in the liver.

CYP3A4 substrates: budesonide, cortisol, dexamethasone, fluticasone, hydrocortisone, hydrocodone, methylprednisone, mometasone, prednisone, prednisolone, androstenedione, DHEA, Estraderm, estrace, progesterone, progestins, testosterone, ethinyl estradiol, desogestrel, etonogestrel, norethindrone, levonorgestrel, itraconazole, ketoconazole, minonazole, voriconazole, amytriptyline, citalopram, clomipramine, imipramine, nefazodone, mirtazapine, sertraline, trazodone, ventlafaxine, alprazolam, diazepam, midazolam, temazepam, triazolam, buspirone, haloperidol, zolpidem, clathromycin, clindamycin, erythromycin, esomeprazole, lansoprazole, omeprazole, pantoprazole, rabeprazole, astemizole, chlorpheniramine, fexofenadine, lortadine, terfenadine, atrovastatin, lovastatin, simvastatin, amlodipine, bepiridil, carbamazepine, cisapride, diltiazem, felodipine, lercanidipine, nicardipine, nifedipine, nimodipine, nisoldipine, nitrendipine, verapamil, ritonavir, saquinavir, indinavir, nelfinavir, lopinavir, nevirapine, delavirdine, efavirenz, amprenavir, bexarotene, busulfan, cyclophosphamide, docetaxel, doxorubicin, etoposide, exemestane, fluvestrant, gleevec, ifosfamide, imatinib, irinotecan, letrozole, paclitaxel, taxol, toremifene, vinblastine, vincristine, vinorelbine, aflotoxin, alfentanyl, almotriptan, amiodarone, aprepitant, benzop;yrene, bromocriptine, buprenorphine, cannabinoids, cafergot, caffeine, cevimeline, cillostazole, cisapride, clopidogrel, cocaine, codeine-N-demethylation, cyclobenzapine, cyclosporine, dapsone, dextromethorphan, dihydroergotamine, disopyramide, dofetilide, dolasetron, donepezil, dronabinol, dutasteride, eplerone, ergotamine, ethosuximide, fentanyl, finasteride, flutamide, galantamine, glyguride, isradipine, levobupivacaine, loidocaine, losartan, methadone, mifepristone, modafinil, montelukast, nateglinide, ondanestron, oxybutynin, pimozide, piglitazone, quetiapine, quinidine, quinine, repaglinide, rifabutin, rifampin, salmeterol, sibutramine, sildenafil, sirolimus,tacrolimus, tamoxifen, tiagabine, tolterodine, topiramate, tramadol, trimetrezate, valdecoxib, R-warfarin, zaleplon, zileuton, ziprasidone, zonisamide inhibitors: clotrimazzole, itraconazole, ketoconazole, fluconazole, ciprofloxacin, clarithromycin, erythromycin, metronidazole, norfloxzcin, delaviridine, indinavir, nelfinavir, ritonavir, saquinavir, acitretin, amiodone, ampernavirf, aprepitant, cimetidine, cyclosporine, danazol, diltiazem, diethyl-dithiocarbamate, efavirenz, ethinyl estradiol, fluoxamine, gestodene, imatinib, isoniazid, methylprednisolone, mibefradil, midazolam, mifepristone, nefazodone, nicardipine, niconazole, nifedipine, northindrone, norfluxetine, oxiconazole, prednisone,quinine, roxithromycin, sertraline, syhnercid, troleandomycin, verapamil, voriconazole ,zafirlukast, zileuton, grapefruit juice, milk thistle, garlic, gallic acid, herbal teas. **Inducers:** aminoglutethimide, aprepitant, barbiturates, carbamazepine, dexamethasone, efavirenz, ethosuximide, glucocorticoids, glutethimide, griseofulvin, modafinil, nafcillin, nevirapine, oxcarbazepine, Phenobarbital, phenyltoin, pioglitazone, primidone, trogliktazone, rifabutin, rifampin, rifapentine, St, johns wort, garlic,licorice [8:Chart 1].

