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Effects of Ozone on Exercising Horses: A Preliminary Report

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ABSTRACT. Air pollution is a problem in metropolitan areas where some racetracks are located. The objectives of this study were to evaluate the effects of ozone, a major component of oxidant air pollution, on treadmill performance by exercising horses and to determine the characteristics and distribution of lesions induced by ozone. Five Thoroughbred geldings ran on a treadmill at speeds up to 16 m s^{-1}. Three were exposed to 0.8 ppm ozone and 2 to 0.25 ppm ozone on 2 consecutive days during a 9 min exercise protocol and a 20 min cooling off period. The exposure regimen simulated a "breeze" and a race on consecutive days during an air pollution episode. All horses performed well during their first ozone exposure, but one horse exposed to 0.8 ppm ozone refused to complete the exercise protocol the second day. Ozone did not alter oxygen consumption ($\dot{V}O_2$) or respiratory frequency (f_R), but did increase heart rate (f_H). Two of the 3 horses exposed to 0.8 ppm had pulmonary hemorrhages and edema. Necrosis and sloughing of type I alveolar epithelium in the centriacinar regions were seen in all horses exposed to 0.8 ppm, but their distribution was not uniform. Lesions in horses exposed to 0.25 ppm ozone were less severe and limited to ciliated cells in terminal bronchioles.

Key words: Exercise; horses; ozone; air pollution; respiratory system; lungs.

INTRODUCTION

Air pollution occurs in many areas of the United States and other countries at concentrations that exceed the National Ambient Air Quality Standards (NAAQS) established by the US Environmental Protection Agency. Oxidant air pollution, with ozone as its archetypical component, is characteristic of smog in the California South Coast Air Basin, which includes the Los Angeles area and 5 major racetracks. In 1988, the most recent year for which data are available, the NAAQS for ozone, 0.12 ppm, was exceeded 178 days. During 78 of those days the concentration was 0.20 ppm or greater.[5] As in previous years, the highest and second highest concentration reported in that air basin in 1988 were 0.35 and 0.34 ppm.[5]

Inhalation of low concentrations of ozone results in physiological, biochemical and morphometric changes in the respiratory system.[20,21] While few physiological changes are reported in resting humans exposed to ozone, exercising humans have significant decrements in pulmonary functions.[1,15] Exercise increases the total dose of ozone inhaled by increasing ventilation, and is a major factor influencing both the total dose and the dose rate.[1,13,15,16] In a review of a large series of studies it was reported[11] that inhalation of ozone during exercise in humans results in reductions in forced vital capacity (FVC) and forced expiratory volume at 1 s (FEV_1), and an increase in airway resistance (R_{aw}). Some studies suggest that these physiological changes may be accompanied by a reduction in performance by trained athletes.[1,9,10,15,16] Although many biochemical

changes follow ozone inhalation, the usual clinical chemistry panels are not particularly useful indicators of damage due to the pollutant.[20,21] While sensitive cells, especially ciliated cells, in all parts of the conducting airways are damaged by ozone, the most significant morphological lesion occurs at the junction of the conducting and exchange areas, the centriacinar region.[6,17,20,21]

Horses, like human athletes, sometimes train and compete during periods of high concentrations of air pollution. Like humans, they significantly increase ventilation during exercise and, therefore, increase the inhaled dose of pollutants and the dose rate. The objectives of this study were to evaluate the effects of ozone on treadmill performance by exercising horses, and to determine the characteristics and distribution of the centriacinar ozone lesion in horses.

MATERIALS AND METHODS

Horses and exercise protocol

The horses used in the exercise portion of the study were 6 Thoroughbred geldings, 5.8 ± 0.8 (SD) years old and weighing 466 ± 19 kg. All of the horses had been trained for racing and several had been raced competitively. Five of the horses had run 3× per week on a treadmill for more than one year and had well documented maximal rates of O_2 consumption ($\dot{V}O_2max$) and O_2 transport variables when running at $\dot{V}O_2max$ (Table 1). The sixth horse had only 6 weeks of treadmill training prior to being used in the study.

The horses ran on the treadmill while wearing a semi-open flow mask attached to a vacuum system. The mask was a 20 cm diameter PVC tee that fit around the horse's muzzle on the long arm of the tee. Straps held the mask to the horse's halter and 2 ropes supported the weight of the mask and its tubing via an overhead pulley system. A rubber-covered bit positioned the mask so that the horse's nares were at the edge of the bias flow of gas in the cross-arm of the tee. A double rubber and foam diaphragm surrounding the horse's muzzle made a gas tight seal. This was checked on the horse by blowing pure He on the outside of the seal and checking for the presence of He on the inside with a Statham He analyzer. The inlet (i.e. upstream) side of the tee was connected to an 8 m length of flexible 20 cm diameter PVC tubing, which in turn was connected to a 2 m length of 25 cm diameter PVC pipe, providing a 10 m length of tubing for gas mixing upstream of the mask. The downstream side of the tee was connected to a 10 m length of flexible 15 cm diameter PVC tubing that was attached to a 20 m length of 25 cm diameter PVC tubing leading to a 25 hp turbine adjusted to pull gas through the system at a rate of 9 300 l (STPD) min^{-1}. At this flow rate, the pressure drop due to resistance in the upstream tubing was < 1 cmH_2O, which did not affect the horses' ventilation even at maximal exercise (i.e. no difference in VO_2max or arterial PCO_2 with or without mask).

