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INTERFACE:
GENES AND THE ENVIRONMENT

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Thoughts and Speculation about the Role of Arsenic in Toxicity and Cancer

Arsenic has been regarded throughout history as somewhat of a mystical metal, and this perception still continues today. During medieval times, arsenic was used as an acute poison for suicide and homicide. In very small doses, however, it was given to treat a number of ailments--including asthma, psoriasis, and trypanosomiasis (Chaga's disease, infection caused by a type of parasite). Although arsenic is usually associated with toxicity, it may in fact be an "essential element" (similar to our daily requirements for small amounts of magnesium, calcium, selenium, etc.). Induced arsenic deficiency impairs growth and reproduction in experimental lab animals and farm animals, with the extrapolated human requirement of 12-25 µg/day. Today, there are quite a few commercial uses of arsenic**:** as a pesticide, as a wood preservative, and in the manufacturing of paper, glass, and semiconductors. The purpose of this brief overview is to examine why arsenic is an environmental concern, to review the chemistry and intracellular actions of arsenic, and to pose questions as to how future genetics research might help make this metal less of a mystery.

IN THIS ISSUE

Human epidemiological studies

Significant exposure of human populations to arsenic comes principally from mining, manufacturing, pesticide use, incineration, and the burning of fossil fuels. In addition, considerable amounts of arsenic in the drinking water have been found in Mexico, Taiwan, China, Chile, Argentina, India and Thailand. The highest levels of human exposure to arsenic are found in ground water--especially in areas where copper and arsenic smelting occurs. Arsenic is a by-product of copper smelting. Populations at particular risk thus include industrialized regions, pesticide and paper and glass workers and manufacturers, and populations living near copper or arsenic smelters (several of which exist in the United States).

Fatal cancers of the bladder and lung have been reported in Taiwan and other countries with arsenic levels of 200 µg per liter of drinking water. The U.S. Environmental Protection Agency (USEPA) has developed its standards for chronic exposure to arsenic in this country, based on the incidence of "blackfoot" disease in Taiwanese exposed to arsenic in the drinking water. Whereas the current U.S. drinking water standard is mandated that it must be less than 50 µg of arsenic per liter, it has been suggested that this concentration might result in 1 out of 100 persons dying from arsenic-induced cancer over a 70-year lifetime. Hence, discussion continues in the United States as to whether the 50 µg/L standard should be lowered.

Clinical toxicity and cancer

Acute arsenic exposure causes irritation of the skin, mucous membranes, and gastrointestinal tract--as well as

anemia (low red cells) and neuropathy (noninflammatory disease of nerves). Chronic exposure leads to decreased pigmentation of the skin, hyperkeratosis (thickening, cracking, peeling) of the palms and soles, cancer of the skin (basal cell and squamous cell carcinoma), and cancers of the urinary bladder and lung, and possibly cancers of the kidney and liver. The USEPA has classified arsenic as a "Group A" carcinogen (known to cause cancer in humans), based on clear-cut evidence of skin and lung cancer in exposed workers.

The mechanisms by which arsenic causes toxicity or carcinogenicity remain unexplained. It is well known, however, that arsenic action is largely "***nongenotoxic***," *i.e.* there is little evidence of damage to DNA, formation of DNA adducts, or mutation in assays such as the "rat liver microsomes/*Salmonella* " (Ames) test. Interestingly, this classification puts arsenic in a similar category to that of dioxin, phorbol ester, diethylstilbestrol, phenobarbital, and dehydroepiandrosterone**;** these are "nongenotoxic" agents that are capable of stimulating cell division in certain cell types, and causing "tumor progression"--rather than "tumor initiation" as DNA-damaging ("***genotoxic***") agents do.

Even more intriguing, arsenic treatment alone does not appear to induce cancer in animals. Although it might appear that arsenic by itself can cause cancer in humans, it is possible that other genotoxic agents (*e.g.* cigarette smoke, radon, dietary mutagens) are participating in human tumor initiation while arsenic acts as the tumor promoter. This discrepancy between human epidemiological studies and laboratory animal studies underscores our need to understand arsenic's biological modes of action as to how it causes its acute and chronic effects on multiple target organ systems.

Because of the highly uncertain clinical exposure estimates (including possible exposures to other carcinogens at the same time), there has been considerable controversy regarding the shape of the dose-response curve in the very-low-dose portion of the curve. This remains an extremely important question, because low concentrations of arsenic are those to which the vast majority of humans are actually exposed. An additional, very real question is the likelihood that different individuals might respond differently to the same level of arsenic exposure. In other words, do we have relatively "arsenic-sensitive" and "arsenic-resistant" individuals comprising the populations that have been studied? And, if so, which gene(s) confer(s) this difference in susceptibility to risk of arsenic-induced toxicity or cancer?

