Issue Number 3

INTERFACE:
GENES AND THE ENVIRONMENT

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Environmental Estrogens: Real or Imagined Threat?

For decades scientists have known that the female sex hormone estrogen is involved in the development of both female and male sexual organs in the embryo and fetus, and that estrogen plays a major role in breast development in the pubescent female and in the 30 or more years of menstrual cycles in women.

Scientists have also known that certain environmental agents can mimic the effects of estrogen. A classic example is the insecticide 2,2-***bis***(***p***-chlorophenyl)-1,1,1-trichloroethane (***p, p'-DDT***), which was banned from use in the U.S. in 1972 after it had been shown to affect egg shell development in several marine bird and turtle species; DDT was found to act as an antagonist of estrogen (***i.e.*** displacement of estrogen from its receptor), leading to the deleterious effect on egg shell formation. Another example is the drug ***diethylstilbestrol*** (**DES**), which was administered early in pregnancy to millions of women between 1948 and 1971 in order to prevent spontaneous abortions. Boys of such pregnancies were then found to have a higher risk of undescended testes and abnormally small penises, whereas girls were found to have a much greater risk of vaginal deformities including an unusual form of vaginal cancer. There have been a couple of newspaper reports that the grandchildren of DES-treated women are also at greater risk for these afflictions.

What other "estrogen-like" environmental chemicals are out there? ***Polychlorinated biphenyls*** (**PCBs**) are chlorine-containing industrial compounds (used in capacitors and transformers, hydraulic fluids, heat-transfer fluids, platicizers and carbonless copy paper). Certain PCB isomers have been shown to be estrogenic. PCBs were responsible for "Yusho" in Japan in 1968 and "Yucheng" in the Taichung province of Taiwan in 1979; these diseases include dermatologic manifestations and metabolic abnormalities caused by the consumption of PCB-contaminated rice oil. ***Polybrominated biphenyls*** (**PBBs**), a flame retardant mistaken as a nutrient by farmers and ingested by livestock in Michigan in 1973, induced effects quite similar to those of heavy PCB exposure. Although no longer allowed to be used in the U.S., PCBs and PBBs are commonly found in human fat tissue and breast milk and in cow's milk. ***Bis-phenol-A*** is a breakdown product of polycarbonate plastics, found in baby bottles and plastic water/juice containers. ***Endosulfan*** is a pesticide used on vegetable crops in the U.S. ***Nonylphenols*** represent a class of chemicals found in polystyrene tubing used for blood transfer, as well as hair-coloring products, spermaticides and other toiletries. 2,2-***bis***(***p***-chlorophenyl)-1,1,1-dichloroethane (***DDE***), the major breakdown product of DDT, is also an estrogenic contaminant of ***dicofol***, an insecticide currently applied to food crops in the U.S. ***Heptachlor***, ***dieldrin***, ***lindane*** and ***kepone*** are additional estrogenic pesticides.

A number of plants that we eat (***e.g.*** soybean; milk from clover-fed cows) contain high levels of certain isoflavones having estrogenic activity, called ***phytoestrogens***. Urinary phytoestrogens are higher in vegetarian women than in women who include meat in their diet.

What putative effects of environmental estrogens have been noted? In addition to DDT effects on egg development, a wildlife biologist in Florida noted 3 years ago that alligator eggs were failing to hatch and that there was a preponderance of abnormally small penises among male alligators. Reports in the ***British Medical Journal*** (1992) and ***The Lancet*** (1993) showed that human sperm counts appeared to have dropped by 30% to 50% worldwide during the last 60 years. One re-analysis of these

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studies (***British Medical Journal***, December 1992) found that sperm counts might have actually increased slightly since 1970, implying that the decline in sperm levels occurred primarily between 1940 and 1970; some have proposed that these data might be explained by increases in venereal diseases and other confounding variables.

Some reports have attempted to link environmental estrogens with male gonad cancer. For example, there appears to be an increase worldwide in testicular cancers during the past 50 years.