Rates of O_2 consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) were measured in the system using the N_2-dilution technique which is accurate within 3%.[7] Each horse wore a pair of surface electrodes affixed to shaved skin on the left neck and right flank for recording electrocardiograms during the run, from which heart rates (f_H) were counted. Pressure fluctuations in the gas collection system were directly correlated with the magnitudes of inspiratory and expiratory volumes (Jones, Birks and Berry, unpublished data) and were used to measure ventilatory frequencies (f_R).

For the ozone studies each horse ran on the treadmill (0% grade) with a similar protocol on 2 consecutive days: an initial warm up trot at 4 m s^{-1} for 3 min followed by 3 min at rest standing on the treadmill. During this interval the mask system was affixed to the horse's face and, if indicated, the flow of ozone initiated. The horse then trotted for 3 min at 4 m s^{-1}, cantered for 3 min at 7 m s^{-1}, then galloped for 3 min at a speed 1 m s^{-1} faster (14–16 m s^{-1}) than that previously determined to elicit that horse's VO_2max.[4]

Table 1. *Physiological and exposure data for horses running at $\dot{V}O_2max$ on a treadmill while breathing ambient air or ozone*

N/A = not applicable

Horse No.	O_3 concentration (ppm)	O_3 uptake at $\dot{V}O_2max$ ($\mu mol\ min^{-1}$)	$\dot{V}O_2max$ in experiment (ml STPD $kg^{-1}\ s^{-1}$)	$\dot{V}O_2max$ in air ± SE (ml STPD $kg^{-1}\ s^{-1}$)	f_H in experiment (min^{-1})	f_H in air ± SE (min^{-1})
7015	0.826	43.2	–	2.56±0.03	210	202±4
7015	0.832	44.9	2.63	2.56±0.03	210	202±4
6019	0.817	50.9	2.75	2.82±0.04	207	205±1
6019	0.820	50.1	–	2.82±0.04	207	205±1
9055	0.803	40.4	2.65	N/A	201	N/A
9055	0.813	53.4	2.83	N/A	210	N/A
6143	0.258	14.5	2.48	2.71±0.08	–	205±2
6143	0.260	14.6	2.50	2.71±0.08	213	205±2
7028	0.263	20.2	2.78	2.64±0.07	210	208±4
7028	0.259	16.2	2.67	2.64±0.07	210	208±4
6090	0.040	0.0	2.91	2.94±0.02	196	206±1
J30251	0.000	N/A	N/A	N/A	N/A	N/A

$\dot{V}O_2$, f_H and f_R measurements were made between minutes 2 and 3 of the high speed run. For each of the horses that had been used in studies[4] of O_2 transport and lactate kinetics, $\dot{V}O_2max$ had been determined within 2–3 weeks of the ozone study (i.e. with continued exercise, 2–3× per week between). The running speed that elicited $\dot{V}O_2max$ varied for each horse (13–15 m s^{-1}), and they were run 1 m s^{-1} faster to ensure that they achieved $\dot{V}O_2max$. Horse 9055, that had not been trained on the treadmill as long as the others, was run at a speed of 13 m s^{-1} in order to ensure that it could complete the protocol.

Ozone exposures

On 2 consecutive days, horses were exposed to ozone only during the 9 min graded exercise test and for the first 20 min of recovery; at all other times they breathed ambient air. Ozone was generated from medical grade oxygen using silent arc discharge ozonizers (Erwin Sander Co., Model IV). Ozone concentrations were measured using an ultraviolet ozone monitor (Dasibi Environmental Corp., Model 1003-AH) calibrated using an absolute ozone photometer (Dasibi Model 1008-PC). Data were recorded on a strip chart recorder and are reported using the UV photometric standard. Ozone was introduced into the bias flow 10 m upstream of the mask, resulting in a uniform gas mixture at the mask (Reynolds number $> 10^5$). Sampling of ozone upstream and downstream of the mask with the opening of the mask sealed (i.e. no horse in line) indicated that there was negligible absorption of ozone by the tubing and mask. Rates of ozone uptake were calculated for the steady-state (i.e. between the

f_R in experiment (min^{-1})	f_R in air ± SE (min^{-1})	Comments
132	129±1	$\dot{V}O_2$ recorder failure
126	129±1	
132	132±1	
132	132±1	Failed to run 3 min
126	N/A	No previous O_2 transport data
123	N/A	No previous O_2 transport data
130	125±3	f_H recorder failure
126	125±3	
138	130±2	
138	130±2	
126	130±3	Only ran one day, ambient air
N/A	N/A	Control, not run ambient air

second and third min) high-speed portion of each horse's data runs (Table 1). They were calculated as the difference in upstream and downstream ozone concentrations multiplied by the flow rate of bias gas.

Details concerning the horses and their ozone exposures are presented in Table 1. One control horse (6090) breathed ambient room air during exercise. Another control horse (J30251) was not exercised nor exposed to ozone.