Chemistry and intracellular effects of arsenic

Arsenic(V), primarily in the form of arsen***ate*** (AsO_4^{-3}) salts, is more common in the soil than As(III), which is mostly present as the salts of arsenous acid (H_3As0_3) and especially arsen***ite*** (AsO_2^-) (***Figure 1***). Both arsenate and arsenite are biochemically active. Arsenate acts primarily as a "phosphate mimetic," entering cells via a ***phosphate transporter***. Once inside, arsenate reacts similarly to phosphate, competing with phosphate to form unstable high-energy intermediates. In this manner, arsenic interferes with energy production and energy-requiring functions of the cell (transport, biosynthesis, replication, etc.).

Although arsenate disrupts energy metabolism, arsenite appears to be even more toxic. The toxicity of arsenite is closely associated with its capacity to bind cellular sulfhydryl groups**;** as such, it may bind to and deplete the major cellular antioxidant, reduced glutathione (GSH; see ***Interface*** issue #5), block mitochondrial electron transport by binding to iron-sulfur proteins, and inhibit dihydrolipoate dehydrogenase (components of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase), thereby perturbing glycolysis and the citric acid cycle. Another enzyme inhibited by arsenite is DNA ligase II, which might be the reason for seeing arsenic-induced chromosomal aberrations.

In humans, and in most laboratory animal species, one or more of the "arsenate methyltransferases" (AsMTs) transfers a methyl group from S-adenosyl-*L*-methionine (SAM) to form monomethylarsenic acid (MMA), and the same reaction repeated can lead to formation of dimethylarsenic acid (DMA). Methylation of arsenic is

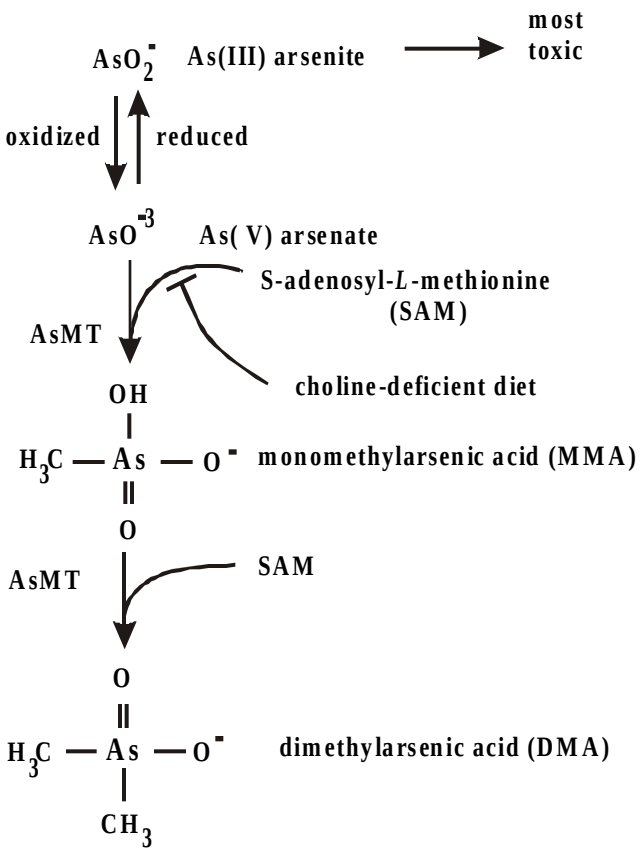

Figure 1. Chemical states of arsenic in the environment and in the cell. Some of these structures are not known for certain.

believed to be a detoxification pathway. Fish are known to chelate the metal in the form of arsenobetaine. Whereas the human, mouse, rat, rabbit, hamster and rhesus (New World) monkey display MMA/DMA formation, the guinea pig, chimpanzee and marmoset (Old World) monkey do not. These ***interspecies differences*** might help in trying to elucidate the mechanisms of arsenic-induced toxicity and cancer.

Poor nutrition might be an underlying reason as to why arsenic-induced cancer is seen most often in lower socio-economic populations. A diet deficient in choline leads to a decreased pool of methyl groups. Thus, it is not surprising (***Figure 1***) that a choline-deficient diet has been shown to block MMA/DMA formation in laboratory animals.

AsMT gene cloning

From the pathway shown in ***Figure 1***, it is likely that genetic differences in the *AsMT* gene(s) responsible for MMA/DMA formation, the purported detoxification pathway, might lead to identification of individuals with high-risk vs low-risk for arsenic-induced toxicity and cancer. In other words, would a low-AsMT activity render an individual more susceptible than a high-AsMT person to elevated, dangerous levels of arsenite in critical cell types, and, therefore, arsenic-induced toxicity and cancer? It also appears likely that more than one form of cell type-specific AsMT might exist (*e.g.* AsMT1 in liver and AsMT2 in skin).

A protein with AsMT activity (for MMA/DMA formation) has been isolated from rabbit liver and has a ~60-kiloDalton (kDa) molecular weight**;** a laboratory in Tucson, Arizona, has purified the corresponding human liver AsMT in hopes of cloning the gene relevant to arsenic toxicity and cancer. Although more than a half-dozen ***methyltransferase*** genes have been cloned to date (some not evolutionarily related to others), the one (or more) gene(s) responsible for MMA/DMA formation has not yet been cloned.

