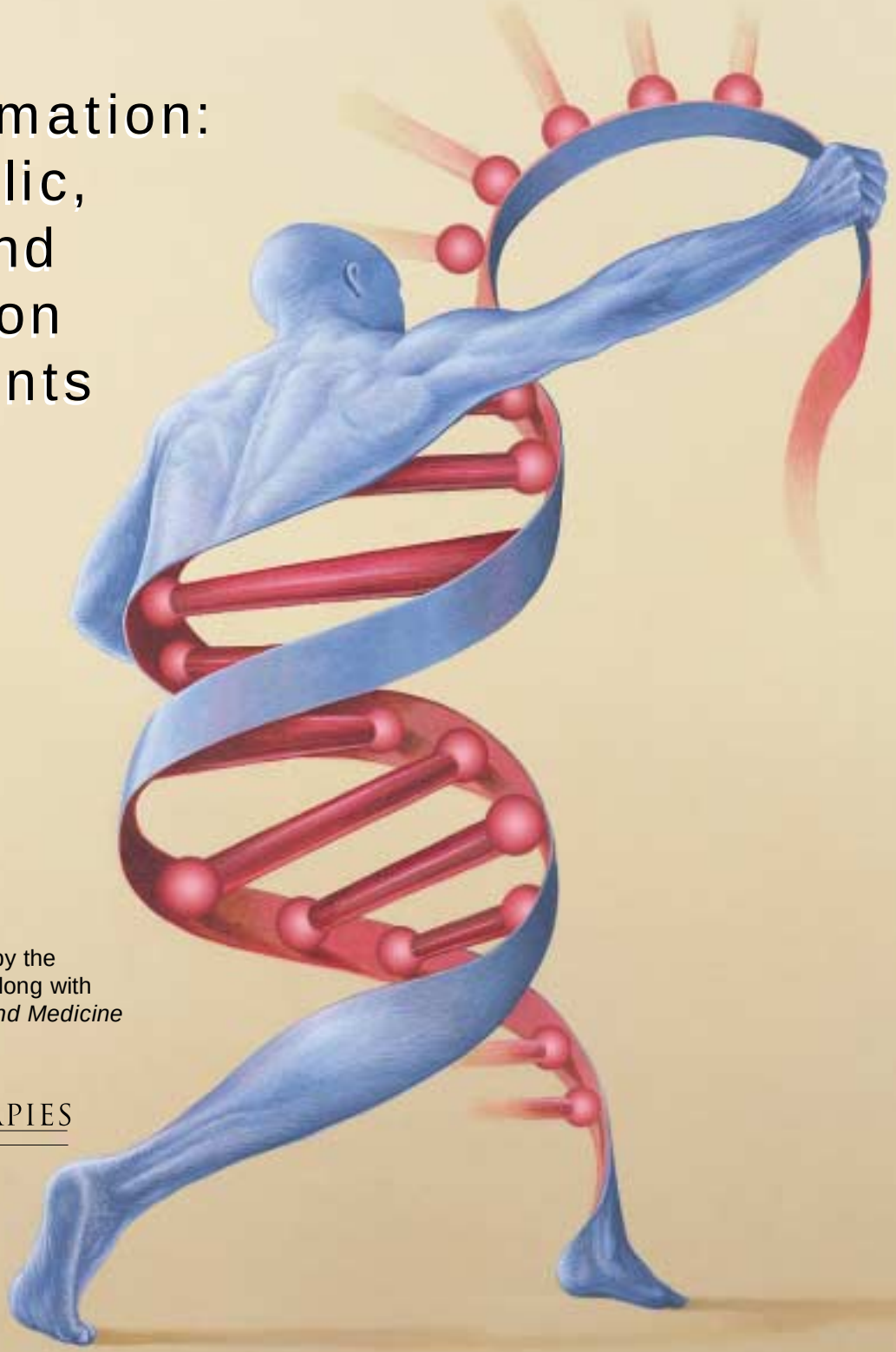
The Proceedings From the 13th International Symposium of The Institute for Functional Medicine

Managing Biotransformation: The Metabolic, Genomic, and Detoxification Balance Points



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Inside

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Presentation Articles Include:

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About the Institute for Functional Medicine:

IFM's mission is to serve the highest expression of individual health through widespread adoption of functional medicine as the standard of care.

- Functional medicine is patient-centered health care that addresses the unique interactions among genetic, environmental, and lifestyle factors influencing both health and complex, chronic disease.
- Our innovative and visionary leadership, model for personalized patient care, and commitment to quality drive our success.
- We commit time, energy, and resources to insure that those we touch feel great value from their experience with us.

Welcome

The 2005 Clemens and Pressman article titled "Detoxification Diets Provide Empty Promises"[1] prompted considerable discussion at the Institute for Functional Medicine (IFM). The daily clinical experiences of our faculty and our practitioner base, and the evidence with which we were familiar at the time, directly challenged the article's conclusion that, "These approaches are contrary to scientific consensus and medical evidence and are not consistent with the principle that diets should reflect balance, moderation and variety." As Dr. Jeffrey Bland points out in his introduction to this *Proceedings for the 13th International Symposium:* "While the principles of balance, moderation, and variety are excellent guidelines for constructing public health policies, they may not be specific enough for constructing the proper diet for a patient with a specific alteration in his or her functional capacity for detoxification."

The Institute decided to investigate further. We devoted more than 6 months to the discovery process for this symposium, reviewing research and discussing the topic with experts in the field. We found a valuable and growing evidence base, developed by both researchers and eminent clinicians, that paints a very rich picture of significant interconnections between diet and the processes of biotransformation and detoxification. Within the presentations from the faculty we assembled for this symposium, you will find robust research and clinical evidence demonstrating the importance of matching the patient's unique genomic characteristics to the appropriate diet, food preparation, and eating patterns in order to induce the appropriate phase I and phase II enzymes responsible for balanced detoxification of exogenous molecules and biotransformation of endogenous metabolic by-products.

The faculty brought to this symposium much that is practical and ready for clinical application—for example, the exciting research from the emerging field of chemoprotection that provides dietary strategies for defense against carcinogenesis. You will also learn about the profound effects of, and possible solutions for, exposure to heavy metals (such as mercury) and other organic and inorganic toxicants that can produce adverse effects, including oxidative stress, inflammation, thrombosis, vascular smooth-muscle dysfunction, dyslipidemia, and mitochondrial dysfunction. The specter of obesity, an epidemic today in the industrialized world, emerges from these pages in a cause-effect relationship with environmental toxins. Toxins can increase oxidative stress, affecting redox signaling, which, in turn, influences gene transcription and signaling pathways controlling insulin resistance, cytokine modulation, and mitochondrial function. Activation of NFκB (a gene transcription factor) is mediated by redox balance and is a final common pathway for obesity and many other chronic illnesses. These actions and reactions contribute to the epidemic of weight gain and resistance to weight loss.

Evidence from years of both bench science and clinical research by the symposium's distinguished faculty demonstrates a very real and important connection between the specificity of patients' genetic/environmental uniqueness and their health status. These presentations substantiate a position that detoxification diets can help fulfill the promise that a carefully crafted, personalized, dietary and lifestyle plan will create significant health benefits for our patients. Working closely with our partner in this project, InnoVision Health Media, the Institute for Functional Medicine (www.functionalmedicine.org) proudly brings you this *Proceedings from the 13th International Symposium on Functional Medicine.*

President and Director of Medical Education
The Institute for Functional Medicine (IFM)

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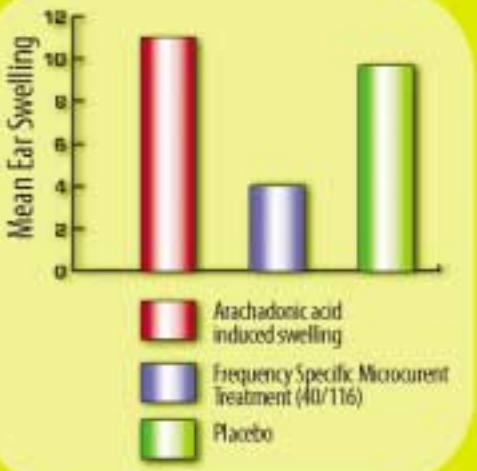

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Dr. Carol

Managing Biotransformation: Introduction and Overview

Jeffrey Bland, PhD, FACN

Jeffrey S. Bland, PhD, is an educator, research professor, leader in the natural products industry, and expert in human nutrition and functional medicine who serves as chief science officer of Metagenics, Inc., in Gig Harbor, Wash. Dr Bland is the founder of, and serves as the founding board chair for, the Institute for Functional Medicine.

A description of the family of human detoxification enzymes, cytochrome P450s, first appeared in the literature in 1962.[1] Until that time it was known that foreign compounds were somehow detoxified by specific physiological processes, but the nature of these processes had not been elucidated. In the absence of an understanding about this superfamily of detoxification enzymes (now known to be generated by 57 genes, many of which show multiple polymorphisms), there was much speculation as to how an individual actually eliminated lipophilic compounds, both exogenous and endogenous. It is now recognized that the enzymes in the CYP450 superfamily have roles not only in the detoxification of drugs and other xenobiotics, but also in the metabolism of nutrients and endogenous molecules such as essential fatty acids, phytonutrients, steroid hormones, and vitamins D and A.[2]

Over the past 40 years, we have learned that what is termed phase 1 activation of lipophilic compounds is carried out by enzymes in the CYP450 family. This phase 1 biotransformation of a molecule creates an activated intermediate that is either directly eliminated from the body or, more commonly, becomes a substrate for one of the phase 2 conjugation enzymes and is then eliminated. The phase 2 conjugases, which include sulfation, amino acid conjugation, glutathione conjugation, glucuronidation, methylation, and acetylation activities, are also highly polymorphic. In both the phase 1 and phase 2 detoxification enzyme families, some enzymes are constitutively expressed and some are inducible. Importantly, certain environmental and nutritional agents have been found to influence the induction and activities of specific phase 1 and phase 2 enzymes.[3,4]

Murray has described some of the effects of diet on detoxification by pointing out that constituents of the diet regulate the expression and function of both CYP450 and conjugation genes, which impact lipophilic compound elimination and may also significantly influence disease pathogenesis. He concludes that "food constituents modulate CYP expression and function by a variety of mechanisms, with the potential for both deleterious and beneficial outcomes."[5] This suggests that diet may have a "detoxifying" influence if the constituents of the diet properly support balanced phase 1 and phase 2 detoxification functions.

Recently, however, Clemens and Pressman suggested that "detoxification diets provide empty promises"[6] because "these approaches are contrary to scientific consensus and medical evidence and are not consistent with the principle that diets should reflect balance, moderation, and variety." While the principles of balance, moderation, and variety are excellent guidelines for constructing public health policies, they may not be specific enough for constructing the proper diet for a patient with a specific alteration in his or her functional capacity for detoxification.

Genetic polymorphisms that result in highly variable individual responses to toxin exposure, to dietary influences, and to drug treatment may be useful in identifying people at risk for many different kinds of diseases and adverse effects.[7] For example, exposure to specific toxins and the absence of proper support for detoxification functions are both thought to increase the risk for neurodegenerative conditions such as Parkinson's disease,[8,9] and in both situations genetic variability is common. It is well known that individuals who consume a poor-quality diet and/or excess alcohol—and concomitantly take acetaminophen—have a much-higher risk of both hepatic and neurological injury from the medication.[10] It is also clinically well established that diet plays an important role in the etiology of hepatic encephalopathy.[11,12] Diet therapy that influences both intestinal and hepatic detoxification enzyme function is part of the standard of care for patients with this condition.[13,14]

Grapefruit juice,[15] red wine,[16] and crucifers[17] have been shown to contain constituents that influence specific CYP450 activity that can alter drug metabolism and elimination. Certain foods and spices (eg, black pepper)[18] can also influence phase 2 activities. Oral supplementation with the amino acid glycine has been found to support phase 2 glycination and glucuronidation,[19] which may improve detoxification in certain individuals. Some of these characteristics have been clinically exploited to develop a nutritional regime that will reduce the rapid first-phase detoxification of drugs, such as with cyclosporine, which is used to prevent tissue rejection in transplant patients.

All of these examples help us understand that specific diets may exert clinically beneficial effects on detoxification function in patients with unique needs. In essence, the emerging understanding of the role that diet plays in influencing detoxification rests on a pharmacogenomic mechanism of action. In a recent article, one suggested approach for minimizing adverse drug reactions was to offer therapy based upon an individual's specific genetic make-up.[20] This calls not only for tailoring the drug therapy to the patient's genetic needs, but also, we suggest, the diet to respond to pharmacogenomic uniqueness. After all, "it is estimated that genetics can account for 20 to 95 percent of variability in drug disposition and effects."[21]

Constituents of food play potentially important roles in defining relative risks for certain diseases through their influence on specific detoxification processes. One interesting emerging example is the role that coffee has in reducing the risk of Parkinson's disease[22] while increasing the risk of myocardial infarction in people with certain detoxification enzyme genotypes.[23]

Constituents of the diet play a role in the metabolism of steroid hormones such as estrogen.[24,25] Estrogen is metabolized by CYP450 and conjugase enzymes whose expression and activities are influenced by specific dietary constituents. Numerous recent studies have indicated that a number of dietary constituents influence the metabolism of estrogen, with the most notable being the isoflavones and lignans from soy foods and the glucosinolate metabolites such as indole-3-carbinol from cruciferous vegetables.[26,27] Indole-3-carbinol has been shown to upregulate CYP1A2 and may influence phase 2 activities. CYP1A2 upregulation, in turn, helps regulate estrogen signaling and appears to decrease the risk of certain cancers.[28,29] An indole-3-carbinol intervention trial with women who had precancerous lesions of the cervix found that there was a statistically significant regression of the premalignant lesions in women treated with 200 mg per day of indole-3-carbinol as compared to placebo.[30] Results such as these strongly suggest that diets containing certain amounts of crucif-

erous vegetables—which deliver useful phytochemicals—can have a salutary influence on estrogen detoxification and excretion.

The clinician is thus challenged to investigate the potentially toxic burdens patients might be exposed to, ranging from pollution or xenobiotics in their homes, work, or local environments; to prescribed or recreational drugs; to the quality of their diets. Each potential exposure raises the questions frequently mentioned by Sidney Baker, MD: "Is there something for which this person has a special, unmet need? Is there something to which this person is having an adverse or toxic reaction (ie, something the person is getting too much of)?" It is now recognized from molecular and cellular biology research that diet and lifestyle choices can influence both the level of exposure to potentially toxic substances and detoxification functions.[31-33] Some key examples of these mechanisms include:[34-37]

1. alteration of the absorption of toxins (ie, fiber intake);
2. alteration of gut microbial function (ie, prebiotics, probiotics);
3. alteration of the genetic expression of CYP 450s and conjugases (ie, glucosinolates);
4. alteration of post-translational, site-specific phosphorylation of CYPs through specific kinase modulation (ie, sulforophane);
5. post-translational and other possible influences on detoxification enzyme function (ie, pH, methylation with folate and vitamin B12, oxidation, and non-enzymatic glycation);
6. modulation of transcription by factors such as orphan nuclear receptors and cell-signaling pathways (eg, PPAR, RXR, T3, 1/25 vitamin D3, Pregnane X, Nrf 2) and by phytochemicals (eg, carnesol, epigallocatechin gallate, curcumin).

In essence, this contemporary view of the role that diet plays in detoxification suggests that specific dietary signals are translated to the genes through a complex process involving reporter gene activation through specific nuclear transcription factors and cell-signaling pathways. These nuclear transcription factors control the cell-specific expressions of various detoxification enzymes. Various environmental substances send "stress" messages to the genome that induce specific detoxification responses. Intracellular reduction/oxidation potential (ie, cellular bioenergetics) plays a role in determining the degree of the response to the toxin. A quick response to a toxic exposure that short-circuits the need to induce protein synthesis in response to a toxic stress signal is mounted through the kinase activation pathway, which in turn is also sensitive to various phytochemicals and dietary factors.

Clearly, the diet–detoxification connection represents a specific example of clinical nutrigenomics. Muller and Kersten defined nutrigenomics as "the understanding that micronutrients and macronutrients can be potent dietary signals that influence the metabolic programming of cells and have an important role in the control of homeostasis."[38] Food-derived molecules of plant origin (phytochemicals) modulate the expression of genes and their post-translational products that control the cytoprotective effects of the detoxification process. It is now apparent that foods can deliver specific phytochemicals that influence detoxification function by a variety of means, including direct ligand interaction with nuclear regulatory factors and interaction with the xenobiotic and antioxidant response elements.[39,40]

The papers you are about to read will explore some of these—and many other—fascinating ideas in greater depth. The IFM symposium itself provided an exciting look at some overarching concepts about the links between diet and detoxification that can now be supported by the emerging science:

1. Numerous genetic differences can influence both phase 1 and phase 2 detoxification functions.
2. Multiple environmental agents and drugs can affect the detoxification process.
3. Many nutrients and phytochemicals can influence both phase 1 and phase 2 detoxification function.
4. The multiple, complex interactions that can involve genetics, detoxification function, and environmental exposures (food, drugs, toxicants) can magnify the effects mentioned in points 1, 2, and 3 above.
5. Dietary influences on detoxification may play a role in the diet-cancer association.

Taken as a whole, the information provided in this series of papers demonstrates that the proper diet for a specific patient can influence detoxification function in a clinically important manner. Our review of this impressive body of evidence suggests strongly that this important topic in clinical nutrition and its relationship to medicine have not been adequately emphasized in either teaching or clinical practice. Detoxification diets may have significant value in promoting more effective physiological responses to toxic stressors that come either from the exogenous or endogenous environments.

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Who Ignores Individuality Fails the Patient

Sidney MacDonald Baker, MD

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TIPPING POINTS

It happens all the time. We find ourselves stuck in traffic wondering what has jammed it up, only to discover when speed is restored that it was nothing but an inscrutable episode concerning the way a certain number of cars and trucks fit the highway and responded to a statistically insignificant wrinkle in their pattern in space and time. Or, we return from going out to dinner and find the fire we had left blazing in the fireplace reduced to embers. Rekindled and restocked with a couple of logs, the new wood smolders over the glowing coals and thick, dark smoke crowds the chimney. Then, strike a match and its tiny, inseminating flame causes the fireplace to burst suddenly into a lively fire, with its hot, clean smoke. As clinicians, we wonder whether we can identify a similar tipping point in a patient's chemistry of energy and detoxification, to purify his or her metabolic fire and kindle the healing process toward which nature has such a strong impulse.

In Chad, Africa, in 1967, I was called to see the 6-year-old daughter of the ambassador of a large Asian country. I was a Peace Corps volunteer at the bottom of the post-colonial hierarchy of medicine among the dozen or so physicians serving the 4 million people of Chad. My job as leader of 7 nurses and lab technicians was to teach and treat Chadians, not members of the diplomatic corps or their families. It was past midnight when the ambassador and his wife arrived at my cubicle in the Peace Corps office with their daughter, Sue, who was somewhere on the boundary between stupor and coma. She had been unremittingly febrile for 3 days, and was, at first glance, a candidate for IV hydration, blood count, thick smear for malaria, and a spinal tap. Encephalitis or meningitis—both common in Chad—appeared to be the top diagnostic possibilities, as her history and physical gave not a shred of evidence for being a benign, localized infection that might have provided some more hopeful traction on the problem. The environment in my cubicle in the 5-room Peace Corps office was ill suited to any of my options, although I did have IV fluids, a microscope, stains, a counting chamber, and an LP setup.

I could not envision a good outcome as I reviewed my findings with Sue's mom and dad. However, I then took an extra long look in her right ear canal where my first examination had encountered some smooth, brown wax that made it difficult to see her ear drum, which, of course, I had hoped would be bright red and give me something to treat—however poorly an earache would have matched the clinical picture. The bead of wax had looked shiny, and I conjured up the novel theory that her very high body temperature had melted the flakey wax more typical of a child. On second examination, the smooth, brown wax turned out to be the backside of a tiny, engorged tick. I drowned it in warmed mineral oil. Sue's parent's helped me set up a lamp to get the right angle on my head mirror so I could extract the tick while Sue was supine. I removed the beast with small bayonet forceps while praying that I not scratch her ear canal and panic her parents with a few innocent drops of blood. All the time I feared I was straying in a fruitless detour from an urgent clinical strategy. Barely had I delivered that bad baby from Sue's ear canal and begun to refocus my senses, when Sue roused herself. Within five minutes she became alert, talkative, and cheerful. By the time she and her parents walked out into the cool night air 30 minutes later, her temperature had dropped from 104.6° F to normal and she had polished off half a liter of IV fluids that I uncorked for her to drink. In those few minutes she had returned to her normal self and I was forever changed.[1]

Sue's particular clinical expression of tick paralysis or tick toxicosis was at the crossroads of something in her individual make up, the location of the tick bite, the species of the tick, and its particular toxin. The lessons I learned at that crossroads were about respect for individuality, the susceptibility of the central nervous system to small toxic exposures, and the imprecision of the boundary between what I had previously thought of as toxic on the one hand and allergic on the other.

LESSONS FROM ACUTE ILLNESS

Most of the lessons medicine has drawn from acute illness provide an imperfect way of thinking about chronic illness, which is what concerns most of us in daily practice, where quick answers such as came that night in Chad are not usually available. Tick paralysis, strep throat, pneumococcal pneumonia, chickenpox, broken arm, and other names we give to acute illness embrace the concept of causation in ways that work very well when it comes to reassuring patients that "we know what you've got." To say that a patient's acute problem is caused by any of the above-named acute illnesses does not defy logic—particularly when the etiologic agent is named in the diagnostic label. In the realm of chronic illness, however, our failure to distinguish between names (diagnostic labels), notions (ideas we form about groups of people), and things (etiologic agents) defies logic when we tell patients that their symptoms are caused by their disease. In reality, depression is the name, not the cause, of sadness. Arthritis is the name, not the cause, of joint inflammation. Lupus is the name, not the cause, of a constellation of sign, symptoms, and lab values. These names of chronic illnesses refer to ideas we form about the similarities of presenting signs and symptoms across many individuals, each of whom, however, has distinguishing features that are cast aside in a diagnostic process that puts individuals into a disease group in order to assign them to a standard treatment. Stop and think about the notion that something called lupus is the cause of symptoms—it makes no sense.

CLINICAL LOGIC

The lesson that we *can* take from acute illnesses, such as Sue's tick toxicosis, is that everyone is different. In a patient-oriented medicine—as opposed to (or complementary to) a disease-oriented medicine—we are deprived of the simplicity of prescription-pad medicine where one can match the treatment to the name of the disease. We are rewarded instead with a clinical logic that rests on more solid semantic and scientific foundation than the thinking that colitis is the cause of gut inflammation. The logic of complementary, functional, integrative, or good medicine begins with the biological reality of individuality and proceeds with the following strategic models that can be posed as questions.

1. Is there something for which this person has a special unmet need?
2. Is there something to which this person is intolerant?

Possibilities of unmet needs include:
- Nutrients
- Accessory nutrients
- Light
- Love
- Rhythmic integration

Possibilities of intolerances include:
- Toxins—elemental, such as lead and mercury; biogenic, such as food; parasites; germs; and synthetic chemicals, most of which are petrochemical products
- Allergens—molds, foods, pollens, dander, dust, and chemicals

THE "WHY" QUESTIONS

Those two questions about unmet needs or unrecognized intolerance have formed the basis for my practice for the past 30 years. In my role as a pediatrician and family practitioner, I am obliged to help my patients onto a mutual path of discovery when they ask questions about whether a chronic condition might benefit from taking supplements or from avoiding allergenic foods or noxious environments. I have found that an extensive questionnaire, including environmental and dietary questions, and a chronological history form are essential tools for letting patients understand the way I think. These documents can be as much an avenue for educating my patients as they have been a way of getting grist for my diagnostic mill. I used to provide a lengthy written philosophical orientation to new patients, but eventually found that the questionnaire was as informative to them about my agenda as it was to me about their situations.

A detailed history is particularly helpful in soliciting the patient's collaboration in trying to answer the "why" questions that lie beneath the first two. That is, if you have an unmet need, why (and how) did it arise?

Is it because you have:
- An excess need genetically, a poor diet for your specific genetic needs, maldigestion, malabsorption, or a tendency to waste certain nutrients?
- Malillumination?
- An inability to give or receive love?
- Or habits that inhibit the healthy meshing of rhythms upon which harmony depends?

If you are sensitive to things that don't seem to bother most people, why and how did you become so?
- Could you be out of balance with respect to the issues raised in the unmet needs question, or could it be because of deficient digestive forces, so that food retains too much of its antigenic identity?
- Could a hypervigilant immune system result from an antigenic load produced by disturbances of the gut microflora or from invasive life experiences?
- Could an exposure to chemicals or molds—especially when under stress—have provoked the well-recognized, but poorly-understood state of sensitization in which the immune system's parallel functions to those of the central nervous system—perception, memory and recognition—have become "hung up" in a maladaptive responsiveness?
- Finally, could endocrine imbalance, such as mild congenital adrenal hyperplasia or acquired adrenal insufficiency[2] have given rise to an easily correctable state of multiple sensitivities?

In my 30 years of practicing by the light of answers to the above questions, I have learned, contrary to expectations set by my medical schooling, that the odds are very good for finding successful answers in a practice that deals with complex chronic illnesses. People who are motivated to seek medical help are more likely than symptom-free people to be "out of balance" within a framework of needing to get or avoid things. They are also likely to be more sensitive in the broadest meaning of that word—including a heightened awareness of and an increased reactivity to stimuli that are unnoticed or are not bothersome to most people. The statistical definitions of "normal" on which we base so much of our laboratory assessment may not work very well in the clinical realm, where we struggle to define the individual patient's need for good things and tolerance for bad things. Disease-based medicine is about how patients fit within definitions forged out of statistical averages. Public policy has made imperfect attempts to grapple with the fact of individual sensitivity.[3,4] When I began to wonder whether addressing these individual quirks might lead to finding remedies for single individuals, I soon discovered in day-to-day practice that it did. A methodical engagement with a patient in a leisurely, intelligent conversation usually yielded answers within the paradigm of my two questions.

WHAT IS "GOING AROUND"?

In the course of practicing medicine, one naturally gets a notion about what is "going around," just as practicing in Chad, Africa, made it clear that malaria, schistosomiasis, intestinal parasites, and other infectious diseases were issues to keep in mind when seeing patients with every sort of presentation, whether typical or not. I believe that what is going around in North America calls for considering unmet needs for omega-3 fatty acids, magnesium, calcium, zinc, and vitamin D, proper exposure to sunlight, and attention to exercise, breathing, and the timing of food and activity in relation to circadian chemistry. I believe that what is going around in North America with regard to allergens has to do with molds and their cousins that make up an undesirable part of the gut microflora as a consequence of antibiotics and dietary sugars and starches. Gluten, casein, and other food sensitivities are common masqueraders. Mild adrenal insufficiency is much more often underestimated than is hypothyroidism and should be brought to mind in a person who has an unusual thyroid story. I believe that what is going around in North America with regard to toxins is a soup of elemental toxins and chemicals—of which the fetal and pediatric toxicity endangers the recent generation of children, whose central nervous systems are extremely vulnerable.

At the moment, mercury is the toxin for which we have the most elegant and persuasive clinical toxicology, but the variable thresholds for its effects in different individuals make the blunt tool of epidemiology more a weapon of defense than discovery.

SEVEN DATA SOURCES

Now, the patient sits before you. You remind him or her that this is a collaborative effort in which the patient's instincts, questions, theories, and intuition all have enormous value in driving decisions. You remind yourself and the patient that the decision at hand is not about an ultimate truth, or the treatment for such and such a disease, or even whether or not the patient has a well-labeled disease. The decision at hand is only what to do next; it will be based upon input from seven sources of data:

1. The outcome of treatments for individuals like this patient
2. The time path in the emergence of problems
3. Biochemical paths of patients like this one
4. History and physical exam
5. Laboratory tests
6. Response to each treatment you try
7. Your intuition and that of your patient

EIGHT LANDSCAPE QUESTIONS

Privately—except in the company of patients with a high level of sophistication—you contemplate the following generic questions about the size, shape, timing, and energy needs of the patient's physiological and biochemical landscape.

Q: What are the organism's largest surfaces? And which variables among the illnesses going around might have a practical impact?

Answer: Cell surface and intracellular membranes, constituting a collective surface area the size of 10 football fields.

Q: What raw materials are used for making most of the message-carrying molecules in the body?

Answer: Essential fatty acids for cell-to-cell communications, cholesterol for steroid hormones, and essential amino acids for neurotransmitters and healthy thyroid functions.

Q: Which individual organ of the body exposes the largest surface to the environment?

Answer: The gut, with the lungs a close second.

Q: Which organ commands the most attention from the immune system?

Answer: The gut, which accounts for about 60% of the immune system's activity.

Q: What organ of the body has the single largest number of individual cells?

Answer: The gut flora, which surpasses the total number of cells in the body by an order of magnitude.

Q: Which organ of the body is most subtly vulnerable to injury and most difficult to repair:

Answer: The gut flora, which suffers enduring changes from antibiotics and maladaptive diet, with profound influences on immunity, endocrine function, energy metabolism, and detoxification.

Q: What is the biggest budget item in the organism's energy expenditure for managing biotransformation?

Answer: Detoxification, which in adults constitutes the body's dominant molecule-joining activity.

Q: When do growth in children and detoxification in adults and children take place?

Answer: During sleep, which should take place during the hours of darkness.

THE LENSES

So far I have referred here and there to certain generic functions of the organism. Here is the complete list: 1) energy chemistry, 2) synthesizing activities, 3) detoxification, 4) membranes/boundaries, 5) messaging, 6) perception, 7) memory, and 8) timing.

This list can be seen as a series of lenses through which pass the impulses that arise in our genetic predisposition. These impulses then filter through our environment before becoming variously deflected, depending on each of these 8 physiologic influences to emerge somewhere in the spectrum of health and disease.

This metaphor lends itself to clinical strategy. It provides me with a way to keep my clinical options grounded in aspects of the patient's physiology and psychology that are amenable to change. It offers a recipe for keeping an open agenda when confronted with the question of detoxification, which lends itself to oversimplification on the part of both practitioner and patient. Both may have a different image of which among the following efforts ranks highest in their hygienic hierarchy: brushing, clipping, combing, cutting, shampooing, picking, scratching, shaving, washing, scrubbing, sweating, blowing, breathing, coughing, sneezing, clearing, burping, defecating, flatulating, discharging, dripping, draining, menstruating, spitting, sweating, urinating, vomiting, wiping, methylating, acetylating, glucuronidating, sulfating, glutathionylating, glycinating, and chelating.

As shown in Figure 1, the 8 lenses appear to be sequential in their arrangement along the trajectory of the impulse arising from the genome and passing through the environment to be modified in various physiologic realms. Each of these realms is amenable to analysis and therapy but none dominates others in its priority or power. Our job as clinicians is to identify the places where we can make the most impact with the safest, quickest, and least-risky or expensive measures. The enormous complexity of the whole system would be overwhelming were it not for two points that I find helpful to keep in mind when addressing my patient's needs. First, the interconnections among the various systems represented in the lenses diagram constitute myriad virtuous—or potentially vicious—cycles. Second, as previously stated, I don't have to know the whole truth; I just have to decide on the next diagnostic/therapeutic step.

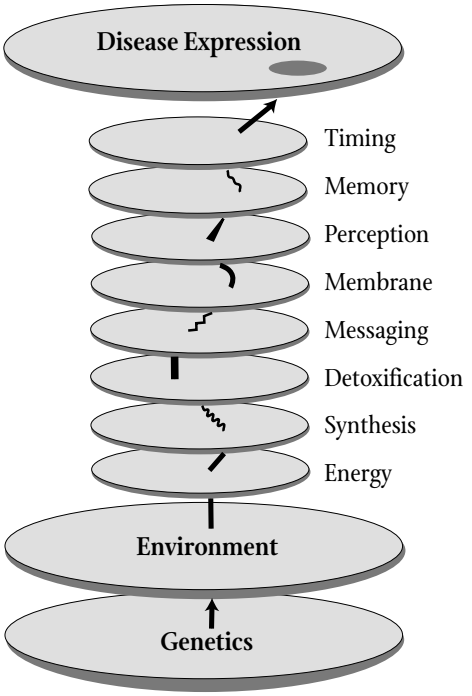

FIGURE 1 The Lenses

The sequential arrangement of the lenses is a concession to the need for graphic representation and the limitations of our imagination about very complex systems. In reality, each is under the mutual influence of all the others. The notion of the lenses is not to be interpreted as though the different physiologic domains are sequential, hierarchical, or independent. A more appropriate diagram would be an octagon or a cube, with lines connecting all the corners (see Figure 2).

VICIOUS CYCLES

The complexity of the relationships among the physiologic domains in the lenses metaphor is not only in their multiple mutual interactions. Within these domains are also cycles such as the citric acid cycle, the urea cycle, and the methionine cycle. In all of these, a virtuous, self-sustaining mechanism can be corrupted, creating a vicious cycle. The bad news on vicious cycles is that they are vicious. The good news about vicious cycles is that, once rebalanced, they return to being virtuous—that is, self-sustaining. Let's take the methionine cycle as an example.

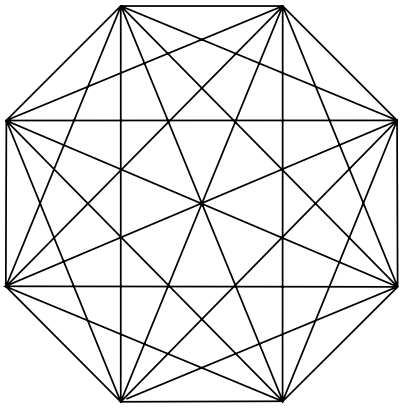

FIGURE 2 The Interconnected Model

Figure 3 shows reduced glutathione (GSH) as one important terminal branch of thiol chemistry. A redox potential sustained by a relatively high balance in GSH to glutathione disulfide (GSSG) is required to support the pathway that begins with methionine. It is a virtuous cycle with high GSH/GSSH. However, with depletion of GSH, a vicious cycle can ensue in which thiol chemistry tends to collapse with an impoverished redox potential.

Figure 4 illustrates a second sort of vicious cycle. Mercury in low concentration is a potent inhibitor of methionine synthase and, thus, can block the synthesis of GSH, a principal detoxifier of mercury. An extension of the example of vicious/virtuous cycles in the physiologic landscape of our patients begins with the cellular methyltransferase box.

Figure 5 expands on the information depicted in Figures 3 and 4 by showing the destination of the methyl groups that come from SAM to transform their acceptors.[5]

AN IMPORTANT ENERGY-DETOXIFICATION LINK

Note that the synthesis of creatine by methylation from guanidoacetate consumes 75% of the methyl groups delivered by SAM. Guanidoacetate is derived from urea-cycle arginine, and creatine's destiny is to become phosphorylated for the ultimate formation of ATP. Note that arginine itself is formed in the very metabolically expensive urea cycle (from which it is snatched before it can have its urea moiety snipped off) to provide the basis for energy transfer via ATP. This is but one of many examples of the links between detoxification—I am now referring to the urea cycle as well as the methionine cycles—and energy chemistry.

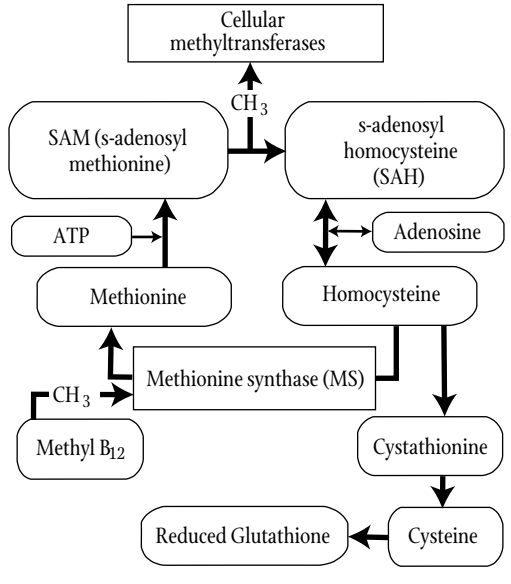

FIGURE 3 The Methionine Cycle

The methionine cycle: Reduced glutathione is needed to provide the redox environment for the synthesis of reduced glutathione.

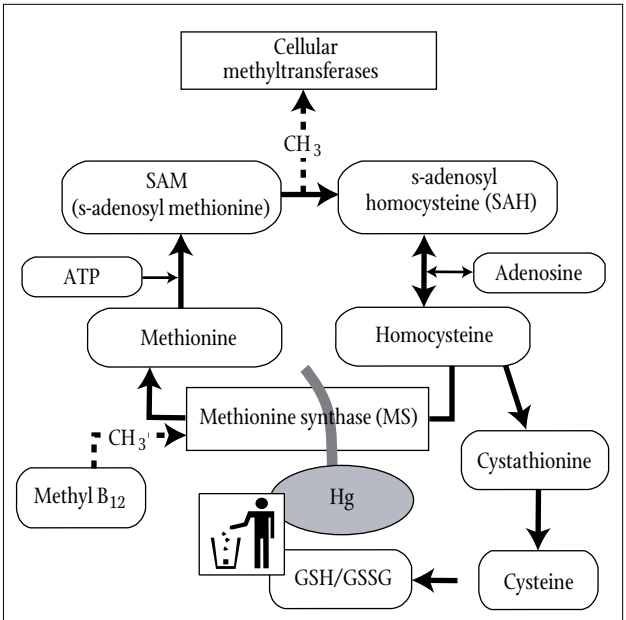

FIGURE 4 Mercury Disturbs the Methionine Cycle

The methionine cycle: Mercury poisons a fundamental step in synthesizing GSH, which is an important detoxifier of mercury.

CLINICAL COMPLEXITY

Let's take some examples of simple toxic exposure to remind us of the complexity of the clinical picture and clinical management in even the most unitary causation of illness—especially when removal of the toxin is not as easy as it was with a tick in a child's ear.

First is the case of the Dartmouth scientist who died 298 days after an exposure to diethyl mercury.[6] Her onset of symptoms followed a latent period of 154 days during which her blood and hair levels fell continuously. Her illness progressed despite DMSA chelation with an increase in urinary excretion of mercury from 257 µg per 24 hours (before chelation therapy) to 39,800 µg per 24 hours. Bernard Weiss comments, "The dose did not ... make the poison, so to speak, in apparent violation of a cherished principle of traditional toxicology."[7]

The second example is the epidemic of Minamata disease in Japan that was the result of industrial poisoning of fishing waters with methyl mercury. The acute symptoms included acral sensory disturbances, ataxia and dysequilibrium, constricted visual fields, neuromuscular impairment, deafness, disturbances of taste and olfaction, and mental disorder. Latency periods of up to 15 years were reported,[8] suggesting, according to Weiss,[9] that aging may unmask toxicity that remained asymptomatic during the acute phase of low-level poisoning.

A third example, also reviewed by Weiss, is an outbreak of methyl mercury poisoning in Iraq in 1971-1972 in which the latency period of 16-38 days did not decrease with higher exposure (as documented by blood levels).

A fourth example is Pink Disease, which resulted from the use of mercury-containing teething powders during the first half of the 20th century. Mortality varied from 5.5 % to 33.3% after an illness characterized by misery, bright pink skin with raw beef hands and feet, desquamation, occasional gangrene, anorexia, and severe behavioral disturbances. It was first described in 1903, but a published description did not appear in England until 1922. A review by Dally[10] traces the tedious path of discovery in which the cause of Pink Disease went unpublished until 1945, unaddressed until the voluntary withdrawal of the teething powders from the market in 1954, and unacknowledged in standard reference books until the 1960s. Arguments were presented that not all children who were exposed to teething powders became ill. In the 1990s it was shown that men with azoospermia were the latent victims of the reproductive effects of Pink Disease in childhood.

The lessons of these examples of single-cause exposures to mercury are that dose and response may have paradoxical relationships; long latency may obscure the cause of symptoms due to poisoning; and refusal of the truth may be a particular barrier to open-minded consideration of toxic causation.

SEVEN PHYSIOLOGIC LENSES—A METAPHOR FOR PATIENT-ORIENTED DELIBERATIONS

With individuality as the watch word, and with those lessons in mind, let us see how a consideration of 7 physiologic lenses may help to organize patient-centered clinical strategy.

1. **Energy.** Energy is the principle of change.[11] The more energy there is, the more change is possible. The special way living things manage energy makes it a primary consideration in the study of living things. In particular, while a person sleeps and the muscles and brain consume less energy, the rest of the body is engaged in repair, healing, and detoxification. During sleep the body maintains a high level of sugar in the blood, which is the body's way of delivering the sun's energy to the liver and all the other organs. As physicians, we have not generally been trained to focus on fundamental questions about energy, or "the chemistry of light," when we approach patients—even when their main complaints include a lack of energy. Recently, however, mitochondrial disease, as indicated by abnormalities in organic acids, has become a fashionable diagnosis, suggesting that a patient's metabolic fire is not burning clean.

