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Effect of *in Vivo* Ozone Exposure to Dorset Sheep, an Animal Model with Low Levels of Erythrocyte Glucose-6-Phosphate Dehydrogenase Activity

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Considerable interest has recently been directed to the possible extrapulmonary effects caused by exposure to ambient ozone. Studies have now demonstrated that ozone exposure may result in chromosomal aberrations in circulating lymphocytes of Chinese hamsters (ZELAC et al. 1971), altered drug metabolism in mice (GARDNER 1979), as well as biochemical changes in red blood cell metabolism in several animal models (GOLDSTEIN et al. 1968; LARKIN et al. 1978; CLARK et al. 1978) and humans (BUCKLEY et al. 1975; FREEMAN et al. 1977). As a result of ozone induced in vivo alteration of red cell function within human subjects (BUCKLEY et al. 1975), CALABRESE et al. (1977) hypothesized that individuals with an erythrocyte glucose-6-phosphate dehydrogenase (G-6-PD) deficiency would be at increased hemolytic risk to elevated ambient ozone exposure.

In order to evaluate such an hypothesis in an experimental setting it would be of great value to have an appropriate animal model with erythrocyte G-6-PD activity similar to the absolute activity range found in the human population. While no such unique animal model is presently known, the literature has revealed that Dorset sheep have an erythrocyte G-6-PD activity comparable in absolute units to a human G-6-PD deficient. It has been speculated that such low levels of G-6-PD activity in the red cells of sheep may be responsible, in part, for their unique susceptibility to copper and primaquine induced hemolysis (NAS 1977; SULL & FORSLIE 1977).

Based on this information, we compared the hematological responses of normal and G-6-PD deficient humans and Dorset sheep to four oxidant stressor agents including paraquat (CALABRESE et al. 1980), nitrite (CALABRESE et al. 1980a), chlorite (MOORE et al. 1980), and copper (CALABRESE et al. 1980b). In general, the G-6-PD deficient red cells of the humans and sheep were significantly more susceptible to the oxidant stressors. However, the expression of oxidant stress differs in that the sheep often exhibited large increases in methemoglobin (MetHb) while the human deficients showed marked decreases in glutathione (GSH). While considerably more research is needed to evaluate the mechanisms by which sheep and human G-6-PD deficient red cells handle oxidant stress, it seemed appropriate to evaluate the effects of in vivo ozone exposure in Dorset sheep over a broad range of concentrations.

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MATERIALS AND METHODS

Twenty adult sheared Dorset ewes between 1.5 and 7 years of age were used. The animals were fed a standard mixed legume and grass forage diet and had been housed in the sheep barn on the University of Massachusetts campus. However, each sheep was moved approximately 1/2 mile to the University of Massachusetts, Division of Public Health Laboratory building and housed in stalls with a room adjacent to the laboratory for approximately one week. This usually included three non-exposure adjustment days, then four consecutive days during which 2.75 hours were spent in the exposure chamber. In the first three exposure days, the sheep were exposed to filtered air while on the 4th day the sheep were exposed to filtered air with ozone. Thus, each sheep served as its own control. The sheep were randomly assigned to one of four ozone exposure concentrations: 0.12, 0.25, 0.50, and 0.70 ppm with five sheep per exposure group.

Immediately before and after the exposure period, blood was drawn from the animal into 5 ml. heparinized tubes. Red blood cell counts were measured using a Royco Cell-Crit 920 (Menlo Park, CA); G-6-PD activity was measured using a Calbiochem-Behring G-6-PD kit, based on the method of Kornberg and Horecker (1953) (Calbiochem-Behring Corp., LaJolla, CA 92037). Decrease in absorbance in the UV range was measured with a Coleman Model 55 Perkin Elmer spectrophotometer and a Coleman Model 5-100 enzyme calculator with a BCD printer. Other assays included glutathione (YUNIS 1969), acetylcholinesterase (ACHE) (ELLMAN et al. 1961), and methemoglobin (BROWN 1973) measurements.

The exposure period was conducted in a 5' x 5.5' x 3' stainless steel and glass exposure chamber constructed by Young and Bertke, Cincinnati, Ohio. Ozone was generated by Welsbach Laboratory Ozonator, Model T-408 (Welsbach Ozone System Corp., Philadelphia, Pennsylvania) using air supplied by a zero-air generator. Ozone levels within the exposure chamber were determined using a Dasibi Model 1003-AH ozone monitor (Dasibi Environmental Corp., Glendale, California). Every ten seconds the ozone concentration was measured and recorded on a Watanabe four-channel multi-recorder (Watanabe Instruments Corp., Tokyo).