How might environmental estrogens alter sexual development and cause cancer? It is well known that levels of the most potent estrogen hormone ***estradiol*** (***Figure 1***), and presence of a functional high-affinity estrogen receptor, are correlated with increases in the risk as well as the progression of human breast cancer. Intriguingly, there is a strong correlation between elevated estradiol 16α-hydroxylase activity in human breast tissue and increased risk of breast cancer. The location of the 16α-hydroxylase is in the human mammary "terminal duct lobular units" (TDLUs), which are common sites of breast tumor formation. Estradiol 16α-hydroxylase is involved in the breakdown of estrogen, which would presumably result in lowered estrogen concentrations. This finding, therefore, seems paradoxical. Why would elevated levels of this enzyme be associated with greater cancer risk?

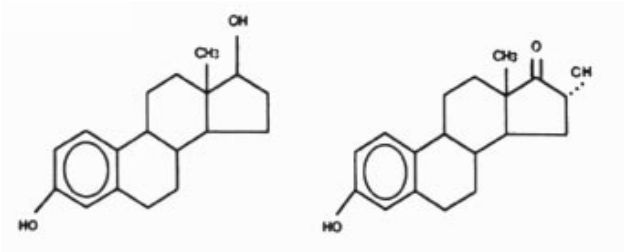


Fig. 1. Chemical structures of estradiol, the most potent ligand for the estrogen receptor (left), and 16α-hydroxyestrone, the oxidative end-product following 16a-hydroxylation (right).

What are the oxidative products of estradiol? There are at least three oxidative pathways of estradiol metabolism: C17-oxidation to ketone, C2-hydroxylation and C16α-hydroxylation. The 16α-hydroxy product readily rearranges nonenzymically to form 16α-hydroxyestrone (***Figure 1***). Interestingly, hormone replacement for menopausal women consists of a cocktail of several estrogens, but primarily 16α-hydroxyestrone. The cytochrome P450-mediated C2-hydroxylation of estradiol is principally carried out by the dioxin-inducible CYP1A2, detectable in the liver of everyone and shown to be inducible in the liver of smokers. In the rat and in cell culture, dioxin or polycyclic aromatic hydrocarbon treatment has been shown to cause decreased estrogenic activity. There are at least three possible explanations for this effect. **(i)** Cigarette smoking women are well known to have lower urinary estrogen levels than nonsmokers. Because dioxin and cigarette smoke dramatically enhance estradiol 2-hydroxylation, exposure to these agents might result in fewer functional estrogen molecules available to bind to the estrogen receptor and, therefore, an ***anti-estrogenic*** response (***Figure 2***). **(ii)** Dioxin also appears to cause down-regulation of the estrogen receptor. **(iii)** It has also been reported that dioxin, when bound to its receptor complex upstream of certain estrogen-responsive genes, actually prevents the proper binding and action of the estrogen receptor complex.

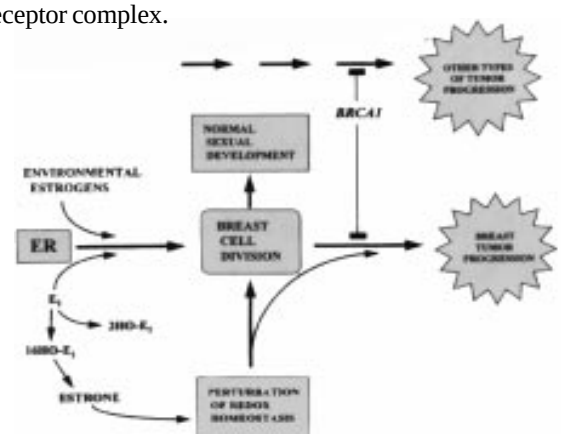


Fig. 2. Diagram by which environmental estrogens might alter sexual development and/or cause breast cancer. ER, estrogen receptor. E2, estradiol. 16OH-E2 and 2OH-E2, 16α-hydroxyestradiol and 2-hydroxyestradiol, respectively. Estrone, 16α-hydroxyestrone. BRCA1, the recently cloned human early-onset breast cancer tumor suppressor gene, also believed to play a role in early-onset ovarian and prostatic cancer.

