Issue Number 5

INTERFACE:
GENES AND THE ENVIRONMENT

CENTER FOR ENVIRONMENTAL GENETICS UNIVERSITY OF CINCINNATI Spring 1995

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Antioxidants: Facts and Fantasies

Musings by a Redox Cellular Biochemist

Recently I started thinking with horror about the number of "oxidative hits" my DNA was taking each day. It's been estimated that our DNA receives 10,000 hits from ***endogenous*** oxidants alone (***i.e.*** just my normal healthy diet, including broccoli and brussel sprouts), not to mention foreign sources (***exogenous chemicals*** and ***ionizing radiation***)! Although almost all of these hits are repaired by our DNA-repair enzymes, about 1 in a thousand has been shown to become "fixed" and lead to ***mutations***. These hits are believed to accelerate such processes as aging, cardiovascular and pulmonary disease, and cancer.

There was no time to lose. At a local health store, I found a shelf of "***antioxidants***"—which might be regarded as anti-missiles that hopefully might intercept most of those daily hits on my DNA. Let's see: on the shelf were vitamins (C, E, and β-carotene), a slew of transition metals (zinc, manganese, cobalt, copper, selenium, chromium), and some enzymes (capsules of superoxide dismutase [SOD] and catalase). Wait a minute! The SOD and catalase proteins won't do well in stomach acid, and they wouldn't get absorbed anyway. And I am aware of almost-daily reports on the toxicity of transition metals. And a recent report even proclaimed that "vitamin E is a complete carcinogen."

I can therefore see in this local health store a world of partial truths and *laissez faire*—marketing at its finest. Are we toxicologists being any more truthful? Carcinogens, teratogens, toxicants: do we live in a world of muck and mire that toxicologists must save? Antioxidants *per se* must be okay, since they are a major component of the vegetables that Mom told me must be eaten before the apple pie. The American Cancer Society and the U.S. Food and Drug Administration agree with Mom and do not advocate a necessity for supplementary vitamins; these agencies maintain that dietary sources of vitamins are sufficient, and the time-proven key to good health is eating a balanced diet with lots of fresh fruits, vegetables and grains—and minimal fat. Still, many, if not most, of us researchers in the field take supplementary vitamins C and E and/or β-carotene, ***just in case***.

It seems like ***anything*** can be healthy or toxic—depending on the circumstances of exposure (dosage, route of entry into the body, genetic susceptibility in the host); this is not only true, but a basic tenet in the field of toxicology. In fact, the Northeast Regional Environmental Public Health Center publishes the BELLE (**B**iological **E**ffects of **L**ow **L**evel **E**xposures) Newsletter (School of Public Health, University of Massachusetts, Amherst, MA 01003). This provocative publication is available free-of-charge, and focuses on the concept of "***hormesis***," ***i.e.*** the phenomenon that low doses of otherwise-harmful physical or chemical agents can actually produce beneficial effects. But—in the case of antioxidants—the reverse is possible: harmful effects might occur from what we normally consider to be beneficial agents. How is this possible and what are the implications? Let's consider some basics.

The Fate of Oxygen in Our Cells

Aerobic organisms have adapted to, and are dependent upon, ambient atmospheric oxygen. Oxygen is distributed to all of our cells, via the lungs and blood, and is used by cellular enzymes such as oxidases and peroxidases to carry out normal cell metabolic functions. In most aerobic cells, oxygen is utilized by mitochondria as the terminal electron acceptor for electron transport during ATP (adenosine triphosphate) energy synthesis.

However, oxygen is a double-edged sword: we can't live without it, yet it is slowly killing us. For example, about 4% of the mitochondrial oxygen produces