Necropsy protocol

One hour after the end of the exercise protocol each horse was walked 200 m to a necropsy room, killed with an overdose of pentobarbital IV, exsanguinated, its chest opened and the lungs photographed *in situ*. The thoracic viscera were removed *en bloc*, the trachea was cannulated, the lungs were inflated

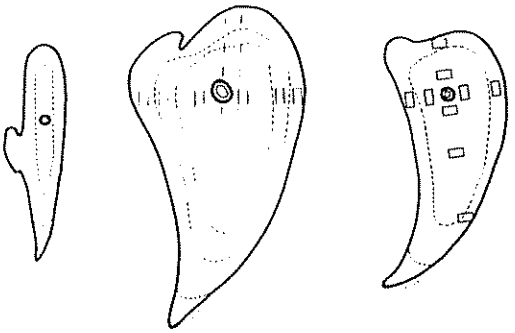


Fig. 1. Sample sites and location of lesions. Schematic transverse sections 1/4, 1/2 and 3/4 cranial to caudal length of a left lung. The position of the largest bronchus in the slice is indicated. The concentric lines indicate zones with different pulmonary arterial blood flows in the study of anesthetized ponies by Jarvis et al.[12] Highest flows were in the central portion of the middle slice, adjacent zones are SD lower flow. Lowest flows were in the subpleural portion of the cranial slice. Sample sites are indicated by the boxes.

with air from a small compressor to 30 cmH_2O pressure and the lungs were examined. The lungs were fixed with either phosphate buffered or cacodylate buffered 0.9% glutaraldehyde and 0.7% paraformaldehyde at a final pH of 7.2 and 550 mOsm.[18] Lungs from all except Horse 7015 were fixed via the trachea at 30 cm fixative pressure. Lungs from this horse had large areas of gross hemorrhage and edema and were fixed via the pulmonary artery at 46 cmH_2O pressure (34 mmHg) to assure fixation of the solid areas and to avoid moving cells and fluids in the air spaces.[18] All pressures were monitored using water manometers. Fixative pressures were maintained for a minimum of 2 hours after which the cannulas were stoppered and the lungs allowed to continue to fix. The time from exsanguination to the start of fixation did not exceed one hour.

The following day the heart, great vessels and mediastinal tissues were removed and the lung volumes determined by water displacement. The fixed lungs were cut transversely into 2 cm thick slices and inspected for lesions. Systematic samples were taken

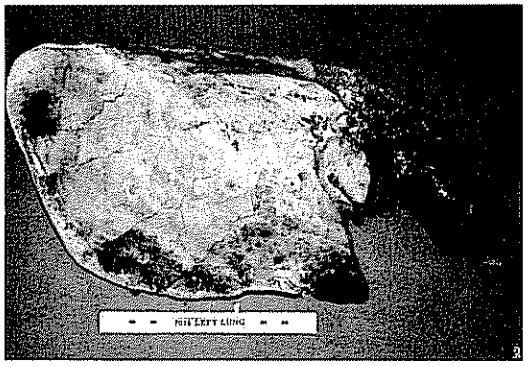


Fig. 2 Medial view of the fixed left lung from Horse 7015. Note the large areas of hemorrhage and edema in the cranial lobe and along the ventral border.

from one lung according to the general scheme in Fig. 1 and from lesions. The precise areas sampled were documented by photographing the slices with the samples individually identified and cut, but left in place in the lung slice. These sampling procedures resulted in approximately 30 sample sites from one lung of each horse.

One paraffin block, up to 20×25 mm, and 2 Araldite blocks, 6×12 mm, from each sample site were embedded, sectioned and stained using routine procedures. Areas of the Araldite plastic blocks containing centriacinar regions were selected and thin sections for transmission electron microscopy (TEM) were cut and stained using routine procedures. Centriacinar regions were examined using a Hitachi H600 TEM. All paraffin and Araldite blocks from all horses were examined by light microscopy (LM) and all sample sites (Fig. 1) from Horse 9055 have been examined by both LM and TEM at this time. Only representative blocks from selected areas of the lungs from the other horses have been examined by TEM to date.

RESULTS

Horses breathed ozone in concentrations of 0.819±0.010 (SD) ppm, 0.260±0.002 ppm, 0.040 ppm (ambient room air) or 0.000 ppm (ambient corral air). Horses breathing either of the higher concentrations of ozone extracted a similar fraction of ozone from the bias flow, 14.4±1.9%, which was approximately the ratio of minute ventilation to bias flow, suggesting that the horses were essentially extracting all of the ozone inspired.

Table 1 shows the results of the physiological measurements made on the horses for the 2 runs at $\dot{V}O_2max$ during which they breathed ozone, and the corresponding data collected previously for the same animals when they ran at $\dot{V}O_2max$ with the same protocol breathing air. Horse 6019 refused to complete the high-speed gallop during its second day of exposure. Two-way factorial analysis of variance (ANOVA) with uneven replicates detected no difference between measured $\dot{V}O_2max$ whether the horses breathed air (2.65 ml O_2 (STPD) s^{-1} kg^{-1}), 0.8 ppm ozone (2.69 ml O_2 s^{-1} kg^{-1}) or 0.25 ppm ozone (2.68 ml O_2 (STPD) s^{-1} kg^{-1}, $p=0.38$, analysis had the power to detect a 5.5% difference at the 0.05 α-level with a 90% probability). There was also no difference in f_R (129 min^{-1} in air, 132 min^{-1} in ozone, $p=0.06$, analysis had the power to detect a 4.2% difference at the 0.05 α-level with a 90% probability), although f_H were

