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The C57L/J Mouse Strain as a Model for Extrapulmonary Effects of Ozone Exposure

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A number of animal and human studies have accumulated that report extrapulmonary effects from inhaled O_3. BUCKLEY et al. (1975) exposed human volunteers to 0.5 ppm of O_3 for 2.75 h and demonstrated increases in red-cell fragility, serum vitamin E levels, and red cell glucose-6-phosphate dehydrogenase (G6PD) activity; while decreases in red-cell acetylcholinesterase (ACHase) activity and reduced glutathione (GSH) levels were evident. HACKNEY et al. (1977) exposed human male volunteers to 0.37 ppm of O_3 for 2.0 h and discovered similar effects. Canadians and Southern California residents were compared in this study for their response to O_3 exposure. Although not all differences reached statistical significance, there were increases in serum vitamin E and G6PD activity while ACHase activity decreased. The levels of GSH were highly variable and ranged from -10.7% to +5.7% difference from controls (Table 1). As might be expected, the human subjects exposed to 0.5 ppm of O_3 (HACKNEY et al. 1975) exhibited more exaggerated effects on blood characteristics measured than when exposed to 0.37 ppm. These results are interesting in that many previous studies exhibiting such extrapulmonary effects in vivo were performed on animals while using high O_3 exposure levels (GOLDSTEIN 1973, GOLDSTEIN et al. 1968, MITTLER et al. 1957). The selection of a suitable animal model to study effects of ozone exposure on blood is complicated by the failure of many species to respond similarly to humans. Rhesus monkeys and Sprague-Dawley rats exposed to 0.5 ppm of O_3 for 8 h a day for 7 days failed to show a change in glutathione metabolism enzymes (CHOW et al. 1975) in contrast to humans (BUCKLEY 1975). Ozone exposed squirrel monkeys failed to demonstrate increases in G6PD activity although other parameters (ACHase, GSH, Osmotic fragility) behaved similarly (CLARK et al. 1978). It is not unusual for animal species including primates to differ from humans in response to toxic substances (COULSTON 1966) and "a necessary step that must not be overlooked by a toxicologist is to determine whether some human data are already available and base the selection of animal species on a similar metabolic and physiologic response," (STARA & KELLO 1979). The purpose of this study was to compare the similarity of the male C57L/J mouse strain to adult male humans in their response to changes in blood and serum characteristics from exposure to low levels of ozone. A similarity in metabolic and physiologic response would indicate the value of such a model in predicting human health effects.

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TABLE 1. Comparison of Human and C57L/J Mice Blood Characteristics Showing Percent Change From Controls Following In Vivo Exposure to Ozone

Subject Type	O_3 Level, ppm	Length of Exp. (h)	Number of Subjects	Serum Vitamin E	ACHase	GSH	G6PD
Human** (Canadian)	0.37	2.0	4	+7%	-11	-2	+6
Human** (Los Angeles)	0.37	2.0	4	+6	- 8	+6	+1
Human** (Total Los Angeles)	0.37	2.0	13	+5	- 8	-11	+7
Human* (Los Angeles)	0.50	2.75	7	+25	-17	-16	+22
C57L/J Mice	0.30	6.0	10	+24	-12	+9	+19

* BUCKLEY et al. 1975

** HACKNEY et al. 1977

MATERIALS AND METHODS

General. Forty C57L/J, 9 month, male mice (Jackson Laboratories, Bar Harbor, Maine) were maintained in individual stainless steel cages in a room with controlled temperature (72≠2°C) and lighting (12 h light and 12 h dark). Animals were randomly allocated into one of four treatment groups with ten animals in each group. Mice were exposed for six continuous hours to 0.0 ppm or 0.32 ppm of O_3, and each of these groups was subdivided into those on vitamin E diet and vitamin E supplemented diet.

Diet. Vitamin E deficient diet (ICN Nutritional Biochemicals, 26201 Miles Road, Cleveland, Ohio 44128) had the following composition: 10% Corn oil, Tocopherol stripped; 4.6% Salt mixture 4164; 66.0% Glucose; 20% Vitamin free casein (plus ICN Vitamin diet fortification mixture without Vitamin E.