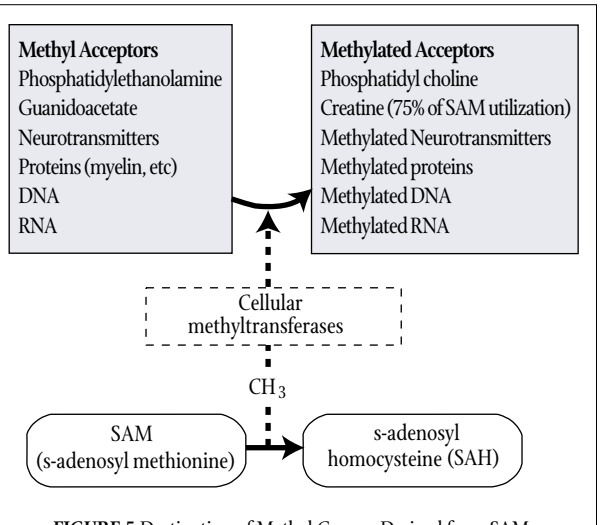

FIGURE 5 Destination of Methyl Groups Derived from SAM

Various inborn errors of metabolism are familiar to pediatricians confronted with severely affected infants who display marked elevation of lactate, pyruvate, and other metabolites in and around the citric acid cycle. More-subtle changes turn up frequently in all sorts of chronically ill patients with impaired capacity to disassemble sugars, fats, and amino acids to retrieve the sun's energy. Such impaired capacity may be part and parcel of a biochemical train wreck into which chronic illness descends in its series of vicious cycles. These physiological changes may provide clues to clinical tactics that can help reinstate the virtuous cycles by adding substances that help or by removing substances that hinder energy chemistry. An example of the former could be a patient with very elevated citrate that normalized with GSH supplementation during a prolonged effort to isolate the factor(s) that might account for the citrate elevation.[12] An unmet need for arginine is another possible cause of citrate elevation.[13] On the other hand, the presumed role of microbial analog inhibitors or agonists of the citric acid cycle could be suspected in an individual who has a mixture of markedly high and low levels of excretion of citric acid cycle intermediaries.[14]

I do not mean by these examples to limit the considerations of energy chemistry to the citric acid cycle. The previous examples citing arginine and creatine suffice to indicate that the lights of energy chemistry blink throughout the whole metabolic landscape. I do mean to carry our attention away from "What diseases make you tired?" to focus on biochemical fundamentals in patients of all kinds, no matter how well or how poorly they fit into some diagnostic box.

2. **Synthesis.** Nitrogen is the element upon which life depends for its complexity. It is the basis for "higher" life found in animals, as opposed to plants, and for consciousness. Dietary essential amino acids come to us prefabricated so that a modicum of enzymatic dexterity is required to create all the shapes and sizes of molecules required for the evolution of consciousness in which our species appears to bear a special responsibility. Sulfur is the element upon which depends the shape of molecules. Sulfur provides the flexible adhesion required to fold and hold the conformation of proteins in all of the moveable keys, locks, hands, pockets, bumps, and hollows needed for enzymatic binding, receptor site rhythms, and the specific stickiness of antibodies with their inventory of immune memories. (Sulfur is absent from the more durable, inflexible templates of genetic memory.)

Methionine brings useful sulfur into the body along with methyl groups on whose placement depends some of the flexibility needed for the specific conformations required for molecular mating. The chemistry of methionine and other sulfur-bearing molecules deserves our clinical consideration as we ponder the synthesizing ability of our patients.[15] Until detailed measurement of thiol intermediaries becomes available from a commercial laboratory, amino acid analysis remains our main tool for assessing levels of methionine and homocysteine that provide clues to disturbances in this chemistry. Amino acid evaluation—akin to analyzing the contents of factory dumpsters in order to assess their manufacturing efficiency—remains the other main tool for assessing synthesizing efficiency in our patients.

3. **Detoxification.** Getting rid of metabolic waste and environmental toxins engages more synthesis of molecules than any other process (other than growth in children) and is consequently a highly energy-dependent process. Just getting rid of the toxic part—ammonia—of recycled protein in the urea cycle accounts for a substantial energy burden. Our body's sanitation department has representatives in all tissues and organs, and its failure is both cause and effect of illness.[16] The metabolic processes involved in thiol chemistry entail nest-

ed vicious cycles, as mentioned previously. Specific aspects of detoxification chemistry are subject to detailed clinical investigation[17-19] but the global importance of detoxification expands its analytical landscape to include questions of the patient's energy and synthesizing efficiency and his or her total toxic and allergic load—especially focusing on the gut and its permeability. Detoxification occurs principally during the dark phase of the circadian cycle,[20-23] so its sleep-dependent, rhythmic pulse calls for attention to the timing of therapeutic intervention.

4. **Messaging**. The mere fact that endocrinology is a medical specialty attests to the clinical relevance of measurements and remedies for messenger molecules. That specialty historically circled its wagons around the pituitary, thyroid, parathyroid, adrenals, and gonads, leaving other pioneers to explore the lands of neurotransmitters, prostanoids, and other actors in the theater of cell signaling. The layperson and many practitioners often do not realize that, in this theater, naturally occurring substances such as amino acids, fatty acids, other nutrients, and accessory nutritional factors provide leverage that is more appropriate to healing chronic illness than steroidal, anti-inflammatory, and blocking agents. The latter work well in acute illness but significantly less well in chronic illness.

Things that can go wrong with the D4 dopamine receptor site illustrate the importance of the receiver's function and ability to provide ongoing transmission of molecular messages. The D4 dopamine receptor site, which is explained in Richard Deth's monograph on attention,[24] is a unique receptor site, but its features still illustrate the point that messaging encounters complexities beyond the mere adequacy of the messenger. The underlying flexibility of the D4 receptor's membrane locale due to its constituent fatty acids is as important as it would be to any receptor. Beyond this, methylation of the phospholipids immediately surrounding the site has a more specific contribution to the activity of the site and constitutes 1 of 4 novel attributes of the D4 receptor.[25] Adjacent neurotransmitter sites become modulated as a result of changes in the membrane locale provoked by methylation. Thus, in contrast to the nearly universal signaling via G proteins, the D4 receptor produces very rapid localized changes in neuronal activity by means of "solid state" modulation of nearby receptors.

A second unique feature of the D4 receptor is that it can directly amplify oscillations in interneuronal circuits so that it participates directly in rhythmic aspects (synchronization) of information transfer. A third feature is the D4 receptor's interaction with folate-dependent aspects of cellular metabolism and energy supply. Finally, dopamine stimulation produces a trophic stimulus to the cell. Unique as the features of the D4 receptor may be, they serve to illustrate the ways that molecular messaging can go wrong or be repaired other than by simply trying to change levels of the signaling molecule.

5. **Membranes and Boundaries**. Just about everything happens on or across multiple surfaces in a human organism that appears whole but is really an integration of compartments from the tiny subcellular to the large bubble of our everyday material reality. In fact, the infinity we associate with the world of consciousness beyond the limits of our everyday sensory experience is not a bad metaphor for the vastness of the boundaries contained within the divisions of our physiology. If the digestive and pulmonary boundary across which we do business with the external world were carried about as an external appendage like a sail or like leaves on a tree, the first clinical question and, perhaps, social greeting would be, "How is your sail?" Except, the health of those tennis court-sized mizzen (the pulmonary epithelium) and mainsail (the digestive epithelium) would be no more a social or clinical secret than the texture and luster of one's skin. The "sail's" texture, integrity, flexibility, and the way it is set to capture its energy source should concern us, even though the digestive membranes' cellular and molecular features are convoluted and hidden from direct observation. Similarly the full extent of the lipid membranes that divide the living intracellular water from its ocean of extracellular water, and form intracellular organelles, should rise to our mind's eye as a surface the size of several football fields.

Travel deeper in your imagination to the molecular level and find yet another boundary consisting of the frontier between the generally reductive molecular milieu and its threatening oxidative surroundings. Are our patients appropriately armed to withstand oxidative stress such as occurs when the cobalt atom in vitamin B_{12} is irreversibly oxidized by nitrous oxide anesthesia (with fatal results in the person with susceptible thiol chemistry)?[26,27]

The evaluation of mucosal, cellular, and oxidative boundaries begins with simply seeing our patients in a way that illuminates these huge and important surfaces, keeping in mind the digestive milieu, the adequacy of essential fatty acids, and the redox potential in the to and fro of glutathione and related mechanisms.

6. **Perception and Memory**. Anatomy fools us into denying that the central nervous system and immune system form a functional unit. If we dissect our language, we see right away that memory and recognition are terms we use with equal facility to describe the principal roles of the brain and immune system. Within the body, these 2 systems appear to be set apart; but, if an alien were to examine a human being with the question "Where is its memory?" the intelligent alien would find it equally distributed between neuronal and lymphoid tissues. Memory of the big world of our senses is in the brain and cognition of the invisibly tiny molecular world belongs to the immune system. The two systems are a functional unit as we take in and respond to signals from our external environment. Some individuals have responses to the external environment that are inappropriately weak or strong. The simplified consequences of such imbalances is illustrated in Figure 6, which shows the extreme consequences of trends created by different patterns of under- or over-responsiveness to external or internal immune stimuli.

If the central nervous system and the immune system are partners in the body's perception and response to external stimuli, then what might be some polarities in the way traumatic life experiences become risk factors for different kinds of illness? Figure 6 illustrates a notion that I have found helpful in communicating to patients the immunological consequences of invasive life experiences on the one hand and loss and grief on the other.[28] The fact that the immune system and the central nervous system share our life experiences is illuminating for people who are burdened by feelings of guilt or shame, which, when understood and lifted, may advance healing.

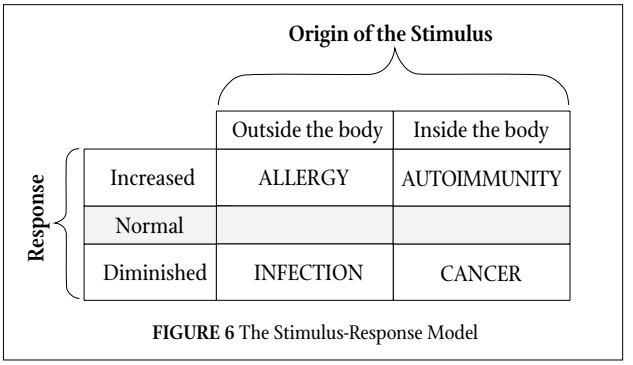

FIGURE 6 The Stimulus-Response Model

7. **Timing**. Individuality is the principal fact that elevates the drama of each patient's illness to the higher slopes of intellectual and emotional effort on our part as we try to teach our patients a new way of thinking about illness.[29,30] Acknowledging functional medicine's respect for individuality, we may avoid semantic arguments if we say that "normal" is a statistical concept that can only loosely embrace a single person's biochemistry or immunology. Just as no individual patient is average, so he or she is not normal except in a statistical sense. When it comes to the temporal domain, however, we are bound by rhythmic imperatives that command humans to heed the universal rules of timing. We all dance to the same beat. Biochemically, we are all different. Rhythmically, we are all the same. In matters of rhythm, "normal" is not a statistical range; it is a relationship embraced by the rules of harmony. There is no normal range for middle C on your piano. Middle C is 256 cycles per second. The harmony of middle C with the next higher C (512 cycles per second) has to do with a precise relationship, not a range of options for either participant in the harmonic relationship. So it is that the meshing of rhythms of pulse, respiration, and all of our other oscillations up and down the scales of our temporal domain, should heed relationships that *fit* when our organism displays the qualities carried in the sense of the word "fitness."

Specific biochemical functions are compartmentalized and sequenced over time, with small waves of activity in every organ and enzyme system in the body being laid out over the big wave of the day-night cycle: the circadian rhythm, which should be considered with the timing of exposure to light; activity; and every therapy, supplement, and nutrient to mesh with the body's timing. Below the day-night frequency of the circadian rhythm are the more rapid oscillations represented by the cardiovascular and respiratory rhythms, brain waves, and periodic conformations of receptor sites and their ligands. Above daily frequency are the longer intervals of menstrual, seasonal, annual, and seven-year cycles. That the timing of these cycles may vary from person to person—more in sickness than in health—does not contradict the fundamental principle that the meshing of these rhythms must follow the strict imperatives of harmony. Physiology invites mechanical metaphor. In the temporal domain, music and dance provide us with a more apt exercise of imagination in which we immediately recognize the difference between consonance and dissonance and keeping time. The relationships between living rhythms obey simple rules that are manifest in our notions of synchrony, resonance, and tuning. The laws of harmony and the day-night cycle of our planet are shared by all of us. We may differ in the ways our biochemistry, environment, or activities and intentions disobey those rules. We do not differ in the hygienic rewards that come from obedience to habits that respect our need to dance in step with the harmonies of our internal and external environments. Awareness of timing is the first step to remediation.

The details of rhythmic integration and related therapeutic opportunities for the healthy meshing of our various rhythms will take a larger part in medicine's future.[31] In that future, medicine will encounter its past—in ancient times, the harmonies of the world were a more conscious part of perceived reality. That reality is one we now consider to be more connected to a spiritual rather than a scientific perception of the world.

CONCLUSION

Laying out a system for thinking about clinical options for individuals with chronic illness does not mean that we have our clinical territory completely mapped. By considering only unmet needs of living organisms to get or avoid certain things—even if one of those things is love—I believe we ignore key factors in healing: imagination, intention, and the attraction toward healing that lives in the implicate order. Some physicians are quick to tell patients, "Do not look for answers" or "There is nothing that will help," effectively amputating hope and blocking imagination and intention. We clinicians regularly witness the forces of hope, imaging, and will in failure and in success. Many of us call upon them, emphasizing nature's strong intention toward healing, cultivating hope, and engaging the patient's will.

I will close by acknowledging two convictions: first, that those who ignore individuality fail their patients; second, that those who do not instill in their patients the seeds of imagining and intention for cure, who do not work to establish a resonance between these forces and nature's strong impulse toward healing, betray the legacy we share as teachers of our patients and students of our own exploration of the landscape of illness.

The map I have offered is helpful when solutions to my patients' problems are not obvious. It is even more helpful when a patient's problem *is* obvious because the biggest cause of mistakes that haunt my office is being blinded by the obvious. When seemingly obvious, I believe I see the patient's problem clearly and become enchanted by my own immediate grasp of a sequence of events that presents a compelling picture from the list of illnesses—be it mild adrenal insufficiency, hypothyroidism, unmet needs for magnesium or omega-3 oils, a problem of mold allergy and yeast dysbiosis, a hidden food allergy, a chemical or heavy metal sensitivity, a gluten sensitivity, a need for hormone replacement, or problems of dyschronism. I attach my ego to my idea. I have learned, however, that I must step back and wonder whether the light I have shed on the situation has also cast shadows, hiding clues that, if considered, would illuminate the problem in a different way.

I remind the patient—and myself—that what is at stake here is not the whole truth of the matter, but only the decision as to what the next treatment step should be. Even so, I prefer not to have to utter the forbidden word "oops," and I therefore carefully review the patient's chronological history form and questionnaire, keeping in mind the questions about the patient's unmet need to get nutrients, light or love, or to avoid toxins or allergens. Then I map my patient's issues against the template of the lenses, and try to remember where I am. When I was in Africa, I knew that, regardless of the problem with which my patient presented, he or she was very likely to have malaria, schistosomiasis, and amoebae that, if ignored, might assert themselves against a weakened carrier. In North America, I recognize that my patients may be "carriers" of unmet magnesium needs, omega-3 fatty-acid deficiency, yeast problems, and heavy metal toxicity, any or all of which may complicate their problems. Such issues are at the heart of a diagnostic paradox that confronts us when we cross the line between prescription pad and integrative or functional medicine. Patients come to us with a great variety of problems upon which we could place myriad diagnostic labels. As we gather experience in the environment in which we practice, we find that even though our patients have "all these different things," some consistently successful strategies emerge; we keep coming back to the relatively short list of causes and remedies.

We who are practicing medicine in the beginning of the 21st century have entered the battle with an old map that pictures the enemy in the false metaphors of disease entities.[32] The scientific medicine that we are evolving recognizes that the individual human being, not the disease, is the fundamental subject of concern and the target of therapy.

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Detoxification Basics

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Almost 50 years ago R.T. Williams first classified detoxification enzymes into phase I (degradative) and phase II (additional) enzymatic reactions. At that time, few of the details we know today were available and very few enzymes were identified. The classification was based on the finding that many xenobiotics, upon entering the liver, are first oxidized (phase I reaction), and then a bulky endogenous compound is added at the site of the oxidation (phase II reaction) before the metabolite is effluxed from the liver into the bile. Remarkably, this classification is still useful, with few exceptions. Recently, as our knowledge of the complexity of the efflux system has increased, scientists have expanded this classification to include the term "phase III" to describe the action of efflux proteins. Efflux proteins belong to the family of ATP binding cassette (ABC) transporters.[1,2]

The phase I enzyme activities include oxidation, reduction, and hydrolysis reactions. Of these, the major players are the cytochrome P450 (CYP) enzymes, which are found across all 5 biological kingdoms. Approximately 50 different human CYP enzymes have been identified. Intriguingly, studying the diversity in CYPs across species allows us to closely map dates of evolution, based on the estimation that about a 1% mutation in the DNA of CYP genes occurs every 4.5 million years.[3,4] From this, one can determine differences in the DNA sequences of divergent CYPs. Plant and animal kingdoms diverged around a thousand million years ago, and vertebrates and non-vertebrates diverged about five-hundred million years ago. Rats and mice only became distinct species some seventeen million years ago. Of the many CYP genes within the human genome, the 3 families expressing CYP 1, 2, and 3 constitute the majority of drug metabolizing detoxification enzymes.[5] These enzymes have broad substrate specificity, so that multiple substrates compete for metabolism at a single enzyme. Chronic exposure (≥ 3-4 days) to a compound that is a substrate for metabolism frequently causes upregulation of enzyme synthesis, resulting in a net increase in 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. The net result of induction or inhibition may be recognized as a drug-drug or drug-nutrient interaction.[6]

Of all the different CYPs, CYP3A4 represents roughly one-fourth of the CYPs in the human liver and is responsible for metabolism of as much as 60% of all drugs, prior to phase II conjugation and phase III efflux. Because of the multiplicity of substrates for CYP3A4, drug-drug and drug-nutrient interactions are common and can be of significant consequence.[7] For example, echinacea taken for 8 days increased clearance of midazolam, a substrate for CYP3A4 and 3A5, by 42%.[8]

Within the CYP1 family, CYP1A1 is highly inducible in the liver, a unique characteristic. CYP1A1 is responsible for bioactivating a number of polycyclic hydrocarbon precarcinogens, such as dimethylbenzanthracene and benzo[a]pyrene, and the carcinogenic heterocyclic amine formed during browning of meats, known as PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine). These activities have done much to persuade the scientific community that CYP1A activity in general may do more harm than good, and that compounds that enhance CYP1A are unsafe.[9] However, the other two members of this family, CYP1A2 and CYP1B1, both metabolize estrogen to potentially less estrogenic products than the major metabolic route (CYP3A4-dependent 16-alpha hydroxy-estradiol formation). Induction of CYP1A2 increases 2-hydroxylation of estradiol relative to 16-alpha hydroxy-estradiol and research is ongoing to determine whether this decreases risk for hormone-dependent breast cancer.[10]

Another key CYP enzyme associated with enhanced toxicity rather than detoxification is CYP2E1. This enzyme metabolizes small volatiles like acetone and amines like nitrophenol, acetaminophen, and aniline. It also carries out S-oxidations to sulfoxides and sulfones. Although it is known to be induced by ethanol, it metabolizes very little ethanol. Ethanol is typically metabolized by alcohol dehydrogenase and aldehyde oxidase to form acetic acid, which then enters normal nutrient metabolic pathways to provide energy. Interestingly, both these enzymes produce NADH2 from NAD. The result is that enzymes associated with the TCA (tricarboxylic acid) cycle that utilize NAD are then reversed, leading to acetone formation from acetyl co-A, lactate from pyruvate, and glycerol from glucose-2,3-phosphate. The latter outcome leads to accumulation of fats and fatty liver.[11]

Ethanol intake may cause acetaminophen toxicity by altering the route of acetaminophen metabolism. Acetaminophen is normally metabolized ~95% by phase II glucuronidation and sulfation, with only approximately 5% undergoing CYP2E1-dependent N-oxidation to the reactive quinone imine. This reactive product is then conjugated to glutathione and leaves the body harmlessly as a urinary mercapturate. However, following several days of ethanol ingestion, CYP2E1 is upregulated and large amounts of the reactive quinone imine are formed, glutathione levels are depleted, and the quinone imine binds to proteins, lipids, and DNA, causing necrosis around the centrilobular area, where the CYPs are located. This interaction between alcohol and acetaminophen is thought to arise from ethanol induction of CYP2E1, such that CYP2E1 successfully competes with the glucuronosyl transferase and sulfotransferase for a greater proportion of the acetaminophen substrate. It appears that alcoholic beverages induce additional CYPs, such as CYP3A4, which then add to the metabolic activation of acetaminophen, aggravating the imbalance between phase I activation and phase II detoxification.[12]

Phase II enzymes are so called because the products of phase I metabolism are frequently substrates for these enzymes. Typically, these enzymes add bulky water-soluble molecules to xenobiotics, often creating inactive products termed conjugates, that are excreted in bile and urine. Major phase II detoxification pathways are glucuronidation, sulfation, glutathione conjugation (and subsequent formation of mercapturates), acetylation, and methylation.[13,14]

A key family of phase II conjugating enzymes includes the glucuronosyl transferases.[15] These enzymes use uridine diphospho glucuronic acid as a substrate, donating the glucuronic acid to bind most frequently at a hydroxyl group, but N- and S-glucuronides can also be formed. The resultant O-glucuronide is typically inactive and excreted via the bile, although the gut microflora contain a beta-glucuronidase activity that can break the conjugate, reversing the hydrophilicity gained from conjugation and permitting re-absorption from the lower intestine

mucosa and uptake via the portal vein back into the liver, where it can be re-conjugated as part of an enterohepatic circulation. Inflamed leucocytes also exhibit glucuronidase activity, which can greatly alter bioactivity of xenobiotics during inflammation.[16]

Both foreign chemicals and endogenous substrates such as estrogen undergo glucuronidation and enterohepatic recirculation. In the liver, uridine glucuronosyl transferases are situated in the endoplasmic reticulum, as are the CYPs. Glucuronidation is a major phase II metabolic path, not just because of its location, but also because the conjugating substrate, uridine diphospho glucuronic acid, is formed from the abundant endogenous intermediate glucose-1-phosphate. The easy availability of glucose-1-phosphate to support glucuronidation contrasts with the vulnerable supply of 3'phosphoadenosyl-5'phosphosulfate and glutathione, required for sulfation and glutathione conjugation, respectively. Both of those systems rely on the sulfate pool, which is easily exhausted in the face of large quantities of foreign substances. Thus, when reactive products are formed during CYP oxidations, such as N-acetyl p-quinone imine from acetaminophen, the glutathione pool can be depleted more rapidly than it can be restored, allowing the reactive oxidation product to bind cellular components such as proteins, lipids, and DNA, causing cell death by necrosis.[17]

About thirty years ago, the first phase-III efflux protein, p-glycoprotein (P-gp), was identified. It is found embedded in the apical membrane of many organs of the body.[18] At the intestine, P-gp is responsible for decreasing bioavailability of many xenobiotics including drugs, toxins, and bioactive food components. When xenobiotics enter the intestinal mucosal cell, a portion of the dose gets effluxed back into the lumen of the gut via the P-gp efflux protein, for which glutathione is required as a co-factor. To determine the role of this efflux system even when a compound is administered intravenously, a small piece of ileum was temporarily isolated in a subject and digoxin was administered.[19] Within 3 hours, 0.45% of the drug was found in this small luminal sac that, extrapolated to the entire intestine, accounted for 11% of the dose.

Like P-gp, another efflux protein—called multidrug resistance-associated protein-2 (MRP-2)—is found on the apical membrane and can decrease absorption at the gut by effluxing xenobiotics. In the liver, MRP-2 plays a key role in effluxing xenobiotic conjugates and sulfated bile acids into the bile duct. More efflux proteins are still being discovered in many different organs and tissues in the body. Important to our understanding of how bioactive food components can affect tissue levels of drugs are multidrug resistance-associated proteins 1 and 3 (MRP-1, MRP-3); they are able to efflux parent compounds as well as glutathione and glucuronide conjugates from organs into blood. Like the phase I and phase II enzymes, phase III efflux proteins are inducible and this may lead to the drug resistance seen so frequently during chemotherapy.

Considering our ability to detoxify xenobiotics across a lifetime, it has been noted that the very young and the very old have lower detoxification enzyme levels—and the elderly also have less ability to respond to the environment by upregulating synthesis of these enzymes. Fortunately, even given the twenty million years since rodents diverged from mammals, a rat's ability to metabolize xenobiotics is very similar to ours, allowing us to use this animal model with confidence during drug development for identifying likely metabolic pathways. Yet there are differences, even among human ethnic groups—partly due to genetic diversity and polymorphisms and partly due to environmental exposure (particularly diet) as reflected in disease risk changes in immigrant groups.[20] A future of personalized medicine will have the ability not only to understand these factors, but also to harness the diet to optimize detoxification.

In considering the vast literature that has been generated over the past 50 years in the area of detoxification enzymes, there are a few key points that we should keep in mind:

1. The detoxification enzymes are multiple, highly inducible enzymes with overlapping substrate specificities, causing drug-drug and drug-nutrient interactions.

2. CYP1A1 and CYP2E1 are particularly known to bioactivate compounds into carcinogens and toxic products, in addition to detoxifying other compounds.

3. Whereas there is an almost endless source of glucose for glucuronidation, sulfate can readily become limiting, compromising the sulfation and glutathione conjugating systems.

4. The drug efflux systems work together with the phase I and phase II detoxification enzymes to rid the body of xenobiotics, and have consequently gained the name "phase III" for their role in clearing detoxified products from the body.

5. Both genotypic and environmental factors cause variation in xenobiotic metabolism and clearance, resulting in different responses to xenobiotics from person to person, and even from time to time in one individual.

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Diet and Detoxification Enzymes

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What you eat certainly affects how you defend yourself from exposure to foreign compounds. We readily accept that exposure to toxic substances from the environment, usually by inhalation or ingestion, may adversely affect our physiology. Similarly, we know that a diet high in salt, sugar, saturated fats, or just too many calories may have adverse effects that lead to a number of chronic diseases, including cardiovascular disease and diabetes. It should not, then, be such a leap in understanding to accept that certain foods will do more than help us avoid adverse health effects; they will actually improve our well-being.

Certain foods can upregulate detoxification enzymes, helping to rid the body of both toxic foreign compounds and toxic endogenous metabolites such as reactive oxygen species that we generate during normal physiological processes. More recently, studies have identified specific foods and food components that enhance our detoxification systems, such as broccoli and the anticancer component within broccoli, sulforaphane.

Change in diet is not simple to accomplish, so it is important to provide patients with clear, rational directives about which foods or supplements are effective in improving health, how foods should be prepared for greatest efficacy, and how much and how frequently these healthy foods should be included in the diet if they are to effectively improve detoxification.

The effect of calorie restriction on health and longevity has been studied many times.[1] Over the years, a body of evidence has accumulated showing that eating less may boost our defense system and substantially decrease our risk for chronic diseases. While not the only result, a major effect of calorie restriction is improved detoxification and clearance of foreign compounds. Interestingly, acute restriction, frequently referred to as starvation, has very different effects than longer-term restriction of calories to 70 or 80% of ad libitum intake. Acute removal of food, or starvation, will rapidly result in insufficient methionine in addition to many other physiological difficulties. Low methionine levels will adversely impact the synthesis of many proteins, including the detoxification enzymes themselves.

Especially affected are those proteins rich in methionine or cysteine—such as metallothionein, the protein responsible for binding and clearing many divalent cations. Insufficient dietary methionine results in an almost immediate loss of S-adenosyl methionine, the starting source for methylation reactions such as DNA methylation and detoxification of catechols through catechol O-methylation; and for production of cysteine, the building block for glutathione. Without glutathione, the body's ability to conjugate reactive electrophiles and quench reactive oxygen species is severely compromised. On the other hand, long-term calorie restriction has been found to be of benefit in maintaining tissue glutathione levels during aging compared to the typical excess of calories in the American diet. Also, calorie restriction supports a more rapid rebound of glutathione synthesis following loss of glutathione after ischemia.[2,3] Given that a key concern as we age is the loss of tissue glutathione levels and, thus, our ability to control oxidative damage, the benefits of calorie restriction are paramount to a healthy aging body.

The story of how dietary components may upregulate the synthesis of detoxification enzymes has developed slowly over the last 30 years, starting with early work by Wattenberg and Conney showing that dietary crucifers altered the clearance of drugs.[4] At that time, the components involved and the mechanisms of improved clearance were unknown. Knowledge slowly grew, taking a great leap forward with the discovery that many dietary components, mostly electrophiles, induce multiple phase II detoxification systems.[5] Talalay and colleagues used the phase II detoxification enzyme quinone reductase as a biomarker for such activity since many other phase II detoxification enzymes were seen to be upregulated in concert with quinone reductase.[6]

Another important step forward was the recognition that quinone reductase and many other phase II enzymes all contain a DNA sequence in the promoter region of the gene, earlier identified as controlled by the synthetic antioxidant tert-butylhydroquinone (BHQ), and given the name antioxidant response element (ARE).[7,8] Apparently, BHQ and other compounds are able to disrupt the cytosolic binding and metabolism of a small protein, Nrf2, which then accumulates and spills into the nucleus, binding to the ARE on multiple genes and triggering coordinated upregulation of all those genes bearing the ARE.[9] As proof of this important mechanism, when the ARE sequence is mutated, or when knock-out mice are generated that do not produce the endogenous factor Nrf2, dietary factors such as sulforaphane from broccoli and curcumin from the curry ingredient turmeric (Curcuma longa) have no effect on synthesis of quinone reductase or other phase II enzymes.[10] Although the ARE is far from being the only regulatory sequence on the promoter region of phase II detoxification systems, it does appear to be the one most frequently affected by dietary components that upregulate phase II enzymes without the concomitant upregulation of phase I enzymes.

Today, knowledge of this intriguing mechanism is still unfolding. Scientific discoveries are explaining why phase II detoxification enzymes and phase III efflux systems are coordinately upregulated by so many food components.[11] There are ARE sequences present on the promoter region of a number of genes expressing phase III efflux proteins.[12] Interestingly, the ARE does not appear to be present on phase I genes. Many dietary components that upregulate phase I cytochrome P450 activities have their effect on cytochrome P450 1A1, through a separate system consisting of a cytosolic binding protein called the aryl hydrocarbon receptor (AhR).[13] Once the AhR is bound to a ligand, the complex travels into the nucleus to bind to a DNA sequence on the promoter region for cytochrome P4501A family members called the xenobiotic response element (XRE). This sequence is also present on many phase II enzymes—including glucuronosyl transferases and quinone reductase—explaining the coordinated upregulation of cytochromes 1A with so many phase II enzymes in response to compounds that trigger the XRE.

A good example is indole-3-carbinol, a metabolite from cruciferous vegetables.[14] Although a very weak ligand for the AhR itself, indole-3-carbinol can generate complexes in the acid environment of the stomach, including 3,3'-diindolylmethane (often referred to as DIM), that bind the AhR avidly. When DIM is bound, the AhR complex is transported into the nucleus where the complex, together with a second protein, binds to the XRE sequence on the promoter region of many genes. This triggers coordinated upregulation of expression of phase I and phase II detoxifi-

cation enzymes. For some genes, the ARE and XRE are in such close proximity, just a few DNA bases apart, that if 2 separate bioactive food components trigger these two at the same time, they can act in an interactive fashion referred to as cooperativity, maintaining prolonged binding and synergy in upregulation.[15] The presence of the XRE on the promoter region of genes expressing phase III efflux proteins is still under investigation.

Having reviewed the mechanisms of action of food components, it appears easy to understand how multiple food components trigger upregulation of detoxification systems and that whole foods and mixtures may provide greater effects than single components. But extrapolating mechanistic studies to animals and then to humans is complex. Utilizing the benefits of this mechanism at the level of the whole body requires attention to many details.[16] Often, such details are missing from the literature since, unlike the required studies prior to the sale of pharmaceuticals, there are no regulations requiring definitions for effective dosing formulations, amounts, and regimens. So little is known about dosing, that often the choice of dose for a clinical trial is not based on sufficient information to make an informed choice. For example, concerns for adverse effects brought 2 beta-carotene studies to a halt.[17] These 2 studies raised blood levels of beta-carotene 6-fold or more above the normal range, whereas 2 studies that were not associated with adverse effects only raised blood beta-carotene levels to about 2-fold normal.

Studies carried out in cell culture must be repeated in whole animals and clinical trials if we are to know whether the bioactive food components of interest even reach the site of proposed activity without being destroyed either in the gut or through metabolism at the gut wall and in the liver. For example, work with curcumin in cell culture has shown effects that are completely different from effects seen in the whole animal.[18,19] Green tea, associated with improved detoxification and prevention of a number of cancers, contains epigallocatechin gallate (EGCG), which is found to be only 1% or 2% absorbed.[20] This is possibly due to destruction in the gut. If this is the case, then how does one compare dosing in digestible capsules with dosing by tea drinking? Furthermore, the question remains whether there is a role for caffeine, a normal component in green tea, in upregulation of detoxification enzymes and prevention of cancer. However, it is clear that caffeinated, but not de-caffeinated, green tea upregulates cytochrome P450 1A2.[21] It also provides greater protection against skin cancer development in mice exposed to dimethylbenzanthracene and UV light than does decaffeinated green tea.[22]

Finally, it is necessary to consider the entirety of science's growing knowledge on the effects, beneficial and adverse, any given dietary component may have on detoxification enzymes—and, thus, on the disposition of both that component and other xenobiotics in the body such as drugs, toxic substances, and other dietary components. Because detoxification enzymes lack specificity, as well as mechanisms that control their expression, drug-drug, drug-nutrient, and nutrient-nutrient interactions can be expected to occur frequently.

Perhaps one of the best known, although still evolving, stories of drug-nutrient interaction is that of grapefruit and other citrus juices.[23] It has been common knowledge for some time that grapefruit juice inhibits the action of intestinal cytochrome P450 3A4, probably due to the presence of furanocumarins such as bergamottin and dihydroxybergamottin, although there are other bioactive components within grapefruit. The effect of grapefruit on phase III efflux proteins is a little more complex, varying from acute effects—when efflux from the intestinal mucosal cell back into the lumen of the intestine is inhibited, ostensibly improving absorption—to chronic effects where this efflux system is upregulated and the organic anion transporting system that moves xenobiotics out to the cell and into plasma is inhibited. Both these actions have a net inhibitory effect on absorption of drugs and bioactive components. Furthermore, orange juice appears to have some of the same effects. When the details are known, determined in clinical trial under strict adherence to protocol and using carefully evaluated dosing and frequency of uptake, it may be possible to take advantage of these effects. Until that time, it cannot be recommended that one takes daily drugs or vitamin tablets with a glass of juice. Since the time that this paper was presented, a manuscript has appeared describing UV-irradiation of grapefruit juice to destroy furanocoumarins and relieve the drug-nutrient interaction with P4503A.[24] Thus, the more we find out about these interactions, the more we may be able to control them.

Currently, the story is similar for many foods and food components: the specific mechanism(s) are studied in cell culture and possibly in whole animals, but not enough is known about effectiveness in humans to provide a general intake recommendation for disease prevention. More translational research is needed if we are to harness the benefits of foods to their maximum.

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Working Up the Toxic Patient: Practical Intervention and Treatment Stategies

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INTRODUCTION

Some problems in clinical healthcare are straightforward. The patient might present with low back pain, having perhaps strained the quadratus lumborum muscle. The patient history matches the physical exam findings and a treatment program is recommended with fairly predictable outcome.

The problems with toxic patients are more complex: they will seldom present with a history of toxic exposure; their symptoms are multisystem and multifactorial; and the findings of the physical exam may provide little confirmation of the intake. Proceeding to lab evaluation can be difficult because few of the indicators at intake provide a clear direction for the clinician to follow. Toxin exposure is hard to detect and the effects are almost impossible to predict with certainty. Toxicity is a highly individual situation based largely on genetics and the strength of the toxic exposure(s). It is the author's experience that we know very little about how the human system responds to multiple interacting toxins; and yet, toxic exposure is a common experience even for "healthy" individuals.

One study[1] found that the combined exposure to toxins of Gulf War veterans created a toxicity cocktail that could not be estimated; the sum of the parts exceeded the whole. In another review article, it was noted that 2 particular PCBs given together have 20 times the capacity to switch the sex of animals than when each is given separately.[2] When endosulfan and dieldrin are combined, they deliver 1600 times the effect of the dieldrin alone. Mercury and lead have been shown to have synergistic effects when combined.[3] Toxic patients are extremely difficult to assess, diagnose, and treat with reliable, predictable effects on their presenting complaints. The clinician has to be thorough, think like a detective, and look at unlikely sources of toxicity and health problems.[4]

HISTORY OF CLINICAL THERAPY

Detoxification therapies applied by the practitioners of the past were not called "detoxification therapy." Their methods of hydrotherapy, fasting, regulated diet, and Nature Cure are, however, similar to many modern detoxification methods.[5] By studying the work of Sebastian Kneipp,[6] John Harvey Kellogg,[7] O. G. Carroll, and John Bastyr,[8] one gets the impression that doctors and healers found that the best results happened in a setting where the patient could be closely watched. Historically, these doctors described their toxic patients as suffering from "auto-intoxication."[9] Medical literature now lends credibility to this diagnosis and to the methods used to treat many chronically ill patients.

Culturally, we tend to think of detoxification as a strategy for dealing with patients exhibiting drug and/or alcohol dependency.[10] This bias is also reflected in the lack of nutritional strategies to care for those who have problems with the toxicity of alcohol and drugs. Ironically, the findings of practitioners who have used nutritional strategies for alcohol detox can be applied to patients with other toxicity-related issues. The liver is the organ that bears the primary role of breaking down any toxin, exogenous or endogenous.[11] The problem of "system overload" is a common consequence of many basic health issues, regardless of current pollution and toxin exposure. For this reason, skillful physicians in all cultures have relied on purification strategies to harmonize and balance biochemistry to induce the body into a pattern of self healing. This strategy is a valuable addition to a medical armamentarium because it allows the patient and doctor to deal with a multi-system/multi-problem approach using multi-system therapies. Toxicity-based clinical problems are embedded within the complex web of an interdependent ecosystem.[12]

WHICH PATIENTS ARE GIVEN DETOX THERAPY?

The most common chronic complaints seen in clinical practice for which there are no known diagnostic criteria are pain, fatigue, and weight gain. Detoxification therapy is indicated in this class of patients.[13] If the patient is acutely toxic, appropriate diagnosis and treatment are indicated.[14] Working up and treating this type of patient is challenging; the chronically toxic patient is even more so.

A complete clinical detoxification program should focus on 3 targets:

1. retrieve gut functioning,
2. reduce heavy metals, and
3. reduce organic chemicals stored in fat tissue.

This type of program can be applied to patients with immune disorders or digestive problems, to cancer patients, and to those individuals who present with psychoneurological problems.[15] Clinically, the best results are achieved with patients presenting with mild cognitive disturbances. Providing assistance in this area can enhance the quality of life in ailing individuals for whom a more conventional medical assessment has not uncovered anything helpful.[16]

This type of clinical detox program can support cellular functioning, improve the filtration capacity of the liver, stimulate the excretion of toxins through the kidneys, bowel, and skin, support gut repair, and improve neuroendocrine balance in the hypothalamic-pituitary system. Studies have shown that poor liver function has a dramatic effect on the level of cognitive functioning.[17] It has been postulated that "recycled" metabolic by-products that are not eliminated by the body contribute to poor cell signaling. The retention of metabolic end-products involves toxic effects on intermediary metabolism. The action occurs at the cell membrane level. As an example, in uremic patients, no single individual compound has been implicated as the uremic toxin.[18]

Reviews of medical literature detailing the negative health effects of toxicity demonstrate that it may be important to help certain patients relieve some of the toxic body burden that modern living imposes.[19,20] Failing to address this issue bodes ill for individuals living in modern society. It is well known that certain chemicals at certain levels damage critical areas of the brain.[21-23] It remains to be seen whether detoxification strategies can avert the social epidemic of toxicity and anticipated increase in chronic degenerative health problems. Our current health paradigm evolved to treat social epidemics of a

different nature.[24] Chronic degenerative disease and poisoning from environmental pollution are not specialties of our healthcare system. Clinical detoxification strategies represent a move or transition in medicine from treating acute disease as the main cause of death at the turn of the 20th century to treating chronic degenerative disease as we enter the 21st. Physicians must develop the skills necessary to cope with this trend.

CLINICAL PROBLEMS AND ASSESSMENT

On presentation to the clinic, the doctor should consider all patients as potential candidates for detoxification therapy. A thorough medical history examining toxin exposure and symptom patterns indicating systemic dysfunction should be taken. In particular, patients with fatigue, muscle pain, immune and neuropsychiatric problems should be thoroughly screened. The dividing line between patients diagnosed with chronic fatigue syndrome and those with fibromyalgia or multiple chemical sensitivity can be difficult to distinguish.[25]

Taking a good family history is absolutely essential. Genetic predispositions can influence the individual's reaction to toxin exposure, and they can also affect the outcome of therapy. It cannot be stressed enough that individualizing your treatment gives better clinical results. Knowing whether your patient has a genetic predisposition such as a sulfoxidation defect offers a chance to focus on prevention of severe chronic degenerative diseases.[26]

Variations in sulfation and sulfoxidation are inherited metabolic polymorphisms. Sulfation is a limited capacity xenobiotic conjugation pathway that is present in many tissues. A significant number of individuals with environmental intolerance or chronic disease have impaired sulfation of phenolic substances from starvation of sulfotransferases for sulfate substrate. The sulfate conjugation of phenolics is an important pathway for the detoxification of catecholamine neurotransmitters, steroids, bile acids, phenolic and aromatic drugs, and xenobiotics. Impaired sulfation may cause tyramine headache due to a poor first-pass sulfation of monoamines. Tyramine is a bacterial fermentation product closely related to catecholamine neurotransmitters, found in cheese, wine, etc.