Once compounds have completed Phase I they will proceed into the Phase II system of detoxification. Phase II pathways provide conjugation through several alternative paths, each contributing to toxins, chemicals, drugs and endogenous compounds. The processes include glutathione conjugation, sulfonation [8:Genovations], peptide conjugation with glycine and taurine, glucuronidation, Acetylation and Methylation. Glutathione conjugation requires adequate amounts of glutathione and compensatory enzymes, e.g., glutathione-S-transferase. This combination can process multiple substrates, providing that all constituents are available in adequate amounts. Substrates such as acetaminophen, penicillin, ethacrynic acid, and tetracycline, and then xenobiotics such as styrene, acrolein, ethylene oxide, benzo pyrinenes, methyl parathion, chlorobenzene, anthracine, tetrachlorovinphos heavy metals-mercury, arsenic, cadnium, lead, petroleum distillates, naphthalene, bacterial toxins, aflotoxin, lipid peroxidase, ethyl alcohol, quercitin, N-acetylcysteine, prostaglaqndins, bilirubin, leukotrene A4 [8]. The complexity and the necessity of glutathione can be seen from the foregoing list. In the autism spectrum population, chronic fatigue, fibromyalgia, allergies, etc., have as a piece of the fundamental issues a problem in the detoxification system and, in particular, glutathione conjugation. The variety of defects can be from insufficient substrates, cofactors or deficient enzymes. Detoxification is only one role that glutathione plays in the body. Glutathione is an antioxidant, helps regulate antigen presentation, and is the most important free radical scavenger of the central nervous system. Functions include: role in DNA and protein synthesis [18], enzymatic activation, transport of amino acids, neurotransmission, neuromodulation [19], prevents neurodegenerative diseases [20], helps maintain blood pressure and glucose homeostasis [21], treats retrovirus infections in the central nervous system [22], helps regulate immune shifts [23] and cell proliferation, prevents mitochondrial [24] destruction by hydrogen peroxide in the cerebral cortex, helps prevent breast cancer, helps modulate carbohydrate metabolism, breaks down oxidized fats, helps prevent cataracts and macular degeneration, prevents Parkinson's Disease [25], decreases multiplication of hepatitis, detoxifies formaldehyde, reactivates Vitamin C and E, protects integrity of red blood cells, detoxifies the bacterial toxins of Clostridium difficile, and many more [26]. Studies by Flagg, Coats and Stall [27] and later studies by Furukaury [28] showed that only local positive effects can be obtained from oral glutathione administration: that of decreasing oral cavity and gastrointestinal tract cancers. Meister showed that supplying up to 3 grams of glutathione orally did not raise cellular glutathione [29]. Dinkova-Kostova *et al*. in the *Journal of Medical Chemistry* showed that the only way to increase intracellular glutathione is by stimulating the enzyme induction. Work by Wibchi et al showed that even with 3 grams of oral glutathione supplementation there was no significant increase in plasma glutathione [30]. Glutathione is critical and its impact on many systems can be seen. A list of problems from glutathione deficiency follows: Idiopathic Pulmonary Fibrosis, hemolytic anemia, spinocerebellar degeneration, peripheral neuropathy, myopathy, amino aciduria, nonspirocytic lymphocytic leukemia, G-6-PD, low CD4 count, non-alcoholic liver disease, recurrent lung infections, polycythemia vera, chronic obstructive pulmonary disease, chronic hepatitis, stomach and intestinal ulcers, myelofibrosis, acute leukemia, Parkinson Disease, Alzheimer's, heavy metal toxicity, ALS, MS, chronic kidney failure and low sperm counts [31,32]. As one reviews the list, it becomes apparent that our body's ability to produce and

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use glutathione is one of the modern problems. This whole process is being challenged by our environmental exposures; it is this specific area that must start to be focused upon to help remove stress from our systems.

Another pathway is sulfation, which is composed of the conjugation of a sulfate to a substrate. These include: drugs--acetaminophen, methyl dopa, minoxidil, metaraminol, phenylephrine, xenobiotics- aniline, pentachbrophenol, terpenes, amines, hydroxylamines and phenols; dietary and endogenous compounds—DHEA, quercitin, bile acids, safrole, tyramine, thyroxine, estrogens, testosterone, cortisol, catecholamines, melatonin, 3-hydroxy coumarin, 25 hydroxy vitamin D, ethyl alcohol, CCK and cerebrosides [8]. The problem in sulfation may lead to environmental illnesses, problems in the nervous system such as Parkinson's disease [33] and other neuron diseases [34,35].

The inorganic sulfates required for sulfation might all be required directly from the diet or through sulfoxidation reactions. Dietary sources would be mostly methionine and cysteine. The compounds that proceed through sulfation are nucleophiolic acceptor substrates and have a high affinity for the SH-groups. Many compounds may go through sulfation at low concentration and when concentrations increase, they move and go through glucuronidation. There is also the complication that some procarcinogens may be activated through the sulfation pathway, such as aromatic hydroxylamines [8:m-5-7].

The next Phase II pathway involves the conjugation with glucuronic acid-glucuronidation. The enzyme UDP-glucuronyltransferase is a super-family of enzymes, which help detox hydroxyl, thiol, amino, hydroxylamines and carboxyl groups [5]. For most people, glucuronidation appears to be a supplemental pathway when sulfation and/or glycination is saturated, however in obese patients glucuronidation is greatly increased and appears to be correlated to body weight [36]. By attaching to the active groups, glucuronidation produces very few genotoxic compounds. A list of some of the substrates follows: drugs—salicylates, morphine, acetaminophen, benzodiazepines, meprobamate, clofibric acid, naproxen, digoxin, phenylbutazone, valproic acid, steroids, lorazepam, oxazepam; xenobiotics- carbamates, phenols, thiophenol, aniline, N-hydroxy-2-napthuleine; dietary and endogenous compounds- bilirubin, estrogens, melatonin, bile acids, Vitamin E, Vitamin A, Vitamin K, Vitamin D, and steroid hormones [8:m-5].

