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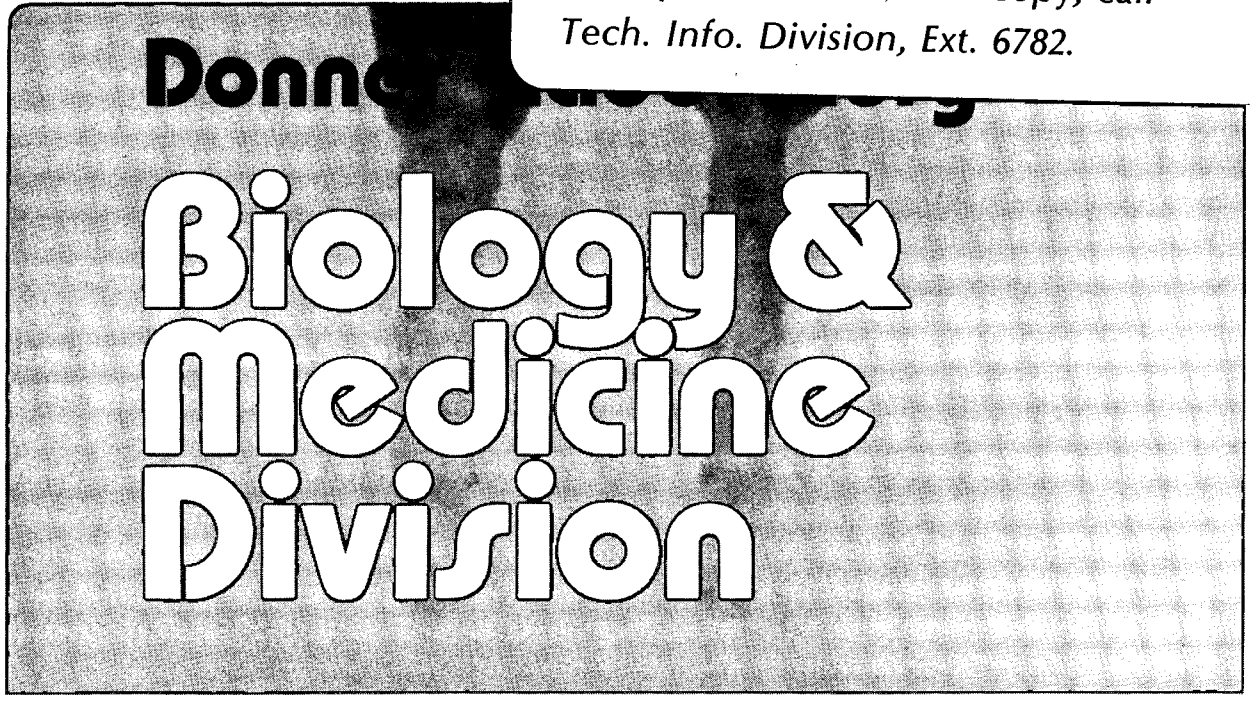

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DISCLAIMER

The Male Disadvantage in Ozone Toxicity

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Many physiological, morphological and biochemical observations demonstrate the toxic nature of ozone[1]; and among these observations, increased mortality in rodents was noted. Sex related differences for rodent survival were observed during a study of the influence of thyroid hormones on the toxicity of oxidant gases, including ozone[2]. Female rats were more resistant to the effects of ozone than male rats. Other relationships of ozone toxicity and thyroid physiology have been examined[3,4], but relationships between sex hormones and ozone toxicity have not been pursued. We report here that gonadectomy and sex steroid treatment further modify ozone toxicity. It is well established that steroid mechanisms are mediated by specific intracellular receptors[5]. In these studies, quantitation of androgen receptors in lungs from male mice showed a 50% reduction after continuous ozone exposure.

Young adult CD1 mice were continuously exposed to ozone (1.5 ppm) for ninety days in large environmental chambers. Results from these studies with normal mice, castrate mice and castrate mice which received steroid replacement are summarized and contrasted in Figure 1. Panel A shows results from intact male and female mice and illustrates the greater sensitivity of the male rodent to ozone ($P<0.025$). The effects of gonadectomy on this response are depicted in Panel B of Figure 1. When the response of intact mice (male and female) was compared to gonadectomized mice (male and female), intact mice survived this toxic environment significantly better ($P<0.005$). When survival rates of gonadectomized male and female mice are compared, the female mouse was significantly more sensitive to ozone toxicity than the male mouse ($P<0.025$). Thus, the absence of endogenous sex hormones produced by either the testis or the ovary significantly affected the animal's ability to survive this ozone environment. Evidently, product(s) of gonadal origin modify the response of mice to this oxidant gas, and this effect is more pronounced in the female than

in the male. Panel C (Figure 1) illustrates survival characteristics of similarly ozone-exposed gonadectomized mice which have been injected weekly with either estradiol (female mice) or dihydrotestosterone (male mice). Survival characteristics were significantly improved in these hormone treated mice when compared to those mice which did not receive steroid replacement. Although survival of steroid treated mice was less favorable than that of intact mice, this may just be a reflection of hormone dosage and/or injection schedule in this experiment. These combined data confirm earlier observations which suggested sex related responses in ozone toxicity[2], and in addition, demonstrate that removal of endogenous gonadal steroids intensifies this response. That this response is related to some product or products of gonadal origin or a possible indirect mechanism affected by these products is supported by the fact that mortality trends are reversed and are normalized by sex steroid treatment.