This biochemical pathway may be the link to explaining some findings (eg, Feingold, B. *Why Your Child Is Hyperactive*. New York: Random House, 1975) that certain children react to food colorings and preservatives. The treatment of depletion or disruption of the sulfate pool may be very important in diet-responsive Feingold patients and autistic patients. Depletion of sulfates might elevate endogenous biocomponents like bile acids and joint glucosamine glycans, leading to primary biliary cirrhosis and rheumatoid arthritis.

Detoxification assessment may provide special considerations for those with neurodegenerative disease, and for prevention in those at risk for such conditions.[27] Patients presenting with chemical sensitivity may be heralding the potential for more serious diseases. Christopher Reading, MD, has evaluated the case histories of over 5,000 patients and strongly advises doctors and patients to draw up family trees showing diagnosed illnesses. It is important to study the various ways certain genetic disorders are inherited. For example, a father cannot pass on an X-linked disorder to his son because his son only gets the Y chromosome from his father, but all the daughters are at risk. Dr. Reading found 1 family where the manic depression was X-linked with an additional X-linked B12 deficiency. The B12 deficiency was later found to be due to wheat allergies, a common inherited trait. He treated this family with vitamin B12 and a gluten-free diet. Their anemia and manic depression resolved.[28] A good family history can even find those at a higher risk for cigarette smoking.[29]

Lab Work-up

After an initial visit, the key strategy is to rule out coexisting disease. Doing a CBC, chemical screen, urinalysis, hair analysis, and serotype are important tests in a screening protocol. Depending on the case, urine amino acids, heavy metal excretion, intestinal permeability, digestive analysis, and food allergy IgG/IgE can be utilized.

Urine Amino Acids

Urine amino acids have been investigated in patients with chronic fatigue syndrome.[30] Essential amino acids provide precursors in the cycle for ATP production, as well as precursors for neurotransmitters. Supplementation with amino acids can significantly affect these processes. The key point is to assess levels of amino acids critical for biotransformation.

Mercury and Heavy Metals

Both mercury and lead body burdens must be assessed because of coexisting toxicity-related health issues. Mercury preferentially disables the body's natural detoxification organs.[31] To assess mercury levels, a provoking or chelating agent is needed—one that has a high degree of binding affinity.[32] DMPS (2,3-dimercapto-1-propane-sulfonate) provides an excellent challenge substance because of its high degree of sulfhydryl bonds.[33] For diagnostic purposes, either IV or oral dosing is appropriate. If testing for mercury, it is appropriate to screen for lead, cadmium, and other heavy metals at the same time. Even though DMPS enhances excretion of a wide number of metals, many clinicians opt to combine chelating agents in the same challenge test. This remains a wide and relatively unexplored area of detoxification medicine.

Intestinal Permeability

Patients with inflammatory bowel disease have up to a six-fold increase in gut permeability.[34] Patients with other chronic immune and digestive problems also commonly have a compromised gut barrier.[35-37] The gut damage and subsequent downstream health problems probably result from local immune-mediated inflammatory reactions to food and dietary antigens.[38] In 1 study, where children were determined to be allergic to foods,[39] intestinal permeability testing proved to be a non-invasive way to monitor patients. There are many protective factors in the intraepithelial intestinal mucosa: lymphocytes, secretory IgA, other immune globulins, mucosal coat, and microvillous membrane. Even though the milk reaction is a local event involving a complex web of protective factors, cow's milk allergy symptoms are commonly found elsewhere besides (but also including) the gut, such as in the respiratory tract and skin. Intestinal permeability can be an excellent measurement for cow's milk food allergies.

THE GUT-LIVER CONNECTION

Since everyone has bacteria in the gut, if the gut is leaking, those patients by definition are leaking bacteria and bacterial toxins. Gut-leaked enteric bacteria and endotoxins play a role in multiple organ failure.[40] Once damage is initiated, it becomes a critical strategy to address liver detoxification.[41]

Bacteria and their endotoxins have a major impact on the host's immune system. Bacterial translocation causes decreased systemic immune responsiveness.[42] Failure of the gut barrier results in further impairment of host defenses, thereby leading to increased survival of translocated bacteria. Endotoxin management is a primary strategy in assessment and treatment. The lipopolysaccharide (LPS) macromolecule of the outer walls of gram-negative bacteria that have died can transit the

gut in patients with intestinal permeability. Endotoxemia has gained favor as an explanation for multiple organ failure with severe trauma and sepsis, and it is associated with Crohn's and neonatal enterocolitis.

Integrity of the detoxification and immune systems is critical in the response to endotoxins. Complications in chronic liver disease can be induced or aggravated by LPS because LPS are scavenged by Kupffer cells; this then depresses p450 and impairs mitochondrial function. Alcohol is known to aggravate LPS toxicity. Strong injurious products are released by macrophages exposed to LPS, causing the need for antioxidants.[43]

Digestive Analysis

An examination of the patient's stool can provide clues as to the extent of dysfunction and dysbiosis in the bowel. A thorough digestive analysis will measure complex sets of interdependent relationships:

- Digestion
- Absorption
- Detoxification
- Immune recognition
- Ecological balance of bacterial flora

Chronic enzyme and HCl deficiency disturbs gut pH and bacterial ecology of the small intestine. It also impairs nutrient digestion and absorption. The humble acts of chewing more thoroughly and eating more slowly can go a long way toward remedying some enzyme deficiencies. Chewing stimulates the secretion of epidermal growth factor, which prevents intestinal permeability.[44]

The ecology of the bacterial flora can be the source of many chronic immune-related diseases. Inhibitors of detoxification might be coming from gut flora.[45] For example, encephalopathy in cirrhotic patients develops after a meal where they cannot metabolize the amino compounds produced by gut flora. The gut flora produces a wide array of chemicals that cause reactions with all organs in the body. In susceptible individuals with reduced hepatic enzymes, these partially metabolized metabolites of gut flora pass into systemic circulation to produce symptoms at distant parts. It is well known that irritable bowel syndrome can occur after surgery, radiation, gastroenteritis, and the use of antibiotics, all of which may change the bowel flora. Some food allergies may not be an immunologic disease but a disorder of bacterial fermentation and enzyme deficiency.

Enteroadherent *E. coli* are present in the stool in a high percentage of patients with a variety of food-related autoimmune problems such as Crohn's disease. Abnormal bacteria are also found in patients with rheumatoid arthritis and ankylosing spondylitis. Human-leukocyte antigen B27 is synthesized by the fecal flora and associated with facultative anaerobes, klebsiella, and proteus.[46]

Food Allergy Testing

Patients with chronic gut/liver/immune problems respond to the identification of immune-mediated food sensitivities and the use of an elimination diet and food rotation program.

Salivary Hormone Testing

Patients under chronic stress frequently have gut/liver dysfunction. Identifying clinically significant hormone abnormalities and treating appropriately facilitates better clinical results.

Organic Acid Analysis

Altered organic acids in the urine can be found in patients with dysbiosis.

DETOX THERAPIES

The essential steps of the clinic-based program that I use are:

- brief water fasting (2 days),
- oligoantigenic diet (5 days) and slow reintroduction of omitted foods,
- saunas and hydrotherapy (1 month),
- nutritional supplements (1 month), and
- chelation therapy for appropriate metals (1-5 years).

Additional tips:

- No supplements should be given during the water fasting except for vitamin C.
- Structuring supplement recommendations for twice-a-day dosing improves compliance.
- Ensure that there is some sort of protein shake for the patient to use, if needed.

Using all these elements together gives reliable results for restoring balance to the system and reducing the toxic burden on vital organ systems.

Fasting

Fasting on water for a short period can be an important clinical therapy for some toxic patients. By stopping all food, the metabolic machinery of the body can focus on cleaning the blood and lymph.

While water fasting may not be suitable for severely compromised patients, research has shown calorie restriction and fasting to alleviate hypertension,[47,48] diabetes,[49] epilepsy,[50,51] and rheumatoid arthritis.[52] Recent research has shown that calorie restriction may be the most powerful way known yet to extend lifespan.[53,54] Studies have shown that high glucose and insulin damage mitochondria, and calorie restriction (fasting) reduces the total amount of oxidative stress within the cellular mitochondria.[55,56]

Fasting may improve liver function. Fasting has traditionally been thought to enhance the liver's ability to clear out metabolic byproducts from the blood stream, and regenerate the liver's ability to function in a healthy way. There are indications from a few animal studies that dietary restriction may help to reduce the risk of age-related diseases associated with impaired lipid metabolism.[57,58] However, caution is indicated because long-term fasting or fasting in a polluted environment can deprive the body of nutrients that are critical to a patient's health. Fasting should be done for short periods of time in a pure environment and, in my practice, I recommend taking vitamin C during fasting in the range of 1 to 4 grams per day.

Fasting may benefit cognitive functioning. Several studies have shown that as severe liver toxicity progresses, the patient fails to break down Valium-like compounds that create a toxic state.[59,60] One might hypothesize a continuum of such effects for patients who are not nearly so ill. Patients who fast do often report a sense of renewal and clearer thinking. Fasting allows the liver to reduce the presence of recycled chemical messengers like adrenalin and other stress hormones, which often have a second chance to restimulate the nervous system when they are not biotransformed and excreted appropriately.

Caloric restriction improves immune function. Caloric restriction, which can be achieved by short-term fasting, appears to have measurable benefit for the immune system.[61,62] It rests the intestines and liver, both key sites of immune function. It is estimated that 60% of our immune system resides in our intestines. By resting this major site of immune function with fasting, the patient's immune function may be potentiated. A fast of 36 or 60 hours significantly increases the

power of white blood cells to destroy pathogenic bacteria.[63] Conversely, eating can depress immune function and have a proinflammatory effect,[64,65] whereas energy restriction may restore the impaired immune response.[66] Studies have shown that a glucose challenge increases the generation of reactive oxygen species (ROS), while nutritional restriction can inhibit ROS generation by leucocytes.[67,68]

Fasting benefits arthritis. It has been demonstrated in research settings that fasting benefits arthritis.[69] The best results in treating autoimmune arthritis are achieved when a short fast is combined with a change to a vegetarian diet, and foods to which the patient is sensitive or allergic are removed.[70,71] Fasting may be involved in changing the bacterial flora in a favorable way for patients with rheumatoid arthritis. Abnormal bacteria or microflora are present in the stool in patients with a variety of autoimmune problems such as Crohn's disease,[72] rheumatoid arthritis,[73] and ankylosing spondylitis.[74] Anaerobic bacterial species such as klebsiella and proteus have been implicated.[75] Fasting may play a role in changing bacterial flora, perhaps by enhancing competition and thereby giving dominance to probiotics. Changes in intestinal flora from a vegan diet have been documented.[76]

Fasting contraindications. A 2-day water fast is safe for most patients. Certain exclusions are important, such as diabetics, hypoglycemics, and severely nutritionally deficient individuals. The biggest risks to most patients are hypoglycemia and orthostatic hypotension with vertigo, sometimes resulting in fainting. Although these reactions are generally harmless, they can cause a fall. Patients should be warned to take extra care in standing up—ie, getting out of bed or a hot bath, or getting up from a chair. If faintness or vertigo does not resolve within a few minutes, patients should contact their practitioner.

There is medical literature to suggest that fasting for a prolonged period of time can diminish the body's stores of glutathione, making it more susceptible to aging and disease. Low tissue antioxidant status is found under dietary restriction because fasting lowers glutathione detoxification in the liver.[77,78] Also, fasting can down regulate phase I detoxification.[79] Therefore, patients who are fasting should be very careful to avoid any chemical exposure, because lack of dietary protein makes the liver unable to process toxins optimally due to lack of inadequate amino acid precursors that are important to the detoxification pathways. (As an aside, patients who are preparing to undergo surgery might have fewer complications to the anesthetic if they were put on a protein-dense regimen instead of clear fluids.[80,81])

Oligoantigenic Diet

After a 2-day water fast, a simple diet of rice, fruit, and vegetables is then followed for 5 days. This is similar to an oligoantigenic diet, used for allergic, behavioral, and digestive problems.[82] This simple diet provides enough caloric input to sustain the patient but is very easy on the intestinal environment to allow optimum rest. The rationale for vegetarian fare is twofold: vegetarian diets contain fewer potential food allergens that can cause activation of the gut-associated lymphoid tissue, and enhanced vegetable intake provides more soluble fiber, bioflavonoids, antioxidants, and complex carbohydrates. Some patients do experience fatigue on this program; if it is not ameliorated with rice- or whey-based protein shakes, it will resolve upon resuming normal protein intake (unless, of course, the patient is allergic to the food being reintroduced).

A high vegetable content is recommended because of its ability to modulate liver detoxification in a beneficial way. This is probably due to the effect vegetables have on the CYP450 enzyme system.[83] Also, vegetables contain a high level of soluble fiber, essential for rebuilding gut integrity. Fiber helps maintain intestinal permeability and fiber could help prevent bacteremia.[84] Vegetables also provide precursors to stimulate liver detoxification.[85] The cruciferous family has the widest range of therapeutic benefits.[86] By choosing organic foods, patients have the benefit of higher nutritional value and lower pesticide content.[87,88] Most patients, doctors and government regulatory agencies do not take the health impact of pesticide residues on food supplies seriously enough.[89] An Israeli study conclusively related a drop in the incidence of breast cancer among Israeli women to a new law prohibiting the use of pesticides. The estrogenic effects of pesticides accelerate breast cancer and other hormone-sensitive cancers, an effect that is magnified when more than one type of pesticide is present or when combined with the consumption of large quantities of alcohol.

Avoiding certain food groups. One man's food is another's poison.[90] Bioactive peptides from foods may act as vasoregulators, growth factors, releasing hormones, or neurotransmitters. Foods generate many reactions that are not true allergies—they may be intolerances or sensitivities. Tyramine in chocolate, for example, causes bouts of headaches in susceptible people because of a genetic inability to detoxify this vasoactive amine before it goes out into systemic circulation. This may be related to genetic predisposition; migraine patients have a low level of monoamine oxidase and phenolsulfotransferase.[91]

Partial enzymatic digestion of reactive food proteins such as gluten and casein may result in the production of opioid-like compounds called exorphins in the gut.[92] These opioid-like compounds can produce behavioral abnormalities such as those seen in food intolerance.

Sauna and Hydrotherapy

Sauna therapy can support the removal of fat-soluble toxins from the body, and has been shown to provide relief of symptoms for patients with toxicity conditions.[93,94] Sauna programs need to be carefully tailored to the individual patient and supervised closely, particularly with more compromised patients.

Hydrotherapy has been employed for hundreds of years because of its ability to stimulate circulation. It used to be the mainstay of traditional naturopathic medicine for the treatment of chronic degenerative disease. One medical study on the effectiveness of hydrotherapy for detoxification proved it to be effective in the treatment of lead poisoning.[95] This study showed an increase in lead excretion of 250%. Cold-water application in winter swimmers has shown an increase in the level of reduced glutathione in red blood cells.[96] There is a study showing that wet sheet pack hydrotherapy produced a statistically significant increase in the level of cognitive functioning.[97] Other research has shown its usefulness in symptomatic relief of many conditions, including rheumatoid arthritis,[98] osteoarthritis,[99,100] chronic heart failure,[101] management of spasticity,[102] and other similar conditions.

Theoretically, application of alternating hot and cold water to the body stimulates regulation of sympathetic tone in the extracellular matrix, and generates a "pumping" action that stimulates circulation of blood and lymph. The extracellular matrix is now understood to influence cellular development, movement, reproduction, and shape, as well as biochemical function. Dr Alfred Pischinger, professor of histology and embryology at the University of Vienna, saw the importance of the extracellular matrix. In 1991, he wrote that the extracellular matrix is the support system for the cell and the foundation substance in which all cells are embedded. The extracellular matrix is made up of collagens and polysaccharides that form proteoglycans. These 2 molecules form a water-filled, gel-like "ground substance" in which the connective tissue fibers are embedded. The condition of the space around a cell is as important to health as what occurs within the cell and in the membrane that encloses it.

Instruct the patient to do the hydrotherapy at home or institute a hydrotherapy option as part of your clinical practice.

Supplements

There is a complex set of variables involved in choosing the appropriate supplements for detox patients. Supplement programs should be adapted to the individual patient's need, using the following general strategies:

- Antioxidants for cellular protection
- Amino acids for phase II detoxification
- Cholagogues (bile stimulants)
- Bile binding
- Replacing probiotic bacteria
- Repairing intestinal permeability
- Vitamins, minerals, and nutritional co-factors
- Cathartics
- Antiparasitics

Glutathione. Glutathione is a primary detoxification chemical in the body.[103] A sick liver does not produce adequate levels of it, thereby accelerating damage and disease.[104] Glutathione enhances immune function and protects cells against free radicals,[105] drugs, and environmental pollutants. Small decreases in mitochondrial glutathione result in cell death. Oral supplementation appears inadequate. In patients who need glutathione, based on history or lab analysis, 500 mg IV several times a week to once a month is a recommended dose. Vitamin C orally and IV is also an effective way to recycle glutathione but, in patients with poor liver function, use glutathione direct.[106]

Lipoic acid has powerful antioxidant abilities extending to both the oxidized and reduced form.[107] It helps the functions of other antioxidants like vitamins C and E, co-enzyme Q10, and glutathione to "recharge" themselves to their active forms. It has the ability to protect organs like the brain and liver from free radical damage.[108] Like other liver-protecting agents, lipoic acid has been effective in treating poisoning from mycotoxins, mercury, lead, carbon tetrachloride, and aniline dyes.[109-110] It has also been used to treat liver disease, alcohol-induced cirrhosis, viral hepatitis, AIDS, glaucoma, and complications of diabetes. The recommended dosage of lipoic acid is 600 mg twice daily.

Milk thistle (*Silybum marianum*) has a group of bioflavonoids collectively known as silymarin. Silymarin is really an antitoxin. No drug can protect your liver the way silymarin can because of its strong action against free radicals and its ability to enhance glutathione production by more than 35%, thus increasing liver detoxification. Its effectiveness has been measured by lowered enzyme markers for nonspecific liver cell inflammation.[111]

Silymarin has been used successfully in the treatment of the following conditions:

- Neurological complications caused by diabetes
- Fatty liver disease in diabetic patients
- Nausea caused by high levels of hormones naturally produced during pregnancy
- Chronic alcoholic liver diseases
- Toxic exposure to industrial chemicals
- Acute viral hepatitis
- Cirrhosis of the liver
- Immune system and liver protection

The recommended dosage of silymarin is 200 mg 3 times daily.

Curcumin. Turmeric (*Curcuma longa*) and its active bioflavonoid curcumin have been used by Indian and Chinese herbalists for thousands of years. It is known to protect the liver, promote bile flow, and act as a powerful anti-inflammatory.[112-114] Curcumin's ability to fight inflammation also makes it helpful as an antioxidant, scavenging free radicals and protecting DNA from oxidant breakage and lipid peroxidation.[115] The recommended dosage is 500 mg 3 times a day.

Green tea (*Camellia sinensis*). Catechin is found in green tea. Green tea contains several polyphenol catechins but the strongest is epigallocatechin gallate.[116] Studies have shown that drinking green tea offers smokers some protection from cardiovascular disease. Its ability to activate detoxification enzymes in the liver has also been shown to provide a defense against cancer. Catechin has a special affinity for the liver, so it can be used effectively in the treatment of liver diseases, hepatitis, and alcohol-related liver syndromes. It also offers protection from bacterial toxins in the intestines and from arthritis and scleroderma. The recommended dosage of green tea is 5 cups a day.

NAC. N-acetyl cysteine is the primary precursor to glutathione. Studies have shown that NAC affects concentrations of glutathione in the blood, helping to provide adequate levels so that the chemicals produced during detoxification don't damage other tissues.[117] Studies have shown it to be helpful in managing HIV and hepatitis C.[118-120] The recommended dosage of NAC is 500 mg 3 times daily between meals.

Probiotics. Antibiotics, steroids, and birth control pills commonly upset the normal bacterial equilibrium in the intestines. Poor diet and chronic constipation are also contributing factors. Reseeding the intestines with favorable bacteria creates an optimum, balanced environment, protecting the intestines and the rest of the body from dangerous bacterial insurgents.[121] An investigation in monkeys demonstrated a marked increase in the proportion of mercury-resistant bacteria in the flora of the intestine and oral cavity soon after installation of dental amalgam tooth fillings, which increased until after the amalgam was removed.[122]

Chelation. After determining the offending heavy metals, select a chelating substance that fits the patient and the problem(s). Time the chelation as a "post" detoxification strategy; patients can only handle so much healing before it makes them sick.

Post-Detox Recommendations

After the 7-day program, it is best to continue the hydrotherapy and/or saunas and the supplement strategies for at least a month. The patient should slowly re-introduce foods, starting with foods least likely to irritate the intestinal mucosa. Since the diet is relatively low in essential amino acids, the introduction of eggs, fish, or lean meat on a daily basis helps to restore proper protein balance. After several days of this regimen, begin adding foods that seem prudent for the individual patient; last, introduce known allergens like dairy products, wheat, and soy foods (one at a time and allowing a day or two between each new food to determine any reactions).

Detoxification therapy can take months in chronically ill patients so patience is a critical ingredient in the care of our poisoned population.

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Diet, Genetic Polymorphisms, Detoxification, and Health Risks

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ABSTRACT

Modulation of detoxification enzymes is one mechanism by which diet may influence risk of cancer and other diseases. However, genetic differences in taste preference, food tolerance, nutrient absorption, and metabolism and response of target tissues all potentially influence the effect of diet on disease risk. Thus, disease prevention at the individual and population level needs to be evaluated in the context of the totality of genetic background and exposures to both causative agents and chemopreventive compounds. Polymorphisms in the detoxification enzymes that alter protein expression and/or function can modify risk in individuals exposed to the relevant substrates.

Diet is a mixture of carcinogens, mutagens, and protective agents that are all metabolized by detoxification enzymes. Genotypes associated with more favorable handling of carcinogens may be associated with less favorable handling of phytochemicals. For example, glutathione *S*-transferases (GST) detoxify polycyclic aromatic hydrocarbons present in grilled meats. GSTs also conjugate isothiocyanates, the chemopreventive compounds found in cruciferous vegetables. Polymorphisms in the GSTM1 and GSTT1 genes result in complete lack of GSTM1-1 and GSTT1-1 proteins, respectively. In some observational studies of cancer, cruciferous vegetable intake confers greater protection in individuals with these polymorphisms; however, in other studies, the converse is observed. A recent study of sulforaphane pharmacokinetics suggests that lack of the GSTM1 enzyme is associated with more rapid excretion of sulforaphane. Many phytochemicals are also conjugated with glucuronide and sulfate moieties, and are excreted in urine and bile. Polymorphisms in UDP-glucuronosyltransferases (UGT) and sulfotransferases (SULT) may contribute to the variability in phytochemical clearance and efficacy. The effects of UGT polymorphisms on flavonoid clearance have not been examined, but UGT polymorphisms affect glucuronidation of several drugs and steroid hormones. Genetic polymorphisms in detoxification enzymes may account in part for individual variation in disease risk but have to be considered in the context of other aspects of human genetics, gut bacterial genetics, and environmental exposures.

NARRATIVE SUMMARY

Numerous factors contribute to variation in nutritional requirements and responses to diet, including sex differences, stage in life cycle, disease states, physical activity level, and environmental exposures. Underpinning this is inherited genetic variation that also influences nutritional status and needs. Genetic difference in taste preference, food tolerance, nutrient absorption, transport and metabolism, and effects at the level of target tissues may all play a role in determining nutritional requirements.

Much of the genetic variation in nutritional requirements is the result of inherited, or germline, variation in genetic code that translates into differences in the amount and/or form of protein expressed. These variations, or mutations, in the genetic code can range from being rare to very common. Rare mutations are often identified because they have high penetrance (ie, they have a major impact on health), whereas the more common variations are usually of low penetrance and often go unnoticed (ie, there is no discernible characteristic or phenotype). Variations with a frequency in the minor allele of >1% in one or more populations are termed "polymorphisms." Variations in the DNA code can occur as a result of single nucleotide substitutions, deletions, insertions, or repeats. Typically, single nucleotide polymorphisms (SNPs) account for much of the low-penetrance variation and may or may not affect gene function. In contrast, base-pair deletions, insertions, and repeats can have profound effects on function.

Cancer risk is determined by a composite of environmental and genetic factors. Factors such as carcinogens, diet, radiation, and viral and bacterial infections can cause somatic DNA mutations in various tissues. Coupled with this, cancer risk is further influenced by genetic variations such as an individual's susceptibility to mutation by these agents and capacity to repair such mutations, as well as his or her ability to destroy transformed cells and prevent metastasis. In considering a linear pathway between exposure and disease causation, a gene that functions on that pathway may be hypothesized to play a role. Genetic variation that changes gene function by increasing or decreasing expression of the gene (ie, affecting the amount of protein produced) or altering the function of the gene product (ie, affecting the protein activity) may be important.

DETOXIFICATION AND CARCINOGENESIS

Biotransformation or detoxification enzymes metabolize a wide range of compounds, including exogenous or xenobiotic compounds such as carcinogens and therapeutic drugs, as well as endogenous compounds such as steroid hormones. In the case of lipophilic carcinogens, metabolism of xenobiotics is often a 2-step process. First, compounds are metabolized by cytochrome P450s (CYPs) to generate reactive compounds. The reactive intermediates can be conjugated by other enzymes such as UGTs or SULTs so that the final product is highly water-soluble and easily excreted in urine or bile or can be conjugated by GSTs to generate compounds that can be further degraded to excretable metabolites. P450s and conjugating enzymes are recognized for their roles in processing carcinogens and, therefore, they play critical roles in the early stages of carcinogenesis. However, given their roles in metabolism of steroid hormones and other endogenous compounds that act as growth factors for cancer cells, there are several other points in the cancer pathway where these enzymes can also play a role. The balance between the activities of the activation and conjugation steps in the pathway are likely critical for cancer risk. Several polymorphic detoxification enzymes are implicated in cancer risk: various CYPs (eg, CYP1A1, CYP1A2, CYP2A6, CYP2E1), *N*-acetyl transferases (eg, NAT1, NAT2), microsomal epoxide hydrolase, catechol *O*-methyltransferase, GSTs (eg, GSTA1, GSTM1, GSTT1, GSTP1), UGTs (eg, UGT1A1, UGT1A6, UGT2B7, UGT2B15), and SULT (eg, SULT1A2).

POLYMORPHISMS AND WELL-COOKED, MEAT-DERIVED CARCINOGENS

The importance of a particular polymorphic enzyme is determined, in part, by its substrate specificity for a particular carcinogen. The association between intake of well-cooked meat and cancer risk in the context of polymorphic detoxification enzymes is an example of this. Heterocyclic aromatic amines (HCA), produced when meat is cooked at high temperatures, and polycyclic aromatic hydrocarbons (PAH), produced when meat is grilled, are both activated by CYP1A1. HCA are further activated and detoxified by NAT2. PAH are detoxified by GSTM1. HCA and PAH induce activating enzymes, such as CYP1A2, but there is a substantial amount of variation in this response.[1] Evaluating this response based on genetic polymorphisms in enzymes responsible for HCA metabolism showed that the CYP1A1 Ile/Val heterozygotes had higher CYP1A2 activity than the homozygote Ile/Ile.[2] No effect was seen for the GSTM1 genotype in this study. More recently, Kiss et al.[3] showed that on a high grilled-meat diet, individuals who were GSTM1-null compared to GSTM1+, or who had the rapid compared to slow NAT2 genotype, had more DNA damage in sloughed colonic epithelial cells.

POLYMORPHISMS AND PHYTOCHEMICALS

Genetic variation in detoxification enzymes can also affect handling of phytochemicals, many of which are of interest for their potential cancer-preventive properties. In plants, phytochemicals provide structure, repel harmful organisms, attract beneficial organisms, serve as photoprotectants, and help plants respond to environmental changes. In order for animals to derive benefit from consumption of plant foods, they have had to develop ways to handle phytochemicals. Phytochemicals regulate gene expression in many cases so as to improve xenobiotic clearance. For example, the isothiocyanates, as well as numerous flavonoids, induce expression of detoxification enzymes with antioxidant response elements (ARE) in their promoter regions by disrupting the Nrf2/Keap1 complex that allows Nrf2 to enter the nucleus of the cell and interact with the ARE.[4]

The impact of phytochemicals in the cancer process is a complex one. On the one hand, phytochemicals regulate the detoxification enzymes such that they may improve clearance of carcinogens and proliferative agents (eg, sex steroids), but at the same time they are increasing their own clearance such that their downstream biochemical effects may be reduced. Genetic polymorphisms that further regulate the expression and activity of the detoxification enzymes may further impact response to phytochemicals. Many classes of phytochemicals are conjugated with glutathione, glucuronide, or sulfate and are excreted in urine or bile.[5-7] Circulating concentrations of phytochemicals vary widely among individuals even in the context of controlled feeding studies,[8-10] possibly due to polymorphic differences in phytochemical metabolism and efflux.

EFFECTS OF GLUTATHIONE S-TRANSFERASE POLYMORPHISMS

Glutathione-S-transferases (GST) are a family of cytosolic conjugating enzymes that catalyze the conjugation of reduced glutathione to sites on a variety of hydrophilic compounds, including common carcinogens and phytochemicals. Of the 4 primary GST classes—alpha, pi, mu, and theta—alpha and mu are the major hepatic GSTs. GSTM1 and GSTT1 have been shown to conjugate isothiocyanates and indoles from cruciferous vegetables, and in turn isothiocyanates have been shown to induce GSTs. Induction in enzyme activity occurs rapidly within several days of cruciferous vegetable consumption and declines with removal of the vegetables from the diet.[11] Several GST polymorphisms have been extensively studied. Mutations in GSTM1 and GSTT1 result in the absence of the functional enzyme. Depending on racial/ethnic group, the frequency of the homozygous GSTM1-null genotype varies from 39 to 63% and that of GSTT1-null varies from 10 to 64%. Despite the complete lack of enzyme, the GSTM1-null genotype only confers a modest increased risk of certain cancers. Epidemiologic studies conducted in the United States suggest that individuals with the GSTM1+ genotypes gain greater protection from intake of cruciferous vegetables compared to those with the GSTM1-null genotype. In contrast, studies conducted in Asian cohorts suggest that GSTM1-null or T1-null individuals may gain greater protection.

Several observational studies have examined the relationship between GSTM1 and GSTT1 genotypes and urinary excretion of isothiocyanates. Seow et al.[12] reported that GSTT1-null individuals had lower urinary concentrations of isothiocyanates than GSTT1+ individuals with similar isothiocyanate intakes. This and other data led Seow et al.[13] to hypothesize that individuals who are null for certain GSTs would less readily conjugate and excrete these compounds, would have greater amounts of isothiocyanates at the tissue level, and would experience more protection. Research I was involved in showed several years ago that, in a controlled feeding study, individuals who were GSTM1-null compared to those who were GSTM1+ had significantly higher serum GST-alpha concentrations in response to a high-cruciferous vegetable diet, suggesting that the lack of GSTM1 may have resulted in more isothiocyanates available to induce expression of GST-alpha.[14] Interestingly, Gasper et al.[15] showed recently in a single-dose feeding of broccoli that, despite slightly higher plasma area-under-the-curve (AUC) for sulforaphane, GSTM1-null compared to GSTM1+ individuals excreted significantly more sulforaphane and its metabolites within the first 6 hours and had a higher percent excretion of dose ingested over the 24 hours. The results of Gasper et al, do not support the hypothesis put forward by Seow et al., and would suggest that we do not yet fully understand the complex relationship between GST genotypes and isothiocyanate disposition.

EFFECTS OF UDP-GLUCURONOSYLTRANSFERASE POLYMORPHISMS

The UGTs are another group of detoxification enzymes that play a major role in phytochemical clearance. Anchored to the endoplasmic reticulum, they transfer a nucleotide sugar to small, hydrophobic molecules. They catalyze the glucuronidation of numerous endogenous and exogenous compounds and are important in maintaining steady-state levels of endogenous ligands involved in gene transcription related to cell growth, differentiation, apoptosis, and cellular homeostasis—all important in the process of carcinogenesis. There are two main families of UGTs involved in detoxification, the UGT1A family and the UGT2B family. Both are highly polymorphic.[16] In vitro, some of these polymorphisms have been shown to have functional effects on the enzyme activities, but these effects are often substrate specific. In vivo, the effects are less clear, but the drug metabolism literature provides some insight into the relevance of the polymorphisms. Although approximately 10% of the top 200 prescribed drugs are glucuronidated, only the UGT1A1 polymorphisms associated with Gilbert syndrome have been associated with altered drug glucuronidation in vivo.[17,18]

The UGT1A1*28 variant, which is distinguished by 7 TA repeats in the promoter region compared to the 6 TA repeat wild type, results in approximately 30% reduced gene transcription of UGT1A1—the enzyme responsible for bilirubin conjugation—and subsequently

higher circulating bilirubin concentrations.[19] The UGT1A1*28 polymorphism is associated with higher AUC for SN-38, a metabolite of the drug irinotecan, and a higher prevalence of grade 4 neutropenia, a toxic side effect of this drug.[20] In contrast, there are also data to suggest that UGT polymorphisms that alter clearance of a drug may increase its efficacy as a chemopreventive agent. Among regular users of nonsteroidal anti-inflammatory drugs (NSAIDs), the UGT1A6*2 (T181A and R184S) polymorphism is inversely associated with colon adenoma risk.[21]

Few studies have examined the interaction between UGT polymorphisms and dietary factors. In one controlled feeding study, Peters et al.[22] reported that individuals who were heterozygous (6/7) or homozygous (7/7) for the UGT1A1*28 genotype had a significant increase in urinary mutagenicity with a 2-week intake of well-cooked red meat; no difference in mutagenicity was observed among the individuals who were homozygous wildtype (6/6). Additionally, it was observed in a cross-sectional study that homozygous UGT1A1*28 individuals who consumed cruciferous vegetables had significantly lower serum total bilirubin concentrations than those of the same genotype who did not consume crucifers; this effect was not observed in the 6/6 and 6/7 genotypes.[23]

Several of the UGTs are integral in the conjugation and excretion of steroid hormones: UGT1A1 conjugates estriol, 17β-estradiol, ethinylestradiol, and catechol estrogens; UGT2B7 conjugates catechol estrogens, estriol, and hydroxylated androgens; and UGT2B15 conjugates primarily the androgens (eg, testosterone, dihydrotestosterone, androstane-3α, 17 β-diol), as well as catechol estrogens. Low-activity alleles associated with greater hormone exposure have been hypothesized to be associated with greater risk of hormone-dependent cancers. In the case of UGTs and breast cancer risk, UGT1A1*28 has been shown to be associated with higher risk of breast cancer in pre-, but not post-, menopausal women in some, but not all, studies, and it has been inversely associated with mammographic density—a biomarker of breast cancer risk—in premenopausal women, and positively associated with mammographic density in postmenopausal women.[24-26] Variants in UGT1A1 and UGT2B15 have been associated with higher serum estradiol concentrations in postmenopausal women.[27,28]

In relation to prostate cancer, prevalence of the UGT2B15 85Asp allele is higher in prostate cancer cases than controls.[29,30] UGT2B15 glucuronidates C19 of testosterone, dihydrotestosterone, and other androgens and is expressed in prostate tissue. 85Asp is associated with a 50% lower UGT2B15 enzyme activity than the 85Tyr. Given that UGTs play a major role in conjugation of a wide variety of compounds, the potential is great that polymorphisms in these genes may impact human health; however, beyond the potential for certain UGT polymorphisms to affect metabolism of certain therapeutic drugs and modulate serum hormones, there is a lot yet to be learned. Specifically, we have yet to determine whether diet modulates UGTs in a genotype-specific manner and to what extent dietary recommendations can be made on the basis of genotype.

IMPLICATIONS FOR INDIVIDUAL- AND POPULATION-BASED RECOMMENDATIONS

The application of available genetic polymorphism data on the individual level to patient counseling and on the population level to making public health recommendations in relation to biotransformation and cancer risk is in the early stages. One of the challenges at the individual level is that SNPs do not act in isolation, but on the background of thousands of other SNPs and environmental factors; there can be as many as 50-250,000 functional SNPs per person.[31] Even within what might be considered high-risk genetic profiles, there are secondary or tertiary genes that modify the effect of the primary variant and even these coupled together do not necessarily translate into contracting cancer. We still lack sufficient data to be able to counsel patients on the ramifications of having a particular constellation of detoxification genotypes.

At the population level, there are other challenges. In theory, it is possible to screen populations to identify high-risk individuals and counsel them about behaviors or exposures or offer possible chemoprevention modalities; however, the feasibility is questionable because many common variants probably contribute to disease risk and it is not cost effective in a public-health sense to target specific groups for interventions from which the whole population might also benefit. As an example, Nicas and Lomax[32] conducted a cost-benefit analysis of genetic screening for susceptibility to the occupational toxicant benzene. Defining susceptibility as CYP2E1 and NQO1 variants, they estimated that 2500 workers would need to be screened to hire 1000 genetically "nonsusceptible" workers to prevent one case of benzene-induced cancer. Variants also can be associated with multiple diseases or can have opposite associations with different diseases. For example, the NAT2 slow-acetylator genotype is associated with increased risk of bladder cancer and decreased risk of colon cancer. Public health interventions invariably must consider the costs and benefits of the entire range of health outcomes and their burden on society.

SUMMARY

There is growing evidence that genetic variation in detoxification enzymes influences response to diet and ultimately the risk of some cancers; however, there still remains a large gap between existing genetic information and its clinical utility. We need more information about general functional information on genotype-phenotype relationships and the impact of diet and other exposures overlaid on these relationships. The effect of genetic variation in detoxification enzymes on handling of dietary carcinogens and phytochemicals also needs to be considered within the context of variation in other aspects of metabolism and disposition of these compounds. Much of the focus to date has been on hepatic activation and detoxification; however, genetic variation in enteric transport and metabolism, distribution and protein binding, and renal and biliary efflux transport also needs to be considered.[33] In addition, many of these compounds are also metabolized by gut bacteria, and the role of differences in bacterial community structure and activity cannot be ignored.

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Xenoestrogens, Biotransformation, and Differential Risks for Breast Cancer

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CANCER INITIATION

Until the last decade, epidemiological evidence of an association between sex steroid hormones and breast cancer risk, based on a retrospective study design such as case-control studies, was generally inconsistent. In spite of the lack of evidence, prospective cohort studies conducted in the last 10 years consistently observed that elevated levels of serum estrogens and androgens preceded the occurrence of breast cancer. In a pooled analysis of epidemiological studies of endogenous hormones and breast cancer in different populations, both estrogens and androgens were strongly associated with an increase in breast cancer risk, with evidence of a dose-response relationship.[1] An etiological link has also been specifically demonstrated between sex steroids and breast cancer development in premenopausal women.[2] Thus, exposure to estrogens is a recognized risk factor for breast cancer.

To understand how estrogens can induce breast cancer, we need to begin by considering natural estrogens and xenoestrogens (Fig. 1). The natural, endogenous estrogens are estrone (E_1), estradiol (E_2), and estriol. Contraceptives and hormone replacement therapy formulations include E_1, E_2, the synthetic ethynylestradiol, and the estrogens obtained from mares, equilin and equilenin. Many of these regimens also include progestins, almost always a synthetic progestin in the United States, rather than the natural progesterone itself (Fig. 1). There is a concern that the use of synthetic progestins, rather than natural progesterone, may increase the risk of breast cancer. Some recent data from a study of over 50,000 postmenopausal women in France support this concern (Table 1).[3]

TABLE 1 Breast Cancer Risk in Relation to Hormone Replacement Therapy[3]

54,598 postmenopausal women
948 primary invasive breast cancer in 5.8 years

Group	Relative Risk
HRT users vs nonusers	1.2
Estrogens alone	1.1
Estrogens + progesterone	0.9
Estrogens + synthetic progestins	1.4*

* The risk with estrogens + synthetic progestins was significantly greater than with estrogens + progesterone ($P < .001$).