An apparently perplexing experiment showed that DMA exposure of rats (that had been pretreated with any of five tumor initiators) enhanced tumorigenesis in the urinary bladder, kidney, liver, and thyroid gland. At first glance, these data might suggest that a high AsMT activity could lead to increased risk of arsenic-induced toxicity and cancer in humans. However, it must be kept in mind that there are many ***demethylases*** in the cell, so what probably happened in this rat experiment was that DMA became demethylated to the toxic arsenate or arsenite form, which then caused tumors. Do inbred mouse strains exist that differ in MMA/DMA formation by factors of 3-fold or 10-fold? To our knowledge, such studies remain to be carried out.

Effects of arsenic on cell growth and DNA repair

Seemingly unrelated findings from numerous laboratories, examining arsenic effects in multiple experimental model systems [***Mutation Res* 386:** 181-361 (1997)], underscore the complexity of action of this heavy metal. For example, arsenite has been found both to stimulate and suppress critical aspects of keratinocyte cell division. Following topical treatment with a tumor initiator, arsenite in the drinking water of mice causes increased production of growth factors and activation of the c-*myc* oncogene in skin of these animals. Arsenic has been shown to induce apoptosis (programmed cell death) in rat T-cell lymphocytes. Arsenite also alters cytosine methylation patterns in the *TRP53* (p53) tumor suppressor gene. Cotreatment with ultraviolet (UV) irradiation greatly augments the DNA-damaging effects of arsenic. In a human T-cell lymphoma-derived cell line, arsenite inhibits the human DNA repair enzyme called poly(ADP-ribose)polymerase (PARP), albeit at concentrations (10 μM AsO_2^-) that might not be regarded as "physiological." Arsenic disrupts mitotic spindle formation and microtubule assembly, which are two likely explanations for aneuploidy (abnormal number of chromosomes) seen in lymphocytes of heavily exposed individuals. Arsenite also induces sister chromatid exchanges (SCEs), which are further indications that this heavy metal especially at high levels can cause chromosomal breaks and rearrangements.

Arsenic is a cause of oxidative stress in the cell

"Oxidative stress" is the unwanted reactive intermediates of oxygen and other unpaired free radicals wreaking havoc in the cell. As previously discussed, arsenite as a known potent electrophile can react with sulfhydryl groups in proteins, as well as GSH (***Figure 2***). There are human polymorphisms in which 20% to 50% of different ethnic groups have either the *GSTM1* or the *GSTT1* (glutathione S-transferase) gene missing. In heavily exposed populations, individuals having the "null allele" for the *GSTM1* or the *GSTT1* gene have been reported to show increased body retention of arsenic (where arsenic exposure had been estimated by determining arsenic + MMA + DMA levels in hair, toe nails, and urine).

Numerous studies, particularly over the past 5 years, have demonstrated that arsenic**:** [a] lowers GSH concentrations, [b] enhances the levels of reactive oxygen species ($O_2^{\cdot -}$), [c] induces metallothionein concentrations, [d] stimulates arachidonic acid metabolism, and [e] increases lipid peroxidation. In general, the potency of As(III) (arsenite) in causing these intracellular changes has been found to be 3-5 times that of As(V) (arsenate), 40-60 times that of MMA, and 100-150 times that of DMA. ***All of these parameters are indicators of oxidative stress***.

These findings thus support the likelihood that arsenic (arsenite, in particular) elicits its cell type-specific toxic and carcinogenic effects by way of oxidative stress (***Figure 2***).

Exciting work during the past decade--from the laboratories of Baeuerle, Karin, Curran, Herrlich, Fornace and others--have shown that, although agents have long been known to induce oxidative stress because they are DNA-damaging (genotoxic), a complex signal transduction pathway ("nongenotoxic," in that it is independent of DNA damage) is able to respond to oxidative stress stimuli as well. This pathway consists of a series of membrane-bound and cytosolic kinases, phosphatases and second messenger molecules**;** because the pathway transmits the signal so rapidly, and is independent of new nucleic acid or protein synthesis, it might be considered somewhat analogous to computer microcircuitry.