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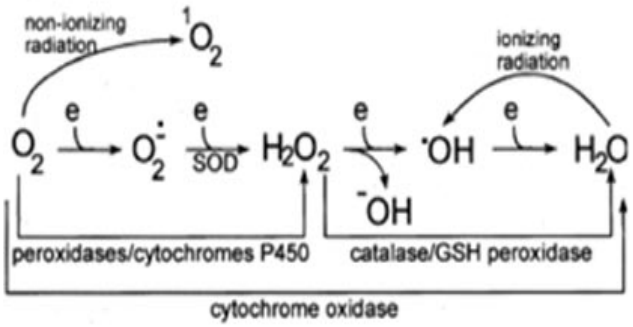
intramitochondrial superoxide. Also, hydrophobic (fat-soluble) endogenous and foreign compounds are oxidized by various cytochromes P450, enzymes which use molecular oxygen and NADPH. In the course of these reactions (***Figure 1***), oxygen undergoes two sequential 1-electron reductions by P450, some of which result in the formation of hydrogen peroxide—either directly (release and protonation of the 2-electron-reduced form of oxygen), or indirectly (release of superoxide, followed by dismutation via SOD). The hydrogen peroxide may be metabolized further to hydroxyl radical by transition metals—such as iron or copper—in a Fenton Reaction. Unlike molecular oxygen, which has limited reactivity by virtue of its electronic spin state, these semi-reduced forms of oxygen (superoxide, hydrogen peroxide and hydroxyl radical), as well as singlet oxygen, are much more reactive with cellular constituents, and hence are called reactive oxygen metabolites (ROMs).



LEGENDS TO FIGURES

*Fig. 1. Metabolism of molecular oxygen (**O_2**) to form reactive oxygen metabolites. A series of 1-electron (**e**) reductions leads, in turn, to the formation of superoxide (**$O_2^{\cdot-}$**), hydrogen peroxide (**H_2O_2**), and hydroxy radical (**$^{\cdot}OH$**). Cellular oxidases, peroxidases and cytochromes may also form **$O_2^{\cdot-}$** and **H_2O_2** directly. **H_2O_2** is degraded by catalase or GSH peroxidase, preventing formation of the strong oxidizing agent **$^{\cdot}OH$**. Cytochrome oxidase is a single iron- and copper-containing enzyme, which sequentially reduces **O_2** to **H_2O** in four 1-electron steps. Finally, non-ionizing radiation forms reactive singlet oxygen (**$^1O_2$**), while ionizing radiation is capable of producing **$^{\cdot}OH$** from **H_2O**.*

How Do Our Cells Fight against ROMs?

In addition to normal physiological ROM production, the diet and the environment contain nasty compounds that interact with cells via a 1-electron (free-radical) mechanism or a 2-electron (***electrophile-nucleophile*** interaction) mechanism. "Electrophile" means electron-, or negative charge-, loving, whereas "nucleophile" means positive charge-loving. Endogenous electrophiles may also be generated during such processes as lipid peroxidation or tyrosine "catabolism" (breakdown). Cellular defense from such an oxidizing environment depends on both **[i]** enzymic (antioxidant enzymes) and **[ii]** nonenzymic antioxidant defenses (free-radical scavengers).

The genes encoding antioxidant enzymes are known to be transcriptionally activated by antioxidants and electrophiles, and have been termed the antioxidant (or electrophile) gene battery (***Figure 2***). These metabolically-linked enzymes scavenge electrophiles and free-radical precursors, and help to enhance reduced glutathione (GSH) levels in cells. GSH, along with ascorbate (vitamin C) and vitamin E, are the major scavengers.

The action of mopping up free radicals and electrophiles results in the oxidation of cellular antioxidants, and this "voltage potential" shift in the oxidation-reduction (***redox***) status of the cell towards the oxidant side elicits an "oxidative stress response" by the cell. Is this response good or bad? The answer depends on the extent and duration of oxidative stress. Toxicity occurs when the rate of production of oxidants exceeds the rate of regeneration of cellular antioxidants—for some duration of time.