Estrogens have been considered epigenetic carcinogens that function by stimulating abnormal cell proliferation via estrogen receptor-mediated processes.[4,5] The stimulated cell proliferation could result in increased accumulation of genetic damage, leading to carcinogenesis.[6,7] Compelling evidence has led to a new paradigm of cancer initiation by

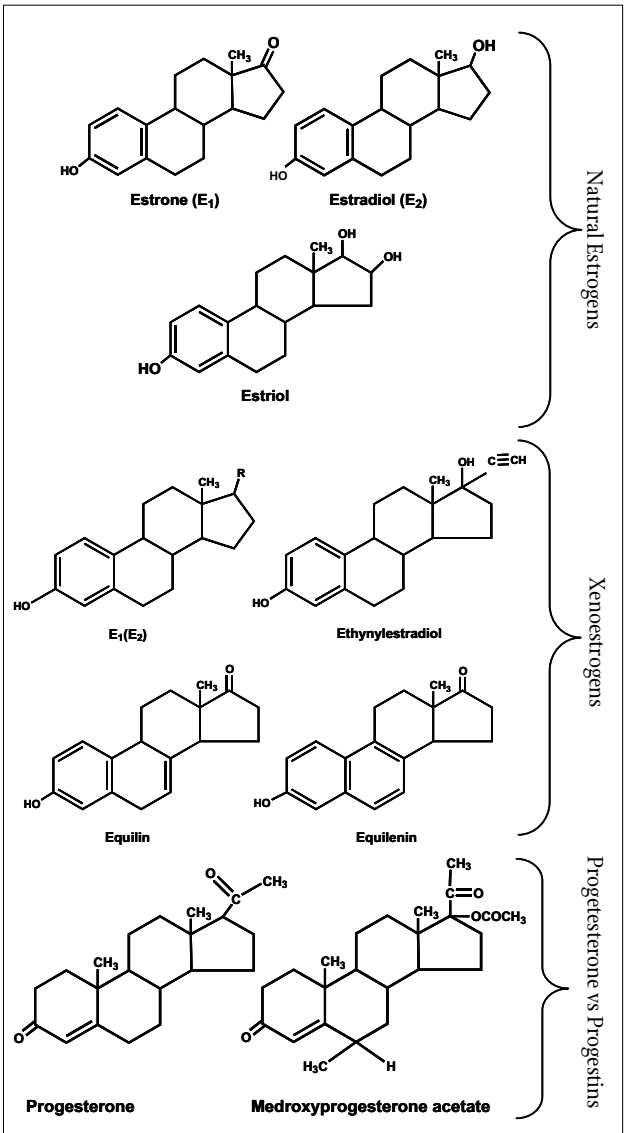

FIGURE 1 Natural estrogens, estrogens in contraceptives and hormone replacement therapy, and progesterone vs progestins.

estrogens. Discovery that specific oxidative metabolites of endogenous estrogens, catechol estrogen-3,4-quinones (CE-3,4-Q), can react with DNA[8-11] led to and has supported the hypothesis that these metabolites can become endogenous chemical carcinogens. Some of the mutations generated by the specific DNA damage can result in the initiation of cancer in breast and other tissues (Fig. 2).[12-15]

Chemical carcinogens, including the estrogens, covalently bind to DNA to form 2 types of adducts: stable ones that remain in the DNA, unless removed by repair, and depurinating ones that are lost from the DNA by destabilization of the glycosyl bond (Fig. 3), generating apurinic sites in the DNA.[16,17] Catechol estrogens (CE) are among the

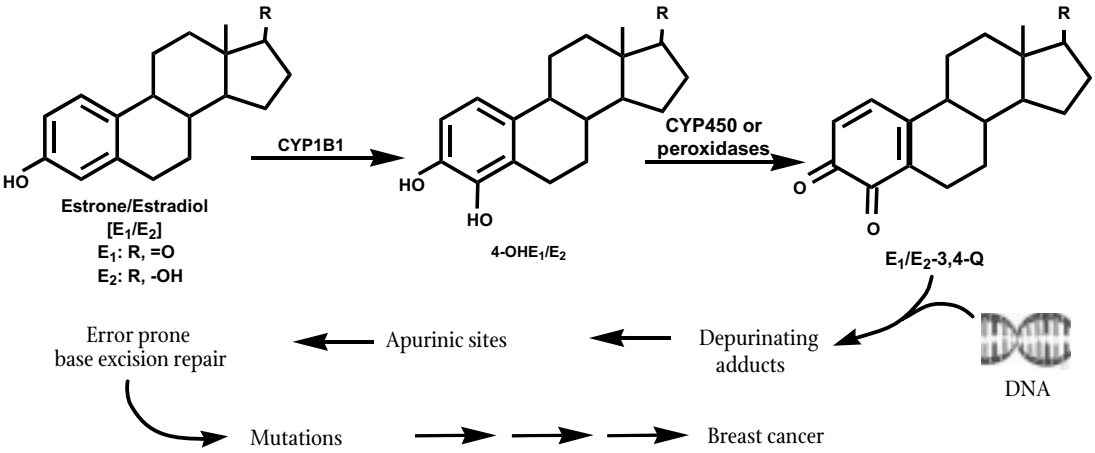

FIGURE 2 Initiation of breast cancer by estrogens.

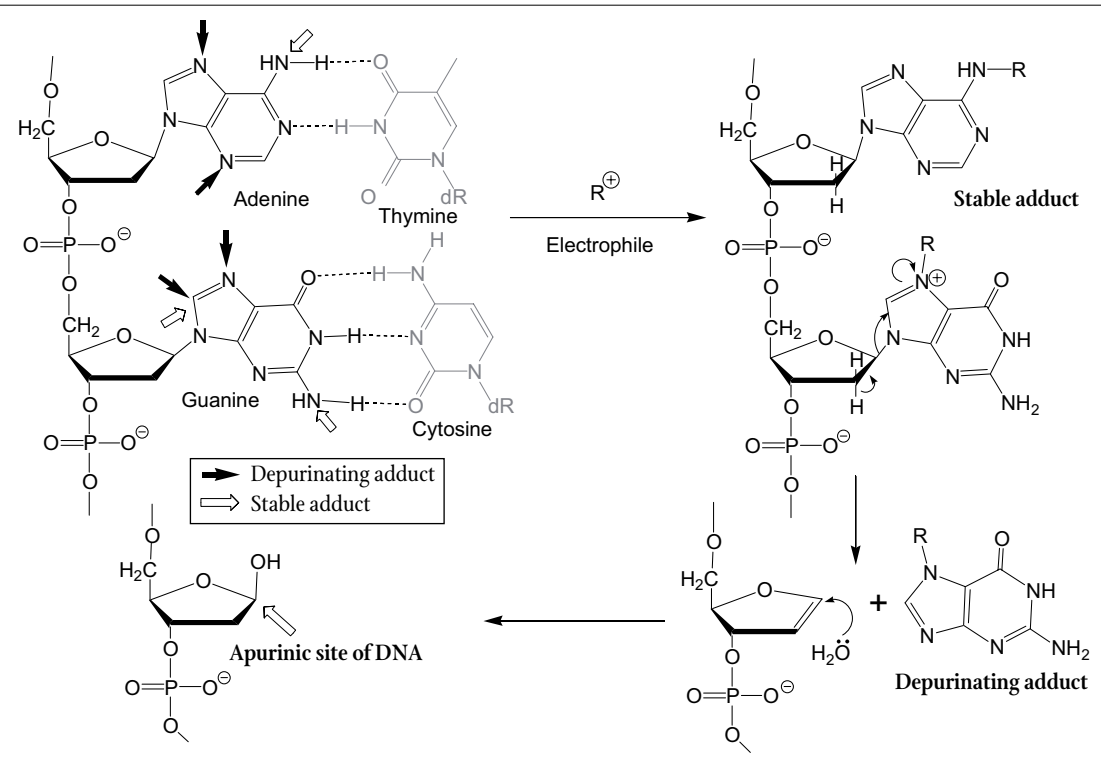

FIGURE 3 Formation of stable and depurinating DNA adducts and generation of apurinic sites.

major metabolites of E_1 and E_2.[18-20] If these metabolites are oxidized to the electrophilic CE-Q, they may react with DNA. Specifically, the carcinogenic 4-OHE1(E2)[21-23] is oxidized to $E_1(E_2)$-3,4-Q, which can react with DNA to form predominantly depurinating adducts (Fig. 4).[24-26] These adducts generate apurinic sites that may lead to cancer-initiating mutations,[27-30] which transform cells, thereby initiating cancer.[31-34] The extremely weak carcinogen 2-OHE1(E2)[35] also forms depurinating adducts (Fig. 4), but to a much lesser extent.[36] The depurinating N3Ade and N7Gua adducts are released from DNA at different rates, the former instantaneously and the latter with a half-life of 3 hours.[37]

E_1 and E_2 are formed by aromatization of androstenedione and testosterone, respectively, catalyzed by cytochrome P450 (CYP) 19, aromatase (Fig. 5). E_1 and E_2 are interconverted by the enzyme 17β-estradiol dehydrogenase. These estrogens are metabolized by two major pathways: formation of CE and, to a lesser extent, 16α-hydroxylation (not shown in Fig. 5). The CE formed are the 2-OHE1(E_2) and 4-OHE1(E_2). The 2-OHE1(E2) are generally the major CE formed. Increases in the level of CYP1B1 and other 4-hydroxylases could render the minor CE metabolites, 4-OHE$_1$(E_2), as the major ones. The CE are generally inactivated by conjugating reactions such as glucuronidation and sulfation, especially in the liver (not shown in Fig. 5). The most common pathway of conjugation in extrahepatic tissues occurs, however, by *O*-methylation catalyzed by the ubiquitous catechol-*O*-methyltansferase (COMT).[38] If conjugation of CE via methylation becomes insufficient, the competitive catalytic oxidation of CE to CE-Q can occur.

Redox cycling via reduction of CE-Q to semiquinones, catalyzed

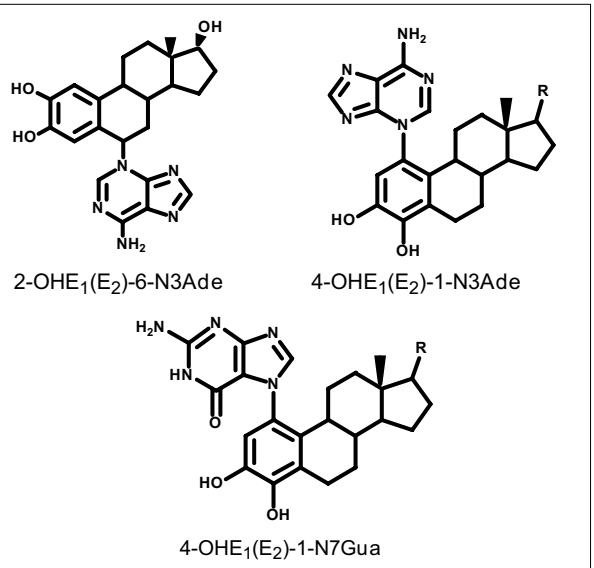

FIGURE 4 Structures of depurinating estrogen-DNA adducts.

by CYP reductase, and subsequent oxidation back to CE-Q by O_2 forms superanion radicals and then H_2O_2. In the presence of Fe^{2+}, H_2O_2 forms hydroxyl radicals (Fig. 5).

The 4-$OHE_1(E_2)$ exhibit greater carcinogenic potency than the 2-$OHE_1(E_2)$, which are borderline carcinogens.[39-41] It is difficult to attribute the greater potency of 4-$OHE_1(E_2)$ to the redox cycling of the 2-$OHE_1(E_2)$ and 4-$OHE_1(E_2)$, because they have similar redox potentials.[42,43] Instead, one can relate the greater carcinogenic potency of the 4-$OHE_1(E_2)$ to the much higher level of depurinating DNA adducts formed by $E_1(E_2)$-3,4-Q, compared to $E_1(E_2)$-2,3-Q.[44] Thus, we think the role of CE-Q in initiating cancer is through formation of the depurinating DNA adducts, 4-$OHE_1(E_2)$-1-N3Ade, 4-$OHE_1(E_2)$-1-N7Gua, and, to a much lesser extent, 2-$OHE_1(E_2)$-6-N3Ade (Fig. 4).

The reactivity of CE-Q with DNA can be prevented by conjugation with glutathione (GSH, Fig. 5). A second inactivating pathway for CE-Q is their reduction to CE by quinone reductase and/or CYP reductase.[45,46] If these inactivating processes are insufficient, CE-Q may react with DNA to form predominantly depurinating adducts (Fig. 5).[47-50] Various results suggest that E_2-3,4-Q may be the major carcinogenic metabolite of estrogens.

IMBALANCE OF ESTROGEN HOMEOSTASIS

The above paradigm of cancer initiation by estrogens hinges on a disrupted homeostatic balance between activating and deactivating pathways (Fig. 5). Several factors can unbalance estrogen homeostasis, namely, the equilibrium between estrogen-activating and deactivating pathways to avert oxidative stress, in particular the formation of endogenous carcinogenic CE-Q and their reaction with DNA (Fig. 5). We think that unbalanced estrogen homeostasis is a condition that precedes the initiation of breast cancer. The effects of some factors

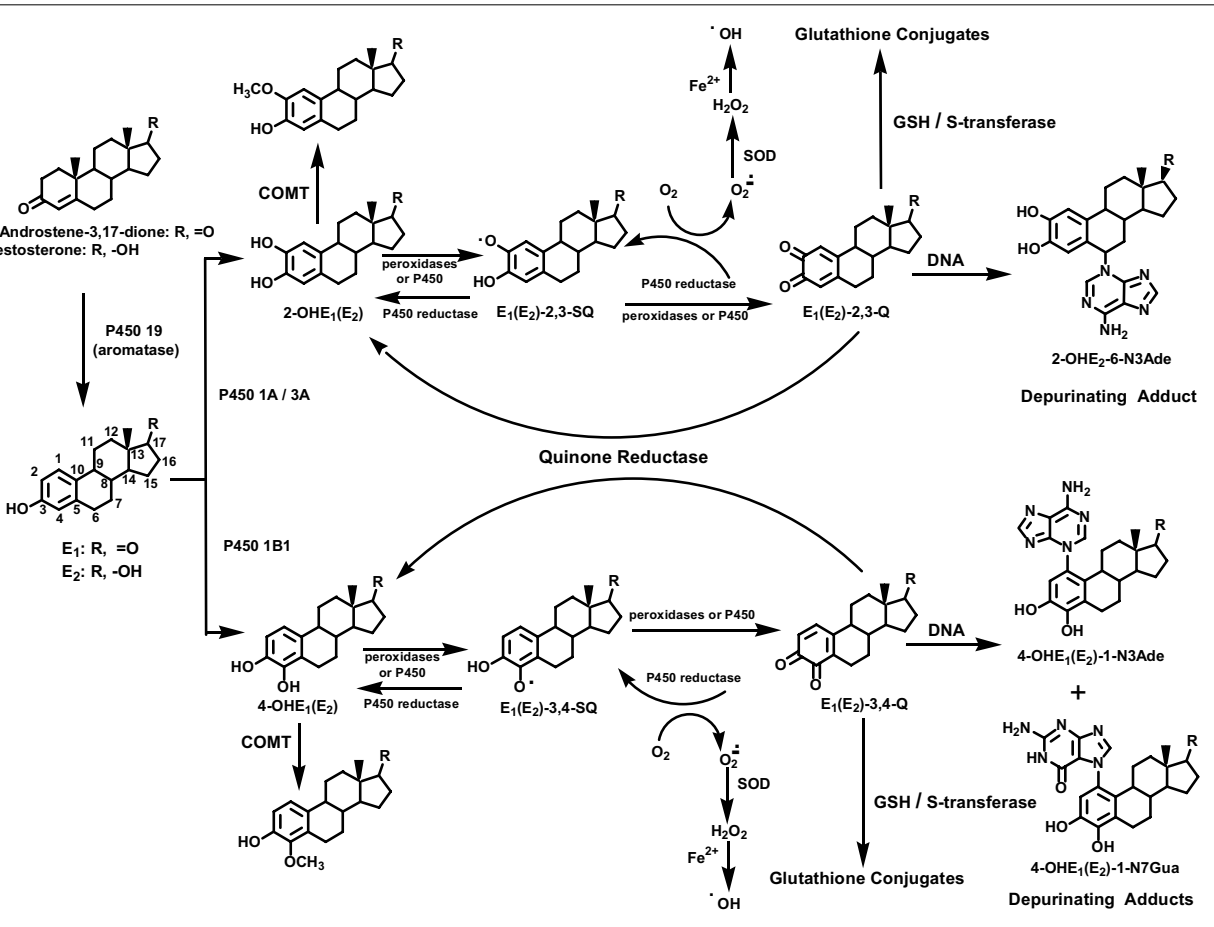

FIGURE 5 Metabolism and DNA adducts of estrogens.

have already been observed in several animal models for estrogen carcinogenesis and in human breast. For example, imbalances in estrogen homeostasis leading to substantial formation of CE-GSH conjugates and depurinating CE-DNA adducts have been observed in the kidneys of male Syrian golden hamsters,[51] the prostates of Noble rats,[52] and the mammary glands of female estrogen receptor-α knockout (ERKO/Wnt-1) mice.[53] A study of breast tissue from women with and without breast cancer provided key evidence in support of unbalanced estrogen homeostasis.[54]

FORMATION OF ESTROGEN-DNA ADDUCTS

As discussed above, when the estrogens are metabolized to CE-Q, they can react with DNA to form predominantly depurinating adducts. For example, in the binding of E_2-3,4-Q to DNA, approximately equal amounts of 4-OHE_2-1-N3Ade and 4-OHE_2-1-N7Gua are formed, but the N3Ade adducts depurinate instantaneously, while the N7Gua adducts depurinate with a half-life of about 3 hours at 37 EC (Fig. 6). One of the reasons why the 2-CE are borderline carcinogens may be related to the poor ability of the CE-2,3-Q to compete with the CE-3,4-Q in binding to DNA (Fig. 7). For example, even with a mixture of 95% E_2-2,3-Q and 5% E_2-3,4-Q, more 4-OHE_2-1-N3Ade and 4-OHE_2-1-N7Gua are still formed than 2-OHE_2-6-N3Ade (Fig. 8).[55] Thus, the formation of depurinating estrogen-DNA adducts appears to be a critical step in the initiation of cancer by estrogens.

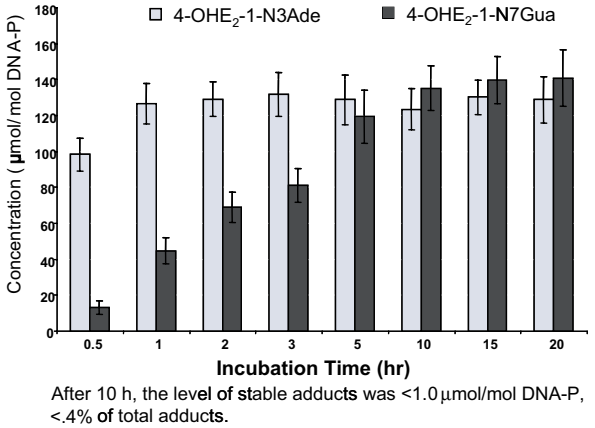

FIGURE 6 Depurinating adducts formed after the reaction of estradiol-3, 4-quinone (E_2-3,4-Q) with DNA.[36]

ESTROGEN-DNA ADDUCTS AND ONCOGENE MUTATIONS

The chief contributor to estrogen genotoxicity in breast cancer appears to be E1(E_2)-3,4-Q, the ultimate carcinogenic form of the 4-CE. An important link in the hypothesis that estrogens are genotoxic would be a demonstration that a major E_2 metabolite, 4-OHE_2, is mutagenic under conditions where it can be metabolized to the putative ultimate mutagenic metabolite, E_2-3,4-Q. Further evidence supporting this hypothesis would be a demonstration of the mutagenic activity of E_2-3,4-Q.

Our studies in SENCAR mouse skin (mice that are extremely susceptible to two-stage skin carcinogenesis) and ACI rat mammary gland (rats that are a cross between the August and Copenhagen–Irish strains) suggest that 4-OHE_2 or E_2-3,4-Q can induce mutations similar to those associated with breast cancer (Table 2). The initial study was conducted in the SENCAR mouse model by administering a single dose (200 nmol in acetone) of E_2-3,4-Q and examining the H-*ras* gene as the target of mutagenesis. We studied early induction of mutations (12 h-3 d after the

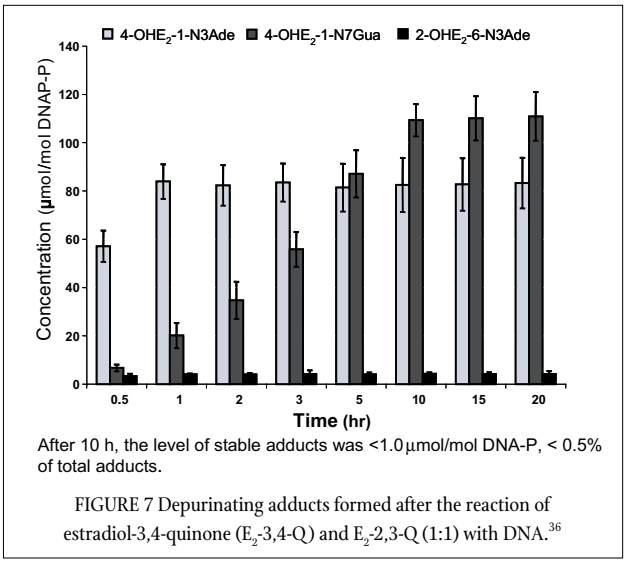

FIGURE 7 Depurinating adducts formed after the reaction of estradiol-3,4-quinone (E_2-3,4-Q) and E_2-2,3-Q (1:1) with DNA.[36]

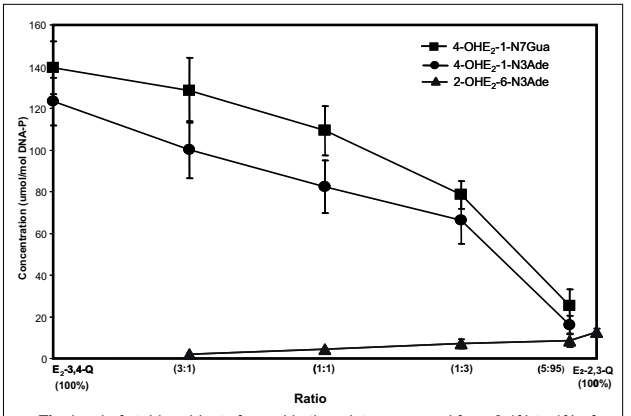

FIGURE 8 Depurinating adducts formed after 10 h by mixtures of estradiol-3,4-quinone (E_2-3,4-Q) and E_2-2,3-Q reacted with DNA.[36]

treatment) to make correlations with DNA adducts.[56] The results show that E_2-3,4-Q induced predominantly A.T to G.C mutations. Next, we examined the ACI rat mammary gland, considered to be a model of breast cancer, for mutagenesis by E_2-3,4-Q. Similar mutations were again observed (Table 2).[57] Approximately equal amounts of the depurinating N3Ade and N7Gua adducts were detected in both tissues. The mutations correlate with the rapidly depurinating N3Ade adducts, while the slowly depurinating N7Gua adducts do not appear to be major sources of mutagenesis in the early period. The depurinating adducts are spontaneously lost from DNA, forming apurinic sites. Since the N3Ade adducts depurinate rapidly, they will induce a rapid burst of apurinic sites in DNA. Exposure of cells to agents that induce abasic sites results in an early, adaptive induction of base excision repair (BER) genes, along with repression of DNA replication.[58] The abundant formation of depurinating adducts and induction of BER genes during mutagenesis suggest that erroneous BER could be the mechanism for induction of mutations.

4-OHE_2 and E_2-3,4-Q have now been shown to be mutagenic in Big Blue rat® cells in culture, which carry a reporter gene.[59] Taken together, the results obtained from mutagenesis studies of 4-OHE_2 and E_2-3, 4-Q support the hypothesis that estrogens can contribute to carcinogenesis by a genotoxic pathway.

TABLE 2 Mutagenesis by E_2-3,4-Quinone

					H-*ras* mutations
	Depurinating Adducts µmole/mol DNA-P		**Stable Adducts µmole/mol DNA-P**	A √G	**Other**
Tissue	4-OHE_2-1-N3Ade	4-OHE_2-1-N7Gua		Total Clones	Total Clones
SENCAR mouse skin[12]	12.5	12.1	0.004		
6 h				5/29	2/29
12 h				4/30	2/30
1 d				7/50	4/50
3 d				3/40	1/40
ACI rat mammary gland[13]	81	90	0.017		
6 h				16/29	3/29
12 h				14/34	6/34

MAMMARY TUMORS IN ERKO/WNT-1 MICE

Experiments using transgenic mice with estrogen receptor-α (ER-α) knocked out (ERKO/Wnt-1 mice) have provided further important evidence for genotoxic effects of estrogen metabolites in cancer initiation. Bocchinfuso and his associates[60] developed a strain of ERKO mice carrying the Wnt-1 gene, which drives the development of mammary tumors. They showed that the ERKO/Wnt-1 mice exhibit a delayed onset of tumor development compared to mice expressing the wild type ER-α, but a nearly 100% incidence of mammary tumors in the absence of ER-α and β (Fig. 9). To directly determine the effect of E_2 in the absence of ER, mice were castrated at 15 days of age; half were treated with silastic implants containing E_2 and the other half with implants of cholesterol. After 100 weeks of observation, the E_2-treated mice developed more tumors (12/15 vs 4/10), which appeared earlier than those in the mice receiving cholesterol implants (50% of tumors at 50 weeks versus 25% of tumors at 100 weeks, $P<.004$) (Fig. 10).[61] Mammary tumors developed even when the mice were treated with both E_2 and the pure antiestrogen ICI-182,780.[62] The mammary tissue from these animals appears to convert little 4-OHE_2 to 4-methoxyE_2, a metabolite that is inactive and cannot be converted to genotoxic metabolites.[63] Overall, these experiments provide evidence that E_2 exerts effects through an ER-α-independent pathway as well as an ER-dependent pathway to produce breast tumors. Presumably, the tumors are initiated by estrogen genotoxicity in an ER-α-independent pathway, followed by proliferation of the initiated cells mediated by an ER-dependent pathway.

PROSTATE TUMORS IN NOBLE RATS

The Noble rat is another animal model in which estrogens play a role in tumor initiation. Prostate tumors are thought to arise in these rats after initiation by estrogen and promotion by testosterone.[64] Indeed, when male Noble rats were implanted with testosterone, only 40% of the rats developed prostate adenocarcinomas; in contrast, 100% of the rats developed the prostate tumors following implantation of estradiol followed by testosterone (Table 3).[65]

CULTURED HUMAN BREAST EPITHELIAL CELLS

To fully demonstrate that estrogens are carcinogenic in the human breast and for testing potential mechanisms of action, an experimental system is required in which the natural estrogen E2 by itself or its metabolites, 2-OHE2 and 4-OHE2, would induce neoplastic transformation of human breast epithelial cells (HBEC) in vitro.66,67 The transforming potential of estrogens on human breast epithelium was evaluated by treat-

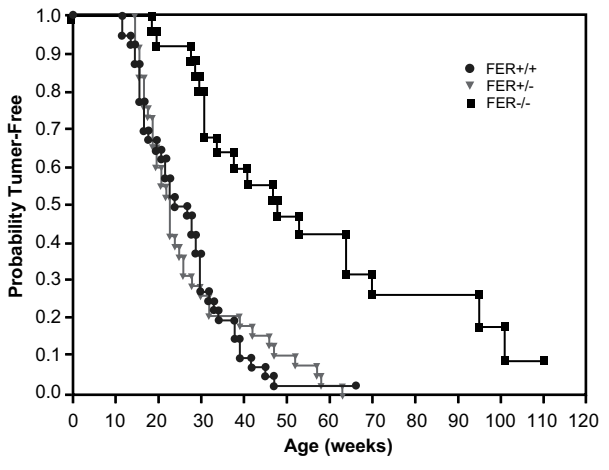

FIGURE 9 Development of mammary tumors in estrogen receptor mice: ER+/+, ER+/- and ER-/-.

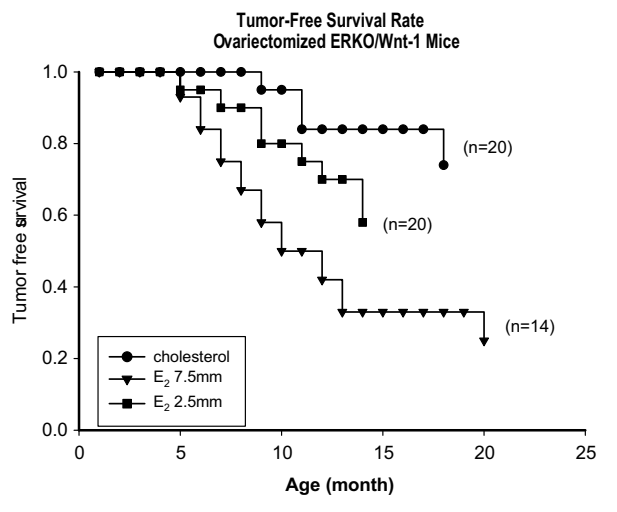

FIGURE 10 Tumor-free survival rate of ovariectomized estrogen receptor-α-knock out (ERKO)/Wnt-1 mice.[61]

TABLE 3 Induction of Adenocarcinomas in the Noble (NBL) Rat Prostate[64]

Treatment	Rats with prostate tumors
Testosterone	40%
Testosterone + estradiol	100%

ing the spontaneously immortalized ER-α negative MCF-10F cells with 0.007 nM, 70 nM, or 1 µg/mL of E2, 2-OHE2, 4-OHE2, 16α-OHE2, or cholesterol.[68] (MCF-10F is a spontaneously immortalized, nontransformed human breast epithelial cell line that does not grow in soft agar or form tumors in nude mice.) Treatments with estrogens were carried out for 24 hours twice a week for 2 weeks to mimic the intermittent exposure of the breast to endogenous estrogens (Fig. 11). At the end of the second week of treatment, and in successive passages thereafter, the cells were evaluated for the expression of phenotypes indicative of cell transformation,[69-72] namely, determination of colony formation in agar-methocel, or colony efficiency, ductulogenic capacity in collagen matrix, invasiveness in a reconstituted basement membrane using the Boyden chamber, genomic analysis by capillary electrophoresis, and tumorigenic assay in severely compromised immune-deficient (SCID) mice.[73,74]

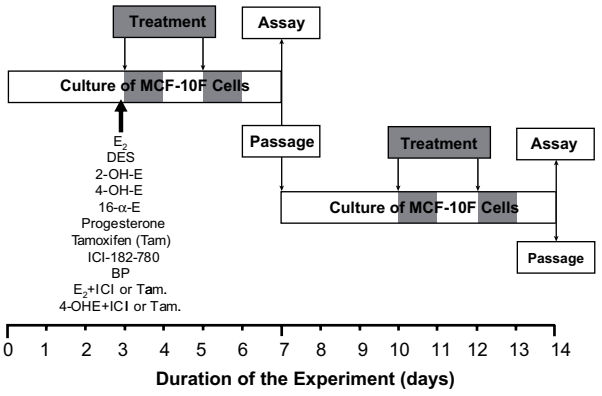

FIGURE 11 Treatment of MCF-10F cells with estrogen to induce transformation.[68]

At all passages tested, MCF-10F cells treated with benzo[*a*]pyrene (BP), E_2, 2-OHE_2, 4-OHE_2, or 16_-OHE_2 formed colonies in agar-methocel that were greater than 80 µm in diameter. Cells treated with cholesterol did not form colonies (Fig. 12). Colony efficiency was dose dependent and in 4-OHE_2-treated cells was greater at the 0.007 nM dose, reaching a plateau at the 2 higher doses. The E_2-metabolite-treated cells formed spherical masses filled by large cuboidal cells. The invasive capacity of MCF-10F cells was significantly increased by E_2 or 4-OHE_2.

Injection of 10-15 x 10^6 control or treated cells in the inguinal fat pad of SCID mice failed to induce tumors up to the ninth passage. To determine whether more aggressive phenotypes could be selected, cells in their ninth passage after transformation with E_2 were seeded in a Boyden chamber, and those cells crossing the membrane were collected, expanded, and designated E_2-70-B2, B3, B4, B5, C2, C3, C4, and C5 for those transformed with 70 nM and 1-B2, 1-B3, 1-B4, 1-B5, 1-C2, 1-C3, 1-C4, and 1-C5 for those transformed with 1 µg/mL E_2 (Fig. 13). These cells were injected in SCID mice for assay of tumorigenicity. Only E_2-70-C3 and E_2-70-C5 were tumorigenic, in 2/12 and 9/10 animals injected, respectively.[75] The tumors were poorly differentiated adenocarcinomas, ER-α, ER-β, and progesterone receptor

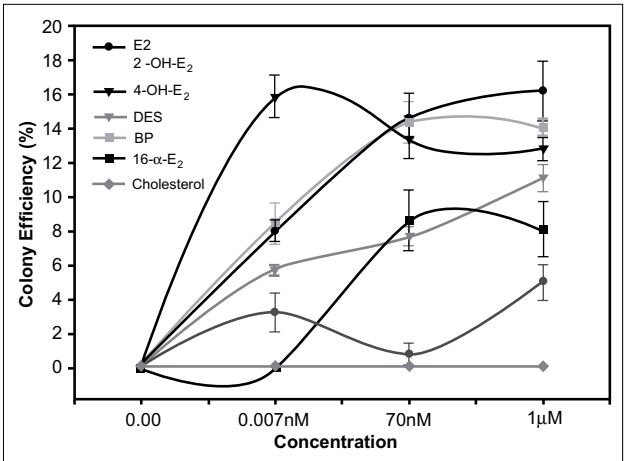

FIGURE 12 Transformation of MCF-10F cells by different concentrations of estrogens.[68]

negative. From the 9 tumors obtained from E_2-70-C5 cells, 4 tumoral cell lines designated C5-A1-T1, C5-A4-T4, C5-A6-T6, and C5-A8-T8 were derived (Fig. 13). Fingerprint analysis confirmed that all these cells originated from MCF-10F cells.

In summary, we have accumulated evidence indicating that E_2 and its metabolites are mutagenic as an early event in the process of transformation of the human breast epithelium. This model of breast carcinogenesis demonstrates clear stages of cell transformation and offers a variety of types of information. This model supports the concept that estrogen is a genotoxic agent inducing transformation and tumorigenesis independently of the hormone ER-∀ pathway.

STUDIES OF BREAST AND PROSTATE CANCER IN HUMANS

If unbalanced estrogen homeostasis leads to excessive formation of E_2-3,4-Q and estrogen-DNA adducts, we would expect to find that estrogen metabolism in the breast is unbalanced. To explore this, nontumor breast tissue was analyzed from women with and without breast cancer.[76] Levels of $E_1(E_2)$ in women with carcinoma were higher than in controls, and the levels of 4-$OHE_1(E_2)$ were nearly 4 times higher in women with breast carcinoma than in women without cancer (Fig. 14). In women with breast carcinoma, 4-$OHE_1(E_2)$ were 3 times more abundant than 2-$OHE_1(E_2)$. Levels of CE-Q conjugates in women with breast cancer were 3 times those in the controls, suggesting a greater probability of CE-Q reacting with DNA in the breast tissue of women with breast carcinoma. Levels of 4-$OHE_1(E_2)$ (P<.01) and CE-Q conjugates (P<.003) appeared to be significantly associated with breast cancer.[77] One established example of this imbalance is the over expression of the estrogen 4-hydroxylase, CYP1B1, in tumors of the breast.[78,79] Therefore, the oxidative pathway that leads to formation of CE-Q is the result of unbalancing 1 or more factors involved in estrogen homeostasis.

The unbalanced estrogen homeostasis was also evident in an analysis of the expression of estrogen-metabolizing enzymes in a small study of breast tissue from women with and without breast cancer (Fig. 15). Expression of the estrogen-activating enzymes CYP19 (aromatase) and CYP1B1 was higher in nontumor breast tissue from women with breast carcinoma than in breast tissue from women who did not have breast cancer. In contrast, the expression of the protective enzymes catechol-*O*-methyltransferase and quinone reductase (NQO1) was higher in breast tissue from women who had not been diagnosed with breast cancer, compared to nontumor breast tissue

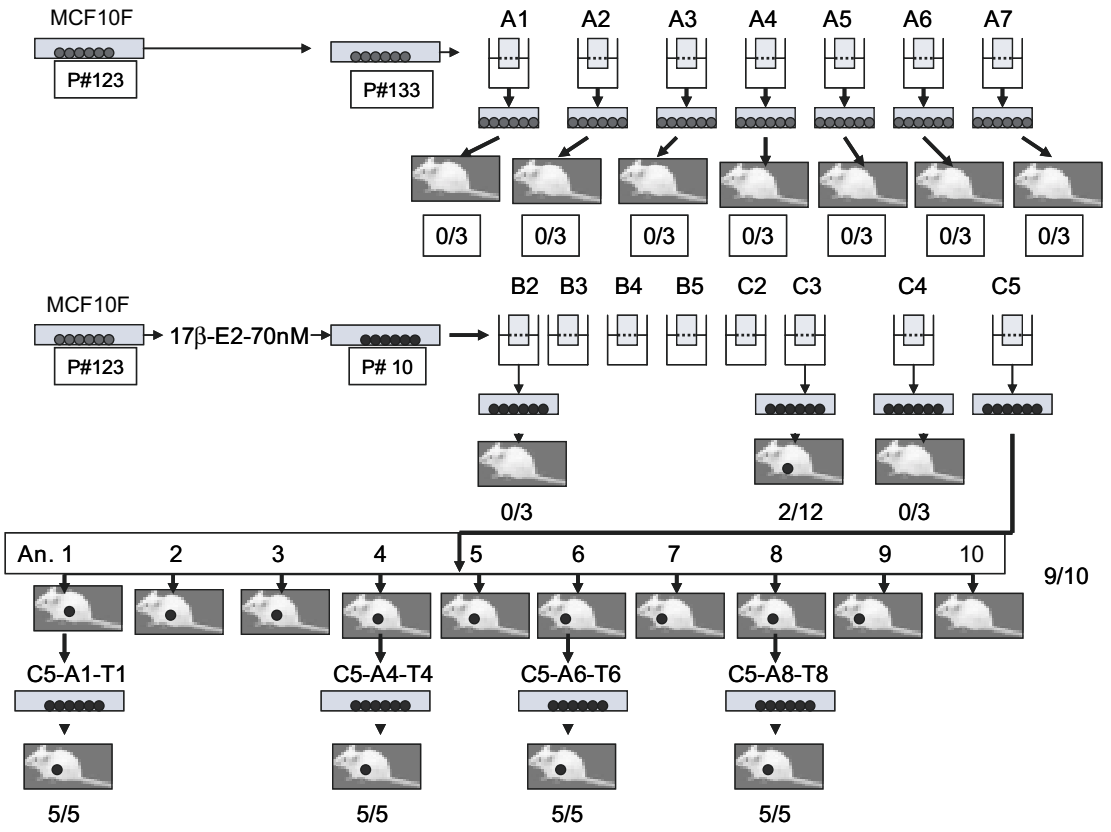

FIGURE 13 Induction of tumors in SCID mice by estrogen-treated MCF-10F cells.[75]

from women with breast carcinoma. These results are consistent with metabolic activation of estrogens to catechol estrogen quinones playing a critical role in the initiation of breast cancer.

To determine whether estrogen-DNA adducts can be detected in human urine, urine samples from men with prostate cancer, benign tumors, or benign prostate hyperplasia, as well as from healthy males, were analyzed in a blind study to determine the presence of 4-$OHE_1(E_2)$-1-N3Ade, one of the major adducts formed by CE-Q.

Urine samples (20 mL each) from 16 subjects were analyzed using several detection methods. In Figure 16, the bars in the first row correspond to the integrated (normalized) area of the absorbance-based capillary electrophoresis electropherogram peaks assigned to 4-$OHE_1(E_2)$-1-N3Ade. Only the samples from the subjects with prostate cancer or urological conditions contained 4-$OHE_1(E_2)$-1-N3Ade adduct, with concentration levels of about 15-240 pmol per mg of creatinine. The identity of the N3Ade adduct in these samples was confirmed by low-temperature (77K) luminescence spectroscopy, as shown by the bars in the second row in Figure 16. An example of the phosphorescence spectrum obtained for sample #11 is shown in the right inset of Figure 16. This spectrum is nearly indistinguishable from the spectrum of the N3Ade adduct standard.[80,81] The amount of this adduct in the 11 samples detected by using low-temperature phosphorescence-based calibration curves was about 10-150 pmol per mg of creatinine. With a detection limit of about 10^{-9} M,[82] no 4-OHE_1-1-N3Ade adducts were observed in the 5 control samples. The observed emission intensity was near the background level.

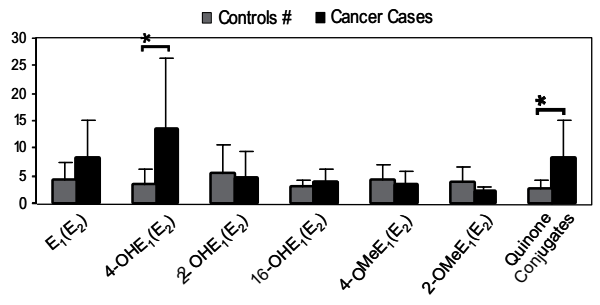

FIGURE 14 Analysis of estrogen metabolites in human breast tissue from women with and without breast cancer. Quinone conjugates are 4-$OHE_1(E_2)$-2-NAcCys, 4-$OHE_1(E_2)$-2-Cys, 2-$OHE_2(E_2)$-(1+4)-NAcCys, and 2-$OHE_1(E_2)$-(1+4)-Cys.

*Statistically significant differences were determined using the Wilcoxon rank sum test, $P<.05$[54]

Finally, ultraperformance liquid chromatography/tandem mass spectrometry (LC/MS/MS) was used for further validation of the above findings using the samples eluted from the immunoaffinity columns, as shown in the third row of Figure 16. Similar adduct distribution is observed in all samples using the 3 different methodologies. An example of the LC/MS/MS obtained for sample #11 is shown in the left inset of Figure 16; the major peak of the LC chromatogram corresponds to the 4-OHE_1-1-N3Ade adduct and indicates that the eluent from the immunoaffinity column was relatively pure. The upper spectrum corresponds to the daughters, *m/z* 135.9 and 296.0, which were obtained from fragmentation of the adduct parent ion, *m/z* 420.1. Thus, 4-OHE_1-1-N3Ade is excreted into the urine of subjects with prostate cancer, suggesting that this adduct may be a biomarker for risk of developing prostate cancer.

