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Air Pollution and Brain Damage

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ABSTRACT

Exposure to complex mixtures of air pollutants produces inflammation in the upper and lower respiratory tract. Because the nasal cavity is a common portal of entry, respiratory and olfactory epithelia are vulnerable targets for toxicological damage. This study has evaluated, by light and electron microscopy and immunohistochemical expression of nuclear factor-kappa beta (NF- κ B) and inducible nitric oxide synthase (iNOS), the olfactory and respiratory nasal mucosae, olfactory bulb, and cortical and subcortical structures from 32 healthy mongrel canine residents in Southwest Metropolitan Mexico City (SWMMC), a highly polluted urban region. Findings were compared to those in 8 dogs from Tlaxcala, a less polluted, control city. In SWMMC dogs, expression of nuclear neuronal NF- κ B and iNOS in cortical endothelial cells occurred at ages 2 and 4 weeks; subsequent damage included alterations of the blood-brain barrier (BBB), degenerating cortical neurons, apoptotic glial white matter cells, deposition of apolipoprotein E (apoE)-positive lipid droplets in smooth muscle cells and pericytes, nonneuritic plaques, and neurofibrillary tangles. Persistent pulmonary inflammation and deteriorating olfactory and respiratory barriers may play a role in the neuropathology observed in the brains of these highly exposed canines. Neurodegenerative disorders such as Alzheimer's may begin early in life with air pollutants playing a crucial role.

Keywords. Air pollution; Mexico City; canines; Alzheimer's; nasal epithelia; BBB; NF κ B; iNOS.

INTRODUCTION

A complex mixture of gases, particulate matter (PM), and chemicals present in outdoor and indoor air produces adverse health effects. Because the nasal cavity is a common portal of entry for such pollutants, the nasal olfactory and respiratory mucosae are vulnerable to damage and well-known targets for air pollutant-induced toxicity and carcinogenicity (55, 85, 86, 91). The nose-brain barrier depends on intact epithelia, including tight junctions and an intact xenobiotic metabolizing capacity (37). Olfactory receptor cell dendrites are in direct contact with the environment, and, thus, pinocytosis and neuronal transport are likely routes of access to the central nervous system (CNS) of potential toxins (75). Olfactory receptor neurons project from the sensory epithelium to targets within the olfactory bulb, the first synaptic relay in the olfactory pathway (75). The mucociliary apparatus of the respiratory mucosa also functions as a barrier to protect against neuronal uptake and transport by trapping insoluble

inhaled material in a layer of secretions that are in continuous movement towards the nasopharynx (85). The contribution of air-pollutant exposure to airway epithelial injury is well documented.

Healthy children and adult populations in Southwest Metropolitan Mexico City (SWMMC)—an urban area characterized by significant daily concentrations of pollutants such as ozone, PM, and aldehydes—have shown extensive damage to the respiratory nasal epithelium (24–26, 27). Children in SWMMC display ultrastructural evidence of deficiencies in nasal epithelial junction integrity, cytoplasmic deposition of PM, and altered mucociliary defense mechanisms (27). Canines living in SWMMC exhibit similar nasal respiratory lesions (unpublished observations, Calderón-Garcidueñas), along with respiratory bronchiolar and myocardial pathology (20, 22). A sustained pulmonary inflammatory process is clearly seen in exposed canines (22), and SWMMC children show radiological and spirometric evidence of lung damage and cytokine imbalance (23). Impaired olfaction, hyposmia, or anosmia are important early changes in neurodegenerative diseases including Alzheimer's (AD) and Parkinson's disease (PD) (58, 66, 114), as well as in aging (57, 59). All layers of the olfactory bulb are affected in

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aging and AD, and olfaction is impaired in the early stages of AD (66, 67).

Aged canines are valuable models of aging (16, 36). Veterinarians have noticed geriatric behavioral changes in pet dogs including decrements in attention and activity, wandering and disorientation, and disturbances of the sleep/wake cycle (36, 98). In aged dogs, beta-amyloid accumulation correlates with cognitive dysfunction; plaques are of the diffuse subtype; and there is no neuritic involvement (36). A threshold effect of plaque development was observed by Russell (99) in 103 laboratory-raised beagles. In dogs kept in outdoor kennels at Davis, CA, and Fort Collins, CO, no plaques were apparent at ages younger than 10 years, but numbers progressively increased to 73% at ages 15 to 17.8 years (99). Weigel (111) described in a cohort of 30 mongrel dogs a subpopulation with increased numbers of β -amyloid-positive diffuse plaques and concluded that only 43% of these mongrel dogs were susceptible to amyloidosis or that only the severely affected subpopulation was exposed to a factor or factors inducing this pathology. Fibers representing an early neuritic change that precedes tau hyperphosphorylation have been described by Satou et al (101) in aging dogs.