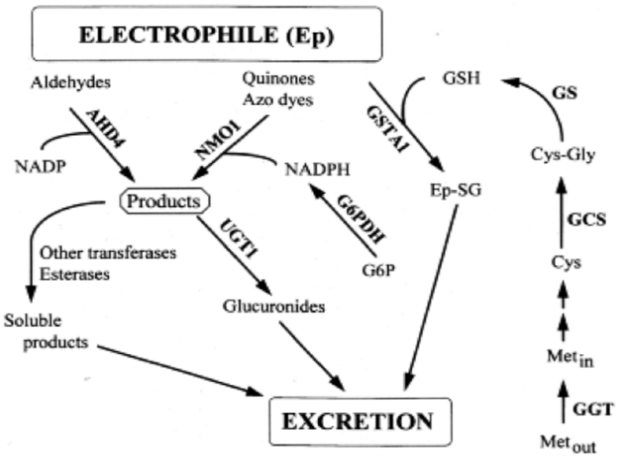


*Fig. 2. Known and postulated members of our proposed "antioxidant (electrophile) gene battery." **Known enzymes:** NMO1, NAD(P)H quinone (menadione) oxidoreductase; AHD4, the mouse Class 3 aldehyde dehydrogenase; GSTA1, glutathione S-transferase class Ya (α); UGT1, UDP glucuronosyltransferase form 1; GCS, γ-glutamylcysteine synthase. **Postulated enzymes:** GGT, γ-glutamyltransferase; G6PDH, glucose-6-phosphate dehydrogenase. Met_{in} and Met_{out}, methionine inside and outside the cell. Cys, cysteine. Cys-Gly, cysteinylglycine. GS, glutathione synthetase. GSH, reduced glutathione (glutamyl-cysteinyl-glycine). Ep-SG, glutathione conjugate of an electrophile. NADP and NADPH, oxidized and reduced forms of nicotinamide adenosine dinucleotide phosphate.*

Thank Mother Nature for GSH!

GSH represents the major regenerating cellular antioxidant. GSH serves a number of physiologic roles—such as storage and transport of cysteine, amino acid transport, metal homeostasis, and protein stabilization and protection. GSH is a "soft electrophile" and reacts both enzymically and nonenzymically as the thiol (**RSH**) or the thiolate anion (**RS^-**) This means that GSH is capable of reacting with many different types of chemicals, including molecules containing double bonds beta to a carbonyl group (Michael Reaction acceptors), azo compounds and organic peroxides (weak oxidants), molecules with a suitable leaving group (nucleophilic displacement),

inorganic oxides acting as strong oxidants, transition metals, and free radicals. By its large variety of interactions, GSH is able to act either as a protectant or as a signal-transducing molecule.

As a protectant, GSH has been implicated in decreasing cellular toxicity—resulting from the exposure to a variety of chemical and electromagnetic agents, which would include halogenated aromatics and aliphatics, quinones and quinone imines, polycyclic aromatic and azo compounds, and ionizing and non-ionizing radiation. GSH also protects against endogenous ROMs, since the selective depletion of mitochondrial GSH is known to be immediately cytotoxic. Furthermore, oxidized vitamins C and E are able to be reduced and regenerated by GSH.

Vitamin C is the major free-radical scavenger in the cytosol and, as such, has a short half-life (meaning we should take it every few hours, for maximal "beneficial vitamin C effect"). Vitamin E is the major free-radical scavenger in the lipid environment of cellular membranes, and, as such, has a long half-life (meaning we can take it once or twice daily, for maximal "beneficial vitamin E effect"). Vitamin E guards against the ROM-mediated loss of membrane functions, including cellular and organellar compartmentation, and the housing of receptors, enzymes and ion/solute translocators.

GSH is also protective by way of its thiol-buffering activity in biological systems. Many enzymes contain redox-sensitive thiols in the form of cysteinyl residues. These residues are in equilibrium with GSH/GSSG (reduced vs oxidized glutathione) via enzymic and nonenzymic pathways. The activities of several such enzymes have been shown to be reversibly stimulated or inhibited, depending on the thiol status of the medium. During oxidative stress, the GSH/GSSG ratio decreases—resulting in formation of protein-glutathione mixed disulfides, which actually protect the cysteinyl group from further metabolism that might result in irreversible damage.

Intranuclear GSH

In addition to the freely-diffusible GSH present in all the cellular compartments, we know that there is a few percent of the total GSH that is tightly bound to chromatin. Since GSH contains a glutamate residue (having a negative charge), it is not surprising that it interacts with positively-charged basic histone proteins. It is thus tempting to speculate that this chromatin GSH might very likely be involved in general DNA protection against those would-be oxidative hits that had escaped all the prior scavenging (***i.e.*** at the cell membrane and throughout the cytoplasm). Such a hit to DNA might also be secondary to peroxidation of nearby lipids, by producing lipid peroxides that could react with chelated iron and forming diffusion-limited hydroxyl radicals in the vicinity of DNA.

Less direct, but more intriguing, is the possibility that chromatin GSH is involved in oxidative signal transduction for gene regulation—by acting as a sentinel and/or a direct regulator of transcription. Activation of the electrophile gene battery is known to be down-regulated, or abolished, by reducing thiols. Thus, chromatin GSH could act as a general redox sensor by maintaining the conformation of DNA or, even more likely, the conformation of DNA-binding proteins. Specificity would be achieved by the nature of the specific DNA-binding proteins and their interaction with specific DNA response elements (motifs) that are known to govern gene expression.

