Our previous studies have demonstrated that nonenzymatic reaction of menadione with thiols in plasma generated reactive oxygen species, resulting in potentiation in the menadione-induced platelet toxicity. Since menadione, one of the representative quinone compounds, has been reported to cause hemolytic anemia in vivo, we hypothesized that erythrocytes could be one of the potential target tissues to menadione in the presence of plasma. To investigate the role of plasma in the erythrocyte toxicity by menadione and to identify reactive oxygen species derived from the nonenzymatic reaction of menadione with plasma thiols, plasma isolated from rats was treated with menadione sodium bisulfite (MSB), water soluble menadione. Treatment with MSB increased oxygen consumption rate as well as luminol- and lucigenin-amplified chemiluminescence in a dose-dependent manner. The chemiluminescences generated by luminol and lucigenin were inhibited by superoxide dismutase (SOD) addition, suggesting that superoxide anions were generated. When erythrocytes were suspended in plasma or buffer, MSB-induced chemiluminescence in plasma was larger than that in buffer, indicating that the presence of plasma increased free radical generation induced by MSB. Consistent with these findings, we observed MSB-induced hemolysis only in erythrocytes suspended in plasma while not in those suspended in buffer. In order to identify the reactive oxygen species associated with cytotoxicity, various radical scavengers were tested to inhibit MSB-induced hemolysis. Addition with catalase and/or mannitol resulted in significant inhibition of hemolysis, while superoxide dismutase had no effect. These results suggest that hydrogen peroxide and hydroxyl radical rather than superoxide appeared to be involved in erythrocyte cytotoxicity although the reaction of plasma thiols with MSB was accompanied by superoxide generation.

**[PA4-2]** [ 10/19/2000 (Thr) 10:00 - 11:00 / [Hall B] ]

## A Study on Endocrine Disruptors: E-Screen Assay of Alkylphenolic Compounds

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It has been hypothesized that environmental estrogens may play roles in the increasing incidence of breast cancer, testicular cancer, and another problems of the reproductive systems. Alkylphenols which are widely used as plastic additives and surfactants have been shown to induce estrogenic responses. We tested 6 alkylphenolic compounds by E-screen assay. E-screen assay is suitable for large-scale screening of suspected endocrine disrupting chemicals. The method introduced by Soto *et al.* is based on proliferative activity of MCF-7 estrogen sensitive human breast cancer cell line. This quantitative cell proliferation assay of MCF-7 cells was performed in the absence and presence of 17β-estradiol (negative and positive controls), and at the range of various concentrations ($10^{-14}$~$10^{-5}$ M) of alkylphenolic chemicals. Cell proliferation yields in the positive control increased up to six-eight fold over those of negative control cells after 144 hr incubation. Among the alkylphenols, 4-chlorophenol($10^{-5}$ M), cyclohexanol($10^{-13}$~$10^{-5}$ M) and 4,4'-isopropylidenediphenol ($10^{-6}$~$10^{-5}$ M), α-naphthol($10^{-13}$~$10^{-12}$ and $10^{-7}$~$10^{-6}$ M), and p-nitrophenol($10^{-14}$ and $10^{-6}$~$10^{-5}$ M) appear to possess estrogen activity. And 4-buthylphenol showed week estrogenicity. The most potent estrogenic chemical was cyclohexanol which was able to stimulate these biological responses to the similar extent as 17β-estradiol itself.

**[PA4-3]** [ 10/19/2000 (Thr) 10:00 - 11:00 / [Hall B] ]