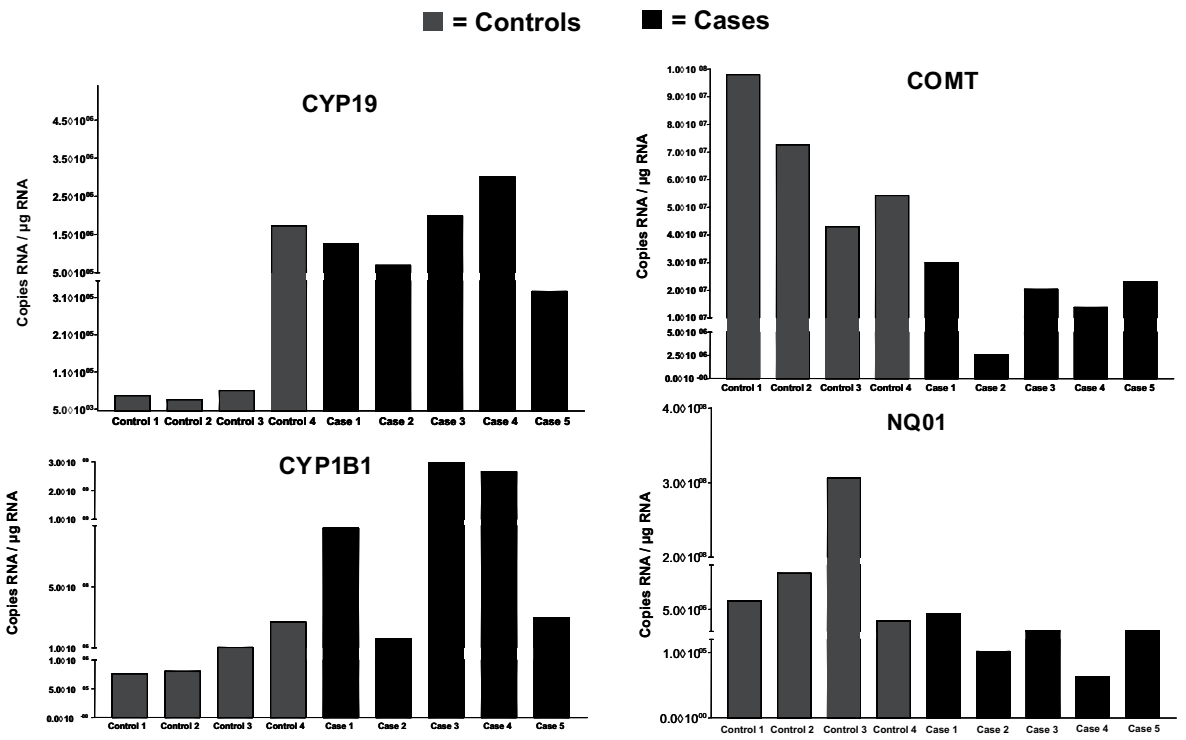

FIGURE 15 Expression of estrogen-metabolizing enzymes in breast tissue from women with and without breast cancer.[79]

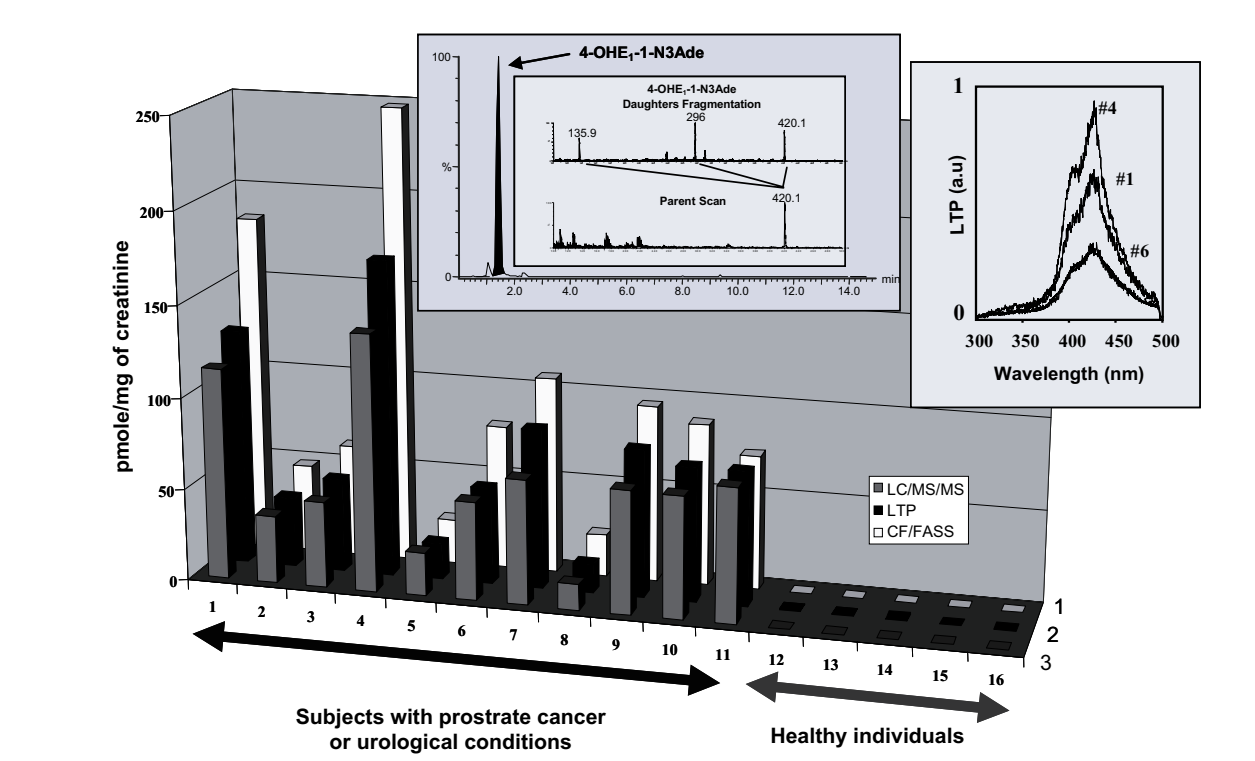

FIGURE 16 Identification of the 4-OHE$_1$-1-N3Ade adduct in human urine samples from men with prostate cancer or urological conditions and healthy men as controls.
Right Inset: The spectra labeled 1, 4 and 6 refer to individual samples 1, 4 and 6, respectively; the red spectrum is that of the standard adduct.
Left Inset: Identification of 4-OHE$_1$-1-N3Ade by LC/MS/MS. The *m/z* 420.1 corresponds to the molecular weight of the parent compound and *m/z* 135.9 and 296 are the fragmentation daughters selected for the unequivocal identification of the adduct.

EXOGENOUS ESTROGENS

We are exposed to exogenous estrogens from many sources, including food and drink. The estrogens in hormonal contraceptives and hormone replacement therapy formulations include both natural estrogens, such as E_2, synthetic estrogens, such as ethynyl estradiol, and estrogens that humans do not synthesize, such as the equine estrogens equilin and equilenin. Typically, most of us think of the hormone replacement formulations as consisting primarily of the equine estrogens, but they actually include a significant component of E_1 and/or E_2. Equilin and equilenin are metabolized to 4-hydroxyequilenin (Fig. 17), which forms adducts with DNA.[83,84] Stable adducts of 4-hydroxyequilenin that remain in DNA unless removed by repair have been studied, but this catechol estrogen can be expected to be oxidized to its 3,4-quinone, which would react with DNA to form depurinating N3Ade and N7Gua adducts, analogous to those formed by the natural estrogens. It is these adducts that we would expect to generate cancer-initiating mutations. This is a line of research that needs to be explored.

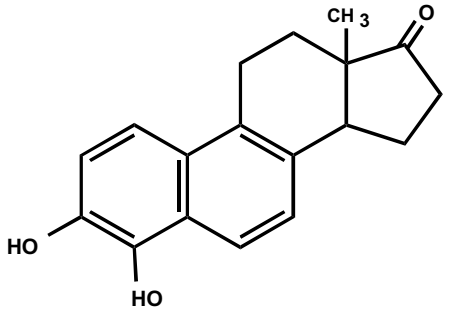

FIGURE 17 The 4-hydroxyequilenin metabolite of equilin and equilenin.[83,84]

The synthetic estrogen ethynyl estradiol is metabolically oxidized to its 2- and 4-catechol estrogen, which, in turn, can be further oxidized to the catechol quinones.[85] Ethynyl estradiol, however, has not been shown to have carcinogenic activity.[86] Presumably, it would be an even weaker carcinogen than the natural estrogens.

PREVENTION OF BREAST AND PROSTATE CANCER

The results of the research described here suggest that prevention of the initiating step (i.e., formation of estrogen-DNA adducts) would be a successful approach to preventing tumor development. We think this could be accomplished in several different ways: (1) preventing formation of catechol estrogen quinones, (2) enhancing reduction of catechol estrogen quinones back to catechol estrogens, (3) scavenging catechol estrogen quinones with glutathione, and (4) limiting the levels of estrogens in the breast or prostate. The aromatase inhibitors have shown some success in reducing the incidence of breast cancer recurrence in postmenopausal women by limiting the levels of estrogens in the breast. In addition, there are a variety of natural products that could be used to modulate the enzymes involved in estrogen metabolism, thereby limiting formation of the estrogen-DNA adducts.

CONCLUSIONS

Breast and prostate cancer are initiated by reaction of catechol estrogen-3,4-quinones with DNA to form depurinating adducts that generate the mutations leading to cancer. These events have been demonstrated with the natural estrogens and may also occur with xenoestrogens. Some of the estrogen-DNA adducts, estrogen-GSH conjugates, and estrogen metabolites may serve as biomarkers for risk of developing breast, prostate, and other cancers. We think they would be detected long before tumors appear. We think breast and prostate cancer can be prevented by using natural dietary supplements to decrease the opportunities for catechol estrogen-3,4-quinones to react with DNA.

Acknowledgements

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Chemoprotection Against Cancer: An Idea Whose Time Has Come

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WHY IS A CHEMOPROTECTION STRATEGY AGAINST CANCER URGENTLY NEEDED?

The process of carcinogenesis begins within a single cell. When challenged by environmental stimuli such as chemicals, radiation, viruses, reactive oxygen and nitrogen species, that cell undergoes a series of genetic changes, gaining growth advantage, circumventing the immune system, and ultimately leading to the development of clinical cancer (following clonal selection and expansion). During this time, many events take place: activation of protooncogenes, inactivation of tumor suppressor genes, and alterations in signaling pathways and apoptosis. Yet, clinically, cancer remains apparently silent for many years, even decades, before diagnosis.[1] In contrast, once a diagnosis is made, it takes only months to at most a few years to determine whether a particular treatment protocol will succeed or fail. The success rate in cancer treatment today still remains very modest, and the number of newly diagnosed cancer cases per year has been increasing steadily and is projected to double by the year 2030, reaching 1.5 million in the United States alone.[2] This devastating prognosis clearly speaks to the urgent need for a radically different strategy in the "war against cancer." We can no longer afford to remain "obsessed with treatment of advanced disease" and "blinded to the promise of prevention."[1,3,4] To focus on developing strategies for protection against cancer is especially important now, as we are experiencing an increase in environmental toxic burden and aging of the population worldwide.

THE ROLE OF PHASE I AND PHASE II ENZYMES IN CARCINOGEN METABOLISM

Most xenobiotics in our environment are not carcinogens themselves. However, upon entry into a biological system, they become substrates for phase I enzymes (mainly cytochrome P450 enzymes)[5] that catalyze their metabolic conversion to form either non-electrophilic metabolites or, in some cases, electrophilic products (ultimate carcinogens) that can damage biological macromolecules and ultimately lead to neoplasia (see Figure 1).

Under aerobic conditions, another source of potentially damaging agents is oxygen itself, through formation of reactive oxygen and nitrogen species (ROS/RNS). DNA, lipids, and proteins are protected against the damaging effects of electrophiles, ROS and RNS by phase II enzymes which, by a variety of mechanisms, including conjugation with endogenous ligands (e.g., glutathione, glucuronic acid) and direct chemical inactivation, detoxify electrophiles and oxidants and facilitate their excretion. Export of the ultimate metabolites is finally achieved through the action of phase III efflux transporters. Consequently, the outcome of an encounter with a potential carcinogen is largely determined by the balance between the activities of phase I enzymes that activate pro-carcinogens and phase II enzymes that detoxify reactive carcinogens. A shift towards the second path is therefore expected to lead to protection against neoplasia.[1,6,7]

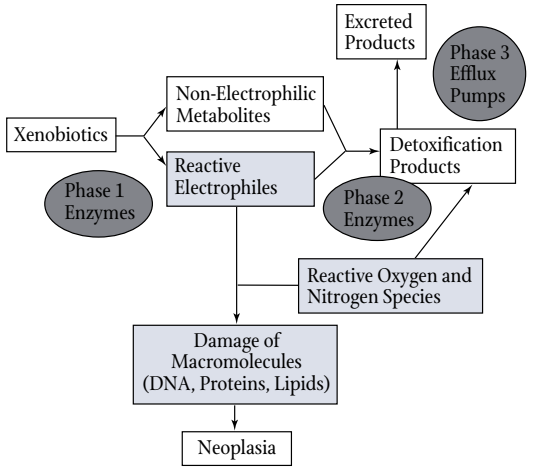

FIGURE 1 Phase I and II enzymes and phase 3 efflux pumps in xenobiotic metabolism

Xenobiotics become substrates for phase I enzymes that catalyze their conversion to either non-electrophilic metabolites or damaging electrophilic products. Reactive oxygen and nitrogen species are also damaging. DNA, lipids, and proteins are protected by phase II enzymes that detoxify electrophiles and oxidants and facilitate their excretion. The final metabolites are exported through the action of phase 3 efflux pumps.

IS CANCER PREVENTABLE?

More than thirty years ago, the laboratories of Frankfurt and Wattenberg reported that the phenolic antioxidants BHA [2(3)-*tert*-butyl-4-hydroxyanisole] and BHT (3,5-di-*tert*-butyl-4-hydroxytoluene) protected rodents against the carcinogenic effects of the polycyclic aromatic hydrocarbon 7,12-dimethylbenz(*a*)anthracene (DMBA).[8] Because BHA and BHT are commonly used as food preservatives and therefore already present in the human diet, this finding provided a strong driving force for the development of the concept of chemoprevention.[9] In the late 1970s, Talalay and Bueding demonstrated that supplementation of BHA in mouse diets leads to induction of phase II enzymes in hepatic as well as extrahepatic tissues (eg, glutathione *S*-transferases, epoxide hydrolase, quinone oxidoreductase 1) (see Figure 2) without any effect on phase I enzymes (eg, cytochrome P450s).[10-12] Furthermore, the discovery that phase II enzymes can be induced selectively by a wide variety of compounds that we now refer to as "inducers," some of which are present in our diet, led to the birth of a new idea in the field of chemoprevention: induction of phase II enzymes as a powerful strategy for protection against carcinogenesis.[13]

Induction of phase II enzymes occurs at the level of their gene expression: all genes encoding phase II proteins contain single or multiple copies of a similar *cis*-acting enhancer element, known as the "antioxidant response element" (ARE), with the consensus core sequence TGACNNNGC.[14-17] In addition to sharing common transcriptional regulation, another characteristic feature of phase II enzymes is that they catalyze enormously versatile chemical reactions that collectively lead to detoxification of various electrophiles and oxidants. Together with housekeeping antioxidant enzymes (eg, catalase, superoxide dismutase) and small mass direct antioxidants (eg, ascorbic acid, tocopherol, glutathione), phase II enzymes consti-

Glutathione *S*-Transferases

γ-Glutamatecysteine Ligase

Glutathione Reductase

Glutathione Peroxidase

Thioredoxin Reductase

Glutathione Conjugate Exporters

NAD(P)H:quinone acceptor oxidoreductase 1 (NQO1)

Heme oxygenase 1

Epoxide hydrolase

Dihydrodiol dehydrogenase

Leukotriene B_4 dehydrogenase
(alkenol/one oxidoreductase)

FIGURE 2 Examples of inducible phase II enzymes

Note that many are related to glutathione, the principal endogenous small molecule antioxidant. All catalyze reactions that collectively result in protection against the toxicities of electrophiles and oxidants.

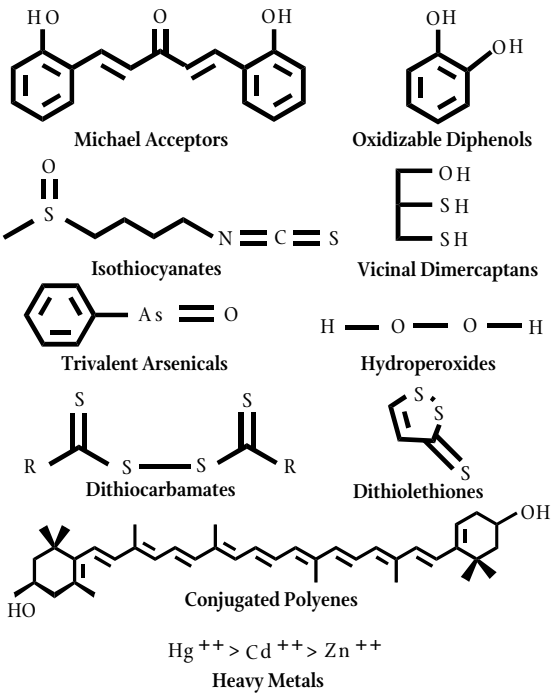

FIGURE 3 Chemical classes of phase II enzyme inducers

Inducers are structurally diverse and share a single common property: the ability to react with sulfhydryl groups.

tute an integral part of the cellular defense. It is perhaps not surprising, therefore, that many phase II inducers were subsequently found to protect against cancer and that a number of compounds that protect against cancer were shown to be phase II inducers.

DEVELOPMENT OF A CELL CULTURE BIOASSAY FOR SCREENING OF POTENTIAL CHEMOPROTECTIVE AGENTS

In order to screen for potential inducers, a microtiter plate bioassay was developed for measuring the activity of a prototypic phase II enzyme—NAD(P)H:quinone oxidoreductase 1 (NQO1) in Hepa 1c1c7 murine hepatoma cells grown in 96-well microtiter plates.[18] Today, we refer to that as "the Prochaska test."[19] The response of NQO1 to a variety of inducers in this assay mimics closely the response of rodent tissues *in vivo*, providing a quick and highly quantitative system for evaluating the potencies of inducers and for screening pure compounds, as well as complex mixtures such as plant extracts, for their inducer activity. The Concentration that Doubles (CD) the activity of NQO1 (CD value) is a characteristic quantitative parameter of this bioassay system and is routinely used to compare inducer potencies. A 2004 review of the application of this bioassay system revealed that phase II inducers belong to at least 10 different chemical classes[20] (see Figure 3):

1. Michael acceptors
2. Oxidizable diphenols and diamines
3. Conjugated polyenes
4. Hydroperoxides
5. Trivalent arsenicals
6. Heavy metals
7. Isothiocyanates
8. Dithiolethiones
9. Dithiocarbamates
10. Vicinal dimercaptans

PLANTS CONTAIN PHASE II INDUCERS

Many phase II inducers are present in plants. Some belong to the Michael acceptor class of inducers, i.e., they have an olefin or acetylene moiety conjugated with an electron-withdrawing group. Based on the naturally occurring analogues, synthetic derivatives have also been obtained in an attempt to improve inducer potency. Some examples include chalcones, curcuminoids, oleanolic, ursolic, and betulinic-acid-derived triterpenoids. Structure-activity relation studies with several chalcone and curcuminoid derivatives revealed that the presence of a hydroxyl group on an aromatic ring at the *ortho*-position with respect to the Michael acceptor moiety improves the inducer potency substantially (from 3 to >200-fold).[20-22] Thus, salicylcurcuminoid (CD = 0.3 μM) is ~25-fold more potent an inducer of NQO1 than curcumin (CD = 7.3 μM). Furthermore, induction of NQO1 correlates with protection against tumor development, eg, salicylcurcuminoid is also a much more potent protector than curcumin in a two-stage skin chemical carcinogenesis model.[23] In this study, at 10 weeks 90% of the control animals had developed tumors. In contrast, tumor incidence was 40% for the curcumin-treated group and all mice from the salicylcurcuminoid-treated group were tumor-free. At the end of the experiment (25 weeks), tumor incidence was 90, 50, and 20% for the control, curcumin-, and salicylcurcuminoid-treated groups, respectively.

PHASE II INDUCERS ARE ANTI-INFLAMMATORY AGENTS

Many phase II inducers that belong to different, structurally unrelated, inducer classes can also inhibit pro-inflammatory responses, eg, cytokine-dependent activation of inducer nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) gene expression. Examples include isothiocyanates, chalcones, curcuminoids, and oleanolic acid-derived triterpenoids. For this activity they require some of the same cellular components (eg, the sensor protein Keap1 and transcription

factor *Nrf2* (see below) that are essential for induction of the phase II response.[24] Furthermore, a detailed structure-activity relation study on ~20 different triterpenoid analogues revealed that their potencies in inducing enzymes (NQO1 in murine hepatoma cells) correlates linearly over 5 orders of magnitude of concentrations with their potencies in inhibiting pro-inflammatory responses (iNOS activation by γ-interferon in mouse macrophages). This correlation strongly suggests that these two processes must be mechanistically related.

THE ROLE OF THE SENSOR PROTEIN FOR INDUCERS, KEAP1 AND TRANSCRIPTION FACTOR NRF2 IN CHEMOPROTECTION

Which is the intracellular sensor for phase II inducers? The enormous structural diversity among these molecules excludes the possibility of a common receptor. In addition, some inducers are electrophiles, some are oxidants, but others are reductants, or have no redox chemistry. Despite this structural and chemical diversity, phase II inducers possess a single common property: they can all react with sulfhydryl groups by oxido-reduction, alkylation, or disulfide interchange. Furthermore, reactivity with sulfhydryl groups parallels inducer potency.[25,26] This property, characteristic for all inducers, led Talalay and his colleagues to hypothesize that the intracellular sensor (perhaps a protein) must possess highly reactive thiols, possibly two cysteine residues in close proximity, to explain the exceedingly high inducer potency of trivalent arsenicals, which are classical reagents for vicinal dithiols.[27] In 1999, Yamamoto and his colleagues reported the isolation of Keap1, the repressor protein of transcription factor Nrf2 which binds to the ARE (in heterodimeric combinations with members of the small macrophage-activating factor family of transcription factors) and activates transcription of phase II genes.[28] The most notable feature of Keap1 that immediately made it "the perfect candidate" for the inducer sensor was its high cysteine content.[29] Murine Keap1 is a zinc metalloprotein[30] that has 25 cysteine residues among its 624 amino acids; the human homologue has 27. Under basal conditions, Keap1 associates with the Cullin3-based E3 ubiquitin ligase complex and binds and targets *Nrf2* for ubiquitination and proteasomal degradation. Inducers chemically modify cysteine residues of Keap1 allowing *Nrf2* to translocate to the nucleus and activate transcription of phase II genes (see Figure 4).

The generation of mice in which the *nrf2* gene has been disrupted[31,32] provided compelling genetic evidence for the protective role of phase II enzymes. Compared to their wild type counterparts, *nrf2* knockout mice have low basal and mostly uninducible levels of phase II proteins and are much more sensitive to the hepatotoxicity of acetaminophen, the pulmonary toxicity of butylated hydroxytoluene, and the carcinogenicity of benzo(*a*)pyrene, aflatoxin B_1, and diesel exhaust.[33] The phase II inducers oltipraz and sulforaphane protect wild type mice against the formation of gastric tumors caused by benzo(*a*)pyrene treatment.[34,35] In sharp contrast, phase II inducers have no effect on either phase II enzymes or tumorigenesis in *nrf2* knockout mice. In addition, these animals develop severe lupus-like glomerulonephritis[36] due to increased levels of oxidative stress and attenuated antioxidant capacity that lead to disturbances in tissue-repairing mechanisms.[37]

HOW DOES KEAP1 SENSE PHASE II INDUCERS?

[^{3}H]Dexamethasone 21-mesylate (Dex-mes), a steroid mesylate that was originally synthesized as a ligand for the corticosteroid receptor, but due to its mesylate moiety is also a phase II inducer, was shown to bind to purified reduced Keap1.[38] Other phase II inducers compete with [^{3}H]Dex-mes for binding to Keap1 and they do so in the order of their inducer potency, e.g., phenylarsene oxide > 1-nitrocyclohexene > 1-chloro-2,4-dinitrobenzene > 2-cyclohexanone > α-methylene-γ-butyrolactone. Because Dex-mes offers the advantage of binding to Keap1 irreversibly, it was incubated briefly with the protein, and following subsequent extensive alkylation with *N*-ethylmaleimide, the modified protein was digested with trypsin. The resulting tryptic peptides were then separated by reversed-phase high-pressure liquid chromatography (HPLC) and analyzed by matrix-assisted laser desorption/ionization (MALDI/TOF) mass spectrometry.[39] The cysteine residues that were consistently labeled were primarily C257, C273, C288, and C297, all located in the central (intervening) domain of Keap1, suggesting that this domain may be of major importance for the repressor function of Keap1. Indeed, subsequent mutagenesis analysis confirmed that C273 and C288 are absolutely required for the ability of Keap1 to repress *Nrf2*.[40-42]

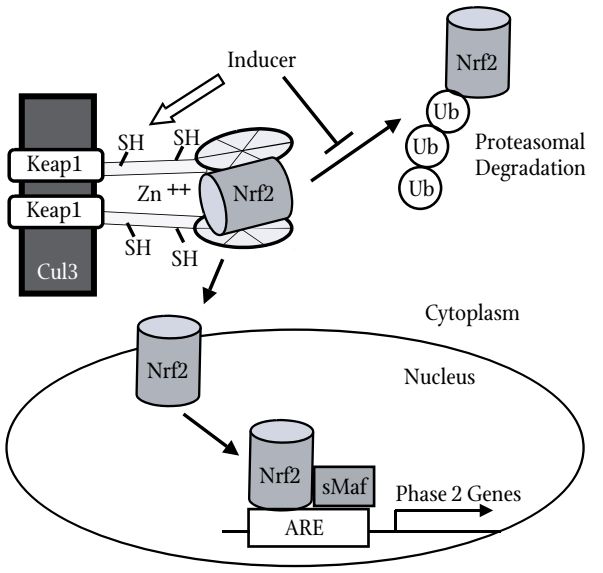

FIGURE 4 Mechanism of phase II enzyme induction. Under basal conditions, the sensor protein Keap1 binds and targets transcription factor Nrf2 for ubiquitination and proteasomal degradation via association with the Cullin 3 (Cul3)-based E3 ubiquitin ligase complex. Inducers modify cysteine residues of Keap1 allowing Nrf2 to translocate to the nucleus, bind to the antioxidant response element (ARE) in heterodimeric combinations with members of the small Maf family of transcription factors, and activate transcription of phase II genes.

CHEMOPROTECTION AGAINST CANCER AND OTHER CHRONIC DISEASES BY THE PHASE II INDUCER SULFORAPHANE

Using the Prochaska test as a bioactivity-guided fractionation, the isothiocyanate sulforaphane was isolated as the principal phase II inducer from broccoli.[43] It was subsequently demonstrated that sulforaphane inhibits tumor development in at least eight different animal models (see Table 1):

1. Zhang and colleagues[44] were the first to demonstrate that oral administration of sulforaphane inhibits the incidence, multiplicity, and tumor burden in the DMBA-induced mammary carcinogenesis model in female Sprague Dawley rats.

2. Sulforaphane was also shown to inhibit the formation of azoxymethane-induced colonic aberrant crypt foci in male Fischer rats.[45]

3. Fahey et al.[35] showed that benzo(*a*)pyrene-induced stomach carcinogenesis in ICR mice was inhibited by feeding sulforaphane in

TABLE 1 Inhibition of Tumor Formation by Sulforaphane in Animal Models

Animal Model	Tumor Type	Sulforaphane Dose and Route of Administration	Reference Information
Sprague Dawley rats	DMBA-induced mammary tumors	75, 100, or 150 mmol/day for 4 days before, to 1 day after the last dose of carcinogen, by gavage	#44: Zhang et al., 1994
Fischer rats	Azoxymethane-induced colonic aberrant crypt foci	20 mmol/day for 3 days before the carcinogen or 5 mmol x 3 times/week for 8 weeks after the carcinogen, by gavage	#45: Chung et al., 2000
ICR mice	Benzo(a)pyrene-induced gastric tumors	7.5 mmol/day for 7 days before, to 2 days after the last dose of carcinogen, by feeding	#35: Fahey et al., 2002
A/J mice	Tobacco carcinogens-induced lung adenoma to carcinoma progression	1.5 or 3 mmol/kg diet for 21 weeks after the carcinogen, by feeding	#46: Conaway et al., 2005
Syrian hamsters	N-nitroso-bis(2-oxopropyl)amine-induced pancreatic tumors	4.5 mmol/kg diet for 3 weeks, including 1 week before and 1 week after the carcinogen, by feeding	#47: Kuroiwa et al., 2005
SKH-1 hairless mice	DMBA/TPA-induced skin tumors	1, 5, or 10 mmol/mouse, topically, twice a week, from 1 week after the carcinogen until the end of the study (15 weeks) or from 7 days before the carcinogen until the end of the study	#48: Gills et al., 2006
SKH-1 hairless mice	UV light-induced skin tumors	1 mmol/mouse, topically, 5 days a week, for 11 weeks starting after completion of the 20-week-UV irradiation schedule	#50: Dinkova-Kostova et al., 2006
Apcmin mice	Intestinal tumors	2.5 mmol/kg diet for 16 weeks, by feeding	#49: Myzak et al., 2006

the diet. Importantly, *nrf2* knockout mice were much more susceptible to the carcinogenic effect of benzo(*a*)pyrene, and sulforaphane had no effect on the tumor development in these animals, providing evidence that, at least in this model, the protective effect of sulforaphane is primarily due to induction of the phase II response, which is ultimately dependent on functional *nrf2* gene (see Figure 4).

4. Conaway et al.[46] showed that sulforaphane and its N-acetylcysteine conjugate inhibit the malignant progression of lung adenomas induced by tobacco carcinogens in A/J mice.

5. Sulforaphane was effective in inhibiting the development of pancreatic tumors when administered before or during the initiation stage in male Syrian hamsters treated with *N*-nitroso-bis(2-oxopropyl)amine.[47]

6. In the two-stage chemical skin carcinogenesis model that involves a single dose of DMBA as initiator, followed by multiple doses of 12-*O*-tetradecanoylphorbol 13-acetate (TPA) as promoter, sulforaphane protected against the development of skin tumors when administered topically during the promotion stage.[48]

7. Importantly, the protective effects of sulforaphane are not restricted to models of chemical carcinogenesis. Myzak et al.[49] showed inhibition of the development of intestinal adenomas in mice in which the adenomatous polyposis coli (*apc*) tumor suppressor gene is truncated, a condition that makes them genetically highly predisposed to multiple intestinal neoplasia.

8. Skin tumor formation was also inhibited in mice chronically exposed to UV light.[50] In this recent study, using a mouse model of UV light-induced skin carcinogenesis,[51,52] topical application of broccoli sprout extracts containing the equivalent of 1 μmol of sulforaphane was shown to inhibit by 50% tumor incidence, multiplicity, and total tumor burden in SKH-1 hairless mice that had been rendered "high-risk" for skin cancer development via prior chronic exposure (20 weeks) to low doses (30 mJ/ cm^2) of UV light.

The findings in the last model described above are highly relevant to humans because UV light is the principal etiological factor contributing to skin cancer development and perhaps the most ubiquitous carcinogen present in our environment. The development of protective strategies against skin cancer is especially needed for the increasing population of solid organ transplant recipients (eg, heart, liver, or kidney) in whom nonmelanoma skin cancer is the most common malignancy in regions with either low (Sweden)[53] or high (Queensland, Australia)[54] solar radiation. In contrast to the general population, the ratio of basal cell carcinoma to squamous cell carcinoma is reversed in transplant patients; squamous cell carcinoma is 65 times more likely to occur as in age-matched controls.[55-58]

It should be noted that sulforaphane and many other phase II inducers are pleiotropic agents for which multiple biological activities have been described that could potentially contribute, independently, or

in combination, to the inhibition of tumor development. Some examples include: induction of cell cycle arrest and apoptosis;[59] inhibition of angiogenesis;[60] inhibition of phase I enzymes;[61] suppression of pro-inflammatory responses;[50,62,63] and inhibition of histone deacetylase.[64]

Although it was isolated from broccoli, the isothiocyanate sulforaphane is not present in plants. Plants synthesize and contain its precursor, the glucosinolate glucoraphanin that co-exists with, but is physically separated from, the enzyme β-thioglucosidase (myrosinase, EC 3.2.3.1). The two come into contact only when plant tissues are damaged, e.g., during plant injury or chewing. The myrosinase-catalyzed reaction results in hydrolysis of the glucosinolate to an unstable aglucone that subsequently spontaneously rearranges to give isothiocyanate, thiocyanate, or nitrile products.[65,66] Importantly, in rodents and humans, in the absence of plant myrosinase, glucoraphanin can still be converted to sulforaphane by the gastrointestinal flora.[67]

Because broccoli and broccoli sprouts are already present in the human diet, they can be used as delivery vehicles for the administration of glucoraphanin and sulforaphane in humans. The pharmacokinetics of extracts of well-characterized broccoli sprout preparations have been examined in human subjects.[68,69] Furthermore, a placebo-controlled, double-blind, randomized clinical study of the safety and tolerance of repeated oral doses of broccoli sprout extracts (containing precisely defined amounts of either glucosinolates or isothiocyanates) in healthy volunteers has been completed and no systematic, clinically significant, adverse effects were observed.[70] A randomized, placebo-controlled, double-blind chemoprevention trial in Qidong Province in the People's Republic of China examined the effect of daily doses of 400-mmol glucoraphanin (in the form of aqueous broccoli sprout extract) on the metabolic disposition of aflatoxin and phenanthrene in 200 healthy human subjects.[71] It was found that the excretion of aflatoxin DNA-adducts and phenanthrene tetraols was lower in the subjects with the highest conversion of glucoraphanin to sulforaphane. Importantly, conversion varied enormously between individuals (between 1 and 45% of the administered dose), highlighting the importance of being able to fully understand and control the factors that determine bioavailability of phase II inducers in the design of future chemoprevention studies.

Sulforaphane is also protective against a number of non-neoplastic conditions (see Table 2):

1. *Helicobacter pylori* infection of human fetal gastric xenografts transplanted in nude mice;[72]

2. hypertension and atherosclerosis in the spontaneously hypertensive, stroke-prone rat;[63]

3. cerebral ischemia in Long-Evans rats;[73] and

4. photooxidative damage of the retina in BALB/c mice.[74]

In a recent human study, *Helicobacter pylori*-infected subjects were randomized into two groups: one receiving 100 g broccoli sprouts (containing the equivalent of 250 mg glucoraphanin) daily for two months and the other, 100 g alfalfa sprouts.[75] At the end of the intervention, there was a significant decrease in *H. pylori* colonization (as evaluated by urea breath test and *H. pylori*-specific stool antigen) and improvement of the degree of gastritis (as evaluated by serum levels of pepsinogen I and II) in the subjects receiving broccoli sprouts, but not alfalfa sprouts. Two months after cessation of intervention, all measured parameters returned to their initial values. Thus, "the case of sulforaphane" has provided proof of principle that inducing the phase II response is a powerful strategy for protecting against cancer and other chronic diseases in the development of which xenobiotic challenge, oxidative stress, and inflammation have been implicated.

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TABLE 2 Inhibition of the Development of Non-neoplastic Conditions by Sulforaphane in Animal Models

Animal Model	Condition	Sulforaphane Dose and Route of Administration	Reference Information
Human fetal gastric xenografts transplanted to nude mice	*Helicobacter pylori* infection	7.5 mmol/day for 5 days administered via catheter	#72: Haristoy et al., 2003
Spontaneously hypertensive stroke-prone rats (SHR)	Hypertension and atherosclerosis	feeding of 200 mg/day, 5 days a week for 14 weeks of dried broccoli sprouts (source of glucoraphanin)	#63: Wu et al., 2004
BALB/c mice	Photooxidative damage of the retina	2.8 mmol/day for 3 days before UV, intraperitoneally or orally	#74: Tanito et al., 2005
Long-Evans rats	Cerebral ischemia	5 mg/kg, intraperitoneally 15 min after the onset of ischemia	#73: Zhao et al., 2006

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The Role of Mercury and Cadmium Heavy Metals in Vascular Disease, Hypertension, Coronary Heart Disease, and Myocardial Infarction

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INTRODUCTION

There is increasing concern regarding the overall health effects of exposure to various heavy metals in the environment. This is particularly true of mercury and less so with cadmium, lead, aluminum, and arsenic. The cardiovascular consequences of mercury and cadmium toxicity have not been carefully evaluated until recently. This paper will critically review the vascular consequences of mercury and cadmium toxicity in humans as it relates to hypertension, generalized atherosclerosis, coronary heart disease (CHD), myocardial infarction (MI), cerebrovascular accidents (CVA), carotid artery disease, renal dysfunction, and total mortality.

MERCURY

Types of Mercury

Mercury exists in 3 basic forms: elemental, inorganic, and organic (Table 1).[1-5] Dental amalgams are the most common source for elemental mercury vapor, which is a stable monoatomic gas. Inorganic mercury, which is a divalent compound, is the toxic species found in human tissue after conversion from the other forms. Organic mercury in the form of methyl and ethyl mercury is primarily from fish, sea mammals, and thimerosal vaccines. Although dental amalgams historically have been the major source of human exposure, fish and sea mammals are becoming an increasingly important environmental source of potential mercury toxicity.[6-9]

TABLE 1 Mercury Types[1-4,44]

1. Elemental	Mercury vapor (Hg°) Stable monoatomic Gas	Dental amalgams
2. Inorganic	Divalent mercury (Hg^{2+})	Toxic species in human tissue after conversion
3. Organic	Methyl mercury (CH_3Hg+) Ethyl mercury (CH_3CH_3Hg+)	Fish, sea mammals Thimerosal vaccines

Mercury Biotransformation and Biomethylation

Mercury from various sources, including elemental mercury from earth sources or inhaled mercury vapor, methyl and ethyl mercury, is converted by biomethylation to inorganic divalent mercury, the toxic form in human organs and tissues (Figure 1).[10] Divalent mercury is soluble and stable in water and undergoes biomethylation to methyl mercury, which is found in high concentrations in certain fish and sea mammals.

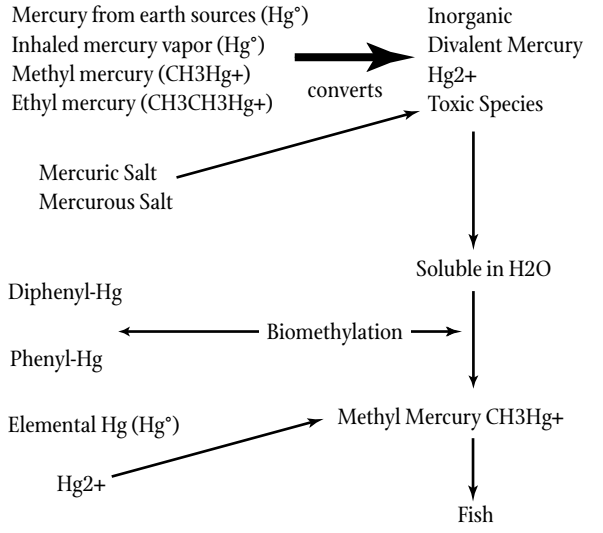

FIGURE 1 Mercury Biotransformation and Biomethylation

The Environmental Protection Agency has determined the safe daily intake of mercury to be less than 0.1 µg/kg/day (about 7 µg/day for a 154 lb person).[11] It is estimated that 1 dental amalgam filling releases about 3-17 µgs of mercury vapor per day. The typical amalgam is composed of 50% mercury, 25% silver, 25% tin, copper, and nickel.[12-14] Fish and sea mammals provide about 2-3 µg per day depending on the type and amount consumed.[15-18] The long-lived, large predatory fish such as swordfish, tilefish, shark, and king mackerel contain about 1 µg of methyl mercury per gram. Pike, whale, bass, tuna, and trout contain about 0.1-0.5 µg of mercury per gram. Nine vaccines containing thimerosal (now off the US market) would give an estimated exposure of 62 µg of organic mercury.[19-22] All other sources of mercury provide about 0.3 µg per day.[23-26]

Important Facts about Mercury

Mercury is the most dangerous of all the heavy metals.[27] It will modify the distribution and retention of other heavy metals.[28-30] Mercury has no known physiological role in human metabolism, and the human body has no mechanisms to excrete mercury actively.[31] Mercury, thus, accumulates during life so that the average 70 kg person has a total body burden of about 13 mg of mercury.[32] Mercury has a high affinity for sulfhydryl groups (-SH), various enzymes and amino acids, N-acetylcysteine (NAC), alpha lipoic acid (ALA), and glutathione (GSH), which provide about 10-50% of the plasma protein antioxidant capacity.[33-35] Lower availability of these antioxidants reduces oxidant defense and increases oxidative stress. Selenium antagonizes some of the adverse effects of mercury by forming a seleno-mercury complex in tissue that is less toxic.[36-43]

Physiological Basis of Mercury Toxicity

Mercury induces mitochondrial dysfunction and oxidative stress.[44-46] The primary mitochondrial dysfunction occurs at the

ubiquinone-cytochrome B region and with NADH dehydrogenase causing displacement of Fe++ and Cu+ ions in the a3Cub center of cytochrome C (Figure 2). This results in depolarization and auto-oxidation of the inner mitochondrial membrane with lipid peroxidation and severe mitochondrial dysfunction. Physiologic consequences include increased hydrogen peroxide, depletion of mitochondrial glutathione by over 50%, increased lipid peroxidation markers, such as TBARS, by over 70%, oxidation of pyridine nucleotides, such as NAD(p)H, and altered calcium homeostasis.[47-49] This severe mitochondrial dysfunction increases oxidative stress and reduces antioxidant defenses, creating significant health implications.