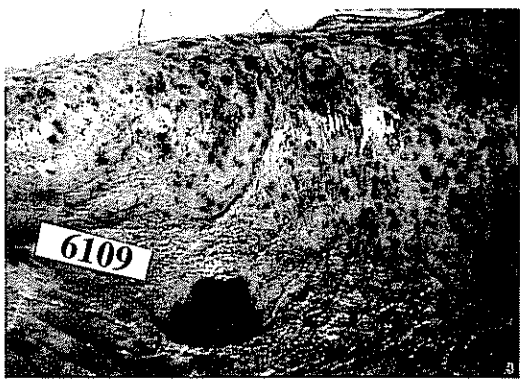


Fig 3 Lateral view of the middle portion of the left lung from Horse 6109. At this stage the lungs have been inflated with air, but were not fixed. Note the multiple hemorrhagic areas; the largest of which was 4 cm in diameter.

significantly higher (210 min^{-1} vs 205 min^{-1}, $p=0.027$) when the horses ran at $\dot{V}O_2max$ breathing ozone rather than air. For the single horse that had previously had O_2 transport data determined and ran in these experiments as a control breathing air, a Student's *t*-test was used to determine if the single values of the corresponding data in these experiments differed from the means of the same variables measured in the previous experiments. Neither $\dot{V}O_2max$ (2.94 ± 0.02 [SE] ml O_2 (STPD) s^{-1} kg^{-1} previously, 2.91 ml O_2 (STPD) s^{-1} kg^{-1} in control run, $p=0.59$) nor f_R (130 ± 3 [SE] min^{-1} previously, 126 min^{-1} in control run, $p=0.62$) differed for the 2 data sets, although f_H was significantly lower in the control run (196 min^{-1} vs 206 ± 1 [SE] min^{-1}, $p=0.005$), in contrast to the ozone-exposed horses, which had higher f_H during these runs when they breathed ozone.

The O_2 transport data for the single horse that had not previously had these data collected were compared with the corresponding data (i.e. breathing air) for the other horses using Student's *t*-tests. No significant differences existed for any of the variables ($\dot{V}O_2max$: 2.74 ml O_2 (STPD) s^{-1} kg^{-1} (single horse) vs 2.73 ml O_2 (STPD) s^{-1} kg^{-1}, $p=0.95$; f_H: 205.5 min^{-1} (single horse) vs 205.2 min^{-1}, $p=0.91$; f_R: 124.5 min^{-1} (single horse) vs 129.2 min^{-1}, $p=0.17$).

At necropsy, 2 of the 3 horses (6109 and 7015) exposed to 0.8 ppm ozone had solid bright red areas of fresh hemorrhage and edema as well as minor dark brown stained areas in the dorsocaudal portion of the lungs (Fig. 2). The fresh lesions were most severe in 7015, in which the entire cranial lobe and parts of the ventral border of the left lung were solid and red (Fig. 2). Other solid red areas, 0.5–3.0 cm in diameter, were in the ventrocranial and dorsocaudal portions of the lungs from this horse. Pink froth came out of the trachea when the lungs were deflated. In the lungs from Horse 6109, the solid red areas were smaller, 0.5–2.0 cm in diameter, but more numerous and more widely distributed (Fig. 3). These hemorrhages were readily apparent on LM. Bronchioles in these areas were slightly thickened. No specific source of the hemorrhage was identified. Lungs from all other horses appeared normal grossly and by LM with the exception in some of minor fibrous tags on the pleura and small areas of bronchiolitis with hemosiderophages typical of earlier episodes of EPIH.

Lungs from the control horses appeared normal grossly and by both LM and TEM.[19] In control centriacinar regions (Fig. 4) type 1 pneumocytes were firmly attached to both the basement membrane and to adjacent epithelial cells. Type 2 pneumocytes were dispersed along and at junctions of the interalveolar septa. Some type 2 pneumocytes occupied interalveolar pores and portions of the cells appeared on both sides of the interalveolar septum. Only few alveolar macrophages (AMs) were found. Ciliated and nonciliated bronchiolar epithelial cells appeared normal. By LM and TEM some centriacinar regions from horses exposed to either concentration of ozone appeared normal (Fig. 4).

Each of the 3 horses exposed to 0.8 ppm had centriacinar lesions (Fig. 5), but the lesions were neither uniform in intensity nor distribution. The changes were not obvious by LM, and TEM was required for all evaluations. Lesions of type I alveolar epithelium were limited to the first 2–3 interalveolar septa immediately adjacent to the end of the terminal bronchiole. Lesions were especially prominent at the tips of interalveolar septa that project into the "core" of alveolar ducts and on projections into alveolar duct bifurcations. Type 1 pneumocytes in the centriacinar region were observed in various stages of necrosis and in some areas they had sloughed leaving the basement membrane bare (Fig. 5). In areas of less severe damage, mild interstitial edema resulted in the epithelium being elevated from the basement membrane. The number of AMs appeared to be slightly increased in centriacinar regions with necrosis of type 1 pneumocytes (Fig. 5). Cilia in the terminal bronchioles of damaged