This report describes early and progressive alterations in the nasal respiratory and olfactory mucosae, the olfactory bulb, and cortical and subcortical brain structures in healthy dogs in SWMMC exposed daily to high levels of ambient air pollutants; canines from a comparable city, Tlaxcala, with low levels of pollution represent controls. Early changes included nuclear factor-kappa beta (NF- κ B)- and inducible nitric oxide synthase (iNOS)-positive cells, vascular changes in cortical small arterioles and capillaries, apoptosis in glial and vascular smooth muscle cells and pericytes, cortical perineuronal satellitosis, and neuronal chromatolysis. These alterations were followed by reactive astrocytosis predominantly in cortical white matter and subpial regions, apolipoprotein E (apoE) immunoreactivity in abnormal lipid vacuoles in blood vessels, and astrocytic processes, nonneuritic plaques, and neurofibrillary tangles (NFT).

MATERIALS AND METHODS

Study Areas

Clinically healthy mongrel dogs from Tlaxcala and SWMMC were studied. The selection of the 2 different cities was based on their concentrations of air pollutants and similar altitudes above sea level. Metropolitan Mexico City extends over 2,000 km² and is located in an elevated valley, 2,250 m above sea level. It is a megacity with 20 million residents and the associated production of air pollutants from automobiles, leakage of petroleum gas, and industrial activity. The climate is mild with year-round sunshine, light winds, and temperature inversions. Each of these factors contributes to create an environment in which complex photochemical reactions produce oxidant chemicals and other toxic compounds. Air quality data are provided by an automated surface network of 33 monitoring stations in and around MC; hourly, near-surface measurements are made of monitored pollutants including ozone, PM₁₀, SO₂, NO₂, CO, and Pb. Mexico City's main pollutants are PM and ozone, with levels exceeding US National Ambient Air Quality Standards (NAAQS) most of the year. The maximal concentrations of ozone precursors

appear downwind of the emission zones toward the southern urban area, southwest and southeast MC (47). According to Fast and Zhong (43), the highest particle concentrations occur regularly in the vicinity of the peak ozone concentrations during the afternoon. Ozone concentrations as high as 0.48 ppm have been measured during severe air pollution (13); the SWMMC atmosphere is characterized by average maximal ozone daily concentrations of 0.250 ppm. An average of 4 ± 1 hr/day with ozone >0.08 ppm is recorded in SWMMC year-round (83.9% of days) (47). NO₂ concentrations do not usually exceed the annual arithmetic mean of 0.053 ppm (4.6% of days), whereas SO₂ levels exceed the 24-hr primary standard of 0.14 ppm in the winter months. Both PM₁₀ and PM_{2.5} exceed their respective annual arithmetic means above the standards (annual NAAQS PM₁₀ 78 μ g/m³ and PM_{2.5} 21.6 μ g/m³ vs standards of 50 μ g/m³ and 15 μ g/m³, respectively) (31, 41). Other pollutants detected in SWMMC include volatile organic compounds (VOC), such as linear and cyclic saturated and unsaturated hydrocarbons (HC); aromatic HC; aldehydes; ketones; esters; and acids and their halogenated derivatives (77). Formaldehyde and acetaldehyde ambient values are in the range of 5.9 to 110 ppbv, and 2 to 66.7 ppbv, respectively (5). Mutagenic particulate matter (108); alkane hydrocarbons (13); benzene (81); various metals such as vanadium, manganese, and chromium (96); and peroxyacetyl nitrate (41) are also detected. Lichens absorb their nutrients from the atmosphere and can be used as sensitive monitors of airborne metals (107); *Parmotrema arnoldii* accumulates lead, copper, and zinc in SWMMC (77). In addition, 500 metric tons of canine fecal material are deposited daily on MC streets (28).

The rationale behind selecting the SW geographical area in MC involved 2 major factors. First, the spacial distribution of pollutants such PM₁₀, O₃, SO₂, and NO₂ within the city reflects the higher concentrations of particulate and gaseous emissions in the northern part of the city where most industries are located. Ozone levels are higher in the south, a residential area, as a result of wind transport of the mass precursor pollutants emitted in the industrial northern and central regions. Second, our studies of healthy children and adults living in SWMMC present evidence of nasal and pulmonary pathology, as well as serum cytokine imbalance in children (21, 23).

Tlaxcala was selected as the control city because its altitude above sea level is similar to that of MC, and studies in canines from this area have shown minimal pulmonary and cardiac pathology (20, 22). Tlaxcala is the capital city of the state of Tlaxcala, located 114 km east of Mexico City at 2,252 m above sea level. It has a temperate climate, an average temperature year-round of 16°C, and 700 mm of annual rainfall. With 63,423 inhabitants, it is a city in compliance with current air pollution regulations.

Atmospheric Pollutant Data

Mexico City's atmospheric pollutant data were obtained from the available literature and a representative monitoring station located in the SW. Tlaxcala's data were obtained from the Subsecretaria de Ecología. SWMMC data represent air pollution patterns corresponding to the ages of the dogs and tissue collection times (2000–2001).