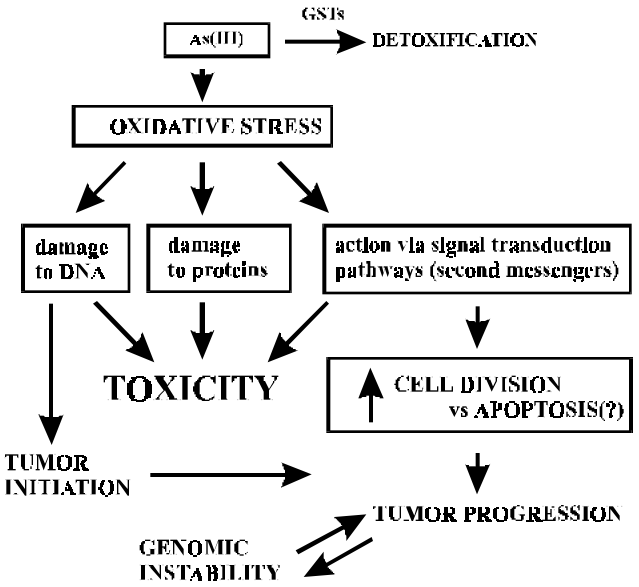

Figure 2. Flow diagram of possible cellular responses to arsenic

Consistent with this putative "oxidative stress signal transduction superhighway" is a very recent paper (from the laboratory of Rapp in Würzburg, Germany) demonstrating that arsenite is able to activate the mitogen-activated protein kinases (MAPKs), the particular ones being termed extracellular signal-related kinases 1 and 2 (ERK1, ERK2). These moieties are known to participate in the c-*jun* N-terminal kinase (JNK)-dependent activation of the *raf*/MEK/ERK cascade that mediates growth- and differentiation-stimulating signals, as well as other parallel pathways involved in response to inflammatory cytokines and environmental stress inducers. In other words, more than a decade of arsenic experiments have implicated the role of oxidative stress, in retrospect**;** and now this very recent paper of Rapp [***J Biol Chem* 273:** 1917-1922 (1998)] proves beyond a shadow of a doubt that arsenite-induced oxidative stress does stimulate this particular nongenotoxic signal transduction pathway.

Arsenic-induced hypoglycemia

One of the older observations and concerns in arsenism among human populations includes hypoglycemia (a lowering of blood sugar). Is hypoglycemia related in some way to the toxic and carcinogenic effects of arsenic, or is this just a red herring? In addition to causing GSH depletion, arsenite also is known to diminish NAD(P)H and ATP pools by the inhibition of enzymes discussed previously**;** these decreases in NAD(P)H and ATP pools lower the capacity of gluconeogenesis (the ability to make more sugar from amino acids and fat)**;** the end result of these liver toxicity effects is carbohydrate depletion and hypoglycemia.

For the past decade the Nebert laboratory in this Center has studied the mutant *14CoS/14CoS* mouse, which exhibits massive oxidative stress, hypoglycemia, and death during the first 24 hours after birth. The newborn behaves as if it were being heavily treated with toxic electrophilic chemicals, but these mice are not being treated with anything! The defect is now known to be due, at least in part, to a deletion of the fumarylacetoacetate hydrolase (*Fah*) gene, which leads to a build-up of endogenous electrophiles**;** the comparable human condition is an inborn error of metabolism called ***hereditary tyrosinemia type I*** (HT1) in which young children usually die with liver fibrosis and cirrhosis. The "oxidative stress response" in the *14CoS/14CoS* mice is believed to involve the dioxin-binding receptor and a battery of genes that are inducible by dioxin-like chemicals, and a subset of those genes that are inducible by potent electrophiles.

The hypoglycemia in these *14CoS/14CoS* mice was first discovered more than 40 years ago by Carl Cori. Hypoglycemia does not appear to be the cause of lethality, because sugar supplementation does not prevent death. The clinical treatment of the HT1 babies with 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (**NTBC**) prolongs their lives by more than 10 years**;** NTBC is an inhibitor of 4-hydroxyphenylpyruvate dioxygenase in the tyrosine degradation pathway, thereby blocking formation of oxidative stress reactive intermediates. Treatment of the *14CoS/14CoS* newborns with NTBC likewise prolongs life in these mutant mice during the neonatal and weanling periods. Whether the hypoglycemia in these mutant mice is related in some way to the massive oxidative stress seen in various organs (particularly liver and gastrointestinal tract)--is intriguing, but not known. Could there be any relationship between this *14CoS/14CoS* model system and the hypoglycemia and oxidative stress seen in arsenic-treated animals or cells in culture?

Conclusions

Arsenate is the most common form of arsenic in soil and water. Arsenate is believed to be detoxified by methylation, and the arsenate methyltransferase (*AsMT*) gene (or genes) that encode the enzymes responsible for

this reaction have not yet been cloned. Since the human thiopurine methyltransferase (*TPMT*) gene is known to be polymorphic, it is reasonable to presume that high-AsMT and low-AsMT individuals might show differences in sensitivity to arsenic-induced toxicity and cancer. To our knowledge, inbred strains of mice have not yet been screened for possible differences in arsenic-induced toxicity and cancer**;** we suggest that such a mouse model system might be helpful in elucidating mechanisms of toxicity or cancer induced by this heavy metal.