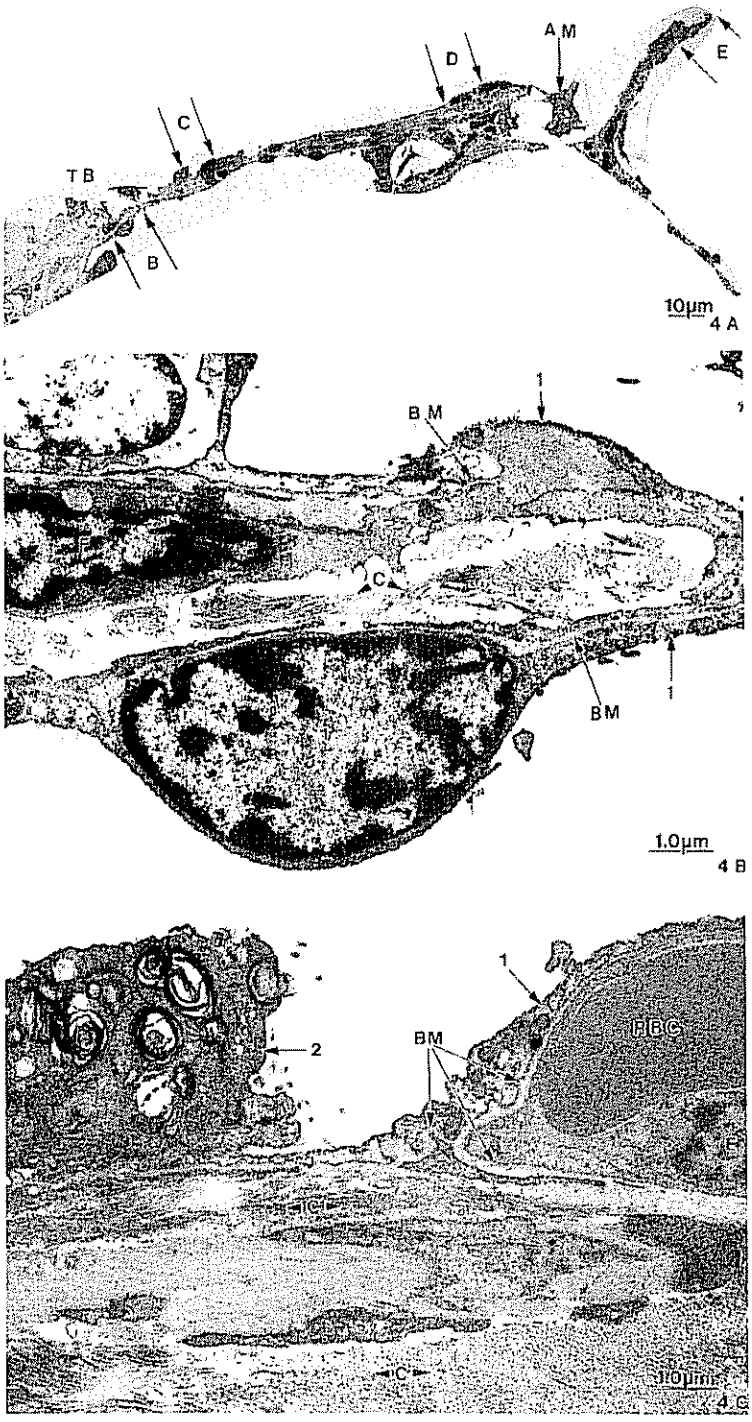
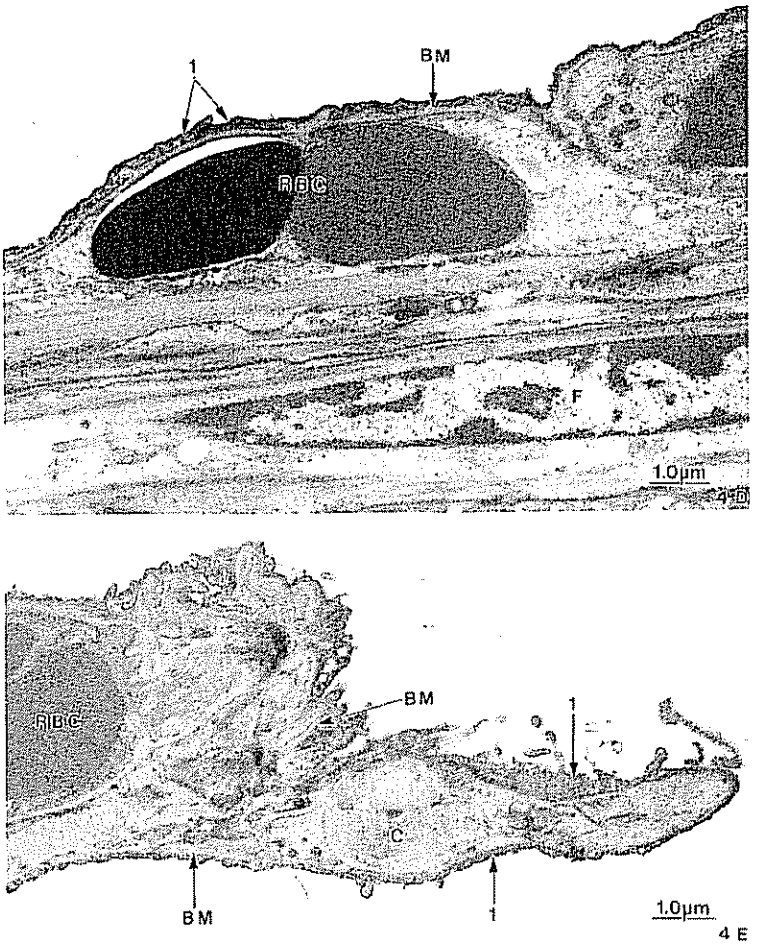