Possible genetic polymorphisms affecting estrogen metabolism. In the mouse, CEG researchers have found by in situ hybridization that basal (constitutive) CYP1A2 levels are detectable in liver and that dioxin-inducible CYP1A2 can be detected in liver, lung, and duodenum. As described in the last issue of ***INTERFACE***, human polymorphisms have been described for both the basal CYP1A2 levels and the dioxin-inducible CYP1A2. Certainly, these polymorphisms are likely to contribute to the "genetic noise" present in any epidemiologic studies about environmental estrogens and sexual development and/or breast cancer. To date, no human polymorphism of the estradiol 16α-hydroxylase has been reported. There is considerable variation in this 16α-hydroxylase activity, however, suggesting that a polymorphism will be found; knowledge of this polymorphism on the DNA level might lead to a simple DNA test that would be beneficial in helping to predict risk of human breast cancer.

A positive association (though not statistically significant) has been observed between DDT levels and breast cancer risk in Caucasian and African-American women, but this association was not seen among Asian women. Such epidemiologic studies would further support the likelihood that genetic polymorphisms contribute to breast cancer.

How might increased estradiol 16α-hydroxylase activity be associated with greater risk of breast cancer? The C16α-hydroxylation leads to formation of the metabolite 16α-hydroxyestrone (***Figures 1 & 2***). Treatment of cells with

16α-hydroxyestrone has been shown to cause oxidative stress and DNA damage. A perturbation in oxidation-reduction (redox) homeostasis can signal particular cell cycle components (***e.g.*** cyclin D1) either to enhance cell division or cause apoptosis (programmed cell death). The result of this can be increased breast cell proliferation, followed by tumor progression (a hallmark of which is genomic instability) (***Figure 2***).

Receptor specificity. If one considers the chemical structures of any "true" endogenous ligand for its receptor, it is virtually impossible to predict which chemical structure might cause an ***agonistic*** or an ***antagonistic*** action by the ligand-receptor complex. For example, estriol and estrone both bind to the estrogen receptor, and their cooperative binding is dependent on both temperature and receptor concentration. It is also possible that 16α-hydroxyestradiol (the product of the 16α-hydroxylase reaction) or 16α-hydroxyestrone (the oxidative product of 16α-hydroxyestradiol) might bind to the estrogen receptor, albeit at one or more orders of magnitude less binding affinity than estradiol. The insecticide kepone—with its chlorinated box-like structure that looks like it is totally unrelated to estrogen—acts as an agonist to the receptor and exhibits estrogenic activity.

Changes in breast development during pregnancy apparently play a role in breast cancer later. Numerous epidemiologic studies have shown that women who have never been pregnant (nulliparous) are more prone to breast cancer. A 1994 study in the ***Journal of the National Cancer Institute*** showed that women who had had abortions after 8 weeks' gestation were at greater risk for breast cancer than women who have gone through complete pregnancies. Dramatic changes in estrogen/progesterone ratios during pregnancy might, therefore, be critical in "fixing," or initiating, the formation of breast tumors—which then progress to malignancy later in life (***Figure 2***).

What does the BRCA1 gene have to do with all of this? Last October the human ***BRCA1*** tumor suppressor gene on chromosome 17q was cloned and identified. The ***BRCA1*** gene is not closely related to any other gene sequence that has been described, although the gene product is most likely a transcription factor. A second hereditary breast cancer locus, ***BRCA2*** on chromosome 13 near the tumor suppressor retinoblastoma (***RB1***) gene, is close to being cloned and characterized (in addition to female breast cancer, ***BRCA2*** likely has a role in male breast cancer). While the ***BRCA1*** gene has gained a lot of publicity, only about 5% of breast cancer can be attributed to a defect in this gene. The risk of breast cancer in ***genetically susceptible women*** (***i.e.*** heterozygotes, or women who carry only one "good" copy of ***BRCA1***) is estimated to be 37% by age 40, 66% by age 55, and 82% over a lifetime. The ***BRCA1*** gene also appears to play a role in early-onset ovarian and prostatic malignancies.

Tumor suppressor genes are believed to "control" normal growth, or to block uncontrolled growth. One could speculate that heterozygous individuals having only one good copy of the ***BRCA1*** gene, who then went on to lose the one good copy of that gene, might include professional golfer Heather Farr (breast cancer at age 28), rock singer Frank Zappa (prostatic cancer at 52), and television actor Bill Bixby (prostatic cancer at age 54).