Epilogue

Well, I bought those bottles of SOD and catalase from the health store. My skepticism about their activity was ill-founded, because an assay in my laboratory revealed that both bottles had lots of enzyme activity. I started taking them last week and have already felt my GSH levels rising. I'm sure it has been several days since my DNA got its last oxidative hit.

Suggested Further Reading

Aruoma OI, 1994, Nutrition and health aspects of free radicals and antioxidants. *Fd Chem Toxicol* **32,** 671-683

Barber DA, Harris SR, 1994, Oxygen free radicals and antioxidants: a review. *Am Pharmacy* **Ns34,** 26-35

Bray TM, Taylor CG, 1993, Tissue glutathione, nutrition, and oxidative stress. *Canad J Physiol Pharmacol* **71,** 746-751

Liebler DC, 1993, The role of metabolism in the antioxidant function of vitamin E. *CRC Crit Rev Toxicol* **23,** 147-169

Rice-Evans CA, Diplock AT, 1993, Current status of antioxidant therapy. *Free Radicals Biol Med* **15,** 77-96

Sohal RS, 1993, The free radical hypothesis of aging: an appraisal of the current status. *Aging* **5,** 3-17

—Contributed by Howard G. Shertzer (with many thanks to Vasilis Vasiliou and Dan Nebert)

Reader Response

Do you wish to contribute an article or news to an upcoming issue of the Interface newsletter? CEG members and others, we would like to know about any awards, publications, invited seminars, and other accomplishments. Letters to the editor are most welcome. Please fill in the information and submit text to address below.

☐ Editorial ☐ People in the News

☐ Letters to the Editor ☐ CEG Members: In the News

☐ SCIENCE LITE ☐ Other

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Changes in the redox equillibrium of tea leaves affect the taste of tea

On the same subject as our lead article, the balance of oxidation and reduction is also important in plants. Like fine wines, each tea has a distinct aroma and taste, certain properties that separate if from the rest, and , interestingly, oxidation of the plant leaves, or lack thereof, are critical in the final taste! Basically there are four distinct categories of the brew:

Black Teas are classic English brews, strong and often drank with the addition of lemon, milk or sugar. They are made by exposing the leaves to air, which turns them to a dark color resulting in a hearty brew (e.g. Ceylon teas, Keeman).

Green Tea are produced by steaming the leaves to prevent oxidation, their fragrance at first being fresh and grassy before changing to very sweet. When brewed, they are lightly colored and fresh tasting and sipped plain (e.g. all Japanese and many Chinese teas).

Oolong Teas are made from partially oxidized leaves. The brew is light brown in color, delicately flavored, and, typically, fruity and mellow, and actually tastes a bit like asphalt (e.g. Ti Kuan Yin from China).

Herbal Teas have become extremely popular because of the belief that they have curative properties (e.g. camomile, peppermint, verbina).

LETTERS

FURTHER DISCUSSION OF VARIOUS TOPICS

[1] "Generally considered safe, pyridostigmine (used by Persian Gulf personnel during the Gulf War) has been used in clinical medicine for decades in patients with myasthenia gravis, in doses up to 6,000 mg/day for life. No significant long-term adverse effects have been noted in these patients. Significant drug interactions that might heighten acute or chronic toxicity were not documented in the U.S. forces. Exposure to pesticides might enhance acute effects of pyridostigmine, but are unlikely to have caused chronic effects at these doses." --*The Persian Gulf Experience and Health*, National Institutes of Health Technology Asessment Workshop Statement (April 27-29, 1994)

[2] "It has been hypothesized that organochlorine pesticides and other environmental and dietary estrogens may be associated with the increased incidence of breast cancer in women and decreased sperm concentrations and reproductive problems in men. However, elevations of organochlorine compounds such as dichlorodiphenyl-dichloroethylene (DDE) and polychlorinated biphenyls (PCBs) in breast cancer patients is not consistently observed. Re-analysis of the data, showing that male sperm counts decreased by more than 40% during 1940 to 1990, indicates that inadequate statistical methods were used and that the data did not support a significant decline in sperm count.