Fig 4 A is a TEM montage of a normal appearing centriacinar region from Horse 9055. TB = terminal bronchiole, AM = alveolar macrophage. Areas B, C, D and E are shown below at higher magnification. 1 = type 1 pneumocyte nucleus and thin regions, 2 = type 2 pneumocyte, BM = basement membrane, C = collagen, F = fibrocyte, E = endothelial cell, RBC = erythrocyte. Note that type 1 or 2 epithelium completely covers the basement membrane.



centriacinar regions appeared shorter than in control areas (Fig. 6). Some ciliated cells of terminal bronchioles had swollen organelles and large vacuoles (Fig. 6). In centriacinar regions with severe lesions in other epithelial cell types, mild degenerative changes were seen in some nonciliated bronchiolar cells. The only damage found to date in the lungs of horses exposed to the lower concentration of ozone (0.25 ppm) has been damage to ciliated cells in terminal bronchioles (Fig. 6).

Lungs that were inflated using the small air compressor had submicron metallic particles on and in the type I pneumocytes and AMs (Fig. 6).

DISCUSSION

The highest concentration of ozone (0.8 ppm) used in this study is more than double the usual ambient high concentration of 0.35 ppm in the California South Coast Air Basin. The lower concentration studied (0.25 ppm) is more relevant as during 1988 ozone concentrations of 0.20 ppm or higher were reported 78 days in the California South Coast Air Basin.[5] As in previous years, ozone concentrations up to 0.35 ppm were reported in that air basin in 1988.[5] Thus, it is likely that horses could be exercised or raced during periods of air pollution at or near the