Are the concentrations of environmental estrogens relevant to humans? Lastly, there have been numerous discussions at several recent meetings, as well as in the literature, as to whether the levels of environmental estrogens are sufficient to be involved in causing changes in sexual development and/or increases in cancer (discussed, for example, in the "Letters" section of ***Science***, 28 October 1994). Investigators such as Bruce Ames, Stephen Safe and Linda Birnbaum say that all of the environmental estrogens are "weak agonists," having poor affinity for the estrogen receptor, and that the "basic pharmacology doesn't add up." On the other hand, investigators such as Larry Hansen and Heiko Jansen suggest that the basic pharmacology might add up, after all. Human blood estrogen levels are found in cycles between 0.03 and 0.50 nanograms/milliliter (ng/ml). Although PCBs have 100- to 10,000-fold poorer affinities for the estrogen receptor, PCBs are present in human blood at levels between approximately 2 and 8 ng/ml. In addition, all ligands of the estrogen receptor are remarkably hydrophobic (not soluble in water). Among the most hydrophobic, DDT bioaccumulates in fat cells and seeps out slowly—explaining the worldwide persistence of this environmental pollutant in animals, even in Antarctic penguins.

It is clear that all of the answers are not yet in. This article was written to demonstrate how exciting, and how controversial, this research field is at the present time.**—Contributed by Daniel W. Nebert**

LETTERS

RESPONSES TO VARIOUS QUESTIONS

Q A *Msp* I polymorphism (which means two or more subsets of some trait in the population), having to do with a *Msp* I site 450 bases downstream of the human cytochrome P450 *CYP1A1* gene, has been shown to be correlated with lung cancer in Japanese cigarette smokers but not in Caucasian smokers. What is the relationship, if any, between the *Msp* I polymorphism in the *CYP1A1* gene and humans having the high- vs low-affinity Ah receptor that you described in issue #2 of *INTERFACE*?

A *This is an excellent question. If there is a direct relationship between Ah receptor affinity and the* ***Msp*** *I site downstream of the human* ***CYP1A1*** *gene, it might have something to do with the efficiency, or the rate, at which the* ***CYP1A1*** *gene is transcribed under control by the Ah receptor complex; more work will need to be done to demonstrate this. Presence of the* ***Msp*** *I site 450 bases downstream of the human* ***CYP1A1*** *gene leads to a 1.9-kilobase (kb) restriction fragment (M2 genotype), while absence of the* ***Msp*** *I site leads to a 2.5-kb fragment (M1genotype). The frequency of the M2 allele in Japan is 0.31, whereas in Caucasians the M2 allele frequency is 0.12. These data indicate that the frequency of the M2/M2 homozygote would be 96 per 1000 among Japanese and only 14 per 1000 among Caucasians. This would suggest that more than 2,700 Caucasians would need to be screened in order to reach the same conclusions that had been made from a Japanese study of 400 subjects.*

The M2 allele has been found by Kawajiri and coworkers in Japan to be in linkage disequilibrium (which means no chromosomal crossovers) with an A—>G polymorphism at codon 462 in the ***CYP1A1*** *gene, resulting in an isoleucine-to-valine (I462V) substitution. This amino acid difference (which is in the enzyme active-site) has been shown by Japanese workers to increase the CYP1A1 metabolism and mutagenesis of benzo[a]pyrene by about 50%. The linkage disequilibrium has not been found to be absolute in the Japanese population studied, however, and, in collaboration with a Bethesda group, CEG researchers have even found a lack of absolute linkage between the M2 allele and the A—>G polymorphism in 15 members of the same 3-generation family! Thus, the final answers about this human* ***CYP1A1*** *polymorphism are not yet in.*

Q **In issue #2 you described a possible link between dioxin exposure and atherosclerotic heart disease. Since the human polymorphism was discovered as the result of the mouse Ah receptor polymorphism uncovered earlier, is there any evidence in mice for an association between dioxin and heart disease?**

A *This is a very good question. A 1986 report by Bev Paigen and coworkers, using various crosses of genetically different mice, showed that 3-methylcholanthrene (3MC)-enhanced atherosclerosis was closely correlated with presence of the high-affinity Ah receptor allele. Their subsequent work in 1987 and 1989 showed two modifier genes,* ***Ath-1*** *and* ***Ath-2****.*