The vitamin E fortified mixture was ordered from ICN to contain 5.0 mg α-tocopherol per 100 lb of chow. The vitamin E content of both mixtures was determined according to the modified method of WINDHAM (1957), i.e. xylene was substituted for petroleum ether in the extraction procedure. The deficient diet was found to contain 0.28≠.02 mg α-tocopherol per 100 lb while the supplemented diet contained 3.9≠0.3 mg α-tocopherol per 100 lb diet. Mice were maintained on the diet for a minimum of six weeks, a period that is known to deplete vitamin E levels in rats and mice on vitamin E diet (BARKER et al. 1973).

Exposure. Mice were placed in individual stainless steel cages and then randomly assigned to one of two 69 x 69 cm stainless steel exposure chambers according to the design of HINNERS et al. (1968). The two chambers were operated simultaneously with control mice (0.0 ppm O_3) in chamber 2 and test mice (0.32≠.01 ppm O_3) in chamber 1. Ozone was produced by a silent arc generator and added to the filtered air entering the chamber. Sampling ports for drawing air for analyzers were located at the rear of the chamber at the same level as the cage shelves (breathing zone). The flow of temperature - controlled filtered air through the chamber was maintained at 3.0≠0.2 SCFM. The temperature of both chambers averaged 72≠3°F with a relatively humidity of 48≠9%. Both chambers were analyzed sequentially at 3 min intervals by drawing samples through a sequential sampling system (MOORE et al. 1979) and delivering the sample, first from one chamber and then the other, to an ultraviolet ozone monitor (Dasibi Environmental Corp., 616 E. Colorado St., Glendale, CA 91205). Consequently, the conditions to which the control and test mice were exposed during any single 6 h exposure period were essentially identical except for the level of ozone directed into chamber 1.

Blood Characteristics Measured. G6PD activity was measured using a Calbiochem-Behring G6PD kit, based on the method of KORNBERG & HORECKER (1955) as revised by MARK (1966) and modified by Calbiochem-Behring (Calbiochem-Behring Corp., La Jolla, CA

92037). This is an ultraviolet kinetic enzyme assay and the change in absorbance 340 nm was read at 30°C on a Coleman model 55 Perkin Elmer spectrophotometer with a Coleman model 5-100 enzyme calculator and BCD printer. Hemoglobin concentration was determined on the hemolysate using the ROYCO hemoglobinometer and G6PD activity was reported in I.U. of G6PD/g Hb in hemolysate.

Glutathione was measured in its reduced form (GSH) based on a colorimetric method of YUNIS (1969) using 5,5' dithiobis-(2-nitrobenzoic acid) (DTNB). The final calculation expresses the GSH content as mg/100 mL of red cells.

Red cell ACHase activity was determined according to the method of ELLMAN et al. (1966). Enzyme activity was measured by following the increase of the yellow color produced by thiocholine when it reacts with the dithiobisnitrobenzoate ion.

Plasma vitamin E levels were determined for mice in each exposure group and for both diets. Free vitamin E was determined by fluorescence according to the method of HANSEN & WARWICK (1966).

The fluorescence was determined with activation at 295 mu and determination at 340 mu.

RESULTS

Mice exposed to 0.32 ppm O_3 showed an increase in G6PD activity controls in both diets. Mice on the deficient diet showed a 24% increase in G6PD activity over controls ($p=0.002$) when exposed to 0.32 ppm O_3 while those on a supplemented diet experienced a 19% increase ($p=0.014$) (Table 2, Figure 1).

Levels of GSH were increased but not significant for mice exposed to 0.32 ppm of O_3 compared with controls for diets. Mice on the vitamin E deficient diet experienced a 13% increase ($p=0.224$) and those on the supplemented diet increased 9% ($p=0.139$) after exposure to 0.32 ppm O_3 (Figure 1).