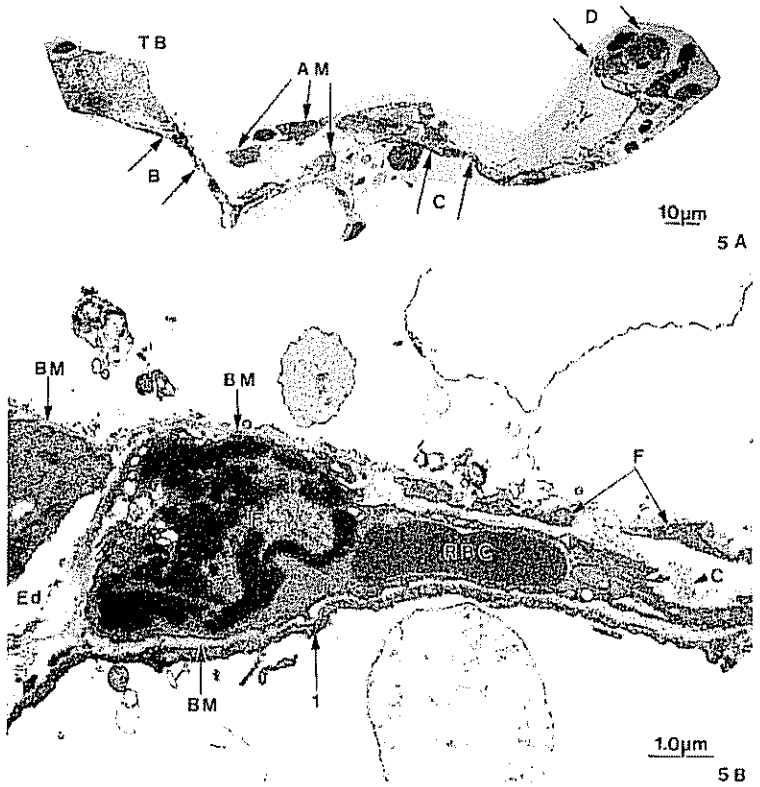


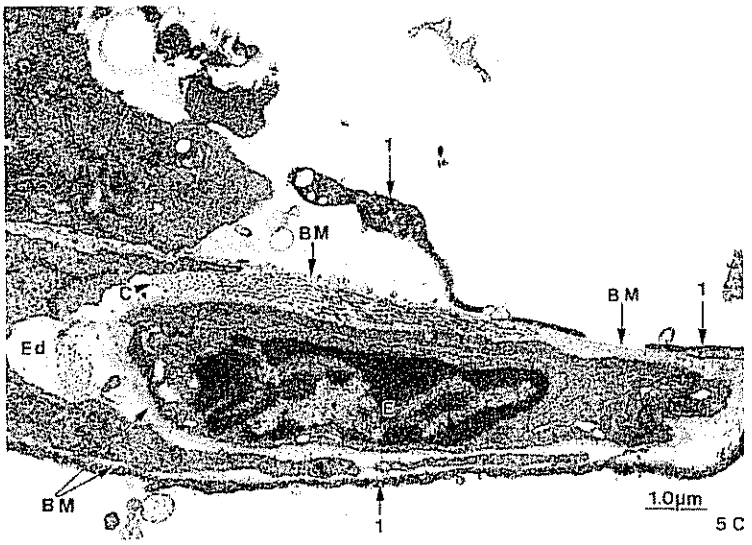
Fig. 5 A is a TEM montage of a centriacinar lesion from Horse 9055. Magnification and symbols are the same as in Fig. 4. Alveolar macrophages, AM, appear to be more numerous than in Fig. 4. Note areas in B, C and D where the type I pneumocytes are missing and the bare basement membrane or connective tissues are exposed to the alveolar air. Edema fluid, Ed, appear to elevate the type I cell from the basement membrane in B and C. SM = smooth muscle.

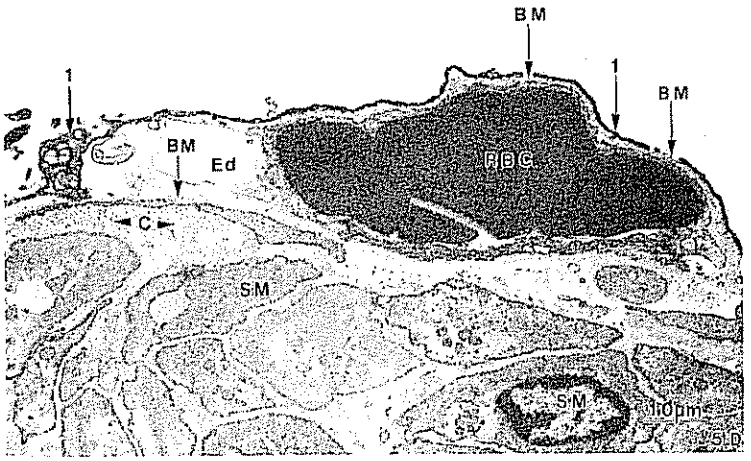
low concentration used in this study, but not at the higher concentration.

The physiological data in Table 1 indicate that Horse 9055, that had not previously had O_2 transport data collected, was physiologically similar to the other horses when running at $\dot{V}O_2max$. Breathing ozone while running, even at an unnaturally high concentration, did not appear to alter $\dot{V}O_2max$ or f_R in the horses, although f_H was slightly higher. This difference did not appear to be an artifact due to conducting the ozone experiment a few weeks after the previous studies when the horses breathed air, as the control horse demonstrated a decrease in f_H during its control run. The ability of the horses to maintain $\dot{V}O_2max$ while breathing ozone during an acute exposure is in contrast to human athletes, in whom aerobic performance decreases.[9] Part of the explanation for this difference in response may be that because the horse's ventilatory cycle is in phase with its strides, it cannot alter its breathing pattern to the same extent that human athletes do when breathing ozone, thus maintaining similar alveolar ventilation whether it is breathing air or ozone.

The observation that one high ozone concentration horse (6019) would not complete the exercise protocol the second day is similar to the observations of Schelegle and Adams.[16] In their study 7 of 10 highly trained athletes did not complete a one hour exercise protocol while exposed to 0.24 ppm ozone. These human atheletes in that study also reported that they felt worse following the second ozone exposure than after the first, third or fourth. There were also greater decrements in pulmonary functions during the second ozone exposure than the first.[8,16] Thus, there is now some evidence that breathing ozone may impair performance by athletic horses as it does human athletes.[20,21]

These are the shortest experimental expo-





sures to ozone which have been evaluated physiologically and morphologically,[6,17,20,21] but the hemorrhage seen in 2 of the 3 horses exposed to 0.8 ppm is a much more severe lesion than those reported for other species exposed while at rest to that concentration. In animals exposed while at rest, gross hemorrhage and edema are only seen at much higher concentrations, 3.0–6.0 ppm, for 4 or more hours.[20] These horses were only exposed to one episode of ozone, consisting of 29 min each, on 2 consecutive days, or less than 1 hour total exposure. By increasing ventilation, exercise increases the dose rate and thus the total dose at equivalent exposure times. These changes appear to have altered the type of lesion (i.e. gross and microscopic hemorrhage and edema) as well as the intensity of the centriacinar cellular lesion. Because gross hemorrhage and edema have not been reported in other species exposed at rest to 0.8 ppm ozone, we suspect that dose rate was the more important factor in changing the type of lesions seen at this