Since the biological interface between atmospheric ozone and the organism is the lung, a direct sex steroid effect within this tissue may be possible. Steroids of adrenal origin do affect lung tissue. It is well documented that glucocorticoids hasten the maturation and development of the fetal lung when administered at appropriate gestational time[6,7]; that glucocorticoid receptors are present in fetal and adult lung tissue[8,9,10,11]; and that, glucocorticoids promote the production of surfactant by fetal lung tissue[12]. Respiratory distress syndrome develops when there is a surfactant deficiency, and the male fetus, unlike the female, is at a disadvantage and does not respond favorably to antenatal glucocorticoid therapy [13]. Sex steroids may also affect fetal lung development[14,15]. Estradiol appears to promote surfactant biosynthesis[16,17], and a recent report shows that dihydrotestosterone retards fetal lung development[18]. Presently the exact role of sex steroids in fetal

lung development is uncertain; and moreover, the physiological importance of this class of hormones to the adult lung remains obscure.

If sex steroids are directly modifying or affecting a lung response, receptors for androgens and estrogens could be expected to reside within lung tissue. Such androgen and estrogen steroid receptors have been identified in adult rat lung tissue[19], and we recently characterized these steroid binding components in adult male mouse lung tissue[20]. To explore a possible relationship between lung androgen receptors and ozone toxicity, intact male mice were exposed chronically to 1.5 ppm ozone; and, androgen receptors within the cytosol of lung tissue were quantitated at 10 days, 17 days and 40 days in control (filtered room air) and treated (ozone) mice. These results are summarized in Table 1. The concentration of dihydrotestosterone receptors in normal male mouse lung was 10.80 ± 0.81 fmol/mg cytosol protein (mean ± SE) and compared favorably with our earlier estimates of 10.25 ± 0.79 fmol/mg (mean SE) for the male mouse[20]. No change in lung androgen receptor content was observed at 10 days or 17 days of ozone exposure, but after 40 days, the androgen receptor content of the lung was reduced by approximately 50 percent when quantitated as fmol/mg cytosol protein. When quantitated as total androgen receptor per lung, the concentration of these steroid binding components is still reduced after ozone exposure. Thus, ozone appears to evoke a real reduction of lung cytosol androgen receptor.

After inhalation of oxidant gases, damage to cells of lung tissue occurs, especially to the type I cells lining the alveoli[21,22]. Lungs become edematous, and type II cells proliferate to reestablish a continuous epithelial lining of the alveolar airspaces. Type II cells may develop into type I cells during normal differentiation and after pulmonary injury by oxidant gases[23,24,25]. A major function of type I cells is gas exchange, and they are

thought to be incapable of replication. Type II cells appear to respond to injury and become more resistant to subsequent injury, and hyperplasia of type II cells accompanies this resistance[26,27]. Concommitantly many enzymatic changes in the lung occur, and these changes may occur in the type II cells as a result of the injury or an expression of the repair process. As a result of ozone inhalation, changes in proteins and enzyme activities[28,29], immunoglobulins, IgG, IgM and transferrin and α1-antitrypsin[30], and lipids[31,32], occur within lung tissue and body fluids. Our current data suggest that steroid receptor mechanisms also may be involved in the lung tissue injury or repair responses after ozone inhalation. From our present data in which androgen binding declines during a period when the total number and total lung percentage of type II cells is increasing[33], it appears that androgen binding may not occur in the type II cell or that the new population of type II cells may be lacking this capability.

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Table 1

Effects of 1.5 ppm Ozone on Lung Androgen Receptors[1]

Treatment	Aver. Lung Wt.[2] (mg)	Androgen Receptors[3] (fmol/mg protein)	Kd (nM)
Control	315	12.34	0.38
0-10 days	340	11.79	0.48
Control	356	9.56	0.34
0-17 days	395	9.76	0.29
Control	345	10.48	0.51
0-40 days	480	5.71	0.94