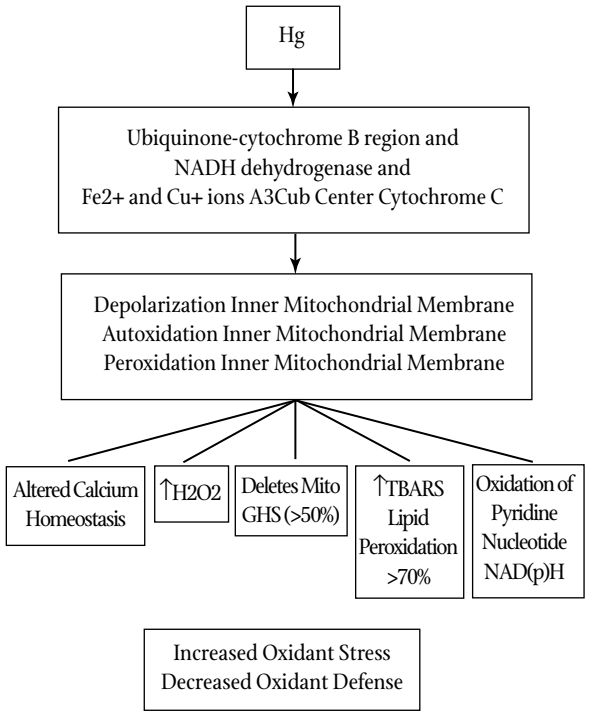

FIGURE 2 Pathophysiologic Basis of Mercury Toxicity, Mitochondrial Dysfunction and Oxidative Stress

The primary 3 sources of mercury-induced lipid peroxidation include the Fenton reaction, affinity for sulfhydryl groups, and selenium deficiency.[50] Mercury serves as a direct catalyst in Fenton-type reactions and as an indirect catalyst via iron stimulation, which increases the production of radical oxygen species and superoxide anion.[51] Mercury's high affinity for sulfhydryl groups (-SH), such as glutathione, NAC, and ALA, which comprise much of the antioxidant capacity of plasma, reduces both membrane and plasma antioxidant defense. Finally, the formation of insoluble complexes of mercury with selenium reduces selenium availability, which is a necessary cofactor for glutathione peroxidase (GPx) activity to break down hydrogen peroxides and various other toxic peroxidation products. Thus, plasma and intracellular antioxidant capacity is reduced.[52]

Vascular Biological Effects of Mercury

Numerous toxic effects of mercury have been demonstrated in vitro and in both animal and human studies (Table 2 provides details with references, and Table 3 provides a summary).

Studies find that mercury:

- Increases free radical production[53-60]
- Inactivates antioxidant defenses[61-63]
- Binds to thiol-containing molecules[64-68]
- Binds to selenium, forming seleno-mercury complexes that reduce selenium availability for GPx activity[69-73]
- Inactivates glutathione, catalase, and superoxide dismutase[74-77]
- Increases lipid peroxidation[78-81]
- Increases oxidation of LDL (oxLDL)
- Increases plasma oxLDL complexes[82]

TABLE 2 Vascular Biologic Effects of Mercury

1. Increases free radical production[53-60]
2. Inactivates antioxidant defenses[53-55]
3. Binds to thiol-containing molecules[53-55,67,68]
4. Binds to Se, forming Se-Hg complex-mercury selenide, which decreases Se available for cofactor with GPx[53-55]
5. Inactivates glutathione, catalase, and SOD[74-77]
6. Increases lipid peroxidation in all organs[78-81]
7. Increases oxLDL27 and oxLDL immune complexes[27]
8. Increases platelet aggregation[83]
9. Increases coagulation/thrombosis: increases Factor VIII PF4 and thrombin and reduces protein C[85,86]
10. Inhibits endothelial cell formation and migration[87]
11. Increases apoptosis[88]
12. Reduces monocyte function and phagocytosis[88]
    - Immune function is impaired
13. Increases inflammation[88]

TABLE 3 Summary of Vascular Biologic Effects of Mercury

1. Oxidative stress
2. Inflammation
3. Thrombosis
4. Vascular smooth muscle (VSM) proliferation and migration
5. Endothelial dysfunction
6. Dyslipidemia (oxHDL and paraoxonase)
7. Immune dysfunction
8. Mitochondrial dysfunction

Thrombosis is potentiated by increased platelet aggregation[83] and by increases in Factor VIII, platelet factor,[84] and thrombin, with reductions in protein C.[85,86] Endothelial cell formation and migration are reduced, which decreases vascular endothelial repair, decreases nitric oxide, and causes endothelial dysfunction.[87] Apoptosis is increased,[88] monocyte function and phagocytosis are impaired,[89] immune function is reduced,[90] and vascular inflammation is increased.[91] There is an increased production and release of superoxide anion from human neutrophils and monocytes,[92,93] depolarization of the inner mitochondrial membrane with severe mitochondrial dysfunction,[94-96] and disruption of plasma membrane lipid integrity by translocation of phosphatidyl serine (PS).[97] Finally, mercury stimulates proliferation of vascular smooth muscle cells[98] and inactivates paraoxonase, an extracellular antioxidative enzyme related to HDL, CHD, and MI risk.[99,100]

In summary, the overall vascular effects of mercury include oxidative stress, inflammation, thrombosis, VSM proliferation and migration, endothelial dysfunction, dyslipidemia, immune dysfunction, and mitochondrial dysfunction. All of these functional

abnormalities have the potential to increase the risk for hypertension and vascular disease.

Clinical Vascular Consequences of Mercury Toxicity

The clinical consequences of mercury toxicity include hypertension,[101-104] CHD,[105-107] MI,[108-110] increase in carotid intimal medial thickness (IMT) and carotid obstruction,[111] CVA,[112] generalized atherosclerosis,[113] renal dysfunction and proteinuria,[114] and an overall increase in total mortality.[115]

Coronary Heart Disease and Myocardial Infarction

In rabbits exposed to inhaled mercury vapor, the cardiovascular and cardiac pathology included bradycardia, thrombosis in small and medium caliber arteries, focal necrosis with thickening of the endocardium of the perivalvular regions, papillary muscles and valves, and endothelial proliferation with inflammatory foci and focal edema, endothelial proliferation, inflammation, and fibrosis of the ascending aorta.[116]

In a case control study in 9 counties of 684 men with their first MI, there was a significant association of toenail mercury content, adipose tissue DHA, and first MI.[117] There was a 15% higher toenail mercury content as assessed by neutron activation analysis (NAA) in the men with their first MI compared to the control group (95% CI; 5-25%). The risk-adjusted OR for MI was 2.16 in the highest vs the lowest quintile (P=.006, 95% CI; 1.09-4.29). The adipose DHA was directly proportional to the mercury toenail content (P<.001) and the DHA content was inversely correlated to MI with an OR of 0.59 in the highest versus the lowest quintile (P=.02, 95% CI; 0.30-1.19). This important study concluded that there exists a positive, monotonic increase in the risk of MI with mercury toenail content above the 0.25 µg/g level, which was even steeper when adjusted for the DHA adipose tissue content. Mercury diminishes the cardiovascular protection of fish consumption. Another study substantiated these results—the highest quartile of DHA with the lowest quartile of mercury was associated with a 67% reduction in CHD (P<.016).[118]

In another large, nested case control study of 33,733 male healthcare professionals between the ages of 40-75 years (Health Professionals Follow-Up Study), no association between mercury toenail content assessed by NAA and CHD was found.[119] However, if dentists were excluded, there was a nonsignificant correlation of toenail mercury and CHD. Also, subjects with the highest tertile of mercury and the lowest serum selenium level had a significant increase in CHD.

Other human studies have shown mixed results.[120-124] One study of mercury miners showed no relationship between CHD and mercury levels.[125] However, another study of European mercury miners showed a significant relationship between mercury exposure and total mortality (increase 8%), hypertension (increase 46%), CHD (increase 36%), renal disease (increase 55%), and CVA (increase 36%).[126] A Finnish study found a significant relationship between hair mercury, 24-hour urine mercury, and cardiovascular events.[127] In patients with hair mercury in the highest tertile (over 2 µg/g) and increased 24-hour urinary mercury, CHD and MI risk was increased 2-fold (P=.005), cardiovascular death increased by 2.9 times (P=.014) and circulating oxLDL and immune complexes to oxLDL increased significantly. The Gothenburg Study showed no relationship between serum mercury content and the number of amalgam fillings and CHD or MI.[128]

Carotid Atherosclerosis

High hair mercury content correlates with increased carotid IMT and carotid atherosclerosis.[129] A study of 1,014 men between the ages of 42-60 years found an increase in mean carotid IMT over 4 years (P=.0007). Each increase of 1 µg in hair content equaled an 8 µmol increase in carotid IMT, a 7.3% increase over the mean. There was a 0.042 mm/4-year difference in the highest quintile versus the lowest quintile, which correlated to a 32% greater increase (P<.05). In addition, mercury hair content was proportional to blood pressure, fibrinogen levels, waist-hip ratio, and low HDL cholesterol; all were significant at P=.0002.

Hypertension

The association of mercury toxicity and hypertension in humans is convincing.[130-133] Mercury miners were found to have significant increases in systolic blood pressure (P<.01) that correlated with lipid peroxidation and overall oxidative stress (P<.01).[134] European mercury miners had a 46% greater incidence of hypertension vs aged-matched controls. Other studies have shown significant correlations with hair mercury content, hypertension, and carotid IMT.[135]

In acute and probably chronic mercury intoxication, mercury binds to the sulfhydryl group S-adenosyl methionine (SAMe) and inactivates this enzyme, which is a necessary cofactor for catecholamine-O-methyl transferase (COMT), the enzyme needed to convert norepinephrine, epinephrine, and dopamine by methoxylation.[136] This results in a clinical syndrome that resembles a pheochromocytoma crisis with malignant hypertension in acute mercury intoxication and significant increases in urinary catecholamines in chronic mercury toxicity. This can be a very helpful clinical clue to mercury-induced hypertension. Mercury also induces renal dysfunction and proteinuria, which contribute to sodium retention and hypertension.[137-140] Studies have shown an increase in renal insufficiency in mercury miners of 55%.[141] Mercury concentrates in the renal tubules and glomerulus and results in proteinuria, fibrosis, and chronic renal insufficiency and dysfunction.[142,143]

CADMIUM

The role of cadmium in cardiovascular disease and hypertension is less convincing than that of mercury due to methodological flaws and study design in most of the published human studies.[144-151] Cadmium exposure is uncommon in most of the population unless there is oral consumption of polluted water or chronic inhalation exposure from cigarettes.[152] Twenty cigarettes will release about 30 µg of cadmium, of which 2-4 µg is actually inhaled.[153] The oral absorption of cadmium in tap water is 13-19% or about 2-4 µg per day.[154] Absorption of cadmium is increased in the presence of low dietary calcium, iron, and protein.[155] Cadmium concentrates in all organs, but mostly in kidney, liver, and pancreas, and has a half-life of over 30 years in renal tissue.[156]

Serum and urinary cadmium reflect recent exposure, but not total body burden.[157] Cadmium binds to metallothionein,[158] substitutes for zinc and copper in metalloenzymes,[159] and has a high affinity for sulfhydryl groups, similar to mercury.[160]

Animal studies show that cadmium toxicity causes aortic and coronary atherosclerosis, reduces cardiac output, alters the cardiac conduction system, reduces ATP, increases cholesterol and free fatty acids, increases blood pressure, and induces renal tubular dysfunction, proteinuria, and chronic renal insufficiency.[161-165] These effects are mitigated by calcium administration.

Human studies attempting to show a relationship between cadmium and cardiovascular disease or hypertension are subject to many methodological errors, so that accurate conclusions are difficult to draw.[166-168] In human autopsy studies, there is a poor correlation between renal cadmium content and hypertension.[169] In those studies where hypertension and cadmium coexist, the mechanisms include

increases in urinary catecholamines, renal toxicity with proteinuria, sodium retention, increased intracellular calcium, and alteration in Na+/K+ ATPase.[170] Cadmium concentrates in the renal cortex and tubules and there reduces the expression of renal cortical CYP4A11.[171] CYP4A11 is involved in the hydroxylation of PUFA and affects sodium balance through 20 HETE. The combination of increased renal tubular sodium reabsorption, direct renal toxicity, and proteinuria increases the risk of hypertension.[172]

Cadmium also increases metallothinein in renal tubular cells and other tissues, which alters intracellular zinc.[173] This reduces zinc-dependent ligand binding to DNA and reduces PPAR expression, and may increase free fatty acids, lipids, glucose, and blood pressure. It is possible that some degree of insulin resistance occurs, which contributes to many of the associated metabolic disturbances noted above.

It is quite likely that chronic high exposure to cadmium in smokers, those drinking polluted water, and those with the CYP4A11 genetic alteration could have cadmium-induced hypertension and cardiovascular disease, but additional human studies are required to confirm this association.

SUMMARY

1. Mercury, cadmium, and other heavy metals have a high affinity for sulfhydryl (-SH) groups, inactivating numerous enzymatic reactions, amino acids, and sulfur-containing antioxidants (NAC, ALA, GSH), with subsequent decreased oxidant defense and increased oxidative stress. Both bind to metallothionein and substitute for zinc, copper, and other trace metals reducing the effectiveness of metalloenzymes.

2. Mercury induces mitochondrial dysfunction with reduction in ATP, depletion of glutathione, and increased lipid peroxidation; increased oxidative stress is common.

3. Selenium antagonizes mercury toxicity.

4. The overall vascular effects of mercury include oxidative stress, inflammation, thrombosis, vascular smooth muscle dysfunction, endothelial dysfunction, dyslipidemia, immune dysfunction, and mitochondrial dysfunction.

5. The clinical consequences of mercury toxicity include hypertension, CHD, MI, increased carotid IMT and obstruction, CVA, generalized atherosclerosis, and renal dysfunction with proteinuria. Pathological, biochemical, and functional medicine correlations are significant and logical.

6. Mercury diminishes the protective effect of fish and omega-3 fatty acids.

7. Mercury, cadmium, and other heavy metals inactivate COMT, which increases serum and urinary epinephrine, norepinephrine, and dopamine. This effect will increase blood pressure and may be a clinical clue to heavy metal toxicity.

8. Cadmium concentrates in the kidney, particularly inducing proteinuria and renal dysfunction; it is associated with hypertension, but less so with CHD. Renal cadmium reduces CYP4A11 and PPARs, which may be related to hypertension, sodium retention, glucose intolerance, dyslipidemia, and zinc deficiency. Dietary calcium may mitigate some of the toxicity of cadmium.

9. Heavy metal toxicity, especially mercury and cadmium, should be evaluated in any patient with hypertension, CHD, or other vascular disease. Specific testing for acute and chronic toxicity and total body burden using hair, toenail, urine, serum, etc. with baseline and provoked evaluation should be done.

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Systems Biology, Toxins, Obesity, and Functional Medicine

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Obesity is not a single clinical disorder. Obesity is a complex chronic illness resulting from the interplay among genetics, environment, and lifestyle. Emerging scientific concepts provide a new basis for understanding the multiple causes of obesity as well as the underlying mechanisms involved in weight dysregulation. While most obesity can be effectively treated for compliant patients, using a focused lifestyle intervention based on a whole-foods, low-glycemic-load, phytonutrient-rich diet combined with exercise and stress management, there are patients who do not respond predictably to normally successful interventions. A novel hypothesis linking environmental and internal toxins to disruptions of key mechanisms involved in weight regulation may explain treatment resistance in obesity. The key biological systems involved in obesity (and all diseases) that are altered by toxins are the neuro-endocrine-immune system, and mitochondrial energetics and redox status. Obesity provides an illustrative example of new navigational tools for diagnosis and therapy of chronic illness based on a paradigm that focuses not on disease or symptoms, but on cause and mechanism. This new framework and methodological approach can be applied to any chronic disease and provides an opportunity to integrate fragmentary scientific discoveries into a cohesive whole that creates a new clinical roadmap.

This paper will explore a novel hypothesis that links obesity and toxins; we will discuss how one particular disease and the effect of one underlying cause can create a clinically relevant, holographic view of physiology. Alterations in thyroid metabolism and receptor function, central appetite dysregulation, inflammation's influence on insulin and leptin resistance, impaired mitochondrial oxidative metabolism, and oxidative-stress-mediated effects via nuclear factor kappa B (NFκB) are all mechanisms by which toxins create alterations in metabolism and finely-tuned weight regulatory mechanisms.

These systems are not discrete entities but systems in the true sense of the word – interlocking, interactive, dynamic, overlapping networks of biochemical and physiological informational spheres of functional relationships. Multiple patterns of genetic, physiological, and biochemical dysfunction are linked to obesity, including genetic polymorphisms, inflammation, mitochondrial dysfunction, oxidative stress, neuro-endocrine-immune dysfunction, especially autonomic disturbances involving the hypothalamic-pituitary-adrenal axis, nutritional deficiencies or excesses, and toxins. The nature, causes, and remediation of obesity can be seen through the prism of any one of these patterns. The focus here will be on how toxins mediate their influence through all these mechanisms.

WEIGHT REGULATION AND TOXINS: UNDERLYING MECHANISMS

The influence of toxins on metabolism occurs through 5 key mechanisms: hormonal regulation, neuro-regulatory mechanisms, immuno-regulatory mechanisms, mitochondrial function, and oxidative stress. Toxins can alter the hormonal regulation of weight, a process that involves insulin, leptin, thyroid, cortisol, adiponectin, resistin, sex hormones, and gut hormones, including ghrelin, peptide YY (PYY), and cholecystokinin (CCK). Toxins alter thyroid hormone metabolism and receptor function leading to lowered metabolic rate. Important neuro-regulatory mechanisms affected by toxins include hypothalamic satiety modulation through effects on peripheral and central inhibitors and stimulators of appetite, including leptin, cortisol, alpha melanocyte stimulating hormone (α-MSH), and neuropeptide Y (NPY). Stress-induced autonomic dysfunction also alters appetite and weight-control mechanisms. Toxins can influence weight through toxin-mediated increases in inflammatory cytokines (TNF-α, IL-6) on the peroxisome proliferator-activated receptor (PPAR) family of nuclear receptors promoting insulin resistance, and on the melanocortin receptor (MCR) system altering central appetite regulation. Counter-regulatory signals triggered by inflammation such as suppressors of cytokine signaling (SOCS) induce leptin resistance. Toxins alter mitochondrial energetics by damaging enzymes involved in fatty acid oxidation and thermogenesis. Oxidative stress influences weight via NFκB-mediated mechanisms of gene transcription that control insulin resistance and inflammation. Other mechanisms may include direct effects of toxins on hepatic control of lipid and glucose metabolism, and on inflammatory cytokines.

CAN FOREIGN MOLECULES CAUSE OBESITY?

It is clear that ingesting foreign molecules can lead to obesity, including medications. While most drugs are not truly toxins, certain drugs can have toxic effects and cause weight gain—psychotropic medications in particular have been shown to promote weight gain. monoamine oxidase (MAO) inhibitors, lithium, valproate, mirtazapine, clozapine, olanzapine, and some selective serotonin re-uptake inhibitors (SSRIs) such as fluoxetine, sertraline, and paroxetine have all been shown to promote weight gain through various mechanisms. Hormones such as megestrol are used to increase appetite in cancer patients. Billions of dollars are pouring into obesity drug research to find the magic molecule that will burn fat or reduce appetite. However, affecting one pathway in a complex cybernetic system will likely fail because of countless counter-regulatory mechanisms. It is clear that medications can affect our weight and may play a role in obesity for some people. But it is important to recognize that, if medications can influence weight, then certainly other foreign chemicals, including environmental toxins, can cause weight gain.

Environmental toxins interfere with metabolism, overload hepatic detoxification systems, disrupt central weight-control systems, promote insulin resistance, alter circadian rhythms, activate the stress response, interfere with thyroid function, increase inflammation, damage mitochondria, and lead to obesity. Most researchers have largely ignored the effects of environmental chemicals on metabolism. Still, a few researchers have started connecting the dots linking

toxins with the obesity epidemic. While research linking environmental toxins and impaired detoxification to obesity remains in its infancy, these factors can no longer be overlooked. Detoxification is a central component in long-term effective weight management and creating a healthy metabolism.

LIVING IN A SEA OF TOXINS: THE PROBLEM

Why should we worry about toxins unless we work with toxic chemicals or spray pesticides for a living? Isn't exposure minimal? Unfortunately, risks of exposure are substantial, pose significant public health risks, and can no longer be ignored. We live in a sea of toxins. Every single person and animal on the planet contains residues of toxic chemicals or metals in their tissues. Eighty thousand new chemicals have been introduced since the turn of the 20th century and most have never been tested for safety or for synergistic actions. The Centers for Disease Control issued a report on human exposure to environmental chemicals. They assessed human blood or urine levels for 116 chemicals (and there were thousands more for which tests were not conducted) as part of the National Health and Nutrition Examination Survey.[1] While they found high levels of toxins in some, and low levels in many more, the study, in isolation, may not tell the whole story. Why? Because these chemical toxins move quickly from the blood into storage sites—mostly fat tissue, organs, and bones—so the blood or urine levels *underestimate* the total toxic load. Both weight gain (because of stored toxins) and the total toxic load can frustrate attempts at weight loss by impairing two key metabolic organs—the liver and the thyroid, by damaging the mitochondria—the site of energy metabolism, by affecting neuroendocrine signaling, and by increasing inflammation and oxidative stress.

FAT AS A STORAGE DEPOT FOR FAT SOLUBLE TOXINS

The Environmental Protection Agency has monitored human exposure to toxic environmental chemicals since 1972 when they began the National Human Adipose Tissue Survey. This study evaluates the levels of various toxins in the fat tissue from cadavers and elective surgeries. Five of what are known to be the most toxic chemicals were found in 100% of all samples (OCDD or octachlorodibenzo-p-dioxin, styrene, 1,4-dichlorobenzene, xylene, and ethylphenol—toxic chemicals from industrial pollution that damage the liver, heart, lungs, and nervous system). Nine more chemicals were found in 91-98% of samples: benzene, toluene, ethylbenzene, DDE (a breakdown product of DDT, the pesticide banned in the US since 1972), three dioxins, and one furan. Polychlorinated biphenyls (PCBs) were found in 83% of the population. A Michigan study found DDT in over 70% of 4 years olds, probably received through breast milk. With the global economy, we may be eating food that was picked a day before in Guatemala, Indonesia, or Asia, where there are not the same restrictions on the use of pesticides as there are in the United States. Many of these chemicals are stored in fat tissue, making animal products concentrated sources. One hundred percent of beef is contaminated with DDT, as is 93% of processed cheese, hot dogs, bologna, turkey, and ice cream.

WHERE DO TOXINS COME FROM?

Exposure to toxins comes from two main sources: the environment (external toxins) and the gut (breakdown products of our metabolism, or internal toxins). Both can overload endogenous detoxification mechanisms.

External Toxins: The Dangers from Without

The external toxins include chemical toxins and heavy metals. The

Testing for Toxins and Detoxification Function

- Genetic testing of detoxification pathways for phase I and phase II SNPs
- Detoxification challenge test (provocations with caffeine, aspirin, acetaminophen)
- Measurement of detoxification enzymes
  - Reduced glutathione
  - Glutathione peroxidase
  - super oxide dismutase (SOD)
- Heavy metals
  - RBC or whole blood
  - Hair analysis
  - Chelation challenge with DMPS or DMSA
- Urinary organic acids
  - Specific compounds measured, including sulfates, pyroglutamate, orotate, and others, can give clues to problems with detoxification pathways.
- Chemical antibodies to various toxins and metals (can occasionally be useful)
- Organophosphates: identified through a 24-hour urine collection test
- Mold and mycotoxin antibodies
- IgG food sensitivity testing
- Celiac testing (IgG and IgA anti-gliadin antibodies, tTG IgA)
- Digestive stool analysis for dysbiosis
- Tests for hidden infections (Lyme, *H. pylori*, etc.)

Practical Suggestions for Patients

Remove Toxins

- Eat organic food and animal products to avoid petrochemical pesticides, herbicides, hormones, and antibiotics.
- Drink filtered water (reverse osmosis or carbon filter).
- HEPA/ULPA filters and ionizers can be helpful in reducing dust, molds, volatile organic compounds, and other sources of indoor air pollution.
- Clean and monitor heating systems for release of carbon monoxide, the most common cause of death by poisoning in America.
- Have houseplants that help filter the air.
- Air out your dry cleaning before wearing it.
- Avoid excess exposure to environmental petrochemicals (garden chemicals, dry cleaning, car exhaust, second-hand smoke).
- Reduce or eliminate the use of toxic household and personal care products (aluminium-containing underarm deodorant, antacids, and pots and pans).
- Remove allergens and dust from your home as much as possible.
- Minimize electromagnetic radiation (EMR) from radios, TVs, and microwave ovens.
- Reduce ionizing radiation (from sun exposure or medical tests such as X-rays).
- Reduce heavy metal exposure (predatory and river fish, water, lead paint, thimerosal-containing products, etc.).

Improve Elimination of Toxins

- Have 1-2 bowel movements a day.
- Drink 6-8 glasses of water a day.
- Sweat regularly.
  - Use exercise to help you sweat regularly.
  - Use steam baths or saunas – infrared saunas may be even more beneficial.

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heavy metals that cause the most ill health are lead, mercury, cadmium, arsenic, nickel, and aluminum. Chemical toxins include volatile organic compounds (VOCs), solvents (cleaning materials, formaldehyde, toluene, benzene), medications, alcohol, pesticides, herbicides, and food additives. Infections (hepatitis C virus) and mold toxins (sick building syndrome) are other common sources of toxins. Our modern refined diet can be considered toxic because it places an extra burden on detoxification systems through excessive consumption of sugar, high-fructose corn syrup (the two most important causes of elevated liver function tests), trans fatty acids, alcohol, caffeine, aspartame, foods made with genetically modified organisms (GMOs), and the various plastics, pathogens, hormones, and antibiotics found in our food supply.

Internal Toxins: Danger from Within

Internal toxins include microbial compounds (from bacteria, yeast, or other organisms), and the breakdown products of normal protein metabolism. Bacteria and yeast in the gut produce waste products, metabolic by-products and cellular debris that can interfere with many body functions and lead to increased inflammation and oxidative stress. These include endotoxins, toxic amines, toxic derivatives of bile, and various carcinogenic substances such as putrescine and cadaverine. Lastly, by-products of normal protein metabolism, including urea and ammonia, require detoxification.

OBESITY AND TOXICITY: IS THERE A CONNECTION?

Effects on Thyroid and Metabolic Rate

Many people reach a plateau during weight loss. After the loss of a few pounds, it is often difficult to shed more weight. What is it that impedes weight loss and interferes with metabolism? A review paper, "Energy balance and pollution by organochlorines and polychlorinated biphenyls,"[2] published in *Obesity Reviews* in 2003 outlines the effects of toxins on metabolic rate and weight regulation via various mechanisms. The authors conclude that pesticides (organochlorines) and PCBs (from industrial pollution) released from the fat tissue, where they are typically stored, during weight loss lower the metabolic rate. The authors go on to conclude that we should lose a little weight to reduce our risk of cardiovascular and degenerative diseases, but not too much because we could poison our metabolism. If there were no way to facilitate endogenous detoxification mechanisms, this would be a sound conclusion; however there are multiple ways to upregulate all phases of detoxification and eliminate both endogenously-liberated and exogenous toxins.

How do the chemical toxins interfere with metabolism? The researchers in the above-mentioned study on the link between chemical toxins and obesity reviewed 63 scientific studies and described many mechanisms. First, people with a higher body mass index (BMI) store more toxins because they have more fat. Those toxins interfere with many normal aspects of metabolism, including causing a reduction in thyroid hormone levels, and increased excretion of thyroid hormones by the liver. Toxins also compete with the thyroid hormones by blocking the thyroid receptors, and by vying for the thyroid transport proteins. Toxins also induce hepatic uridine diphospho-glucuronosyltransferase (UDPGT), which catalyzes glucuronidation of T4 for excretion in bile. T3 concentrations and resting metabolic rate are inversely related to organochlorine levels. Thus, it is clear that organochlorine pesticides and PCBs lower thyroid hormone levels, interfere with their function, and slow the metabolic rate.

Toxins Alter Mitochondrial Function, Redox Status, and Cytokine Function

In addition, toxins damage the mitochondria, increase oxidative

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- Regular exercise, yoga, or lymphatic massage can improve lymph flow and help flush toxins out of your tissues into your circulation so they can be detoxified.

Increase Fiber Intake

- Eat more beans, whole grains, vegetables, fruits, nuts, and seeds.

Feed Your Gut with Healthy Bacteria

- Taking probiotics such as lactobacillus and bifidobacter species helps normalize gut flora and reduce endotoxins (toxins produced by imbalances in gut bacteria).

Foods and Phytochemicals that Boost Detoxification

- Try to eat at least one cup of cruciferous vegetables daily.
- Eat a few cloves of garlic every day or take a garlic supplement.
- Try decaffeinated green tea in the morning.
- Try fresh vegetable juices including carrots, celery, cilantro, beets, parsley, and ginger.
- Try prepared herbal detoxification teas containing a mixture of burdock root, dandelion root, ginger root, licorice root, sarsaparilla root, cardamom seed, cinnamon bark and other herbs.
- Eat high-quality, sulfur-containing proteins – eggs, whey protein, garlic, onions.
- Consume citrus peels, caraway, and dill oil (they contain limonene).
- Consume bioflavonoids in grapes, berries, and citrus fruits.
- Eat cruciferous vegetables (cabbage, broccoli, collards, kale, Brussels sprouts).
- Consume dandelion greens to help liver detoxification, improve the flow of bile, and increase urine flow.
- Eat celery to increase the flow of urine and aid in detoxification.
- Consume cilantro, which may help remove heavy metals.
- Consume rosemary, which has carnosol, a potent booster of detoxification enzymes.
- Consume curcuminoids (turmeric and curry) for their antioxidant and anti-inflammatory action.
- Consume burdock root for aid in detoxification.
- Consume chlorophyll in dark green leafy vegetables and in wheat grass.
- Take pycnogenol (found in grape seeds) in supplement form for support of detoxification and circulation.

Supplements for Detoxification

The Basics

- Take a high potency multi-vitamin and mineral formula.
- Take extra-buffered vitamin C 1000-4000 mg a day with mineral ascorbates in powder, capsule, or tablets during periods of increased detoxification. (This can cause loose stools. If it does, just reduce the dose or stop.)
- Take milk thistle (silymarin) 70 to 210 mg a day.
- Supplement with essential fatty acids (omega-3 fatty acids), 1000-2000 mg a day.

Additional Supplements (use under medical supervision)

- N-acetylcysteine 500 to 1000 mg a day
- Amino acids (taurine 500 mg twice a day, glycine 500 mg twice a day)
- Alpha-lipoic acid 100 mg to 600 mg a day
- Carnitine 1000 to 2000 mg a day in divided doses
- Bioflavonoids (citrus, pine bark, grape seed, green tea)

stress, and reduce their ability to burn fat and calories by inhibiting thermogenesis through effects on fatty acid oxidation. Organochlorines alter skeletal muscle oxidative enzyme activities. Enzymes of electron transport are inhibited by toxins, specifically 3-hydroxyacyl-CoA dehydrogenase (HADH) and cyclooxygenase (COX), both markers of fatty acid metabolism. Toxins also lead to decreased capacity for fatty acid utilization in skeletal muscle.

Oxidative stress is both a cause and effect of obesity. Toxins increase oxidative stress and affect redox signaling. Redox signaling influences gene transcription and signaling pathways controlling insulin resistance, cytokine modulation, and mitochondrial function. Activation of NFκB (a gene transcription factor) is mediated by redox balance and is a final common pathway for obesity and many other chronic illnesses.[3] All of these actions cause both weight gain and resistance to weight loss.

Toxins may also influence metabolism and obesity through cytokine-mediated mechanisms. Toxins activate neutrophils.[4] Increases in tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) induced by toxins promote insulin resistance via effects on PPAR and NFκB.[5] Leptin resistance is also triggered by inflammation via SOCS.[6]

Detoxification Enzyme Polymorphisms and Obesity

The effect of toxins on an individual is determined, in part, by the polymorphisms of phase I and phase II detoxification enzymes. Highly prevalent single nucleotide polymorphisms (SNPs) of glutathione transferase enzymes predispose to increased toxic loads. Detoxification of heavy metals is an important task for the body. It depends on specific proteins and enzymes that bind the metals and transport them out of the cells. In one recent study, mice bred without the protein (metallothionein) that is necessary for heavy metal detoxification gained more weight over their lifetime than mice that could eliminate the metals. They were more sensitive to the effects of toxic metals and oxidative stress.

Toxins Impair Central Appetite Regulation

Toxins have many effects. Besides directly lowering thyroid hormone levels, metabolic rate, and fat burning (fatty acid oxidation), they can damage the mechanisms by which hormonal and neuro-regulatory signals control our appetite and behavior. These signals are finely choreographed and sensitive to environmental inputs. To briefly review, the hypothalamic appetite-control system is centered in the arcuate nucleus. It receives peripheral feedback from leptin, insulin, PYY, and adiponectin. Central inhibition of food intake is regulated by pro-opiomelanocortin (POMC) and cocaine-and amphetamine-regulated transcript (CART). Central stimulation of intake is modulated by neuropeptide Y and agouti-related peptide (AgRP).[8] The melanocortin system and its receptors, MC3R and MC4R, play a crucial role in appetite control. Specifically, α-MSH binds to MCR, suppresses appetite, stimulates the thyroid axis, and increases energy expenditure, brown fat temperature, and sympathetic activity. It is inhibited by TNF-α. Other downstream control sites also exist in the related areas of the brain. Reward centers also play a role and are targets for new drug research including the endocannabinoid and serotonin receptors.[9]

Leptin resistance is found in obesity. Leptin's inhibitory effect on appetite is impaired by toxins, leading to leptin resistance and increased hunger. Hyperleptinemia increases mitochondrial reactive oxygen species monocyte chemoattractant protein-1 (ROS MCP-1). Leptin induces inflammation in a feed-forward cycle. Toxins may inhibit satiety effects of leptin, leading to increasing hyperleptinemia.

Researchers treated rats with a neurotoxin that damaged another critical appetite control system (the melanocortin system).[10] The toxin lowered levels of alpha-melanocortin-stimulating hormone (α-MSH), which acts as a brake on appetite.[11] This pathway may be a missing link in understanding the effects of toxins on obesity through the interaction of α-MSH[12] and TNF-α[13] and PPAR. α-MSH is a central and peripheral inhibitor of TNF-α, IL-1, and IL-6 via inhibition of NFκB and cytokine-mediated gene transcription. TNF-α downregulates genes that are required for normal insulin action, has a direct inhibitory effect on insulin signaling, and induces elevated free fatty acids via stimulation of lipolysis. A key effect of TNF-α is the negative regulation of PPARγ, an important insulin-sensitizing nuclear receptor. Neurotoxins also may directly inhibit α-MSH, leading to hyperphagia and increased body weight.[14,15]

Human studies complement research from animal studies. One study examined prenatal and breast milk exposure to PCBs and DDE (a by-product of DDT).[16] Researchers followed 594 children who had their prenatal and breast milk exposures to PCBs and DDE measured. At puberty, children with the highest exposures were larger, and girls were an average of 12 pounds heavier. In a second study, a group of researchers from Laval University in Quebec found that, during weight loss, those who released the most organochlorines from their fat stores had the slowest metabolism after weight loss.[17] Their explanation for the decreased thermogenesis, after taking into account all other possible factors, was the exposure to pesticides. In yet another study, the rise of toxins during weight loss in men inhibited normal mitochondrial function and reduced their ability to burn calories, retarding further weight loss.[18] Weight loss seems to prevent further weight loss, and one of the key mechanisms may be the release of internally-stored toxins that occurs during weight loss.

Hormone Disrupters: Hormonal Chaos

The dance of hormones is critical for balancing your metabolism. Environmental chemicals and heavy metals are well known hormone disrupters. A Tufts University professor, Sheldon Krimsky, in his book *Hormonal Chaos, the Scientific and Social Origins of the Environmental Endocrine Hypothesis*, has extensively reviewed the research in this field. Low levels of these toxins, levels far below what are considered acceptable by the Environmental Protection Agency, interfere with our normal hormone balance, including sex hormones, which may lead to early puberty in girls and an increase in hormonal disorders. Toxins can affect many of the major weight-control hormones including thyroid, estrogens, testosterone, cortisol, insulin, growth hormone, and leptin. Toxins interfere with our stress response (our autonomic nervous system), and alter the normal circadian rhythms[19] that control our eating behavior. These connections were explored at a conference co-sponsored by the National Institute of Environmental Health Sciences and Duke University entitled, *Obesity: Developmental Origins and Environmental Influences*.[20] While we still have much to learn about this connection, we can no longer ignore the effect of environmental toxins on weight. It is certainly not the only factor in our obesity epidemic, or in any one person's struggle with weight, but it must be considered in the evaluation and treatment of obesity.

Fatty Liver: Cause or Effect in Weight Gain

Non-alcoholic steatohepatitis (fatty liver) is the most common liver disease in America, affecting 20% of the population. The major cause is not medication, a virus, or pollution. It is the most abundant toxin in our diet: *sugar*. Increases in sugar or refined carbohydrate consumption increase insulin and insulin resistance, which leads to

the accumulation of fat in the hepatocytes. Increased fat inside the hepatocytes is produced from sugar, refined flour products, and high fructose corn syrup. The sugar is turned into intracellular triglycerides. Excess sugar calories also increase oxidative stress and further damage the mitochondria. Damaged mitochondria can't effectively burn fat or calories, which leads to a slower metabolism and more weight gain. A fatty liver further impairs detoxification. A fatty liver is also an inflamed liver; it is called non-alcoholic steatohepatitis (NASH), a form of hepatitis caused by insulin resistance. A fatty liver produces more inflammatory cytokines, free radicals, and leads to more mitochondrial damage. Fatty liver impairs optimal hepatic detoxification of endogenous and exogenous toxins.

OPTIMIZING DETOXIFICATION: A NOVEL STRATEGY FOR THE MANAGEMENT OF OBESITY

While still a hypothesis, the emerging evidence forms a plausible link between toxins and obesity. To review, toxins alter metabolism, interfere with key weight-control mechanisms, disrupt endocrine function, damage the mitochondria, increase inflammation and oxidative stress, lower thyroid hormones, and alter circadian rhythms and the autonomic nervous system. Using a comprehensive approach to obesity, including the assessment and treatment of toxin-mediated effects, it is necessary to address this multi-faceted disorder affecting two-thirds of Americans. Simple lifestyle choices, as well as medical detoxification, can reduce exposure to toxins and enhance mobilization and elimination of stored and external toxins.

Amino Acids, Nutrients, and Phytonutrients in Detoxification

The detoxification system relies on the right balance of protein, fats, fiber, vitamins, minerals, and phytochemicals to be effective. All these play a role in facilitating the elimination of toxins. For example, adequate protein is required to supply the amino acids used by the liver to provide glycine, cysteine, and glutamine to synthesize glutathione, as well as amino acids critical for many phase II detoxification pathways including methylation, acetylation, glucuronidation, and glycination. Glutathione is the most critical antioxidant and detoxifier in the body, and one that is easily depleted in the face of chronic exposure to toxins. Many phytochemicals enhance detoxification pathways.[21] These include many pigmented plant foods such as cruciferous vegetables (broccoli, kale, collards, Brussel sprouts, cauliflower), green tea, watercress, dandelion greens, cilantro, artichokes, garlic, ginger, rosemary, turmeric, citrus peels, and even cocoa. Polyphenols found in berries, green tea, and cocoa enhance the genetic expression of γ glutamylcysteine synthetase, which increases intracellular glutathione concentration.

Hyperthermic Therapy

"Regular use of a sauna or steam bath may impart a similar stress on the cardiovascular system [as exercise], and its regular use may be as effective a means of cardiovascular conditioning and burning calories as regular exercise."

W. Dean. Effect of sweating. *JAMA*. 1981;246:623.

Heat therapy is an underutilized treatment in medicine. It helps balance the autonomic nervous system, reduce stress, lower blood glucose, increase caloric expenditure, and enhance excretion of pesticides and heavy metals through the skin. Sauna therapy is an established treatment for chemical poisoning. While more research is needed, a review paper on "thermal therapy" suggests many promising effects including a reduction of inflammation and oxidative stress,[22] as well as weight loss.[23] In a 2-week study of 25 obese adults, body weight and body fat were reduced after sauna therapy for 15 minutes at 60 degrees Celsius daily, for two weeks, in a far-infrared sauna. One case report described an obese patient who couldn't exercise because of knee arthritis and who lost 17.5 kg, decreasing body fat from 46% to 35% after 10 weeks of sauna therapy. Sauna therapy has many benefits, including increasing autonomic balance through increases in heart rate variability, reduction in cardiac arrhythmias, and reduction of oxidative stress, as well as mobilization and excretion of toxins.

Practical Implications in Obesity: Elimination of Toxins and Maximizing Detoxification

In the face of the toxic environment of the 21st century, and with the reality that all living species contain increasing levels of environmental toxins with widespread biologic effects, it is clear that both new research to elucidate the mechanisms by which toxins affect health and novel clinical strategies for detoxification are needed. What follows is an overview of a comprehensive clinical approach to identifying and eliminating toxins (in the broadest sense of factors that affect weight and metabolism), as well as maximizing endogenous detoxification mechanisms.