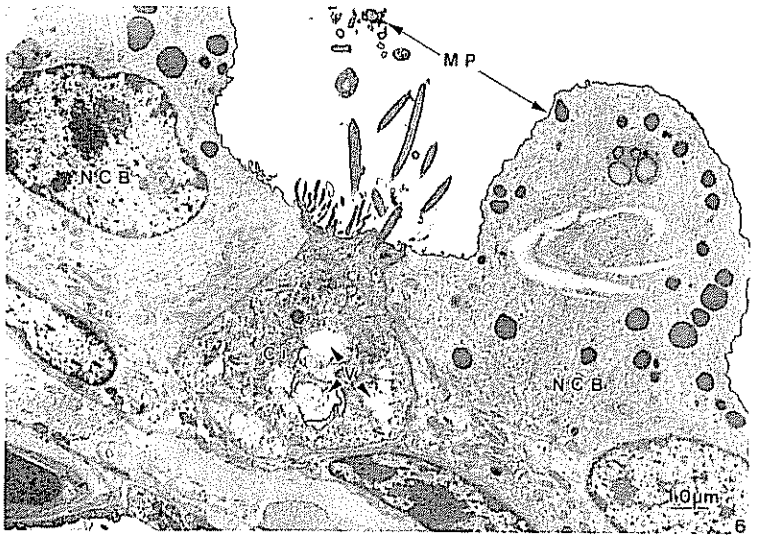


Fig 6. Ciliated and nonciliated epithelial cells in a terminal bronchiole from Horse 6143. Ci = ciliated cell, NCB = nonciliated bronchiolar cell, V = vacuoles, MP = metallic particles. Note the large vacuoles in the ciliated cell. Clefts in the supranuclear position of nonciliated bronchiolar cells are common in horse lungs fixed via the airways. Metallic particles from the air compressor coat the luminal surfaces of the epithelial cells.

concentration. It is important to note that the fresh hemorrhages in these 2 horses were much more extensive and had a different distribution than hemorrhages in exercise-induced pulmonary hemorrhages (EIPH).[14] Thus, it appears unlikely that a single episode of ozone during a breeze and a race on consecutive days would have any influence on the occurrence of EIPH during those runs. However, the effects of multiple episodes of ozone could be quite different.

The severity of the epithelial lesions in horses exposed to the highest concentration, 0.8 ppm, appear due to both the high dose and the high dose rate. The lesions were much milder in horses exposed to the lower concentration of 0.25 ppm. While effects of very short-term exposure of animals with or without exercise have not been reported, necrosis of ciliated epithelial cells and of type 1 pneumocytes has been reported to occur following the shortest exposure previously studied (i.e. exposure of rats to 0.5 ppm ozone for 2 hours[17] or monkeys to 0.8 ppm for 4 hours[6]). Because shorter ozone exposure times have not been studied, the time required for necrosis and sloughing of type 1 pneumocytes or of ciliated cells is not known. Cells probably continue to slough for some time after they are terminally damaged by ozone. The maximum amount of bare basement membrane has been reported to occur after 12 hours of continuous exposure to 0.8 ppm ozone.[6] Thus, a longer time between the start of even a short ozone exposure and fixation of the lung might result in more bare basement membrane than we saw. Inflammation, characterized by increased numbers of neutrophils (PMNs) and AMs, both within air spaces and tissues, is also an early component of the centriacinar ozone lesion.[6,17]

The centriacinar ozone lesion in horses is similar to that seen in other species.[20,21] While there are several reports of variations in intensity of centriacinar ozone lesions following long-term exposures,[3,20,21] this observation has not been reported following short-term single exposures.[6,17] Variations in intensity of centriacinar lesions might be due to the inhomogeneity in the distribution of ventilation or to differences in acinar size, either of which would alter the dose to centriacinar cells of some acini.

The significance in horses of these very small lesions, which can only be seen using TEM, may be greater than their size implies. Repeated daily exposures result in extension of the damage to type 1 pneumocytes deeper in the acinus.[3] Hyperplasia of nonciliated

bronchiolar cells and of type 2 pneumocytes starts early in ozone exposures[6,17] and becomes a prominent feature of chronic ozone exposures.[20,21] The net result of these processes is the remodeling of distal airways converting alveolar ducts to respiratory bronchioles.[3,20,21] Injury to type 1 pneumocytes is accompanied by an increase in collagen[2] and collagen content of lungs is increased following ozone exposure.[20,21] Remodeling of centriacinar airways should have an effect on ventilation of acini. Increases in collagen probably change the compliance of that specific portion of the lung. Changes in ciliated cells could be related to changes in clearance and the inflammatory response may also result in lung damage. In all species studied, repeated ozone exposures of resting animals results in a smoldering bronchiolitis[20,21] and bronchiolitis has been associated with EIPH.[14] Thus, while the lesion from an individual ozone exposure is very small and might be considered by some to be of little or no consequence, the potential consequences of repeated exposures are probably similar to those in other species and significant.[20,21]

Although it is relatively easy to determine the distribution of pulmonary blood flow (B_F) during exercise using microspheres, measuring the distribution of ventilation in horses during exercise presents technical problems. If $\dot{V}_A/\dot{Q}$ inequality is minor during exercise and shunt nearly absent, the distribution of ventilation must follow the same pattern as perfusion. Ozone is a potential marker for the distribution of ventilation as the intensity of centriacinar ozone lesions should correspond to the ventilation of that specific portion of the lung. The most severe ozone lesions should be found in areas with highest perfusion, as those areas should correspond to areas of highest ventilation and dose of ozone. The sampling scheme used here was based on observation of pulmonary B_F in horses during anesthesia[12] because there are no data available on pulmonary B_F in horses during exercise. While the distribution of centriacinar lesion was not shown to be uniform, a relationship of the intensity of centriacinar ozone lesions to the distribution of pulmonary B_F has yet to be demonstrated.

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