control mice were kept in the same room and did not develop any neurological disease. The incubation periods correspond to survival times assessed according to the criteria in (25).

29. Whole brain hemispheres were fixed in buffered 10% formalin. Pieces of brain were then embedded either in paraffin for immunohistochemistry (7-μm sections) or in Araldite (4-μm sections) for fine morphological examination. Antibodies were a polyclonal antibody to mouse glial fibrillary acidic protein (GFAP) and a horseradish peroxidase–conjugated secondary anti-body (Dako). Seven PrPres+ and six PrPres− brains were examined. Spongiform lesions and gliosis could not be seen in any brain region of PrPres− mice. The absence of localized PrPres deposits was confirmed by PrP immunohistochemistry.

30. Whole brain hemispheres were fixed overnight with a solution of 1% glacial acetic acid and 1% formalin in 0.12 M phosphate buffer (pH 7.4). After 1 hour postfixation with 2% osmic acid, they were stained en bloc with uranyl acetate and embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate before examination with a Philips CM10 electron microscope.

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earlier work showed synergistic responses to weakly estrogenic chemicals in turtles that were treated early in development (4). The study by Ramamoorthy et al. was performed in the uterus of female mice that had already undergone sexual differentiation. Our contention has been that developmentally exposed animals are more likely to demonstrate synergistic responses to estrogenic chemicals. Nonetheless, a careful inspection of the data provided by Ramamoorthy et al. indicates that diethylstilbestrol and toxa酚ine, at the lowest doses used, appeared to have induced the progesterone receptor, an estrogen-specific marker in mice, in a synergistic manner; no indication of this effect was seen when measuring uterine weight or uterine peroxidase activity. This suggests that some estrogen-dependent phenomena are better markers than others for revealing synergistic responses. Consistent with this idea is the observation that a combination of estradiol and 3,4,3',4'-tetrachlorobiphenyl synergistically induces pS2, an estrogen-regulated protein, but not another estrogen responsive marker, in the human breast cancer cell line, ZR-75-1 (5). Indeed, synergy observed in one cell line (ZR-75-1) was not seen in another (MCF-7) in the same study (5); this underscores the importance of cell type in determining estrogenic responses.

A mechanism underlying these synergistic effects remains to be determined. One of our working hypotheses is that under conditions in which the ER tends to exist as a monomer, the binding characteristics of two interacting molecules are different from that observed at high receptor concentrations. We contend that this low ER experimental condition better approximates ER concentrations found during early development [the ER content of uterine epithelial cells is low in fetal or newborn mouse (6) or rat (7), a period critical for estrogen-associated disorders (8)]. During these sensitive periods, chemical interactions resulting in synergy may occur at conditions in which critical ligand-receptor or receptor-receptor combinations occur.

Synergism between weakly estrogenic chemicals may not be universal, as Ramamoorthy et al. suggest. However, synergy in biological systems has a long history. Synergy has been observed between steroid hormones, different nuclear receptors (9), membrane; and nuclear receptors (10), drugs and hormones (11), and temperature and hormone response (12). Synergistic interactions have also been observed between drugs and temperature (13) and weakly estrogenic compounds (4). Our discovery of synergy of natural and synthetic estrogens was made by observing the effects of these compounds on the sexual development of turtle embryos. We demonstrated synergy between a combination of two polychlorinated biphenyls (4), and, more recently, two steroidal estrogens (14). We also have recently reported that the binding of chemical mixtures to the estrogen receptor from the American alligator occurs in a synergistic manner (15). Our laboratory has shown that a combination of phytoestrogens produced a synergistic response in yeast (16). In addition, in cell culture studies of fish hepatocytes (17) as well as mammalian cells (18), mixtures of weakly estrogenic chemicals were shown to act synergistically in stimulating estrogenic responses appropriate to the species. These findings together suggest that the synergistic action of weak estrogens may be phylogenetically conserved and therefore fundamental.

We currently are evaluating the occurrence of synergistic interactions of chemicals with the ER in different yeast strains, mammalian cells, and biological systems. We have noted synergy in some yeast strains, but not others, as well as an apparent relationship to ER concentrations (19). We have likewise found a synergistic interaction between ovarian steroidal estrogens in both a yeast-based assay and the developing turtle (14). These latter studies both confirm and extend our previous report (1) and suggest a mechanism for synergy. We look forward to the continued clarification of this important issue.

John A. MacLachlan
Steven F. Arnold
Diane M. Klotz
Bridgette M. Collins
Peter M. Vonier
Tulane-Xavier Center for Bioenvironmental Research, New Orleans, LA 70112, USA
Louis J. Guillette
Department of Zoology, University of Florida, Gainesville, FL 32611, USA

Redox Stabilization of the Atmosphere and Oceans and Marine Productivity

Philippe Van Cappellen and Ellery D. Ingall provide a coupled biogeochemical box model to investigate whether negative feedbacks between the global cycles of phosphorus and oxygen might have stabilized the amount of atmospheric O2 during the Phanerozoic (1). We have duplicated these results (1), but have found that slight modifications to the treatment of tectonic uplift and resultant weathering rates dramatically affect the outputs of the model.

Van Cappellen and Ingall set the rate of O2 consumption during weathering to be proportional to the global rate of uplift. The rate of O2 production is highly sensitive to marine reactive P availability through interactions with the carbon cycle. Van Cappellen and Ingall assume that the rate of P input to the oceans depends only on the size of the terrestrial lithospheric reservoir of this element and not on weathering rates. This assumption virtually decouples the rate of oxidative weathering from that of P transfer to the oceans on time scales of tens to hundreds of millions of years and accounts for the rapid depletion in atmospheric O2 in the model after an increase in uplift rate (Fig. 1). It seems more likely that the flux of P to the oceans also depends on the rate of uplift. Today, refractory, detrital P phases account for less than 25% of the total solid-phase P in most marine sediments (2), and changes in total continental P weathering rates have apparently led to comparable changes in the chemical weathering of P phases over at least the last 100 million years (Myr) (3). When the model (1) is run with P and Fe oceanic inputs coupled to
Potency of Combined Estrogenic Pesticides


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