Exposure to 0.32 ppm of O_3 produced decreases in ACHase activity for both diet groups. The decrease of 19% in ACHase activity for mice on the vitamin E deficient diet was significant ($p=0.00$). Mice on the supplemented diet exhibited a 12% reduction in ACHase activity ($p=0.044$) (Figure 1).

Levels of free vitamin E in the plasma of C57L/J mice were determined for those mice exposed to 0.0 and 0.32 ppm of ozone that were also on vitamin E supplemented and deficient diets (Table 3).

TABLE 2. Mean Values for Selected Blood Characteristics of C57L/J Mice On Vitamin E Deficient or Supplemented Diets Exposed to 0.0 ppm or 3.2 ppm of O_3 for Six Hours

Blood Characteristic Measured	Level of O_3 Exposure (ppm)	Vitamin E (-)	Vitamin E (+)
G6PD Activity (I.U. g/HB on hemolysate)	0.0	9.0≠1.2	8.2≠1.5
	0.32	11.2≠1.6	9.8≠1.0
GSH (mg/100 mL red blood cells)	0.0	63≠16	65≠3
	0.32	71≠12	71≠9
ACHase (moles hydrolyzed/min/ RBC x 10^{-16})	0.0	3.1≠0.4	3.0≠0.4
	0.32	2.5≠0.2	2.6≠0.3

a. Vitamin E (-) corresponds to those groups of mice maintained on a vitamin E deficient diet whereas vitamin E (+) corresponds to these mice on a vitamin E supplemented diet.

b. Average value and standard deviation based on ten mice per cell.

TABLE 3. Levels of Free Vitamin E in Plasma of C57L/J Mice Exposed to 0.32 ppm O_3 and Controls on Vitamin E Deficient or Supplemented Diets.

	Ozone (0.0 ppm)	Ozone (3.2 ppm)	
Vitamin E (-)	5.2 ≠ 1.5	10.14 ≠ 1.5	$p \leq 0.005$*
Vitamin E (+)	8.9 ± 3.4	12.0 ≠ 1.3	p=0.40
	p = 0.25	$p \leq 0.005$	

* p values indicate probability of difference for column or row.

Mice on a vitamin E deficient diet exhibited marked reductions in plasma levels of free vitamin E for the control (0.0 ppm of O_3) and exposed (3.2 ppm O_3) mice when compared with similar groups on a vitamin E supplemented diet. However, this difference was statistically significant only for the O_3 tested group ($p \leq 0.005$). The effect of ozone exposure was to increase the level of free vitamin E in the plasma for animals on both diets. Mice on a vitamin E deficient diet exhibited an increase from 5.2 to 10.1 µg/mL free vitamin E ($p \leq 0.005$), while mice on a vitamin E supplemented diet exhibited an increase of 8.9 to 12.0 µg/mL. This latter difference was not significant, however, (p=0.40) and may be due to the variability in measurement.