Ed Benditt's research in the 1980's suggested that atherosclerotic plaques are similar to tumors, in that they are monoclonal and might arise by a mutational event in a single cell. CEG researchers have found that the CYP1A1 enzyme exists in endothelial cells of mouse arteries throughout the body, suggesting that CYP1A1-mediated reactive metabolites of 3MC could be capable of causing mutations directly in arterial endothelial cells. Mutations are the result of DNA damage, ***i.e.*** *genotoxicity.*

A nongenotoxic mechanism of atherosclerosis might also be offered. Not only dioxin (which is virtually not metabolized), but also polycyclic hydrocarbons such as 3MC and benzo[a]pyrene, have been shown by CEG research to act via an Ah receptor-mediated nongenotoxic signal transduction pathway that includes the ***Fos*** *and* ***Jun*** *proto-oncogenes. This pathway might also be involved in cell type-specific differentiation, programmed cell death (apoptosis), and proliferation (cell division). Somewhere in here might be the relationship between dioxin (which is hardly metabolized at all and is not genotoxic or mutagenic in the Ames* ***Salmonella****/liver microsomes in vitro test) and cardiovascular defects seen in the dioxin-exposed population of Seveso, Italy.*

Q **Dioxin has been shown to be carcinogenic in laboratory rodents, and to be associated with endometriosis in monkeys. However, how strong is the evidence for dioxin-induced disease in humans?**

A *This subject remains controversial, as one can find from reading the latest draft by the U.S. Environmental Protection Agency (EPA) and the objections by scientists to the latest EPA conclusions. For your further reading enjoyment on this subject, may I refer you to the following news articles and letters-to-the-editor:* ***Science*** *263, 1545-1546 (18 Mar 94);* ***Science*** *264, 1071 (20 May 94);* ***Nature*** *371, 272 (22 Sept 94);* ***Science*** *266, 349-352 (21 Oct 94);****Science*** *266, 1141-1145 (18 Nov 94); and* ***Science*** *266, 1628-1629 (?? Dec 94).*

CEG Members in the News

Grace Lemasters spoke on "The prevalence of work-related disease in carpenters" at the NIOSH Symposium on Efforts to Prevent Injury and Disease Among Construction Workers (Cincinnati, Ohio), July 1994.

Glenn Talaska accepted an invitation to participate on a national committee sponsored by the Health Resources and Services Administration (HRSA; Washington, D.C.) to develop model internship programs for environmental health students. He was also interviewed by and quoted in the October 1994 issue of Discover magazine, in which an article entitled "Fast Enzymes and Cancer" described his recent work.

Gwen Choi successfully passed the specialty board exams and achieved Diplomate status in the American College of Laboratory Animal Medicine (ACLAM) July 1994.

Vicki Hertzberg has been elected to chair the Biometrics Section of the American Statistical Association, beginning in 1995 for a three-year term. She presented the paper "Occupational epidemiology in the era of total equality: Challenges for exposure assessment" at the Joint Statistical Meetings (Toronto, Canada), August 1994.

Jonathan Bernstein presented a talk entitled "Occupational asthma caused by low molecular weight chemicals" at the National Workers' Compensation and Occupational Medicine Conference (Hyannis, Massachusetts), July 1994.

Gordon Livingston was co-author of a paper recognized during the presentation of the Alice Hamilton Science Awards for Occupational Safety and Health at NIOSH (Morgantown, West Virginia), April 1994. The paper, entitled "Cytogenetic effects of formaldehyde exposure in students of mortuary science," was published in Cancer Epidemiology Biomarkers and Prevention (1993) and received an honorable mention as one of 16 papers nominated for the award honoring the first American physician to devote her life to the practice of industrial medicine.

Carol Rice has joined the Occupational Studies Branch at the National Cancer Institute in Bethesda MD, for a one-year sabbatical beginning 1 September 1994. She has recently been appointed to the External Advisory Board for tetraethyl lead study being conducted at The Johns Hopkins University. In addition, she has been appointed to the General Motors/United Auto Workers' Occupational Health Advisory Board, which assists the National Joint Committee in the evaluation of hazards, review of applications for research funds, and recommendations for actions to monitor or reduce hazards.