A broad-based and comprehensive strategy for addressing the obesity epidemic is needed,[24] including the implications of new research linking toxins and obesity. Toxins have their impact through effects on endocrine function, the immune system and cytokines; central neuro-regulatory systems; and mitochondrial and oxidative stress. Strategies for treatment of obesity need to be inclusive of research on meal timing, meal composition, glycemic load,[25] phytonutrient content, reducing inflammation, balancing autonomic function by reducing stress,[26] improving sleep habits and duration, as well as treatments aimed at enhancing mitochondrial function and balancing redox status. In addition, minimizing exposure to toxins and enhancing detoxification can be an integral part of obesity management, especially in treatment-resistant patients.

A comprehensive detoxification strategy[27] should include the identification and removal of infections, limiting endogenous toxicity by improving digestive function, enhancing blood and lymphatic circulation, facilitating phase I and II detoxification pathways, and addressing the toxic effects of stress.

The first step is a thorough clinical evaluation for a history of toxic exposures, including amalgams, fish, mold, occupational exposures, and pollution or chemical contamination of water, air, or food. The toxic effects of occult infections, allergens, and medications also need to be considered.

Reduction of dietary toxins or chemicals can be helpful in reducing overall toxic load; these may include *trans* fatty acids, processed foods and suspect additives (aspartame, high fructose corn syrup), sugar and refined flours, salt, caffeine, charbroiled meats, and alcohol. Identifying and eliminating common food allergens such as gluten, dairy, eggs, soy, corn, and yeast may be helpful in reducing the effects of inflammatory cytokines on weight regulation. Minimizing unnecessary medications such as acetaminophen and non-steroidal anti-inflammatory or acid-blocking medications can prevent depletion of hepatic glutathione and reduce altered gut function. Recommendations to eat organic food, drink filtered water, and use an air filter can further limit overall toxic exposures. Common household or environmental exposures can be limited by considering the causes of sick building syndrome (mediated through the effects of mycotoxins), garden chemicals, household cleaners, dry cleaning sol-

vents, second-hand smoke, plastics and phthalates in food and water containers, toxic molds common in basements and bathrooms, and UV radiation, which can be limited by sunscreen and sun glasses. Heavy metal exposure is also common, including mercury from fish, amalgams, water, latex paint, vaccines, and contact lens solutions; lead from old paint, blinds, and canned foods; and aluminum common in deodorants, antacids, and baking powder. Addressing occult infections is also important; consider H. pylori, chlamydia, viruses, Lyme disease, chronic fungal sinusitis, periodontal disease and infected root canals, as well as intestinal dysbiosis from yeast, parasites, and bacteria. Psychosocial stressors can exacerbate the effects of other toxins and affect central and peripheral appetite control mechanisms.

Optimizing digestive function is important through the elimination of common food allergens and medications, re-inoculation with beneficial flora (probiotics), and the use of specific nutrients for gut repair, including essential fatty acids, zinc, and glutamine. Regular elimination is critical to excrete toxins through the bile and can be facilitated by fiber, magnesium, vitamin C, and charcoal. Enhancing blood and lymphatic circulation can be accomplished through aerobic exercise, yoga, massage and body work, sauna and heat therapy, as well as skin exfoliation and brushing. Facilitation of endogenous detoxification systems can be accomplished through diet and strategic supplementation, including the use of specific nutrients, amino acids, and herbs. Useful strategies include a high-potency multi-vitamin and mineral (enzyme cofactors), buffered vitamin C, and regular intake of phytonutrient-rich foods that facilitate phase I and II detoxification (Brassicas, alliums, lemon peel, green tea, watercress, cocoa, pomegranate, cilantro, and artichoke). Detoxifying herbs include milk thistle, green tea, and dandelion. Additional supplements that can be helpful include N-acetyl cysteine, α-lipoic acid, amino acids, and bioflavonoids. Probiotics, omega-3 fatty acids, and adequate monounsaturated oils are important. Adequate fluid intake to facilitate renal toxin excretion is also important. Finally, an increased intake of plant foods can alkalinize the urine, which helps facilitate toxin excretion.

SUMMARY

By recognizing the role of toxins in obesity and altered function of the neuro-endocrine-immune and mitochondrial and redox systems, and by creating a comprehensive strategy for both the reduction of exposure to and elimination of toxins, as well as the development of effective clinical strategies, treatment resistance in obesity may be more successfully addressed. Further research is needed to explore the clinical relevance and the mechanisms that underlie this hypothesis and to examine clinical detoxification methods. Through the prism of functional medicine, a context and road map exist for tackling many treatment-resistant and complex chronic diseases, including obesity.

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Children's Cognitive Health: The Influence of Environmental Chemical Exposures

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INTRODUCTION

Over the past forty years, concern has grown that some of the 80,000 chemicals used commercially could be exerting adverse effects on children's health. Many of these chemicals were synthesized for the first time within recent decades, suggesting that the body's detoxification mechanisms, the results of thousands of years of evolution, might not be effective in limiting their impact. The potential for exposure is substantial, as the US Environmental Protection Agency (US EPA) estimates that 2.5 billion pounds of chemicals are emitted yearly by large industrial facilities. At the same time, it is remarkable how limited are the data on the toxicities associated with most of these chemicals. The US EPA maintains the Integrated Risk Information System (IRIS), which serves as the repository of the consensus scientific opinions on chemical toxicity. Yet IRIS lists only 550 chemicals (www.epa.gov/iriswebp/iris/stand-al.htm), indicating significant lacunae in the knowledge needed to estimate and manage the risks associated with current exposures.

For many chemicals, most of the available data pertains to occupational exposures. The amount of data available regarding the potential effects of chemicals on children's brain development is much more limited. It was not until the 1990s that the US EPA published guidelines for registrants with regard to testing in animal models of the developmental neurotoxicity of certain chemicals, primarily organophosphate pesticides, for application in human risk assessments (US EPA OPPTS Health Effects Test Guideline 870.6300; www.epa.gov/EPA-TOX/1998/May/Day-14/t12303.htm).

At present, for many of the chemical exposures of current concern with regard to children, little or no data are available on either the extent of exposures or the neurological effects. This is true for exposures associated with living in proximity to hazardous waste sites, emissions from municipal waste incinerators, solvents, groundwater pollutants such as arsenic and manganese, and widely used materials such as phthalates (plasticizers) and polybrominated diphenyl ethers (flame retardants). More information is available about population exposures to potential neurotoxicants such as pesticides, dioxins, elemental mercury, and fluoride, but detailed data are lacking on potential effects of such exposures. The data available can be characterized as "considerable" only for the so-called "big three": inorganic lead, methylmercury, and polychlorinated biphenyls. Fortunately, recent initiatives undertaken by the US Centers for Disease Control (US CDC) are addressing these issues, issuing a periodic National Report on Human Exposure to Environmental Chemicals, based on the National Health and Nutrition Examination Survey (NHANES). The Second Report, issued in 2003, provided data on 116 chemicals, 89 of which had never before been measured in a nationally representative sample of the US population, including many that would be expected to affect brain function. In the Third Report, issued in July 2005 (http://www.cdc.gov/exposurereport/), data were provided on 148 chemicals. This effort, while important, represents only half of the challenge. The other half involves the difficult task of determining the dose-response relationships associated with these chemicals, since the mere presence of a chemical in blood or urine does not mean that it is affecting health.

METHYLMERCURY

Mercury is a heavy metal that is present in the environment as a result of both natural processes and human activities (referred to as anthropogenic sources). The natural sources include volcano emissions and the weathering of rock containing mercury ore. The primary anthropogenic sources are the combustion of fossil carbon fuels, particularly from coal-fired utility boilers; other such sources include municipal, medical, and hazardous waste incineration.[1] Mercury can travel long distances in the atmosphere and contaminate sites far from its point of release. Furthermore, the complex biogeochemistry of mercury fate and transport creates uncertainty in efforts to apportion the relative contributions of these processes to global mercury pollution. The US EPA estimated that 50 to 75% of the total yearly input of mercury into the environment is anthropogenic[2]; the United Nations suggests that it accounts for more than half of the inputs (http://www.chem.unep.ch/mercury/Report/GMA-report-TOC.htm).

Mercury exists in the environment in several different forms, including metallic, inorganic, and organic; interconversion between forms can occur. The form of mercury of greatest concern with regard to seafood consumption is methylmercury (MeHg). Methylmercury results when mercury in other forms is deposited in water bodies and biotransformed through the process of methylation by microorganisms. It bioaccumulates up the aquatic trophic food chain as smaller organisms are consumed by larger organisms. Because methylmercury is persistent, this biomagnification process results in the highest concentrations in large long-lived predatory species, such as shark, swordfish, and tuna. Methylmercury levels can also be high in marine mammals such as whales and in animals that feed on marine life, such as polar bears and sea birds. Consumption of marine life is the major route of human exposure to methylmercury.

The devastating effects that high-dose exposure to methylmercury can have on neurological development were first recognized following a decades-long poisoning episode that occurred in the region of Minamata Bay in southern Japan as the result of industrial discharge of mercury salts. Women who consumed methylmercury-contaminated fish from the area gave birth to children with what came to be called Congenital Minamata Disease (CMD), which includes growth disturbances, primitive reflexes, movement and coordination disorders (cerebellar ataxia, chorea, athetosis, dysarthria), sensory impairments, cerebral palsy, and mental retardation. Because of the delay in identifying methylmercury as the cause, it was not possible to determine the critical dose required to produce CMD. It was noted, however, that the mothers of some children with CMD appeared to be asymptomatic or to suffer only mild, transient paresthesias. Another episode of mass poisoning occurred in Iraq in the 1970s, when, rather than being planted, mercury-treated seed grain was ground into flour and consumed.

In this episode, as well as in Minamata, it was apparent that the critical doses necessary to produce severe, debilitating neurological outcomes in the fetus were far lower than those necessary to produce effects in adults, resulting in the recognition that the pregnant woman is the critical population subgroup. Autopsy studies of the brains of affected individuals revealed a striking age-dependence in the distribution of methylmercury-associated lesions. In individuals exposed only in adulthood, the distribution was highly focal, primarily in the cerebellum, calcarine fissure of the occipital cortex, and post-central gyrus, as might be expected given the specific clinical signs of adult methylmercury poisoning. In the individual exposed prenatally, however, lesions were diffusely distributed throughout the brain.[3] This is most likely because methylmercury arrests mitotic cells in metaphase, thus disrupting cell proliferation and migration in the brain. The abnormalities observed include reduced cell densities, islands of heterotopic neurons, glial proliferation, incomplete myelination, and disturbances in brain cytoarchitecture.

Recognizing the devastating effects of high-dose exposure to methylmercury, investigators were led, beginning in the 1980s, to ask whether milder neurological effects are associated with the lower-dose in utero exposures to MeHg that are more typical within the general population of seafood consumers. Based on the Iraqi study, the WHO identified maternal hair levels of 10 to 20 micrograms/gram (or parts per million, ppm) as the range within which the risk of adverse neurodevelopmental outcomes such as delayed walking and talking began to rise.[4] Several longitudinal prospective studies involving the recruitment of birth cohorts were undertaken to evaluate this conclusion, most importantly in New Zealand,[5] the Faroe Islands (located in the Northern Atlantic Ocean),[6] and the Seychelles Islands (located in the eastern Indian Ocean).[7] These populations were selected for study because of the prominence of seafood in the diet. For example, the women who enrolled in the Seychelles Islands study reported eating an average of 12 fish meals per week. In addition to frequent fish consumption, the Faroese also periodically consume pilot whale, which contains high levels of methylmercury. The New Zealand and Faroe Islands studies have generally been interpreted as demonstrating inverse associations between prenatal exposure to methylmercury and children's neurodevelopment, while the Seychelles Islands study has not. In the Faroe Islands study, cord-blood mercury level was inversely associated with children's scores on tests of attention, language, and memory. In follow-up evaluations at age 14 years, children's hair mercury levels were positively associated with delayed responses on brainstem auditory evoked potentials.[8] Inverse associations between children's outcomes and maternal hair mercury levels, which averaged between 4 and 5 ppm, were also observed. In the New Zealand study, maternal hair mercury levels greater than 10 ppm were associated with a doubling of the risk of IQ scores below 70.

The apparent inconsistencies in study findings have posed a challenge to risk assessors attempting to establish intake guidelines for methylmercury. Some risk assessors have chosen the Seychelles Islands study[9] or used an integrative strategy that took into account the results of all three studies.[10] Adopting a precautionary approach, the US EPA elected to base its derivation of the Reference Dose (RfD) for methylmercury on the Faroe Islands study. [The RfD is defined as "...an estimate of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime."] One consideration motivating this choice was that it would result in a guideline that is more protective of the population than would a guideline based on the Seychelles Islands study.[11] Using the benchmark dose method to determine a point of departure and incorporating uncertainty factors, a critical dose of 5.8 micrograms/liter of cord blood (equivalent to a maternal hair mercury level of 1.2 ppm) was identified. By making a variety of toxicokinetic assumptions, the US EPA established an RfD of 0.1 micrograms/kilogram bodyweight/day as the methylmercury intake that, over a lifetime, should not produce adverse effects.

In 2004, the US Food and Drug Administration and the US EPA offered a joint advisory regarding fish consumption by pregnant women, women considering becoming pregnant, and young children (http://www.cfsan.fda.gov/~dms/admehg3.html). This advisory recommended the avoidance of four types of fish that, on average, have the highest levels of mercury: shark, tile fish, king mackerel, and swordfish. Furthermore, it suggested that these population subgroups can eat up to 12 oz (two average meals) a week of a variety of fish and shellfish that are lower in MeHg (e.g., shrimp, canned light tuna, salmon, pollock, catfish). It noted that because albacore or "white" tuna tends to have more MeHg than canned light tuna, up to six ounces of albacore tuna can be consumed per week. Finally, those who consume fish from local lakes, rivers, and coastal areas were encouraged to check local advisories for guidance and, if none were available, to eat ≤ 6 oz per week of such fish and to avoid eating any other fish that week.

With regard to the distribution of mercury burdens within the US population, in the NHANES 1999 survey, women of child-bearing age (16-49 years) had a median hair mercury level of 0.2 ppm, although 8 to 10% of women had levels that were consistent with mercury intake above the RfD (1.2 ppm).[12] Moreover, the strong influence of fish consumption on mercury burden was evident. More than 25% of women who reported consuming 9 or more fish meals per month had a burden that indicated mercury intake above the RfD, as did 10 to 25% of women who reported consuming 5 to 8 fish meals per month.[13]

Overall, the consensus view of "how much mercury is too much" has declined steadily since 1970 and has been accompanied by concomitant changes in the regulatory standards. It can be expected that this process will continue as additional research, using more sensitive methods of exposure and outcome assessment, is conducted.

LEAD

Lead, a useful metal that has been mined and smelted by humans for millennia, has been recognized as a potent toxicant for nearly as long. Interestingly, recognition of children as the subgroup of the population that is at greatest risk from excess exposure occurred only a little more than a century ago. Voluminous research conducted over the past half century has catalogued a wide array of processes by which lead produces neurotoxicity, including apoptosis, excitotoxicity, impaired cellular energy metabolism, impaired heme synthesis, oxidative stress, lipid peroxidation, impaired first and second messenger systems, and many others.[14] The relative importance of specific mechanisms of neurotoxicity is likely to be dose-dependent. At the lower doses characteristic of community-level exposures, it is thought that lead's disruption of the role of neurotransmitter systems in the sculpting of the brain is important. Specifically, by increasing the slow tonic (normal basal) release of neurotransmitter and inhibiting the release evoked by depolarization, the presence of lead in the neuronal environment increases the level of background "noise" in excitatory synapses, disrupting activity-dependent plasticity at developing synapses, including the process by which neuronal connections are selectively pruned (eg, organization of "whisker-to-barrel" sensory pathway in rodents).[15]

As was the case with methylmercury, the view of "how much lead is too much" has declined dramatically since the 1960s, when pediatric textbooks identified a blood lead level in a child of 60 micrograms/deciliter (µg/dL) as the upper limit of "normal." In retrospect, this seems remarkable, given that the risk of encephalopathy is increased at 100 µg/dL and death occurs at 150 µg/dL. It is somewhat less surprising, however, in light of the high prevalence, at that time, of broad lead levels of 40 µg/dL or more among poor children living in inner cities.[16] Based on a steady accretion of epidemiological studies documenting adverse effects at lower and lower levels, the value used to define "elevated" was decreased to 40 in 1971, 30 in 1975, 25 in 1985, and 10 in 1991. Fortunately, recognition that levels formerly regarded as safe were, in fact, associated with increased risk of adverse effects resulted in governmental initiatives that produced rapid and substantial declines in children's exposures to lead. The most important of these were a ban on the amount of lead used in residential paints and the elimination of the use of lead as a gasoline additive. Whereas the median blood lead level of US preschool children was 15 µg/dL in the late 1970s, with 88% having a level of 10 µg/dL or greater, this level now stands at under 2 µg/dL, with 2% having a level greater than 10. This still represents an unacceptably large number of children with exposure to a toxicant that is known to reduce cognitive function. Moreover, in many US cities, the prevalence of levels greater than 10 µg/dL still exceeds 10%, primarily among poor minority children, reflecting the continued socioeconomic bias in the occurrence of this disease.

Many public health advocates are urging the US CDC to reduce once again the definition of an elevated blood lead level. Much of the impetus for this is provided by the results of analyses that pooled the data from a set of 7 prospective studies conducted in four countries.[17] These analyses indicated that the inverse association between children's blood lead levels and their IQ scores holds even at levels below 10 µg/dL. Moreover, it appears that the slope of the inverse association is even steeper below 10 µg/dL than it is above 10 µg/dL. In these pooled data, over the range of 1 to 30 µg/dL, children's IQ scores declined 9.2 points, but as much as 6.2 points of this decline occurred in the range of 1 to 10 µg/dL.

The importance of the magnitude of the changes in children's cognitive function observed in association with exposures such as lead is frequently questioned. How important is, for example, a shift of several points in IQ, a change that would likely not be readily discerned. In part, this perspective reflects a failure to acknowledge the distinction between individual and population risk. Whereas a loss of 5 points in an individual's IQ might be inconsequential, a shift of 5 points in the mean IQ score within an entire population (eg, from 100 to 95) would have large implications. If the other characteristics of the IQ distribution remain constant, such a mean shift implies a doubling of the number of individuals with scores 2 or more standard deviations below the mean and a halving of the number with scores 2 or more standard deviations above the mean.[18]

Even for chemicals as well-studied as lead, detailed answers are lacking to many important questions of toxicological as well as public health importance. Among the unresolved issues are the functional form of the dose-effect relationship, particularly whether it is linear or supralinear at levels below 10 µg/dL, the critical window(s) of vulnerability (prenatal, early postnatal, concurrent, cumulative exposure), the factors that influence prognosis of lead-associated injuries, characteristics of the "behavioral signature" injury and its dependence of dose, timing, and chronicity, and a unified understanding of neurobiological mechanisms of injury.

An issue that has stimulated particular concern is chemical exposures that might disrupt endocrine-mediated processes by mimicking or antagonizing natural hormones, so-called "endocrine disrupting chemicals." It is known, for example, that gonadal hormones are important in producing sex-specific regional differentiation in the brain and the expression of sexually-dimorphic reproductive and nonreproductive behaviors. Exposure to some environmental chemicals interferes with these modulatory effects of sex hormones on brain development and behavior. Some pesticides and phthalates (plasticizers) are anti-androgenic, with developmental exposure of male rats producing a feminization of social behavior (play).[19] Bisphenol A, a chemical used in the food industry and dentistry, is estrogenic, with developmental exposure of female rats producing masculinization of play and sociosexual exploration.[20]

It is a consistent observation that, at chemical burdens typical of children's environmental exposures, there is substantial inter-individual variability in the response of individuals at a given level of exposure. In order to make risk assessments as accurate as possible, it is important to understand all the sources of this variability. It could result from imprecision (ie, misclassification) in the measurement of the exposure biomarker or in the extent to which it characterizes the dose at the critical target organ, or represent the most appropriate exposure averaging time for the health endpoint of interest (i.e., concurrent, age-specific, cumulative). For example, in the case of lead's neurotoxicity, we are most interested in the amount of lead in the brain, the critical target organ. Because this cannot be measured in humans, the exposure biomarker most often used is blood lead, yet only about 5% of an individual's total body burden is in the blood compartment. Moreover, most of the lead in blood is tightly bound to erythrocytes, whereas the most important toxicologic fraction of the blood compartment is the lead in plasma, due to its access to soft tissues such as the brain. Thus, using blood lead as an index of exposure is likely to result in a considerable, but unknowable, amount of exposure misclassification, and thus likely underestimation of lead's neurotoxicity. Similarly, with respect to methylmercury, the exposure biomarker most commonly measured is hair mercury, a compartment that is a considerable toxicokinetic distance from the brain, which is the compartment of greatest interest. Another component of variability is likely to be true variability in response, reflecting biological processes that are not captured by the terms included in our statistical models.

Some of the apparent inter-individual variability in response almost certainly reflects factors that systematically render some more vulnerable and others less vulnerable to toxicant exposures. One class of such factors is genetic polymorphisms that modify the association between external dose and internal biomarkers (toxicokinetic variability) or between the biomarkers and health outcomes (toxicodynamic variability). Few such polymorphisms have been identified, however. In the case of lead, studies have shown that individuals with a variant allele of the heme pathway enzyme, amino levulinic acid dehydratase, have higher blood lead, but lower bone lead levels, and, at a given lead level, have reduced renal function and an increased risk of amyotrophic lateral sclerosis. Individuals with a variant allele of the vitamin D receptor have higher blood lead levels and increased blood pressure. In children who carry this allele, the slope of the association between floor dust lead and blood lead is steeper than it is among children with the wild-type allele. The E4 allele of apolipoprotein has been shown to increase the neurobehavioral toxicity of lead in adults.[21] Other alleles that have been investigated are nitric oxide synthase and the HFE protein (hemochromatosis).

Non-genetic factors that appear likely to influence response to toxicant exposures include nutritional status and social characteristics. Again, most of the work exploring these issues has been conducted on lead. Calcium and iron are known to influence lead absorption and might influence toxicity as well. Animal and human studies suggest that being reared in an environment that provides less cognitive stimulation increases the toxicity of lead. In one study, rats were lead-exposed during gestation and lactation, and their spatial learning was assessed using a water maze at 50 days of age. Some of the rats were raised in groups in cages that contained objects to explore ("enriched"). Others were raised alone in empty cages ("isolated"). The performance of the enriched, lead-exposed rats was indistinguishable from that of the enriched, non-exposed rats, but the isolated, non-exposed rats learned more slowly than either of these groups. The isolated, lead-exposed rats did not show any improvement in performance over the learning trials. The better performance of the enriched lead-exposed rats was accompanied by changes in brain biochemistry; increased induction of BDNF mRNA expression in the hippocampus was observed, as well as recovery of deficits in gene expression of the NR1 subunit of NMDAR (N-methyl d-aspartate receptor) in the hippocampus (CA1-CA4) and granule cell layer of dentate gyrus.[22] In children, observational studies have shown that the magnitude of neurobehavioral deficits evident at a given blood lead level is greater among children who are socioeconomically disadvantaged,[23] and that the extent of recovery from early deficits is greater among more socioeconomically advantaged children.[24] Some evidence suggests that the effect of a chemical exposure on brain plasticity might provide a sensitive index of toxicity. For instance, in rats, prenatal exposure to methylazoxymethanol acetate reduced the magnitude of their response to an enriched postnatal environment, operationalized as the change in the thickness of the occipital cortex. The dose needed to produce the same reduction in cortical thickness directly was >10 mg/kg, but a dose of 1 mg/kg was sufficient to observe the same reduction in the capacity for experience-dependent cortical plasticity.[25]

Achieving success in characterizing the extent and the bases of inter-individual variability in susceptibility to toxicants will permit significant implications for the risk assessments of the toxicants. It will allow for a quantitative rather than qualitative evidence-based characterization of relative subgroup susceptibility, which will enable risk assessors to move beyond the practice of setting exposure standards by dividing a "no observed effect level" by ad hoc "one size fits all" uncertainty factors (e.g., 10, 100) in order to provide a margin of safety for susceptible subgroups.

ENVIRONMENTAL CHEMICAL EXPOSURES AND PSYCHIATRIC MORBIDITY

In recent years, investigators have begun to expand the scope of the health endpoints evaluated as potential consequences of toxicant exposures in children to include non-cognitive brain-based disorders. The psychiatric sequelae of high-dose, usually occupational, exposure of adults to various metals have long been recognized. The syndrome of erethism, resulting from exposure to inorganic mercury and the origin of the phrase, "mad as a hatter," is characterized by irritability, excitability, emotional lability, extreme shyness and avoidance of strangers, sudden anger, fatigue, memory loss, insomnia, and, in severe cases, to depression, manic depression, hallucinations, delusions, and suicidality. Manganese exposure is associated with mania, insomnia, hallucinations, aggression, incoherent speech, inappropriate affect, and emotional lability, while trimethyl tin exposure is associated with alternating bouts of rage and depression, sleep disturbance, fatigue, memory loss, and apathy.

Most of the epidemiological work on toxicants and psychiatric morbidity has focused on lead. In adults, case studies have suggested associations between high-dose exposure and depression, and also affective or schizophreniform psychosis. A facility in which tetraethyl lead was manufactured was known as the "House of Butterflies" because of the hallucinations suffered by workers. In occupational studies, greater depression, irritability, interpersonal conflict, fatigue, anger, tension, and decreased libido have been noted in lead workers, compared to controls. Environmental or pharmacologic interventions that reduce workers' blood lead levels have sometimes been found to reduce the severity of their mood disturbances. Finally, some reports suggested improvements in the clinical status of psychiatric patients following chelation therapy.[26-28]

A recent case-control study suggested that higher levels of in utero lead exposure, reflected in higher levels of amino levulinic acid in archived samples of maternal serum from the 2nd trimester of pregnancy, were associated with an increased risk of schizophrenia.[29] It is on the association between lead exposure and behavior disorders in children that the greatest data are available. Many studies have demonstrated that higher exposures are associated with increased distractibility, impulsivity, poor organization skills, inability to follow directions, low frustration tolerance, and a lack of persistence.[30-32] Recently, several studies have shown that adolescents with higher exposures are at higher risk of increased aggression and juvenile delinquency,[33-35] with some speculating an association between lead and homicide.[36,37] Experimental studies with animal models support the plausibility of this association. The threshold eliciting predatory attack behavior in cats decreased following a lead challenge, increased during a washout period, and decreased in response to a second lead challenge.[38] In another animal study, lead-exposed rhesus monkeys engaged in less play, particularly social play, than controls, and in more self-stimulation and fear grimacing.[39] These impaired social interactions persisted after cessation of exposure.

TREATMENT FOR CHEMICAL-INDUCED MORBIDITIES

Given the accumulating evidence that environmental chemical exposures are contributing to neurodevelopmental morbidities in children, the issue of whether these morbidities are amenable to treatment has become of paramount importance. Chelating agents have been administered to lead-poisoned children since the 1950s in spite of little published evidence that such interventions were effective. It was only in 2001 that the results of the first randomized trial of chelation were published, and the results were disappointing. This was the Treatment of Lead-Exposed Children (TLC) trial, which enrolled 780 12 to 33 month olds with a baseline blood lead level of 20 to 44 µg/dL. Children were randomized to receive either a placebo or the oral chelator succimer. Although blood lead level declined significantly faster in the succimer group following initiation of treatment, after one year the mean blood lead levels in the two groups were equivalent. Moreover, the scores of the two groups on a large number of neurodevelopmental tests were not significantly different three years following treatment40 or at 7 years of age.[41] The findings from observational studies of children with lower exposures to lead are consistent with those of the TLC trial in suggesting that the neurodevelopmental morbidities are persistent and possibly permanent.[42-44] The clear implication is that a primary prevention strategy is necessary if lead-associated morbidity is to be reduced. Waiting to identify and treat children who have been overexposed will not be effective.

SUMMARY

The potential exists for developmental exposure of children to myriad chemicals, many of which are known to be neurotoxic. Some, such as the organophosphate pesticides, are specifically designed to attack the central nervous system. Despite the known and suspected risks associated with such exposures, critical aspects of the dose-response relationships are unknown or, at best, poorly characterized for the overwhelming majority of chemicals. Among the major knowledge gaps for most chemicals are the critical window(s) of vulnerability, the threshold or "no observed adverse effect level," and the host/environmental characteristics that modify individual vulnerability. Investigation of the role of genetic polymorphisms in determining vulnerability has barely begun. In the real-world, children are not exposed to a single chemical at a time but to complex mixtures of chemicals, and we have only a minimal understanding of the way in which exposures might interact with one another. Effective medical/environmental treatments for the adverse effects associated with chemical exposures are largely unknown, rendering primary prevention of exposure the most effective strategy for protecting children.

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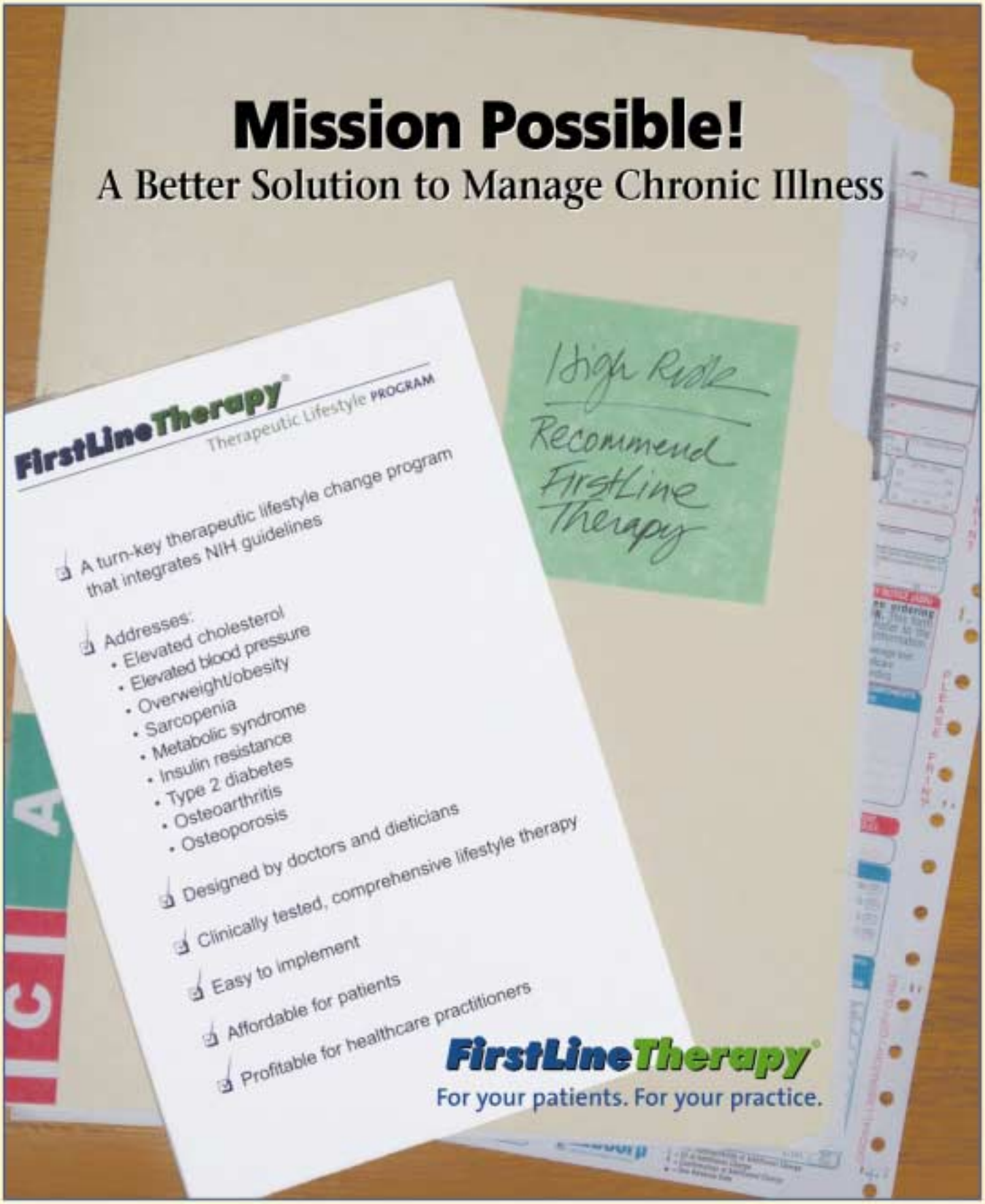

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Molds and Mycotoxins: Beyond Allergies and Asthma

Michael Gray, MD

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GENERAL CHARACTERISTICS OF MOLDS

In the last 10 to 12 years, I have seen close to 400 patients with histories of adverse effects from exposure to microenvironments in which mold has been a prominent feature. There are clearly some common factors across various species, but those discussed here represent only a small number of the species about which we are concerned.

We are almost invariably exposed to multiple species of molds when we are dealing with indoor environments. Unlike many other areas of toxicology with much more research, we often don't know what molds are contributing to which symptoms. We certainly know, however, that we're looking at a patient with a syndrome that is very real. When we listen to what patients tell us about their symptoms and signs, and we look at their laboratory reports, we get a composite picture of the impact of the multiple toxins associated with different species of molds.

Some of the common features are that hyphae and spores are involved, and most of these organisms are multi-nucleated. Hyphae consist of thread-like filaments that release enzymes for the degradation and absorption of specific substrates:

- components of wood, organic debris, or tissue;
- proteins to amino acids;
- carbohydrates and polysaccharides to simple sugars;
- fats to triglycerides; and
- DNA and RNA to nucleic acid bases.

Spores are produced on specialized hyphae. Spore production is dependent on a number of environmental variables:

- light,
- oxygen levels,
- temperature,
- nutrient availability, and
- moisture.

Spores serve as the primary means for dispersal and survival. Mold spores can remain dormant for months or even years. Spores are often capable of withstanding extremely adverse conditions. In fact, spores from *Stachybotrys*, a very well-known organism because of the work of Dearborn in association with hemorrhagic pneumonitis in infants,[1] as well as subsequent work in other environments where we're finding it to be a prominent and concerning organism,[2] tolerate 550°F, acids, bases, and ozone.

Typical molds, particularly the so-called filamentous molds or terrestrial molds, grow through a series of phases where they form hyphae. Their spores are usually found at the end of the hyphae, but they can also just break apart with little bits of DNA in the hyphae. Yeasts and several other organisms and fungi will do this when there are multiple spores just along the hyphael structure. The spores can remain dormant for prolonged periods of time (months or even years), and when they hit the proper environment, they'll germinate. Germination requires specific environmental factors to exist—availability of nutrients, oxygen, and moisture levels. There have been some experiments that grew spores from sedimentary rock shaved over a petri dish. The life form is incredibly stable. It should remind us that DNA, by itself, is an incredibly stable molecule under most normal circumstances.

Hyphae have a function in the life of these organisms, other than just being a place to produce spores: they engage in exodigestion. Enzymes are released through hyphae and these enzymes degrade the nutrient material they're resting upon, which could be some form of carbon-based organic debris, or it could be human tissue. The proteins get broken down into amino acids, the carbohydrates and polysaccharides into simple sugars, fats into triglycerides, and the DNA and RNA to DNA bases. This all happens outside of the organism, unlike the process in the eukaryotic cells that most animals—including humans—are made of, which digest nutrients by bringing them into the cell interior through phagocytosis, marrying them with a packet of enzymes in the lysosome, and then digesting them there.

Moisture is probably the key factor in the life of most of these organisms. As moisture disappears, they desiccate and die, but the DNA and the spores are left behind as the potential seed for a new generation. In the desert environment, for example, that means only the most tenacious molds have survived.

Molds tend to have short life cycles. Within a matter of 24 to 36 hours, they can come to life, develop a colony, and then, as it gets very dry, desiccate. We usually associate molds with damp environments, but they are also ever present in the desert. In fact, they are ever present in the air because, whenever the wind kicks up, the dust in the soils and in the environment contains fragments of spores and of colonies that have broken apart. This process goes on in all outdoor and indoor environments. Molds are pervasive across the continent. The only exception is in the arctic zones where they are frozen out.

Molds can withstand extreme and adverse conditions. Although we are often told that we can clean moldy environments by using bleach, problems arise from chlorinating the toxins we are trying to get rid of. You can easily get rid of the growing organism, but getting rid of the spore can be very problematic.

CLINICAL PRESENTATIONS OF SOME MOLD-RELATED ILLNESS

Shifting our attention to an illness that clinicians see in great abundance, there has been a dramatic change in the understanding of chronic sinusitis. Prior to 1999 and early 2000, we all pretty much assumed and were taught that sinusitis had perhaps a 6% to 7% involvement of fungi. It turns out that work done by Ponikau and Sherris[3,4] at the Mayo Clinic has changed that estimate dramatically. The prevalence of the disease is now thought to be quite substantial.

In addition to linking fungi to rhinitis, we know that they are significantly associated with asthma. At Mayo, 250 consecutive patients were evaluated after presenting to their ear, nose, and throat clinic with diagnoses of rhinitis and rhinosinusitis. They were able to culture specimens using rhinoscopic techniques and found that 95% of those patients grew mold, or fungus, as the primary organism. When they looked at the distribution of the bacterial flora in the cultures, it was similar to that seen in the general population, significantly identifying the fungi and molds. Their work has been replicated several times,[5,6] with multiple species found in each individual.

Sherris worked a little bit further on the ultra-structure and the histology. Because fungi and molds attract eosinophils, these infections set the stage for the development of allergic fungal rhinitis. Eosinophils play an important role in allergic interaction with the environment. People begin to experience nasal allergy symptoms, but it's truly an allergic fungal rhinitis.

A chapter by Dennis in *Mold and Mycotoxins*, a textbook edited by Kilburn and released in September of 2004, related his experience treating 650 cases of rhinitis over a decade. He emphasized that unless you 1) treat the fungus and 2) look at the environment the people are living in, you have little hope of solving the problem for the patient. If there are more than 4 colony-forming units settling on a mold plate per hour in the rooms and environment in which they're living, working, or studying, you'll never cure the disease. You'll only provide a temporary remission. You also need to treat for a prolonged period of time. For example, if you stop treatment when the infection disappears in a dermatomycosis, it usually returns within a month because the spores are not affected by any of the antifungals, and so you have to basically exhaust the supply of the spores. The same principle applies with rhinitis.

I saw a 27 year old autistic male who presented in September 2004 with very severe rhinitis and nasal polyps so proliferated that you couldn't really see into the nasopharynx. He was scheduled for a procedure in late October to have his nasal mucosa stripped. We asked the surgeon whether he would wait while we tried an anti-fungal spray (a 2% preparation of ketoconazole). The surgeon agreed, and we treated this young man. In early February, I received a letter from the surgeon saying that he had scoped the patient again, and all of the polyps had receded. His ears were cleared of recurrent otitis, and his sinuses had cleared of recurrent sinusitis. The young man had had these problems for years, had become a head-banger, and was extremely agitated most of the time. His whole demeanor changed. We didn't solve the autistic problem, but we certainly made major gains. The only problem was that the treatment was stopped after 3 months. Within a matter of another 3 months, he had problems again and needed re-treatment. The Mayo Clinic protocols recommend 8 months of therapy and they use amphotericin. I use 2% ketoconazole or 2% itraconazole with good effect. I usually treat this condition empirically because it takes a month to get a culture grown, whereas within two weeks of treatment, your patient can tell you whether or not they're improving. Keep the treatment going for 7 or 8 months.

MYCOTOXINS

According to biologists, mycotoxins are secondary metabolites because they don't seem to contribute to the growth of the organisms. I disagree. Although they're not actually contributing to metabolism, it isn't really true that they're secondary, because mycotoxins represent the organism's mechanism for competing in their microenvironment. Every antibiotic we use, except sulfurs, are produced by fungi. All of the anti-fungal agents that we use are produced by fungi. In the transplant world, CellCept is a pharmacologic version of mycophenolic acid, which is produced by *Penicillium* species of several types. It is basically sold to suppress the immune system so that a person doesn't reject an organ. If, in fact, it's a potent immune suppressant, it's also a powerful adaptive mechanism for the fungi because their survival depends on the inability of the organism that they are parasitizing to deal with them. Therefore, I would disagree with the notion that mycotoxins are secondary metabolites. They are clearly species-specific survival mechanisms of the first order, and they are very significant in terms of veterinary, human, and plant pathology.

Among the more important mycotoxins are aflatoxins, citrinin, ergot alkaloids, fumonisins, ochratoxins, mycophenolic acid, patulin, satratoxin (only associated with *Stachybotrys*), trichothecenes in general as a group, and zearalenone. They're rather challenging to classify because of their diverse chemical structures and their biosynthetic origins. They have myriad biological effects and there are several different classification schemes. Clinicians tend to talk about them in terms of the organs they target. Cell biologists talk about them in terms of generic groups: teratogens, mutagens, carcinogens, or allergens. Organic chemists classify by the chemical structure and biochemists by their biosynthetic origins. Physicians will also associate them with particular illnesses—St. Anthony's fire, stachybotryotoxicosis. Mycologists group them according to which organisms are producing them. There are various advantages to each of the approaches.

Mycotoxins can be categorized as acute or chronic, and they can generate very obvious toxic responses when they are acute. When the effects are chronic, it's a little bit more difficult for us to recognize them because there can be prolonged latencies and subtle effects. There is reasonable medical and biological certainty that mycotoxins can be carcinogens, immune suppressants, and neurotoxic agents. They can induce asthma, hypersensitivity pneumonitis, and other proinflammatory and sometimes irreversible effects. The range of physiologic effects is substantial and significant.

There is a huge literature on this field, even though some aspects are still being debated. Unfortunately, in papers from the American College of Occupational and Environmental Medicine in August of 2002 and, more recently, from the American Academy of Allergy and Immunology, the only concerns that are deemed "legitimate" with regard to fungi and molds are allergies and asthma. I believe this is an erroneous picture; there is, indeed, significant pathology well beyond allergy and asthma.