Humans are exposed to both natural and industrial chemicals which exhibit both estrogenic and anti-estrogenic activities. For example, bioflavinoids, which are widely distributed in foods, and several industrial compounds, including organochlorine pesticides and various phenolic chemicals, exhibit estrogenic activity. Humans are exposed to chemicals which inhibit estrogen-induced responses such as the aryl hydrocarbon receptor (AHR) agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin and related chlorinated aromatics, polynuclear aromatic hydrocarbon combustion products, and indole-3-carbinol, which is abundant in cruciferous vegetables. Many of the weak estrogenic compounds, including bioflavinoids, are also anti-estrogenic at some concentrations.

A mass balance of dietary levels of industrial and natural estrogens, coupled with their estimated estrogenic potencies, indicates that the dietary contribution of estrogenic industrial compounds is 0.0000025% of the daily intake of estrogenic flavinoids in the diet. Moreover, dietary levels of anti-estrogen equivalents (industrial or natural) are significantly higher than the estrogen equivalents of organochlorine pesticides. Therefore, the suggestion that industrial estrogenic chemicals might contribute to an increased incidence of breast cancer in women and male reproductive problems is not plausible." --Save SH, 1995, Environmental and dietary estrogens and human health: Is there a problem? ***Environ Health Perspect* 103**, 346-351.

[3] Speaking at an April meeting of the American Chemical Society in Anaheim, California, Marcus E. Brewster (a chemist at Pharmos Corp. in Alachua, Florida) described a new anti-cancer agent, tamoxifen methiodide (TMI). TMI dissolves more easily in water than the estrogen antagonist tamoxifen, which blocks estrogen's stimulation of cell division in many breast tumors. The increased water solubility of TMI impedes its ability to enter the brain and disturb central nervous system functions. Large decreases in the size of mammary tumors occurred in 90% of the mice treated with TMI.

An unexpected beneficial effect was that TMI strengthens bones without overstimulating the uterus, although the mechanism for this action is not clear. Tamixofen does not have this effect. Clinical trials of TMI may begin within the year.

SCIENCE LITE

The Rabbit, the Fox and the Wolf -- A Fable

One sunny day a rabbit came out of her hole in the ground to enjoy the weather. The day was so nice that the rabbit became careless, and a fox sneaked up behind her and caught her. "I am going to eat you for lunch!" said the fox.

"Wait!" replied the rabbit, "You should at least wait a few days."

"Oh yeah? Why should I wait?"

"Well, I am just finishing writing my Ph.D. thesis."

"Hah! That's a stupid excuse. What is the title of your thesis, anyway?"

"I am writing my thesis on 'The Superiority of Rabbits over Foxes and Wolves.'"

"Are you crazy? I should eat you up right now! Everybody knows that a fox will always win over a rabbit."

"Not really. Not according to my research. If you like you can come to my hole and read it for yourself. Then, if you are still not convinced, you can go ahead and have me for lunch."

"You really are crazy!" But, since the fox was curious and felt that he had nothing to lose, he went with the rabbit into her home. The fox never came out again.

A few days later, the rabbit was again taking a break from writing, and sure enough, a wolf came out of the bushes and was ready to eat her.

"Wait!" yelled the rabbit, "You cannot eat me right now."

"And why might that be, you fuzzy appetizer?"

"I am almost finished writing my Ph.D. thesis on 'The Superiority of Rabbits over Foxes and Wolves.'"

The wolf laughed so hard that he almost lost his hold on the rabbit. "Maybe I shouldn't eat you. You are really sick in your head. You might have something contagious," the wolf opined.

"Come read it for yourself. You can eat me after that, if you disagree with my conclusions."

So the wolf went to the rabbit's hole and never came out again.

The rabbit finished writing her thesis and was out celebrating in the lettuce fields. Another rabbit came by and asked, "What's up" You seem to be very happy."

"Yup, I just finished writing up my dissertation."

"Congratulations! What is it about?"

"It is titled 'The Superiority of Rabbits over Foxes and Wolves.'"

"Are you sure? That subject doesn't sound right."

"Oh yes, you should come over and read it for yourself."

So they went together into the rabbit's hole As they went in, the friend was the typical graduate student abode -- albeit a rather messy one after the student having just written a thesis. The word processor with the controversial dissertation was in one corner, on the right there was a pile of fox bones, on the left there was a pile of wolf bones, and in the middle **sat a large lion.**

The moral of the story: "the title of your dissertation doesn't matter; all that matters is who your thesis advisor is.