Jeffrey Whitsett received the first Julius H. Comroe Jr. Award in pulmonary physiology and presented a keynote address to the Federation of the American Societies for Experimental Biology (FASEB), (Anaheim, California), Spring 1994.

Dan Nebert was invited to organize one of the 59 symposia and act as co-chairman of that session during the 7th Annual Meeting of the International Union of Pharmacology (IUPHAR). Five speakers participated in this symposium on "Signal transduction and drug metabolism" (Montreal, Canada), July 1994. In addition, Dan Nebert was elected in October 1994 to the rank of American Association for the Advancement of Science (AAAS) Fellow, to be awarded next February in the AAAS Annual Meetings in Atlanta.

Alvaro Puga will lecture on "The role of molecular genetics in risk assessment" at the Fifth International Symposium on Biological Reactive Intermediates (Munich, Germany), January 1995.

David Warshawsky participated as an invited speaker, lecturer and official reviewer of a dissertation defense at the University of Kuopio (Kuopio, Finland), October 1994.

Lisa Loberg, graduate student of **Kathleen Dixon**, presented her work on cellular responses to ionizing and UV radiation at the First Round Table Meeting on Ataxia Telangiectasia Research, sponsored by the A-T Children's Project (Boca Raton, Florida), August 1994.

Evolutionary Link Between Mushrooms and Animals!

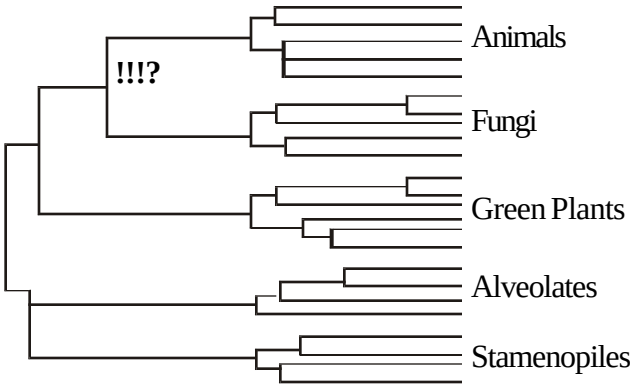
Recent studies of the evolution of ribosomal RNAs and animal origins have classified all eukaryotes into five "kingdoms" and placed the fungi as more closely related to animals than to green plants or the other two kingdoms. Do you realize what this means?

(a) It is now clear from this phylogenetic tree that fungi must have feelings, just like animals.

(b) Vegetarians must reconsider their priorities. For example, mushroom soup and morel mushrooms-without-steak will now be off limits.

(c) The agency of Animal Rights Activists (ARA) is now changing its name to Animal and Fungal Rights Activists (AFRA) and will be picketing and otherwise harassing fungal scientists around the country who are not treating mushrooms in a sufficiently humane manner.

(d) At the University of Cincinnati, the Department of Laboratory Animal Medicine (DLAM) has now changed its name to the Department of Laboratory Animal and Fungal Medicine (DFLAM). Furthermore, for those experimenting on fungi, it used to cost only $0.02 per mushroom per day. However, since fungi are now known to be related evolutionarily to animals, this price will immediately increase to $0.32 per mushroom per day (and is likely to double in price again, as of next July 1st).



RECENT CEG-SPONSORED SPEAKERS

OCTOBER 26, 1994
Anil Menon, Ph.D
Assistant Professor of Molecular Genetics, Biochemistry and Microbiology
"The role of genetics in understanding susceptibility to environmental agents"

NOVEMBER 4 1994
Professor Wolfgang Huber
Institute of Tumorbiology and Cancer Research
University of Vienna
A-1090 Vienna, Austria
"Estimation of human risk by exposure to the plasticizer di-(2etyhlhexyl)phthalate"

NOVEMBER 9, 1994
Scott W. Burchiel, PhD
Assistant Dean for Graduate Studies and Research
Professor of Pharmacology, Toxicology and Immunology
The University of New Mexico College of Pharmacy
Albuquerque, New Mexico
"Alterations in Ca^{2+}-dependent signaling pathways in lymphoid and non-lymphoid cells by PAHs: potential applications to immunotoxicity and human breast cancer"

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"

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