Arsenite appears to act as a ***tumor promoter*** rather than tumor initiator. Arsenite is also electrophilic and has been shown to induce numerous subcellular parameters involving oxidative stress. The genotoxic and, in particular, the ***nongenotoxic*** effects induced by such oxidative stress, will very likely play a central role in arsenic-induced organ- and cell type-specific toxicity and cancer. The very recent exciting findings--that arsenite is able to activate two protein kinases, termed ERK1 and ERK2, which participate in the JNK-dependent activation of the *raf*/MEK/ERK cascade that is known to respond to numerous environmental stress inducer electrophiles--should now lead to rapid advances in our molecular understanding of arsenic-induced toxicity and cancer.

-----Contributed by Dan Nebert and Howard Shertzer, with special thanks to Richard Weinshilboum for valuable discussions

CEG - SPONSORED SPEAKERS

Gloria M. Petersen, PhD
Associate Professor,
School of Hygiene and Public Health
The Johns Hopkins University
Department of Epidemiology
7 January 1998 ***"Genetics of colon cancer"***
8 January 1998 ***"Risks in genetic testing: socio-legal implications"***

Robert L. Strausberg, PhD
Assistant to the Director
Head, Strategic Technologies Division
National Cancer Institute
21 January 1998 ***"The genomics revolution: Forging a new era in biological and biomedical research"***

CEG Members in the News

Grace Lemasters received a Cincinnati Women's Leadership Award for 1998 in the area of Research and Technology. She also presented a talk entitled ***"Cytogenetic effects of jet fuel exposure in aircraft maintenance workers"*** at an "International Conference on Jet Fuels" (March 1998, San Antonio, Texas)

Dan Nebert was an invited speaker at a Symposium on "Action of Natural and Synthetic Environmental Chemicals on Nuclear Receptors," during the Annual Meeting of the German Society of Pharmacology and Toxicology (March 1998, Mainz, Germany).

Alvaro Puga chaired a session and gave a presentation at the "First International Colloquium on Transcription Factors as Therapeutic Targets" (January 1998, Luxembourg).

Rakesh Shukla and Pilot Project Recipient Dr. J. Broderick submitted a proposal to CIDR (Center for Inherited Disease Research) dealing with genome-wide scanning for cerebral aneurysms. The Workshop hosted by the Genetic Epidemiology and Biostatistics Facilities and Services Core entitled "Marker-based Human Genetic Epidemiologic Analysis" (February 1998) was tremendously useful in this endeavor.

Nancy Steinberg-Warren received a UC Strategic Enrollment Management grant on "Enhancing minority recruitment into the genetic counseling profession."

David Warshawsky conducted a symposium with Joseph Landolph (USC), entitled "Molecular and Cellular Biology of Chemical Carcinogenesis" and also delivered a talk entitled ***"DNA adduct formation and oncogene activation by N-heterocyclic aromatics"*** at the 37th Annual Meeting of the Society on Toxicology (March 1998, Seattle, Washington).

SCIENCE LITE

Humor from a Molecular Biology Laboratory

One sunny morning a PhD graduate student, a postdoctoral fellow, and a professor were walking through a small park on campus next to the lake, on their way to the cafeteria, when they found what looked like a very old antique oil lamp. They rubbed the lamp and, to their amazement, a Genie appeared in a puff of smoke. The Genie said, "I usually grant only three wishes, so I'll give each of you just one wish."

"Me first! Me first!" exclaimed the graduate student. "I want to be in the Bahamas, driving a speedboat, with a gorgeous woman who sunbathes topless." And ... Poof! He disappeared from the campus...

"Me next! Me next!" shouted the postdoc. "I want to be in Hawaii, relaxing on the beach, with my arm around a professional hula dancer and a large Mai Tai drink in my other hand." And ... Poof! He also disappeared...

"And you are last, Oh Master," the Genie said to the professor.

The professor answered, "I want those two back in the lab after lunch."

"Entering the Century of the Environment"

When Jane Lubchenko (Oregon State University) gave her Presidential Address at the 1997 Annual Meeting of the American Association for the Advancement of the Sciences (AAAS), she described the 21st Century as the "Century of the Environment" and urged scientists to forge a new social contract [***Science*** 279: 491-497 (1998)]. The major goal is to move toward a more sustainable biosphere--one that is ecologically sound, economically feasible, and socially just. We, as one of the more than two dozen ***Centers of Excellence*** funded by the National Institute of Environmental Health Sciences (NIEHS), hope to help lead the way in meeting her challenge!

See? ... People were talking about "Bioinformatics" before the 1990's!

"The modern age has a false sense of security because of the great mass of data at its disposal. But the valid issue is the extent to which people know how to ***form*** and ***master*** the material at their command."