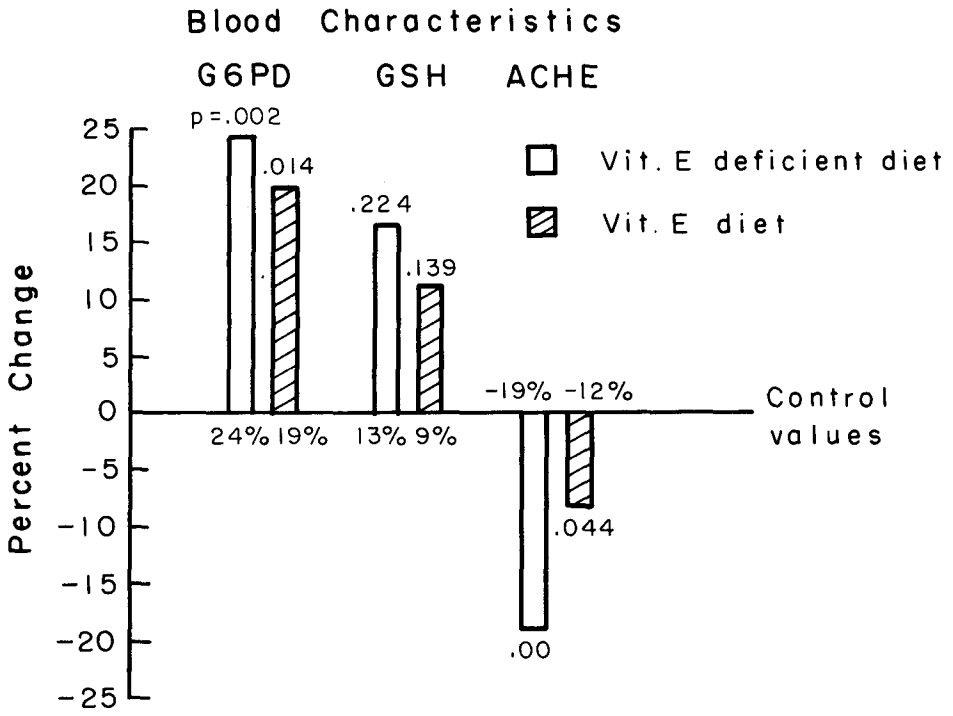

Figure 1. Percent change from controls of blood characteristics of C57L/J mice exposed to 0.32 ppm O_3 for six h.

DISCUSSION

Humans exposed to ozone in the range of 0.37 to 0.50 ppm of ozone have demonstrated increases in levels of serum vitamin E and G6PD activity while exhibiting decreases in ACHase activity. The levels of GSH were increased in some cases and decreased in others (Table 1). Studies were performed on both Canadian subjects and residents of Los Angeles, California (BUCKLEY et al. 1975; HACKNEY et al. 1977). A similarity to humans in physiologic response is demonstrated by the C57L/J mouse strain which also showed increases in serum vitamin E levels and G6PD activity and decreases in ACHase activity. The increase in GSH levels in mice is consistent with one of the human study groups (Table 1) but GSH levels appear to be highly variable with respect to O_3 exposure.

In addition to similarities in physiologic response between humans and C57L/J mouse strain, there are also metabolic similarities. The C57L/J mouse strain has similar erythrocyte G6PD structure and activity to humans (BEUTLER 1972, HUTTON 1971, MOORE & CALABRESE 1980). The normal activity range of G6PD for humans is 5 to 9 I.U./g Hb (KORNBERG & HORECKER 1955, MARK 1966) and the

activity for the C57L/J mice is 8.2 ≠ 1.5 I.U./g Hb on a vitamin E supplemented diet at 0.0 ppm O_3 exposure (Table 2). The level of erythrocyte G6PD activity is important to the response of the red cell to oxidant stress. Persons with G6PD deficiency are at increased risk to developing hemolytic anemia from oxidant compounds (BEUTLER 1972) and may be more susceptible to ozone exposure (CALABRESE et al. 1977). Therefore, levels of G6PD activity are important in considering an appropriate animal model. The selection of the mouse strain is critical since strains fall into 3 distinct categories of G6PD activity including high, medium, and low (HUTTON 1971). The C57L/J mouse strain represents the 'low' category of G6PD activity in mice and has approximately 33 percent of the G6PD activity of the high strains.

The mature red blood cells of humans and mice are anucleated while mouse erythrocytes have a smaller mean cell volume and slightly less hemoglobin than the normal adult human male. The red cell count is higher in mice while hematocrits are very similar to humans (WINTROBE 1974, JOCELYN 1972). The life span of circulating erythrocytes in mice is one-fifth to one-third (i.e. 26-46 days) of humans, suggesting a more rapid rate of erythropoiesis (WINTROBE 1974). Thus, even though the normal blood picture of inbred mouse (Mus musculus) varies somewhat from humans, the similarities in G6PD enzyme structure and activity, are relevant when considering ozone exposure. Further, the physiologic response of C57L/J mice to ozone exposure is qualitatively and quantitatively similar to humans and therefore the C57L/J mouse represents a potentially useful mocel for studying effects of certain blood characteristics following in vivo exposure to ozone via inhalation.

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