There was approximately one air change per minute within the chambers. This permitted the maintenance of a stable thermal environment with the chamber temperature ranging between 24.5-28°C while RH was 55% ± 10.

A polyvinyl chloride cuffed endotracheal tube (Center Vet Supply, Leominster, Massachusetts) 5mm in diameter and 17cm in length was completely inserted into each nostril and the cuff inflated before the sheep was placed in the chamber. This was done in order to reduce the magnitude of ozone extinction along the surface area of the prolonged nasopharyngeal tract of the sheep. The data were analyzed using replicated analysis of variance and Tukey's T-test. The three control day data were averaged to compare

with the one ozone exposure day. In Figures 1-5 the control data are represented by the baseline against which treatment data are compared.

RESULTS

The ozone exposure was found to affect a number of hematological changes indicative of oxidant stress. Of particular relevance was the marked decrease in GSH levels at 0.50 ppm ($p=0.05$) (Figure 1) and 0.70 ppm, the decreased ACHE activity at 0.70 ppm (Figure 2) and a generally consistent dose-dependent increase in MetHb (Figure 3). In addition, each level of ozone exposure resulted in a reduced red blood cell count although no dose trends were evident (Figure 4). G-6-PD activity displayed a progressive increase in activity up through 0.50 ppm but at 0.70 ppm there was a slight reduction (Figure 5).

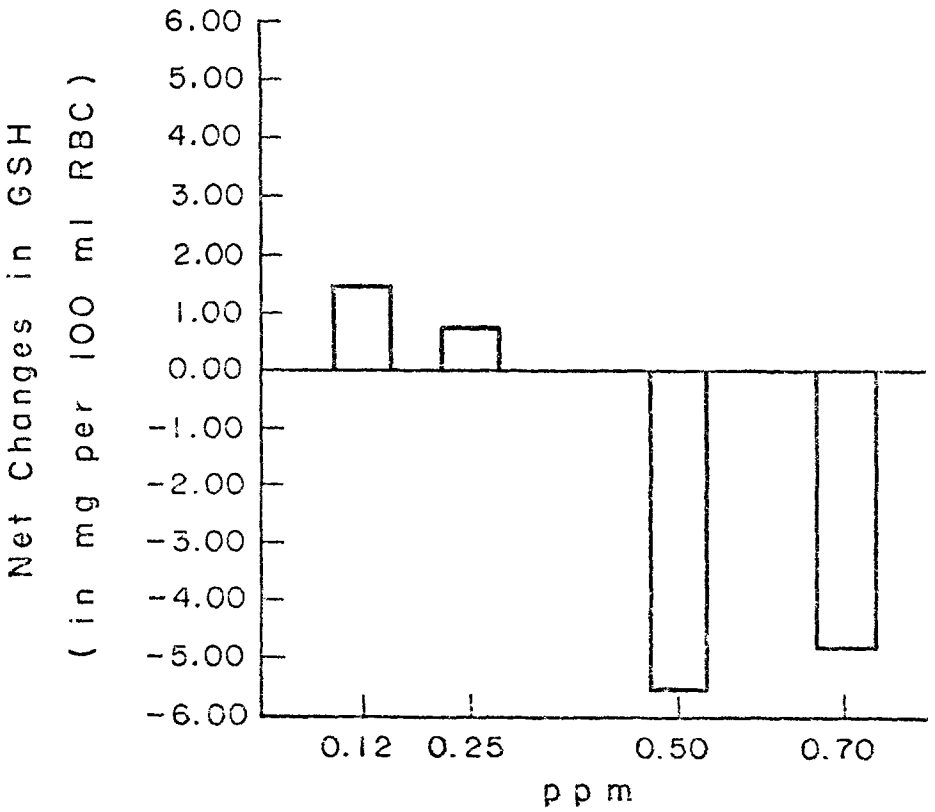

Fig. 1. Effect of ozone on Erythrocyte GSH levels.