--— Goethe, 1832

Genomic Mismatch Scanning (GMS)

In case you missed it, another major breakthrough has been reported (it seems like human genetics research has about one major breakthrough each month now, if not each week)! GMS is a technique that enriches for regions of "***identity by descent***" (IBD) between two individuals--without the need for genotyping or sequencing [***Nature Genet*** **18:** 200-202 and 225-230 (1998)]. Chromosomal regions of IBD, selected by genomic mismatch scanning, are mapped by hybridization to a microarray, containing ordered clones of genomic DNA from the chromosome(s) of interest. Cheung et al. demonstrated the efficacy of GMS by studying congenital hyperinsulinism (HI), an autosomal recessive disease quite prevalent in Ashkenazi Jews. The combination of GMS, and hydridization of IBD products to a chromosome-11 microarray, correctly mapped the ***HI*** gene to a 2-Mb (2 million base pairs) region--proving that linkage disequilibrium mapping is possible without genotyping (the kind of linkage analysis that we discussed in the Lead Article of the last issue of ***Interface***).

"...And Lead Us Not Into Temptation, ..."

In a recent issue of ***J Personality Social Psychol***, Baumeister and coworkers reported a test to measure self-control. Volunteers at Case Western Reserve University (Cleveland) were told to skip a meal, and they would be tested for "taste impressions of radishes, and memory." The volunteers were asked to eat radishes and write down their impressions of that taste, and then work on a confounding mental puzzle for as long as they could. The groups included those facing radishes alone, radishes plus ***chocolate-chip cookies*** (*i.e.* the temptation), or no food. The subjects were left alone in the room, but observed through a 2-way mirror. Some refused to look at the cookies, some went as far as picking up a cookie and smelling it, but no one cheated and ate a cookie.

Those facing the cookies gave up more easily than both a group facing the radishes alone and a group asked to perform without being offered any food at all. The conclusion was that "resisting temptation can be mentally fatiguing."

LETTERS TO THE EDITOR

RESPONSES/COMMENTS TO VARIOUS QUESTIONS

Q **There was a recent report in the newspaper that the frequency of *BRCA1* mutations (believed to contribute to breast cancer) was found to be much lower than previously thought. In what scientific journal did this publication appear, or has it appeared yet?**

A *Actually, there are two recent reports back-to-back in the same journal issue: Newman et al., **J Am Med Assn 279:** 915-921 and Malone et al., 922-937 (1998). Earlier studies of **BRCA1** gene defects --suggesting that mutations were as high as 60% of young women with breast cancer--might have been "skewed," because the patients were extracted from pools of high-risk families with multiple early-onset cases of breast cancer. Finding solid predictors of breast cancer may be more elusive, however, than just selecting one or two genes for analysis. The Newman et al. study in North Carolina found that only three of 211 breast cancer patients selected randomly (1.4%) had **BRCA1** mutations, and the Malone et al. study in Seattle found mutations in 12 of 193 subjects (6.2%) under the age of 35 who had breast cancer. These studies indicate that "general population screenings for the gene mutation" might not be as necessary as previously believed (there was a lot of hype 1-2 years ago). Of course, this low incidence of **BRCA1** defects in the general population differs considerably from that among Ashkenazi Jewish women. Imagine the poor family physician, being bombarded with requests for such genetic tests and trying to explain all this to his/her patients!*

COMMENT ***BRCA1*** **(and *BRCA2*) are examples of "reverse genetics"--in which [a] the chromosomal location of a gene (responsible for some portion of inherited breast cancer in young women) was first pin-pointed, [b] "actively transcribing" genes in that chromosomal region were isolated, and, finally, [c] mutations in a particular gene were shown to be correlated with the disease. As discussed in several previous issues of *Interface*, however, the function of these two genes is still being sought. Understanding these functions might help with cancer therapy. In the past year, at least seven studies suggest that *BRCA1* and *BRCA2* both participate in the cell's response to DNA damage [reviewed in *Cell* 92: 433-436 (1998)], including interactions/coactivation with the TRP53 (p53) and TRP21 (p21) proteins. If breast cells cannot respond normally to the every-day occurrence of DNA damage, this might explain how *BRCA1* and *BRCA2* defects lead to abnormal growth and, ultimately, breast cancer.**

COMMENT **Your articles on the genetic differences in metabolism of Prozac and Halcion, and the possible genetic differences in response to Fen-Phen treatment, were intriguing, simply fascinating! Keep writing about topics as exciting as these!**

COMMENT **For the past 5 or 6 years there have been major concerns that "environmental estrogens" might be causing "endocrine disruption" in alligators, salamanders, and perhaps other species (*cf.* issue #3 of *Interface*). In the 1 Dec 97 issue of *Am J Epidemiol*, Pauline Mendola and coworkers studied 2,200 premenopausal women and found that the subset of 280 women who reported eating Lake Ontario fish more than once a month "tend to have menstrual cycles that are 1 day shorter, on average, than those who don't." Lake Ontario has high concentrations of toxic pollutants, especially the polychlorinated hydrocarbons (PCBs) that are considered to "exhibit estrogenic effects." Whereas this small (but statistically significant) decrease in length of menstrual cycle is not enough to jeopardize fertility or health, it "may indicate that potential endocrine disruptor effects on human populations are being caused by environmental pollution."**

COMMENT **In issue #10 of *Interface*, we reported the finding of a National Research Council (NRC) Panel that "no adverse effects on cells or animals were found at electromagnetic force levels that are typically measured in house**

electrical wiring or in houses located under power lines." In issue #12, we discussed some of the further controversy about this research field, as many scientists disagreed with the conclusions of this expert panel. An excellent, lengthy review on "extremely low-frequency electromagnetic fields (ELF-EMFs) has just appeared in *FASEB J* 12: 395-420 (1998). Containing 166 references, this review presents a very balanced, objective, and scientifically rigorous analysis of all the data, problems with reproducibility, and both sides of the issue.