Acetylation is the next pathway to be considered; this process is carried out by two-acetyltransferase enzymes- NAT-1 and NAT-2. The action of these enzymes is to transfer/remove acetyl groups usually to hydroxylamines or amines. The specificity of these two enzymes is very different, with NAT-2 showing a greater variety of medication substrates and the capability of handling aromatic amines. **Drugs metabolized include:** clonazepam, dapsone, mescaline, isoniazid, hydralazine, procainamide, benzidine, sulfonamides and promizole; xenobiotics- 2 aminofluorine, anilines; dietary and endogenous compounds- serotonin, PABA, Histamine, typtamine, caffeine, choline, tyramine and coenzyme A [8:Genovation]. This provides a look at cancer risk, providing indicators for lung, colon, bladder, head and neck cancers.

The next Phase II pathway is conjugation with amino acids: glycine, taurine, asparagines or glutamine. Most of these are secondary pathways: drugs—salicylates, nicotinic acid, chlorpheniramine, brompheniramine; xenobiotics—benzoic acid, phenylacetic acid, napthylacetic acid, aliphatic amines, organic acids, propionic acid, capryllic acid dietary and endogenous substances- bile acids, cinnamic acid, PABA, plant acids, stearic acid, palmitic acid, myristic acid, lauric acid, decanoic acid, butyric acid [8].

The final pathway is Methylation; this is of great importance for the metabolism of endogenous compounds as well as drugs and xenobiotics. There is a large family of methyl transferases; one of the most important is catechol-O-methyltransferase (COMT). If there are errors in COMT, then there is an increased incidence of: depression, bipolar disorder, ADD/autism and alcoholism. COMT can also prevent the formation of quinines, which are highly genotoxic. These reactions require a co-substrate, which is S-adenocylmethionine. The substrates are: drugs- thiouracil, isoetharine, rimiterol, dobutamine, butanephrine, eluophed, morphine, levaphanol, nalorphine xenobiotics-paraquat, beta carbolines, isoquinolines, mercury, lead, arsenic, thallium, tin, pyridine; dietary and endogenous compounds-dopamine, epinephrine, histamine, norepinephrine, L-dopa, apomorphine and hydroxyestradiolos [8].

When considering the detoxification system, the possibilities for malfunction are staggering. All systems depend on the detoxification of the products, by-products and protection from outside infiltration. The more the toxic exposures, the greater the burden as this occurs, then the accumulation of toxins begins and these will be stored in different tissues; the effects and accumulation becomes additive, and then the interplay between reactions begins and expands exponentially. In looking at heavy metals, the work of Dr. Boyd Haley from the University of Kentucky shows that the effects of metals on the central nervous system are increased exponentially rather than additive. This then magnifies the toxicity of each metal involved. With aluminum being the third most common element in the earth's crust [5:756-60], we are exposed in the food supply, soy [37], aluminum cookware, aluminum foil, and vaccines. The exposures from vaccines were Thimerasol/ethyl mercury, which has been reduced or replaced with aluminum and formaldehyde. Dr. Boyd Haley has done extensive research into links between Alzheimer's and autism and the link to heavy metals and their neurotoxicity. These can be reviewed at ALTCORP.com. The multi system effects of heavy metals must then be seen through the light of their accumulation and the accumulation of other toxins. Along with viewing the direct levels of the antioxidants we can now peer into the DNA: we can look at specific "snips".

Why would we want to do this? This information allows us to view any predisposition that is present. The heart of where life occurs is at the intersection of genes and environment; this tells us exactly where we are, where we have been, and what are the long-term issues that we must address going forward. The information available today allows us to look at the Phase I cytochrome P450s and to look at several pathways in Phase II. We can look at Methylation, Acetylation, glutathione-S-transferase and superoxide dismutase. This information allows us to prepare for and even prevent catastrophic events in the future. Genetic information allows us to be proactive and very personal in the quest for physical health. This is but one step in the process of health and healing. We must rejoin the spiritual, mental and physical aspects to reach true health. The ways we

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begin to address these issues have to be multifaceted. It is through these processes that we can begin to understand the basis of the problems. We must move past medication as a band-aid and look for the true problems, the causes and the cures.

Our present "medication-for-symptoms medical system" is no longer acceptable. As it has been said that genes only create possible predisposing issues, and that if the "right" stimulus is never encountered then the problem is only theoretical, then, too, we must apply this idea of environmental insult to the medical treatments we employ. Any detoxification protocol must be undertaken with due diligence, ensuring that the patient's body will be able to handle the possible consequential effects of a given detoxifying agent's action upon other physiological pathways and systems.

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