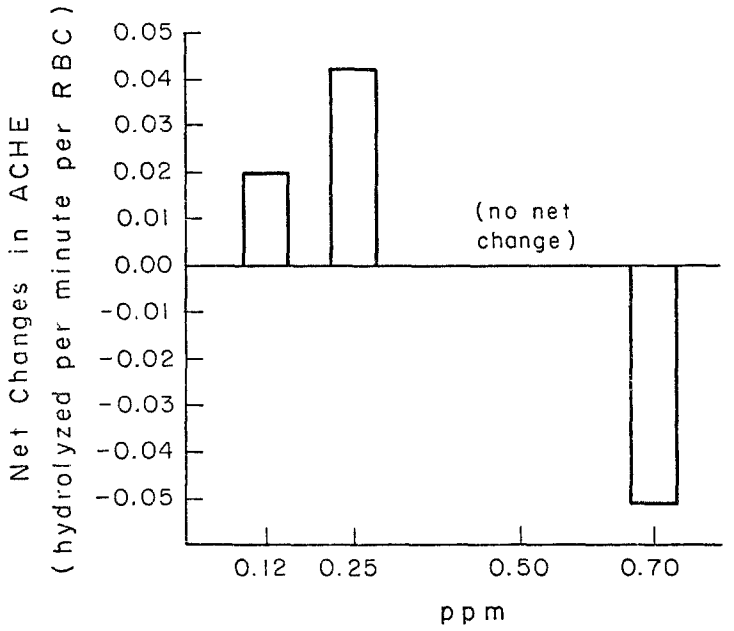

Fig. 2. Effect of ozone on Erythrocyte ACHE activity.

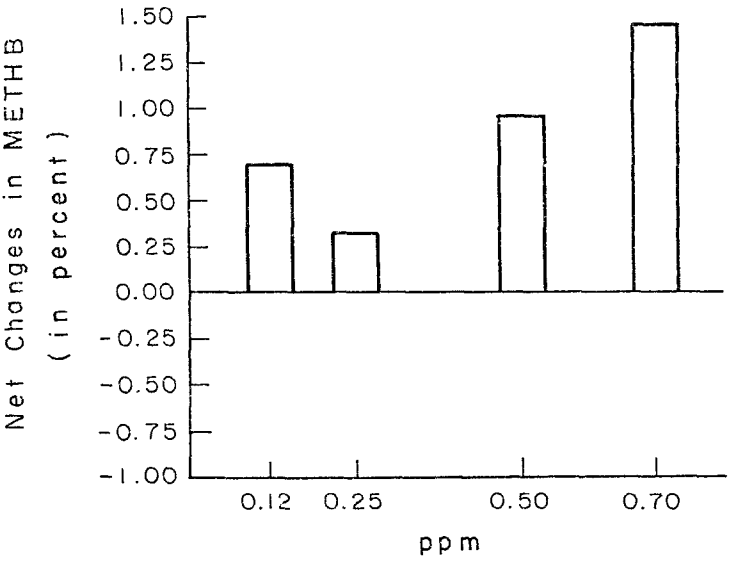

Fig. 3. Effect of ozone on METHB formation.

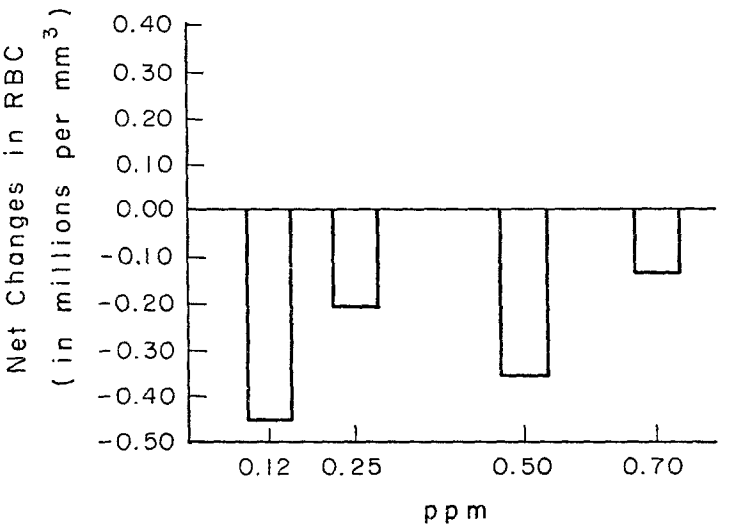

Fig. 4. Effect of ozone exposure on RBC counts.

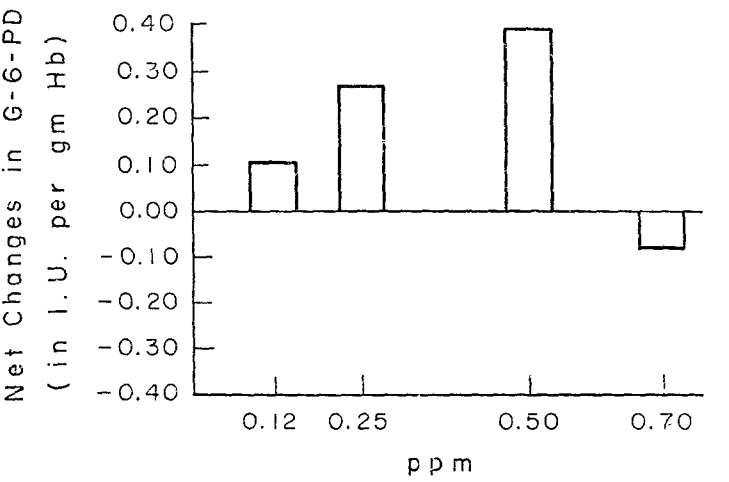

Fig. 5. Effect of ozone exposure on Erythrocyte G-6-PD activity.