Q **There was a newspaper report about a scientific study that can explain some of the male-female differences in "social graces," but it was not explained well at all. Do you know what I'm talking about, and what is the science behind it? Did this study "correlate the genotype with phenotype," as you described so elegantly in issue #12?**

A *The Skuse et al.[**Nature** **387:** 705-708 (1997)] paper identified the first known case of imprinting (DNA methylation, "turning off" a gene) on the human X chromosome. Normal females have two X chromosomes (one inherited from each parent), and normal males have an X (from the mom) and a Y chromosome (from the dad). Skuse and coworkers studied women with Turner syndrome (who have a single X chromosome, making a total of 45 instead of the usual 46 chromosomes), comparing those with the maternal X^m chromosome to those with the paternal X^p chromosome. The X^m girls were scored as having the more clinically significant social difficulties (such as disruptive or offensive behavior) than the X^p girls. On a test of "behavioral inhibition," X^p females had scores similar to normal X^m/X^p females, who in turn scored better than normal X/Y males. Further studies were done on females in whom only part of the X^p chromosome was missing. These results demonstrate that there must be an imprinted gene on the X chromosome (which escapes X-chromosome inactivation) that affects social functioning and related cognitive abilities (which fork to use when, where to place your napkin, how to use your soup spoon, etc.). This study also challenges the recent prevailing belief that gender differences are largely culturally determined. Moreover, this study forewarns that we will find other provocative genes "out there," including genetic differences in performance on IQ tests and military combat!*

The Increasingly Urgent Need for Standardized Nomenclature of Genes

Recently, ***Nature*** [vol. **389:** 1 (1997)] devoted its opening article to point out the urgent nomenclature problems facing molecular biology. As the Human Genome (HUGO) Project completes its identification and sequence of all of the 60,000 or 100,000 human genes during the next 6-8 years, there screams the need for a standardized approach. Not only would such a system help avoid two or more laboratories from naming the same gene two different things, but it should help other colleagues in related fields as well as incoming graduate students if a gene has only one name. The Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB), the International Union of Pure and Applied Chemistry (IUPAC) and the IUPAC-IUBMB Joint Commission on Biochemical Nomenclature (***JCBN,*** stemming from a 1993 meeting in Marseille, France) have served the scientific community to help classify and name enzymes (from all organisms) since the 1950's and, in the last decade, have begun to classify genes. The JCBN has representatives from ***Swiss-Prot*** and the ***Nucleic Acid Database*** on their committee. The JCBN continues to search for satisfactory financial backing, and welcomes initiatives from groups in the research community to organize nomenclature within specialized areas. The Genomic Data Base ***HUGO/GDB*** Nomenclature team (London) is working around the clock in the naming of human genes**;** slightly more than 8,000 human genes have been named-- meaning that we are perhaps about 10 per cent of the way so far!

And that is just human genes. Already 15 genomes have been sequenced to date (a summary of 14 of these appeared in issue #12 of ***Interface***), and dozens more genomes will soon be sequenced. During the next 5-10 years, all the genes will have been identified (***and will require names!***) from**:** the small nematode *Caenorhabditis elegans*; the small plant *Arabadopsis thaliana*; the mouse *Mus musculus*; the fruit fly *Drosophila melanogaster*; zebrafish; rat; agriculturally important plants such as rice, wheat, corn and barley; and agriculturally important animals such as chicken, sheep, pig, goat, cow and horse. ***Ancestral genes***--present 600 million or 100 million years ago--will have diverged into all the animal species, or into all the plant species, listed

above**;** it therefore stands to reason that each of these ancestral genes has given way to a superfamily and that all members (orthologues, the same gene in different species) in that superfamily should be given some standardized "root" in their naming. The power of an organ-ized nomenclature system cannot be underesti-mated, nor should the need for adequate resources to establish and maintain it. Further information can be found at **http://www.gene.ucl.ac.uk/nomenclature/guide-lines.html** and **http://www.genetics.nature.com/nomen/nomen_article.html** and sites crosslinked therein.

Observations by a Biologist

Evolutionary link between crustaceans and insects

Attending a science fiction movie with my four children recently, I watched the interplanetary (human) combat troops fighting some ***arthropod*** (jointed, segmented) thing that looked like a cross between a very large mechanical cockroach and a crab. And then I wondered how insects (flies, beetles) could not be evolutionarily related to crustaceans (*e.g.* crabs, brine shrimp). But the evidence, until very recently, has not been over-whelming.