Veterinarians deal with these issues on a regular basis. They see a wide array of illnesses and are attuned to them because many of the feeds that animals eat, both in agricultural and pet settings, are susceptible to infestations by molds and fungi. I'm reminded of an incident that occurred when a friend had a goat that suddenly became ataxic and blind. She rushed it off to the vet. The vet took one look at it and said that the goat had eaten moldy hay, gave her thiamin and a tube of probiotics, and told her to inject the thiamin 4 times a day and put a 2-inch stripe of the probiotic on the goat's tongue 4 times a day, and within a week, it should be better. In a week, it was walking normally, the blindness was gone, and the goat had recovered. While we may be debating about whether or not molds and fungi affect the central nervous system in humans, the vets are seeing the effects in animals.

There are actually a number of well-known manifestations with which we are already familiar. Human ergotism is associated with the Salem witch trials because of ergot and rye. Stachybotryotoxicosis was an epidemic that affected tens of thousands of animals and hundreds of people in the Soviet Union in the 1930s, when there were early snows in the winter that covered the crops. When they uncovered them in the spring, in the middle of the Depression, and used the silage, horses and cows began dropping like flies because of the impact of the *Stachybotrys* organism and its associated mycotoxins. In that context, they became ataxic and had hemorrhagic GI effects. The animal handlers were also significantly affected by the exposure. The curse of Tutankhamen is another incident with which we are somewhat familiar. When they opened up the tomb, the first 35 to 40 men who entered the tomb died within 3 weeks from the effects of aflatoxins.

Aflatoxin B1 is possibly the most potent mutagenic agent known to humankind. Studies out of China have shown that it's capable of inducing every form of mutation and every form of DNA damage that we know to be possible. There are several different types of *Aspergillus* organisms that produce it. They are encountered with great frequency in indoor environments associated with *Aspergillus* growth. *Aspergillus* and *Penicillium* are both common. They're rarely delineated, identified, or separated because we generally use microscopic methods of identification and culture, but if one employs DNA by PCR, we will know specifically what's present. Although the test is expensive, there are circumstances in which it makes good sense to use it.

In addition to carcinogenicity and mutagenicity, aflatoxin B1 causes aflatoxicoses. Chronic aflatoxicosis can involve cancer, immune suppression, and various slow pathological conditions associated with DNA adduct formation, which ends up interfering with protein synthesis. Patients who are genetically null for glutathione *S*-transferase (GSTM1-null) are at a dramatically greater risk for the impact of the mutagenic effects of the aflatoxin, because GST is required in phase I detoxification of aflatoxin. If GST is not present, the more toxic and more mutagenic form remains. At least 40% of the general population is GSTM1-null.[7] Liver cancers are a common aflatoxin-associated condition.[8,9] We are now able to test urine for mycotoxins and we're finding aflatoxin with regularity, often months to years after the patient's exposure ended. This should be of great concern to all of us. It is not a trivial issue.

Fumonisin B1, first described in 1988, is the most abundantly produced member of the fumonisin family. It's produced by a number of *Fusarium* species, by *Alternaria*, and by several other species. The International Agency for Research on Cancer (IARC) has evaluated it as a probable human carcinogen. It affects animals by interfering with single lipid metabolism and is associated with leukoencephalomalacia, or "hole in the head syndrome," in horses and rabbits.[10] It can cause pulmonary edema and hydrothorax in swine. Its hepatotoxic and carcinogenic effects are seen in the livers of rats. It is also indicated as a possible cause of esophageal cancer. It's been hypothesized that a cluster of anencephaly in spina bifida cases in southern Texas was related to fumonisin contamination in corn products.[11]

With ochratoxin, primary effects are associated with the enzymes in phenylalanine metabolism. It inhibits the enzyme involved in the synthesis of phenylalanine TR&A complex, and it inhibits mitochondrial ATP production, stimulating lipid peroxidase. Ochratoxin targets the kidney, and is also a liver toxin, immune suppressant, and a teratogen.[12] Of all the species studied so far, humans have the longest half life for ochratoxin. Ochratoxin is now one of the substances that we can detect in urine and other human fluids using enzyme-linked immunosorbent assay (ELISA) techniques—spinal fluid, nasal secretions, sputum, and tissue samples. Ochratoxin is primarily associated with the Aspergillus species and was involved in an epidemic referred to as Balkan endemic nephropathy,[13] which is a progressive, chronic nephritis. Ochratoxin contamination of food and the presence of ochratoxin in human serums were more common in families with nephropathy and urinary tract tumors than in unaffected families. It's thought that the gene associated with phenylketonuria occurs in higher frequency because of an advantage against ochratoxin poisoning in these endemic areas. And ochratoxin is a risk factor for testicular cancer.[14]

Patulin, a colorless, crystalline antibiotic produced by several molds, was originally considered for medical use, but was discarded because of toxicity. It was reclassified as a mycotoxin and, in spite of relatively minimal data, suggested standards at the moment are for a maximum of .4 mg per kilogram. The adequacy of the data supporting that standard may be questionable. In my opinion, most special limit values published by the US Occupational Safety and Health Administration (OSHA) and the U.S. Environmental Agency (EPA) are not true health-based standards, and, in general, do not have strong toxicologic support for the levels selected. We ought to be cautious about accepting those types of standards in the absence of clear data supporting safety.

Trichothecenes are another major group of mycotoxins—140 different sesquiterpenoid metabolites and compounds. They are produced by several different species of molds, including *Stachybotrys*. Generally speaking, if you have an environmental sample that demonstrates the presence of trichothecenes, you can be reasonably certain that *Stachybotrys* has been in the environment. The hydrogen esters delineate type A from type B, and type B contains a ketone. Deoxynivalenol[15] is a trichothecene compound often known as vomitoxin. Hyperemesis is a symptom in animals and people who have been exposed. *Fusarium* is probably the major group that produces the non-macrocyclic trichothecenes. Trichothecenes are extremely potent inhibitors of protein synthesis, interfering with initiation, elongation, and termination stages of synthesis. Between the adduct formation and the interference with protein synthesis, the aflatoxins, ochratoxins, and trichothecenes can be considered radio mimickers, meaning they are able to do damage to DNA in a rather random fashion, and they are also able to interfere with protein synthesis to a significant degree. The illnesses that are associated with them can look very much like radiation sickness.

The Defense Department recognizes these effects, as discussed in a chapter of *Medical Aspects of Chemical and Biological Warfare* (1997), the collective work of 5 major military healthcare and research think tanks. Chapter 34 is devoted specifically to trichothecenes, and the authors acknowledge its ability to induce multisystem disease that can involve all major organ systems.

There is an illness known as alimentary toxic aleukia (ATA) that is associated with the T2 toxin, which is one of the trichothecenes. When you look at the GI tract of exposed animals, and in cases of human illness from the same agents, you'll find that the entire GI lining is denuded of white cells and the rapidly replicating lining cells. Symptoms of the disease include inflammation of the skin, akin to St. Anthony's fire, vomitoxin damage of hemophoretic tissues, and any other tissue that has relatively rapid replication. Acute phases are accompanied by necrosis in the oral cavity, bleeding from the nose, mouth, and vaginal vault, and CNS disorders.

Some of these species have been looked at as antimicrobial or chemotherapeutic agents, but without success due to their tremendous neurotoxic effects. A good review of indoor molds and toxic fungi from an infectious disease perspective was published in *Clinical Microbiology Reviews* in 2003.[16]

Satratoxin, associated with some occupational illnesses among workers exposed to moldy hay, has also received attention concerning illnesses connected with the presence of *Stachybotrys* in building materials with a high cellulose content. A major shift in construction may be responsible for a lot of the new proliferation of mold amplification in the paper on dry walls.[17] When we make paper *pulp*, it's a wood product; unlike making plywood, where the bark is stripped from the tree before they veneer it, the whole tree is ground up. Microscopic studies of dry wall paper show that the mold spores are already there. All you have to do is add water. We know that within 15 to 20 years almost all polyvinyl chloride pipe is going to deteriorate, forming little pinholes that become droplet sources for water or fine sprays inside the wall where you don't see it. The mold will then grow on the

paper on the interior of the wall. I've seen many examples where we open up a wall and, from floor to ceiling, the cavity is literally filled with multiple species and colonies of molds.

A report on *Stachybotrys* and its association with pulmonary hemorrhage or hemosiderosis was published first in the mid 1990s in the *Morbidity and Mortality Weekly Report*, but a couple of years later, a retraction was printed, driven by debate within the agency about whether a causal effect was proved.[18] Species of *Stachybotrys* taken from the homes that were involved with the infant cases didn't produce as much mycotoxin in the laboratory as was thought to be needed to cause the illness. However, if you grow species of mold in ideal laboratory conditions, and they're not stressed, the organisms are not going to produce as much mycotoxin as they do under wild conditions. It was also suggested that the smoking habits of the parents caused the problem. As Dr. Dearborn accumulated more cases of hemorrhagic pneumonitis, all of which were associated with *Stachybotrys* present in the homes and increased mold growth, he was able to show that if you controlled for the smoking issue, the odds ratio was still 17:1 for increased risk of hemorrhagic pneumonitis when *Stachybotrys* was present.[19] The findings were supported by studies done by Rand in Nova Scotia, where he instilled *Stachybotrys* organisms into the lungs and pulmonary tracts of mice, and duplicated the pathology in the small airways.[20] In addition to that, Ruth Etzel, a pediatrician who for 12 years was the senior epidemiologist at the Centers for Disease Control, solicited cases of hemosiderosis from the American Academy of Pediatrics. She was able to demonstrate that a relationship exists.[21-23] However, the Centers for Disease Control has not re-amended their earlier retraction although they continue to publish updates, the most recent of which was in 2004.[24]

Zearalenone is another mycotoxin of interest that's associated with *Fusarium*. There are some data suggesting that it is not a significant problem, and recommendations have been made suggesting that we ought to be able to set a limit for exposure, but I think there's other evidence that raises significant concern. Moldy grain consumption is associated with hyperestrogenism in swine.[25] That's been known since the 1920s. Modern work shows that dietary concentrations of zearalenone, as low as 1 part per million, can lead to hyperestrogenic syndromes, again in swine. Higher concentrations can lead to disruptive conception, abortion, and other problems, and reproductive problems have also been observed in other species—cattle, sheep, etc. In human beings, it has been associated with endometrial hyperplasia, endometrial dysplasia, endometrial cancer, and possibly endometriosis.[26] To emphasize, zearalenone is a very powerful estrogenic, and it is commonly associated with various species of mold occurring under conditions of mold amplification.

Sterigmatocystin is a carcinogenic mycotoxin associated with *Aspergillus* species and *Bipolaris*. It causes necrosis of the liver and the kidney, has inhibitory effects on orotic acid incorporation into nuclear RNA and is, therefore, a concern for affecting protein synthesis.

Gliotoxin is associated with *Candida albicans.* Gliotoxin is immune modulating, antiphagocytic, and can induce apoptosis inappropriately in different tissues. It can also interact with transcription factor and can impair glutathione metabolism, perhaps affecting chemical sensitivity.

We have been aware of the issue of bioterrorism in recent times. Since the 1940s, U.S., British, and Soviet microbiologists have recognized the potential for mycotoxin use in chemical warfare. There were allegations that the Iraqi scientists had developed aflatoxins as part of their bioweapons programs in the 1980s, and toxigenic strains of *Aspergillus flavus parasiticus* were alleged to have been used to produce several thousand liters of concentrated toxin. Unlike aflatoxins, trichothecenes can react immediately on contact, and exposure to even a few milligrams of T2 toxin may be lethal.

SICK-BUILDING SYNDROME

From an allergic standpoint, molds play a major role and have been implicated for years in sick-building syndrome, or building-related illness. The most common genera that we see are *Alternaria, Aspergillus, Coelosphaerium,* and *Penicillium*. The Society for Occupational and Environmental Health held a conference in February 2003 and posted the papers on their website at www.soeh.org/July04pdfs/Clinician Report.pdf. J. David Miller out of Canada has shown that, if you do sampling in ambient air across the continent of North America, *Coelosphaerium* generally predominates. *Aspergillus* and *Penicillium* are rarely present in concentrations greater than perhaps 1 to 2% of all molds present. When you get a sample in an indoor environment, and are looking for mold amplification, the presence of *Aspergillus* and *Penicillium* in significant amounts is the index. There are other species, of course. Air sampling—in fact, every mode of sampling—has its biases. *Stachybotrys* spores form a gelatinous mass, and they don't take to the air very often. In the desert, however, we do see it in the air. Finding even a single *Stachybotrys* spore in an air sample should be considered significant, because it implies that somewhere, in a cavity in that building, *Stachybotrys* has a greater presence.

In addition to the fact that dry wall manufacture and use have proliferated significantly and we have stopped using lath plaster, we've also tightened up our buildings. The new air that's coming into a building is 10 to 15% of what circulates when you can't open windows and let in fresh air. If we used energy exchange devices, we could ensure a lot more air flow through our buildings, still save energy, and not have as much moisture accumulating. Moisture is the precondition for the growth of these molds. We have accepted a standard CO^2 concentration of 1000 parts per million, where outdoor air is generally 250 to 300 parts per million. What we're saying, in essence, is that it's OK to concentrate all of the volatiles that are in the building by a factor of 3 and still maintain our presence in these buildings. That's problematic.

We usually associate degraded air with irritating symptoms and we know that headache, fatigue, irritation of the skin, and non-specific hypersensitivity reactions peculiar to odors, taste sensations, etc., are associated with what really should be referred to as sick buildings. We should also recognize that the potential toxicity of mycotoxins may raise the risk for other illnesses looming within the indoor environment. Although sick building syndrome was supposed to mean that there was no specific etiologic factor identifiable, cogent data implicating molds and mycotoxins are available[27] including fact sheets available online from the EPA (http://www.epa.gov/ iaq/pubs/sbs.html) and the National Safety Council (http://www.nsc.org /ehc/indoor/sbs.htm).

CLINICAL PRACTICE RESULTS

Figure 1 compares the complaints of patients in my general practice to those of patients who had come in specifically with documentation of exposure to mold and damp indoor environments. Fatigue and headaches lead the list, but all of these symptoms should focus us on two general areas—excessive inflammation and neurotoxicity.

One of the arguments often raised in litigation environments is that patients are malingering. We tested that argument by looking at the frequency of these same symptoms, across all patients with a history of mold exposure, and then divided by the 71 litigants and 138

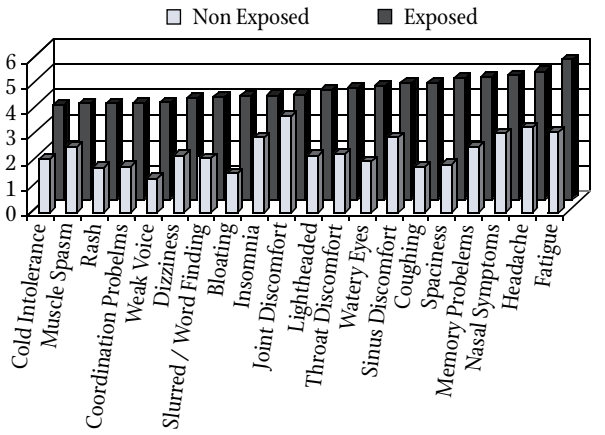

FIGURE 1 Symptom Profiles Comparison: 156 Mold-Exposed Patients vs. 28 Reference Patients

TABLE 1 Symptoms: Litigant vs. Non-Litigant Patients (a = $P<.05>.01$)

Symptoms	All Patients N =209 Mean+SD	Litigants N = 71 Mean + SD	Non-litigants N = 138 Mean + SD
Fatigue	5.8+1.9	5.6+2.1	5.9+1.8
Headache	5.2+1.9	5.2+1.8	5.2+1.9
Nasal Symptoms	5.1+2.2	5.1+2.1	5.1+2.2
Memory Difficulty	5.1+2.1	4.8+2.2	5.2+2.1
Sinus Discomfort	4.7+2.1	4.9+2.0	4.7+2.3
Spaciness	4.8+2.3	4.8+2.1	4.5+2.2
Watery Eyes	4.6+2.1	4.7+2.1	4.5+2.2
Coughing	4.6+2.2	4.9+2.0	4.5+2.2
Throat Discomfort	4.5+2.1	4.8+2.1	4.3+2.1
Lightheaded	4.4+2.2	4.7+2.2	4.2+2.2
Slurred Speech	4.5+2.3	4.2+2.3	4.6+2.4
Joint Discomfort	**4.4+2.3**	**3.9+2.1**	**4.6+2.3**[a]
Dizziness	4.3+2.1	4.5+2.0	4.2+2.1
Bloating	4.2+2.2	4.0+2.2	4.3+2.2
Weakness	4.2+2.3	4.0+2.2	4.3+2.4
Insomnia	4.1+2.2	4.2+2.2	4.1+2.2
Weak Voice	4.1+2.2	4.2+2.1	4.0+2.2
Rash	3.9+2.2	3.8+2.2	3.9+2.2
Coordination Problems	4.0+2.2	3.9+2.1	4.0+2.2
Spasms	4.0+2.2	3.7+2.0	4.1+2.2

non-litigants. There was absolutely no statistically significant difference between the groups in frequency of symptoms reported, except for joint discomfort, in which the non-litigants actually predominated (see Table 1).

When we did immunologic profiling on these patients, we found a paradox. On one hand, we were seeing dramatic evidence of hyperactivation of a variety of factors and cell types. On the other hand, certain cell types and mitogen responses were decreased. The B-cell elevation certainly would be expected if you're exposed to a potentially infectious agent, and the T-cell activation, in general, reflects a response to potential infection by the immune system. The question was, why were we seeing the decreased factors, as well? Now that we've reviewed the toxicity of just a few of the many mycotoxins, it's clear that we're looking at immune systems that are stimulated by a

TABLE 2 Mycotoxin Exposure Produces a Mixed Immune Effect

Increased immune activation:	Immunosuppression:
• B-cells	• CD3-CD16+CD56 Natural Killer Cells
• T-cell activation CD3+CD26+(TA1) excess CD3+HLA-DR+ excess	• CD8+CD11b+ Human Leukocyte Complement Receptors
• Suppressor Cell Activation CD8+CD38+ excess CD8+HLA-DR+ excess	• Mitogen Response Phytohemagglutinin (PHA) and Concanavilin A (Con A)
• Interleukin 2 Receptors on T-cells	
• Several Autoimmune Markers	
• IgM, IgG, IgA, IgE to specific molds & mycotoxins	

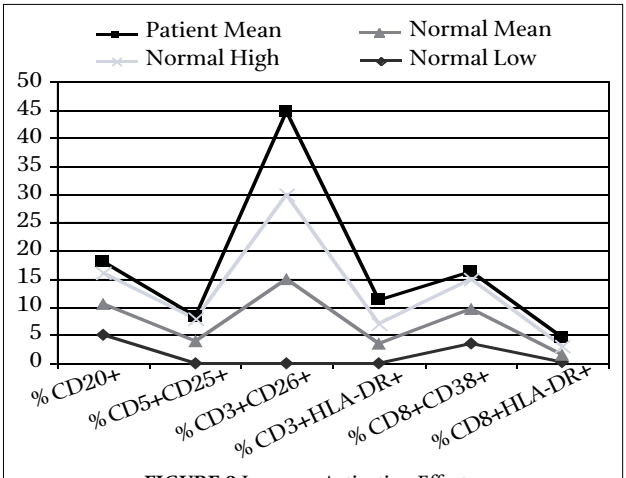

FIGURE 2 Immune Activation Effects
Comparison of mycotoxin exposed patients to normal ranges indicating significant elevation of B cell & markers of activation on T cells

potential infection while immune-suppressant toxins that favor the survival of the organisms involved are also active (see Table 2 and Figures 2, 3, and 4).

To give you an idea of how dramatic this was, the lower three lines of Figure 2 represent the 95% confidence interval on 2 markers of activation, the HLADR and the CD26. The T regulatory cells used to be called the suppressor cells, and these, again, are 2 markers of activation. The line on the top is the mean score for the population of patients (n=297) who had documented exposure to fungus and mold. We're looking at six factors that are above the 96% confidence interval. There is a 2.5% probability that these findings represent a normal result. Therefore, it's a 97.5% probability on each one of these factors that the person is truly abnormal.

Looking at the natural killer cell population in Figure 3, we see a significant drop in the mean score for the mold-exposed patient; human leukocyte complement receptor presence on T regulatory cells is also dramatically decreased. Looking at lymphocyte stimulation by phytohemagglutinin (see Figure 4), the mean score expected was 145% of the cells at the end of the test period, and we're down to 106%. For concanavilin stimulation the range of 94 to 354 should generate a mean of 224, but the mean score for the mold group was 108. Again, something is blunting the ability of these cells to divide. I suggest that

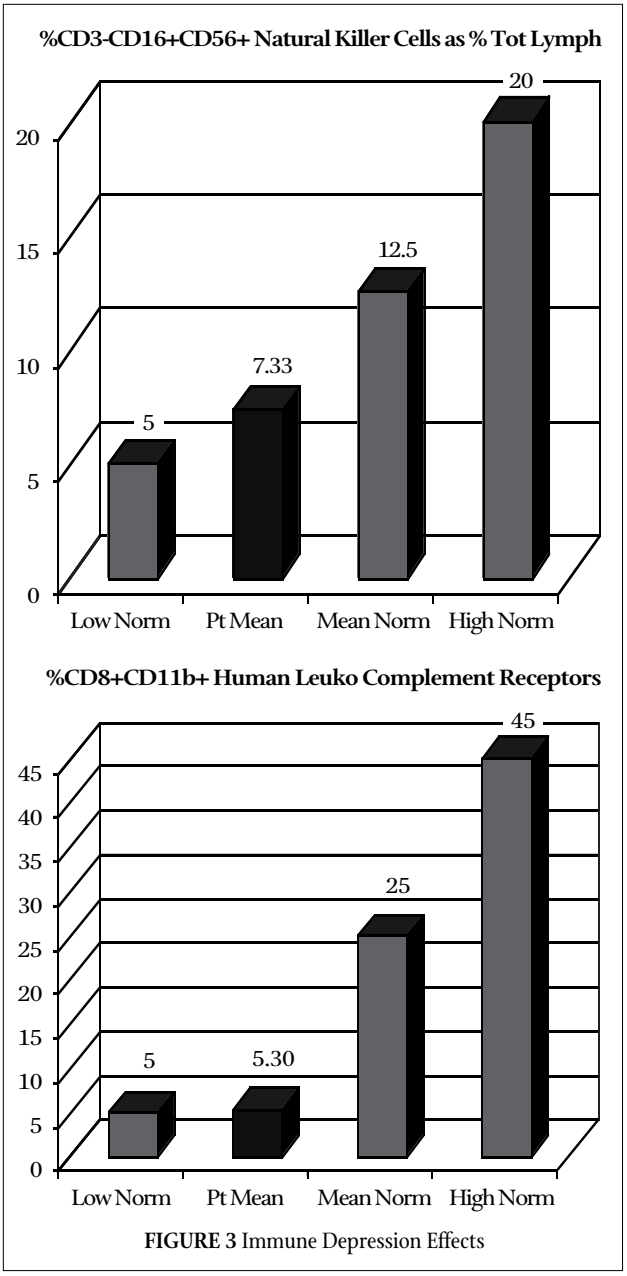

FIGURE 3 Immune Depression Effects

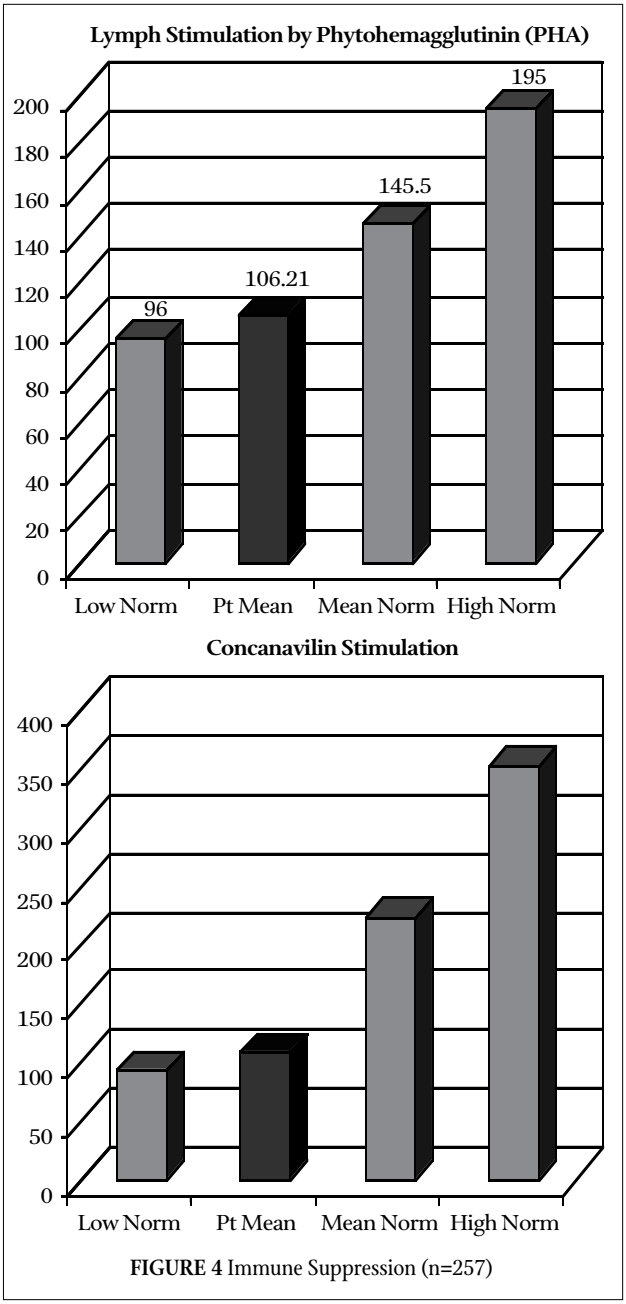

FIGURE 4 Immune Suppression (n=257)

we think about the agents that interfere with protein synthesis.

We also looked at 13 different autoimmune markers in these patients. CNS and peripheral nervous system antibodies targeting the myelin were elevated in all of the patients, as compared to controls. Interestingly, in researching the control population, I was surprised, because 28, 15, 20%—these are pretty high numbers for what you would expect to be only a 5% abnormality. It turned out that the lab controls were medical students so they weren't a true control population. Still, the odds ratios for the mold-exposed population compared to this control group were quite substantial. We need an odds ratio of 2 or better to go into court and say that there's a significant excess of abnormality in the populations that we're looking at. Antinuclear antibodies showed a 32% abnormal rate in this group. Anti-smooth muscle antibodies were abnormal in 33% of the group. Apoptosis was increased, which we considered as an index of oxidative stress.

Shifting from the immune system to the pulmonary system, we remember that we need particulate sizes between 5 micron and 0.05 micron or respirability. The *Stachybotrys* spore is 5 micron in diameter. Most spores are between 1 and 5 micron. Only 30% of the toxic burden is present on the surface of the spores. Some 70% of the toxic burden of mycotoxins and other digestive enzymes coming from these organisms is on the sub-micron size particulate matter, which is not even measured in most air sampling. There are probably 300 to 400 times more particles and surface area involved in those sub-micron size particles, which are distinctly respiratory, meaning they penetrate to the deepest regions of the lung and the alveoli. If that's where most of the action is in terms of the particulates coming from these organisms, passive filtration is useless. We see pictures of people wearing a surgeon's mask, or a paper mask, or even a gas mask with a particulate canister on it. Fifty years of research in the Archives of the National Institute for Occupational Safety & Health (NIOSH) tell us that a half

day's beard growth is enough to break the seal on those masks and that the respirable particles will penetrate. There's also a similar duration of research from NIOSH telling us that if you're dealing with particles of this size, you do not have the ability to filter them, unless you use a forced air device, because you could not breathe through a mask fine enough to catch these particles.

Practically, nothing is going to make it safe to enter a building that was submerged for a week in water during hurricane Katrina in New Orleans. The National Resources Defense Fund sent a team of environmental hygienists there several months ago. According to the American College of Governmental Industrial Hygienists, the standard is 250 to 300 spores per cubic meter of air for indoor air safety. The NRDF abandoned the standard because in the New Orleans parishes that were submerged, the indoor and outdoor air averaged 675 *thousand* colony-forming units of mold per cubic meter of air.

I have seen my first case of a nurse who volunteered for 2 weeks (1 week after Katrina) in Bay St. Louis, MI, 45 miles from New Orleans. She spent 2 weeks telling people that, according to the guidelines, all they had to do was use 10% bleach on these homes to clean up the mold, but remember that bleach and chlorinating hydrocarbons makes these organisms more mutagenic, more carcinogenic, and more lipid soluble. She returned unable to work. She is now cognitively impaired, sniffles continuously, and has significant respiratory compromise. She used to be a triage nurse in an OB/GYN clinic at the main hospital in the city. I expect that we may see a great deal many similar cases—perhaps in epidemic proportions.

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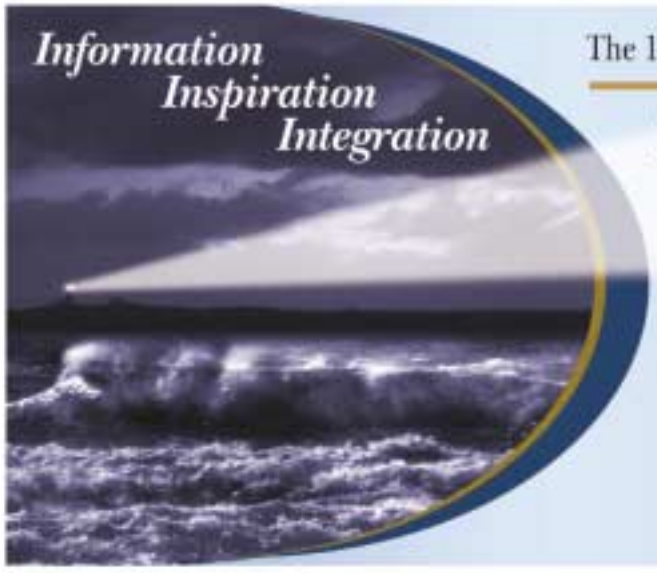

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Components of Practical Clinical Detox Programs—Sauna as a Therapeutic Tool

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When the term "sauna" is used, it typically refers to the Finnish sauna. The Finnish sauna is a wood paneled room with wooden benches and a radiant heater that keeps the temperature between 176° and 194° F at face level, with a humidity of 50-60 g H_2O vapor/M3. A person does 2-3 sauna sessions of 10-20 minutes each, which can then be followed by cold immersion.

CARDIOVASCULAR EFFECTS OF FINNISH SAUNA:[1]

- Peripheral circulation increased by 5-10% in one study, accounting for 50-70% of the cardiac output.[2]
- Circulation to muscles, kidney, and viscera decreases.
- Metabolic rate increases.
- O_2 consumption increases.
- Water loss occurs with maximal cutaneous circulation.
- Heart rate may increase to 100 bpm in persons who frequently sauna and to 160 bpm in persons unaccustomed to sauna baths.
- Systolic blood pressure can remain unchanged, increase by 9 to 21 mm Hg, or drop by 8 to 31 mm Hg, depending upon many individual factors.
- Diastolic blood pressure can remain unchanged or drop by 6 to 39 mm Hg.

Saunas have been used as a treatment for various forms of heart disease and are not associated with increased rates of sudden death or myocardial infarctions.

- Only 1.7% of the 6,175 sudden deaths in Finland occurred within 24 hours of taking a sauna.[3]
- In a 10-year follow-up of 102 men, 80 of them began saunas again within 2-24 weeks post MI. As would be expected from men with established cardiac insufficiency, 60% of them reported angina during normal daily life, but only 2% of them reported chest pains while in the sauna.[4]
- Taking saunas twice a week resulted in an increased ventricular ejection fraction (7-8% improvement) in 19 men.[5]
- 114 hypertensive men who did twice-weekly saunas after receiving coronary bypass surgery were rewarded with a reduction in blood pressure.[6]
- 46 hypertensive males doing saunas twice weekly for 3 months showed decreased blood pressure, from an average of 166/101 mm Hg to 143/92 mm Hg.[7]
- A protocol involving 15 minutes of far-infrared (FAR) sauna, followed by 30 minutes of bed rest, daily for 2 weeks was studied in persons with at least 1 coronary risk factor.[8] After 2 weeks, those who did the sauna had significantly lower blood pressure than those who did not (110 vs. 122 mm Hg). In addition, those who did daily sauna also had lower urinary levels of 8-epi-PGF2alpha, indicating lower oxidative stress.

Saunas have also been used with benefit for the treatment of congestive heart failure (CHF).

- After 10 sauna sessions over 14 days (15 minutes at 60° C [140° F] followed by 30 minutes of bed rest covered with a blanket), the at-risk group enjoyed a significant improvement in their brachial artery dilation (untreated with nitroglycerin) that approached, but did not quite reach, the dilation of the healthy men.[9]
- 20 CHF patients (New York Heart Association functional class II or III) underwent the same sauna protocol, with an additional 10 CHF patients treated only with bed rest as the control group.[10] This group also experienced improvement in the endothelium-dependent dilation of the brachial artery after only 10 sessions of sauna, while the control group showed no change. In addition, 17/20 patients in the treatment group reported an improvement in their clinical symptoms.
- Interestingly, a study in Germany utilizing daily hot and cold water applications (traditional hydrotherapy) also provided significant symptomatic improvement in 15 class II and III CHF patients.[11]
- Thirty (20 cases and 10 controls) class II and III CHF patients who were experiencing at least 200 premature ventricular contractions in a 24-hour period were studied.[12] After 10 sauna sessions, the study group had a dramatically lower 24-hour average number of PVCs (848) than the control group (3097), who received only the bed rest with blanket. Prior to treatment, the 24-hour average of PVCs in the treatment group was 3,161.

PHYSIOLOGIC EFFECTS OF FINNISH SAUNA

- Increased plasma cortisol, corticosteroids, growth hormone, TSH, and prolactin[13]
- Bronchodilation
- Muscle relaxation and decreased activity of neuromuscular system
- Loss of water and electrolytes (Na, K, Cl), compensated through hormonal regulation via kidneys of aldosterone secretion
- Lipolysis

MOBILIZATION THROUGH DIAPHORESIS

Numerous compounds are released in the sweat.

- The minerals sodium, potassium, magnesium, and chloride are all excreted via sweat,[14] along with iron[15] and chromium.[16]
- Sodium and chloride have the greatest losses (155 and 137 meq, respectively), while magnesium and potassium are excreted in much lower quantities (13 and 16 meq, respectively).
- Copper and zinc are released in high amounts in sweat (avg. for copper 550 µg/L for males and1480 µg/L for females; for zinc 500/1250 for males and females, respectively).[17]
- Nickel and lead were found in lower levels than copper and zinc.

- Manganese, cadmium, and aluminum have also been found in the sweat in much lower amounts than Cu and Zn.[18,19] With protracted sweating from exercise, the levels of iron and zinc in the sweat were lower in the second hour than in the first.[20]
- Cadmium and nickel levels in the sweat have been found to be higher than corresponding levels in the urine, making sweating a prime route of depuration for cadmium or nickel toxicity.[21]
- Sweating has also been explored as a valid method for reducing antimony levels in persons with high Sb exposure.[22]
- In persons whose blood lead averaged 8.62 µg/dL, the level in the sweat averaged 5.2 µg/L, which was about 25% that of the urine.[23]
- Dermally absorbed lead was released in both sweat and saliva, but did not show up in the blood.[34]
- A study on the toxicokinetics of lead states that soft tissue lead, not blood lead, is the source for lead released in sweat.[25]
- Only 1 published study was found regarding mercury and sweat. This study did not give a measurement of mercury in the sweat, but noted that in a mercury-poisoned person the mercury blood level continued to drop during the sauna portion of the protocol (which followed chelation therapy).[26]

A large number of medications have been detected in the sweat:

- Amphetamines (and metabolites)[27]
- Methadone and its metabolites[28]
- Antiepileptic drugs[29]
- Phenytoin, phenobarbital, and carbamazepine were measured after it was noted that a number of hospitalized patients had lower serum levels of phenytoin during a particularly hot summer.

TISSUE PENETRATIONS BY WAVELENGTH

- IR-A (Near infrared–0-3,000 nm)—tissue penetration deepest up to 5 mm
  - Makes it to subcutaneous layer
  - Dissipates heat from surface the best
  - Beyond 3mm—best heat transfer
- IR-B (Mid infrared—3,000-5,500 nm) – tissue penetration to about 0.5 mm
- IR-C (Far infrared—5,500-7,000 nm) – tissue penetration of about 0.1 mm (despite claims that IR-C has the deepest penetration)
- Radiant heaters as used in traditional saunas have all three wavelengths present (IR-A, IR-B, IR-C).

SAUNA USE IN CLEANSING PROGRAMS

L. Ron Hubbard, founder of Scientology, developed and promoted the use of the Hubbard Purification Rundown as a method of reducing environmental chemicals in an individual. The components of his 3-6 week protocol include doing all of the following daily:[30]

- Physical exercise for 20-30 minutes
- Sauna, 140-180° F, done in 30 minute sessions for a total of 2 1/2-5 hours daily
- Increasing doses of niacin each day and a multivitamin
- H2O, NaCl, K replacement
- Oil, 1-8 Tbsp
- Balanced meals and adequate sleep

Ten electrical workers who did 3 weeks of the Hubbard protocol were studied.[31] They experienced a 7.8% drop in adipose pesticide levels and a 4.7% drop in PCB levels. In the 3 months after treatment ended, the pesticides continued to be cleared from the workers' bodies. At the 3-month follow-up, the mean total drop in pesticides from pre-treatment levels for the treatment group was 21.2% (2.3% for PCBs).

Dr William Rea, director of the Environmental Health Center, Dallas (EHC-D), has also published data on the therapeutic use of thermal chambers. Dr Rea's protocol included on a daily basis:

- Chemical-free living facilities
- Use of specially constructed, less chemically polluted heat chambers; 140-160° F for 2 hours
- Exercise before the sauna
- Massage after the sauna
- Niacin—up to 3,000 mg
- Vitamins, minerals, amino acids, given orally and IV
  a. IV vitamin C–15 g daily, with another 2-8 g orally
  b. Several other vitamins and minerals
  c. Glutathione

In Dr Rea's clinic, the results of treating 156 chemically sensitive females and 54 males were reported as follows: 86% of the participants had improved symptom pictures; 57% of those with abnormal balance and 31% of those with autonomic nervous system disorders (as measured via an Iriscorder) improved.[32] Sixty-three percent of those undergoing the cleansing program had reductions in their serum toxin levels; of those without any noted reduction, 18% showed an increase and 19% remained the same.

Dr Gerald Ross, who worked with Dr Rea for many years, gave a presentation in 2003 that described sauna therapy utilized as part of a drug addiction/criminal rehabilitation program. When a 2-year follow-up was done, it was found that 23% of those who failed to complete the program had criminal records, while only 13% of those who did the entire program re-offended.[33]

During many years in private practice, I used the following as a daily cleansing/detoxification protocol with patients:

- Exercise 15-20 minutes
- Thermal chambers, 120-130°; 3 60-minute sessions with 10-minute cool-downs in between
- H2O (bottled spring) and electrolyte replacement
- Ginger/yarrow tea
- No niacin
- Flax oil, 1/2 to 1 oz daily
- Psyllium
- Constitutional hydrotherapy (using the protocols of Harold Dick, ND, and Andre Saine, ND) with dichromatic green light
- Liver herbs, 1 capsule daily containing *Chelidonium, Chionanthus, Arctium lappa, Taraxacum, Urtica dioica, Arctostaphylos uva-ursi,* and *Silybum marianum*
- Colonic irrigations with purified H2O (triple treatment)
- Herbal, bacterial implants
- Body work, including craniosacral, visceral, trigger point, shiatsu, and massage
- Constitutional homeopathy
- Acupuncture
- Nutritional workup
  1. Dietary avoidance of adverse food reactors
  2. Dietary avoidance of most toxic foods
  3. Dietary avoidance of sugar

After the first 10 years of doing the cleansing program we did an outcome study.[34]

The results are shown in Table 1.

TABLE 1 Results of 15 or more sessions of the Crinnion Cleansing Program

Complaint	Worse	No Change	Slight	Mod./ Good	Great	Total
MCS	0	2	1	8	13	25
Autoimmune	0	0	0	4	12	16
Neurologic	0	3	2	4	6	15
Fatigue	0	1	0	6	7	14
Cancer	0	2	0	2	4	8
Allergies	0	0	1	5	1	7
General Cleansing	0	0	2	5	0	7
Musculoskeletal	0	0	2	2	1	5
Dermatological	0	0	0	3	1	4
Respiratory	0	0	0	0	3	3
GI/Liver	0	0	0	1	2	3
General Debility	0	1	1	0	1	3
HIV/AIDS	0	0	1	1	0	2
Addictions	0	0	0	0	1	1
Totals	0	9	10	41	52	112
Percent	0	8	9	36.6	46.4	

SUMMARY

Saunas can be used very effectively for certain cardiovascular problems and as a means to enhance the mobilization of fat-soluble xenobiotics. When saunas are used to reduce blood pressure and enhance blood flow and cardiac functioning, only short sauna sessions (15 minutes) are necessary. When one wants to enhance the mobilization of heavy metals and chemical xenobiotics, longer sessions are needed and those should be medically monitored. But, for either use, saunas are safe and effective and should be used more frequently to benefit the health of our patients and ourselves.

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