1 Groups of mice were exposed to 1.5 ppm ozone as described in Figure 1.

2 Average weight was obtained by dividing the total pooled weight of the lungs by the number in each group.

3 After the mice were anesthetized, the chest cavity was opened, and the lungs were perfused with ice cold 0.01 M Tris-HCl, 0.0015 M Na_2EDTA, and 0.01 M Na_2MoO_4, pH=7.4 (TEM buffer) via the right ventricle until blood was cleared and the lung tissue was pale white. Perfused lungs were trimmed of adhering tissue and placed in TEM buffer (4^oC) until homogenized. Lung tissue from 10-20 mice was pooled, blotted on absorbant paper, weighed and homogenized in a volume of TEM so that the final cytosol concentration was 8-10 mg/ml. The homogenate was centrifuged at 105,000 x g to yield a

cytosol fraction. Cytosol (0.25 ml) containing 1-2 mg protein, was mixed with increasing quantities of [^{3}H]-dihydrotestosterone, 10 pM to 5 nM, and the final incubation volume was 0.3 ml. A parallel series of tubes, identical except for the presence of 100 nM nonradioactive steroid was assayed simultaneously to quantitate nonspecific binding. Samples were incubated overnight at 4^{o}C. Bound and free steroid were separated by charcoal (0.625% Norit A, 0.0625% Dextran T-70) in TEM. After tritium quantitation, receptor concentrations were calculated from Scatchard analysis of the data (35).

Figure 1. Survival results from three experiments are illustrated in which the effects of chronic ozone exposure (1.5 ppm) on mice were studied. Male and female mice were caged separately, 8 per stainless steel wire cage. Construction of these cages was such that a free flow of ozone was present within the environmental chambers. No bedding was used in the cages, and they were replaced with fresh sterilized cages, food and water three times a week. Environmental chambers were of stainless steel construction and had two large glass doors which freely admitted light on a 12 hour light/12 hour dark schedule. Ozone was generated by an OREC ozone generator using oxygen as a gas source; and, chamber ozone content was measured daily with a spectrophotometric ozone analyzer (Dasibi Model 1003-AH). Mortality differences were analyzed by Chi square techniques[34]. To study the effects of castration on this response, mice were ovariectomized or orchidectomized between 35-45 days of age and allowed to recover for two weeks before exposure to ozone began. Steroids (estradiol or dihydrotestosterone) were injected into gonadectomized mice in an ethanolic saline solution once a week (25 μg/0.1 ml/mouse) for the duration of the experiments. Panel A = control mice, Panel B = gonadectomized mice, and Panel C = gonadectomized mice which received sex steroid injections. Male mice are represented by ▲ and female mice by ● symbols.

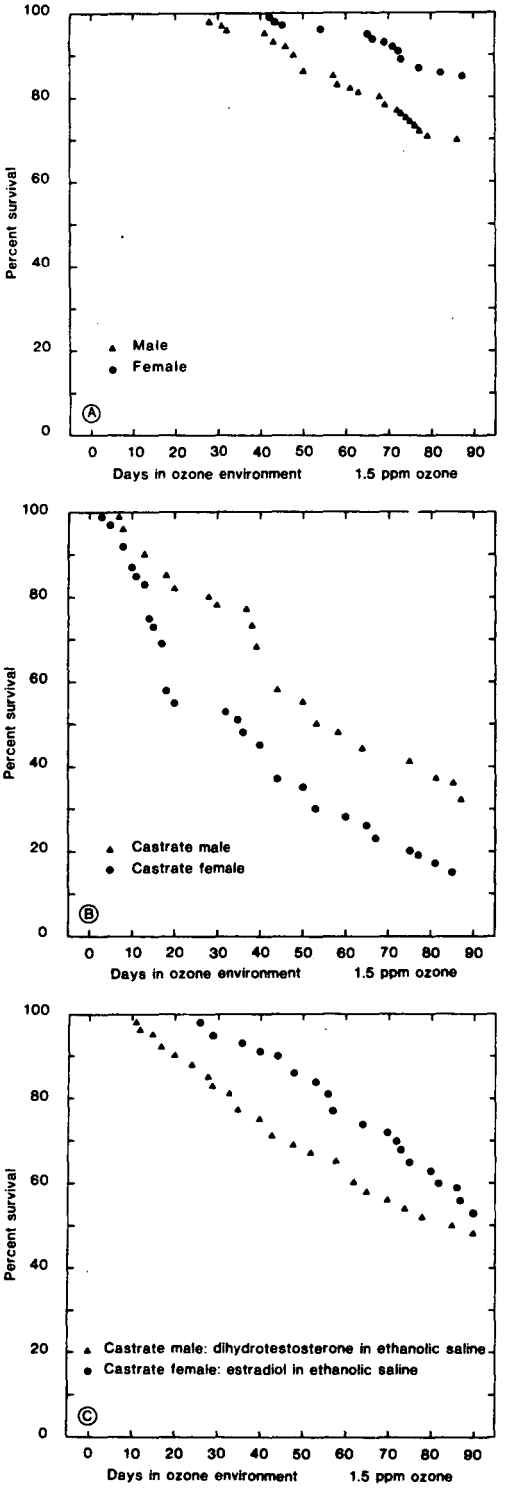

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Figure 1

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