Boore *et al.* [***Nature* 392:** 667-668 (1998)] and others have been studying the complete ar-rangement of 37 genes in mitochondrial DNA (mtDNA) of twelve metazoans (invertebrates, creepy crawly things). Insects and crustaceans were found to share a derived location for the gene encoding mitochondrial leucine transfer RNA [designated *L(UUR)*], as compared with the gene's primitive location in a chelicerate (*e.g.* scorpion, horseshoe crab), four myriapods (*e.g.* centipede, millipede), an onychophoran (*e.g.* velvet worm), and several non-arthropod metazoans (*e.g.* earthworm, snail). The simplest explanation for this complex gene rearrangment is that a single translocation of the *L(UUR)* gene occurred in a common Arthropoda lineage which led--after it split from the other lineages shown--to insects and crustaceans (***Figure 3***).

If true, some features shared by insects and myriapods (*e.g.* tracheal system for respiration, unbranched legs, Malpighian tubules for excretion) then become examples of ***convergent evolution***--perhaps as adaptations to life on land. Again, this is an example of the interaction of genes and the environment. As the environment changes, so can the genome to help support that organism in its new ecological niche. After 10 or 40 generations of humans living in a smoggy city, perhaps a resistant subline will develop--one that does not develop watery eyes or asthma in response to urban pollution or cigarette smoke!

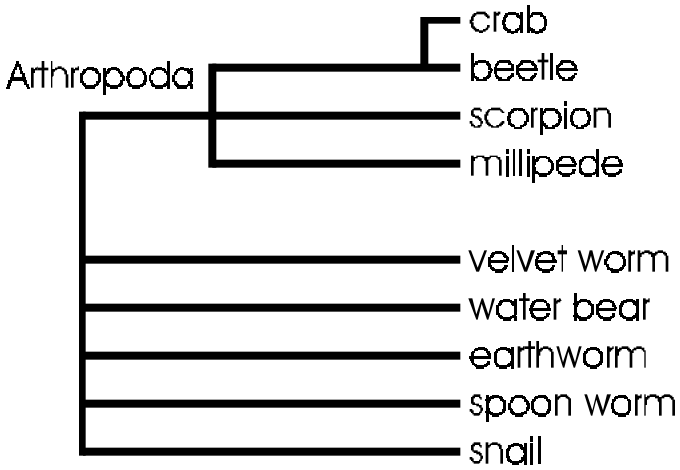

Figure 3. Simplified diagram of divergent evolution, concern-ing only the relative location of the *L(UUR)* gene for 153 taxa. This phylogenetic tree was derived from (going from ***top*** to ***bottom*****):** four crustaceans, 134 insects, two chelicerates, four myriapods, one onychophoran, one tardigrade, four annelids, one echiuran, and two gastropods. One common name for each of these nine classes is given ***at right***. The arthropod phylum is shown as diverging into four classes ***at top***.

Genotype-Phenotype Correlations: Caution!

Several studies have demonstrated a correlation between vitamin D receptor (VDR)-associated variation in osteoporosis (thinning of the bone) and risk of prostate cancer [***Biochem Biophys Res Commun*** **242:** 467-473 (1998) & refs therein]. None of the ***VDR*** gene variants, however, affects VDR function (*i.e.* binding of vitamin D_3)--suggesting quite strongly that these markers in and near the ***VDR*** gene might be in linkage disequilibrium with a nearby gene (which could be even a million base pairs away) that is actually responsible for the variation in osteoporosis and prostate cancer risk.

This is a good lesson for genetic epidemiologists**:** even though you might demonstrate "a strong correlation between a biomarker and a disease," be sure to prove that mutations (loss of function, gain of function, etc.) in that gene under consideration are actually responsible for the phenotype (trait)! The same thing has been going on with studies showing a correlation between nucleotide changes in the human *CYP1A1* gene and risk for cigarette smoke-induced lung cancer in Japanese, but not Caucasian, populations [***Pharmacogenetics*** **7**: 435-440 (1997) and refs therein].

The 18th International Congress of Genetics

The lack of logic in planning the location of international genetics society meetings continues. The 7th International Congress of Genetics was scheduled for Moscow in 1937**;** however, the triumph of Lysenkoism over genetics led to the imprisonment and disappearance of geneticists in the U.S.S.R. between 1932 and 1937 and, ultimately, cancellation of the Congress by the Communist Party. The 18th International Congress of Genetics is now scheduled for Beijing in 1998. There are serious concerns over "the eugenic legislation in China." Because "no country has yet enforced a eugenic policy that was not racist, sexist and class-biased," the Genetical Society of Britain has withdrawn from the International Genetics Federation, stating that silence by the American and Russian societies toward the Chinese eugenics law "is cowardly in the face of what promises to be the death of ... (human genetics) ... in China and the greatest perversion of genetics the world has yet seen." [***Nature Genet*** **15:** 1-2 (1997)]

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