Meeting Report

International Genetic Epidemiology Society
Fourth Annual Meeting, Snowbird, UT, 1995

John J. Milvihill, president of the International Genetic Epidemiology society, gave his address on "The ecogenetics of human cancer: Inspiration and perspiration." He proposed development of Cooperative Genetic Disease Study Groups (GDGs) between institutions with an organizational structure that includes a headquarter, data analysis and statistical functions, and spearate PI's for interinstitutional studies. This year's meeting had three sessions with one fully devoted to "Gene-Environmental Interactions." Abstracts from this conference are available in the ***Journal of Genetic Epidemiology*** **12(3)**, 1995.

- Toward the Cloning of Lead Resistant/Susceptibility Alleles - IL Cartwright
- Target Disruption of the $p27^{K1P1}$ Gene: A Possible Tumor Suppressor - TC Doetschman
- Interaction of the Environmental Estrogens with the Estrogen Receptor: Implications in Risk of Breast Cancer - S Khan
- Can Quantitative Trait Linkage (QTL) Be Used to Find Genes Responsible for Differential Sensitivity to Environmental Agents?--AG Menon
- Molecular Drive: Ability of Environmental Chemicals to Drive Evolution in a Vertebrate Species--DW Nebert
- Aryl Hydrocarbon - Induced Lymphocyte Apptosis as a Determinant of the Immune Repertoire--CL Sidman

CEG Members in the News

The Alumni Association of the University of Cincinnati College of Medicine "Reunion '95," (May 1995), celebrating the 175th Anniversary of the college and the University, included a scientific session of six speakers. Three of these -- **Jeff Whitsett**, **Peter Stambrook** and **Dan Nebert** -- are CEG members. Jeff and Dan had also been invited speakers at the alumni reunion scientific session last year.

Iain Cartwright has recently had the following paper published: Robulski KR, Cartwright IL, 1995, Multiple interacting elements delineate an ecdysone-dependent regulatory region with secondary responsive character. ***J. Mol Biol* 249:** 298-318.

Kathleen Dixon was invited to speak on "Mutagenesis in a mammalian in vitro DNA replication system," at the Gordon Research Conference (New London, New Hampshire), June 1995.

Joanna Groden was invited to speak on "Inherited susceptibility to cancer," at Medical Grand Rounds, University of Cincinnati (Cincinnati, Ohio), March 1995, and on "The biology of APC, a human tumor suppressor," at the University of Toledo (Toledo, Ohio), May 1995.

Dan Nebert has been invited as a plenary speaker at the 2nd International Congress on "Hazardous Waste: Impact on Human and Ecological Health," (Atlanta, Georgia), June 1995. He has also been invited as a platform speaker at the Symposium on "Gene Families, Structure, Function, Genetics and Evolution," at the 8th International Congress on Isozymes (Brisbane, Australia), June 1995.

Alvaro Puga will speak on "Alteration of calcium homeostasis and arachidonate metabolism by dioxin," at the 15th International Symposium on Dioxin and Chlorinated Compounds (Edmonton, Canada) August 1995. He will also give a plenary lecture on "Modulation of oxidative stress response by Y-box factors," at the 2nd Xenobiotic Metabolism and Toxicity Workshop of Balkan Countries (Ioannina, Greece), October 1995.

Carol Rice has been asked by the Mine Safety and Health Administration (MSHS) to serve on the National Silica Advisory Board, which is charged with evaluating the procedures to evaluate silica exposures.

David Warshawsky will give a lecture on "Comparison of benzo[a]pyrene-DNA adducts with 7H-dibenzo[c,g]carbazole-DNA adducts in target and nontarget organs of mice exposed chronically by topical application," at the 15th International Symposium on Polycyclic Aromatic Compounds (Belgirate, Italy), September 1995.

RECENT CEG-SPONSORED SPEAKERS

FEBRUARY 22, 1995
Muin J. Khoury, MD, PhD
Deputy Chief, Birth Defects and Genetic Diseases Branch
Division of Birth Defects and Developmental Disabilities
Center for Environmental Health and Injury Control
Center for Disease Control
Atlanta Georgia
"Epidemiologic approaches to gene-environment interactions"

APRIL 10 & 11, 1995
John Cairns, DM
Emeritus Professor, Harvard School of Public Health
Clinical Trial Service Unit
Harkness Building
Radcliffe Infirmary
Oxford OX2 6HE, England
"Control of the human environment and the prevention of untimely death,"
"The causes of spontaneous mutation and their role in human cancer"

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