DISCUSSION

This experiment has shown that a single, short term in vivo exposure (2.75 hours) to ozone (i.e., 0.12, 0.25, 0.50, 0.70 ppm) affected several hematological parameters in female sheep. These findings are clearly indicative of oxidant stress as shown by the occurrence of GSH and RBC decreases and MetHb increases. These results indicate that ozone is capable of producing systemic oxidant stress on the red blood cells in dosages as low as only 0.12 ppm which is the current US EPA one hour ambient ozone standard. Further, these results are consistent with the findings of CLARK et al. (1978) and BUCKLEY et al. (1975), who reported that ozone (0.37-0.75 ppm for 2.75 to 4 hours) induced decreases in GSH levels and increases in RBC fragility in monkeys and humans, respectively. In addition, this study represents the first report that ozone exposure resulted in consistently decreased RBC counts as well as increased MetHb levels. It must be emphasized that the ozone induced biochemical changes were statistically insignificant for all parameters and concentrations evaluated with the sole exception of changes in GSH at the 0.5 ppm level. Consequently, while it is possible to explain these changes entirely to chance, it must be recognized that nearly all changes were consistent with the occurrence of ozone induced oxidant stress as predicted.

The sheep were expected to be more sensitive to ozone than normal red blood cells because of their relative deficiency of G-6-PD. However, when the data from this study are compared to the BUCKLEY et al. (1975) in vivo study in which normal humans were exposed once to 0.50 ppm for 2.75 hours, it appears that the sheep approximate but do not exceed the normal human blood response to ozone with respect to changes in GSH.

The sheep may not be as sensitive to ozone as predicted, when sensitivity is measured by decreases in GSH levels, for several reasons. Possibly the ozone was extinguished before reaching the blood. This study attempted to address ozone extinction in the nasopharyngeal tract by inserting polyvinyl chloride endotracheal tubes into the sheep nostrils before placing the sheep in the chamber. This presumably reduced the amount of surface area inside the nasopharyneal tract with which the ozone could react. This was considered preferable to tracheostomizing the sheep, which would have considerably shortened the breathing distance of the ozone to the lungs. It was felt that such an alternative would have made the animal model less realistic in simulating human exposure. The sheep would have been rendered more susceptible to infections and a confounding variable would have been introduced by the anesthesia used during the operation.

Sheep may have an adaptive mechanism which reduces an oxidative stress response when exposed in vivo to ozone. VENINGA & LEMSTRA (1975) have reported that mice exposed to 0.20 ppm ozone for two hours significantly increase hepatic ascorbic acid synthesis. WINTERBOURN (1979) reports that human G-6-PD deficient erythrocytes

exposed in vitro to acetylphenylhydrazine are protected from Heinz body formation by ascorbic acid. Although sheep red blood cells are G-6-PD deficient, sheep do synthesize ascorbic acid in the liver which may exert such a protective effect as discussed by Winterbourn. Further research is needed to determine whether ozone does enhance ascorbic acid synthesis in sheep and whether this response would be adaptive.

It should be emphasized that simply because the sheep did not appear more sensitive than normal humans with regard to ozone induced decreases in GSH does not mean that they aren't more sensitive. Sheep have been shown to express oxidant stress from paraquat, copper, chlorite, and nitrite more by increased levels of MetHb as compared to changes in GSH (CALABRESE et al. 1980, 1980a, 1980b; MOORE & CALABRESE 1980). In fact, the occurrence of ozone-induced MetHb in the sheep is the first report to our knowledge of this phenomenon, thereby inferring that sheep are more sensitive with regard to this indicator of oxidant stress than normal humans.

In conclusion, sheep demonstrated systemic hematological responses when exposed in vivo to ozone. Of particular importance are ozone induced dose-related MetHb increases and RBC decreases immediately after exposure to only 0.12 ppm, and GSH decreases at the highest two concentrations (0.50 and 0.70 ppm). While the changes in ozone induced parameters were quite modest and statistically insignificant it represents one of the first attempts to evaluate an animal model with an erythrocyte G-6-PD deficiency similar to human deficients and may hopefully stimulate the search for other models simulating potential human high risk groups to ozone.

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