TABLE 1.—Physical descriptions of Tlaxcala control and SWMMC canines.

Age	Control (gender)	SWMMC (gender)	Average weight
24–48 h	0	4 (2F/2M)*	275 ± 120 g
2–4 wk	0	3 (1F/2M)*	554 ± 190 g
1 and 3 mo	0	2 (M)*	460 g and 7 kg
8–12 mo	2 (1F/1M)	2 (M)*(F)	11.2 ± 2.4 kg
>1 yr to <3 yr	2 (1F/1M)	4 (2F/2M)	12.16 ± 5.74 kg
4–5 yr	1 (F)	4 (2F/2M)	20.3 ± 5.2 kg
6–8 yr	2 (F)	11 (7F/4M)**	22.0 ± 8.6 kg
11 and 12 yr	1 (F)	2 (1F/1M)	37.33 ± 11.01 kg
Total	8	32	

*Animals raised at the animal facility outdoor/indoor kennel.

**Two males ages 6 and 7 also raised at the animal facility.

Canine Population

Physical descriptions of the mongrel dogs used for this study (18M/22F)—8 from Tlaxcala and 32 from SWMMC—are presented in Table 1. The younger SWMMC dogs (≤ 1 year) and 2 of the older dogs were whelped and continuously housed in an outdoor-indoor kennel. The remaining older (> 1 yr) SWMMC dogs and all the Tlaxcala dogs were home-raised and kept continuously outdoors. These dogs were periodically seen by a veterinarian, received all of the applicable vaccinations, and were dewormed regularly. They were fed twice daily with a combination of owners' choice commercial dog chow and home food leftovers and had free access to potable water. These dogs had no contact with other animals, lived permanently in the selected residential area, and received no exposures to local toxins such as paints, metals, and solvents. Dogs kept in an outdoor-indoor kennel were housed 1 dog per run, fed once a day (Purina Dog Chow, Ralston-Purina, Cuautitlan, Mexico), and provided water ad libitum. They were under close clinical veterinary observation during their entire lives, and at no time was there any evidence of overt respiratory, cardiovascular, or neurological disease. None of the dogs had been outside their residential area or lived in a different city.

The study protocol was reviewed and approved by the Basic Research Committee of the Instituto Nacional de Pediatría in Mexico City. Husbandry was in compliance with all regulations of the American Association of Laboratory Animal Certification (AALAC). We applied the dementia index suggested by Uchino (105) to all participant dogs, except those younger than 3 months. Euthanasia was conducted in accordance with established guidelines and applicable animal care-and-use regulations (92).

Necropsy and Tissue Preparation

Animals were examined by a veterinarian before they were euthanized. A brief clinical exam included cardiac and pulmonary auscultation, an abdominal and peripheral lymph node palpation, and a neurological examination. Whole blood was taken for a complete complete blood count (CBC). Each dog was sedated with IV xilazine (Rompum, Bayer, Leverkusen, Germany) and then deeply anesthetized with sodium pentobarbital (Salud y Bienestar, Mexico). A gross external description was noted after their demise; animals were weighed; and a complete necropsy was performed. Immediately after death, the skull was opened and the olfactory bulbs and brain removed. Nasal tissues including the nasoturbinates, maxilloturbinates, and ethmoturbinates

(including the olfactory epithelium) were carefully dissected; the anatomical regions were identified; and tissues from specific anatomical areas were labeled separately. Separate sets of these tissues and the olfactory bulbs were immediately immersed in 2 types of fixatives for EM and LM, respectively: 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1M phosphate buffer at pH 7.4, and 10% neutral formaldehyde. The base of the skull was inspected for gross abnormalities. The brain was carefully examined, and alternating right and left cerebral hemispheres were quickly frozen and stored at -80°C for future studies. The uncut hemisphere and the remaining portions of the brain stem and cerebellum were fixed in 10% neutral formaldehyde for 2 weeks. Paraffin sections for light microscopy (LM) were then cut from the following regions: frontal (sigmoides anterior and posterior), postcruciate gyrus, gyrus coronalis, gyrus ectolateralis, posterior suprasylvian gyrus gray and white matter, hippocampus, entorhinal cortex, olfactory bulbs, cerebellum, caudate nuclei, thalamus, hypothalamus, midbrain, pons, and medulla. The location of the sample in each of these regions was standardized in every brain. The trachea, extrapulmonary bronchi and lungs, and heart were excised intact from the thoracic cavity, and the abdominal cavity was opened and examined. In a group of 14 dogs (7 from each city) matched by age and gender, frontal lobe samples were processed for electron microscopy (EM). Samples of liver, spleen, and kidneys were obtained and immediately immersed in 10% neutral formaldehyde. Other sections were cut from the caudal and cranial lung lobes, peribronchial and peritracheal lymph nodes, right and left ventricles, interventricular septum, atrium, and pancreas.

Paraffin sections 5- μm thick were cut and stained routinely with hematoxylin and eosin. Special stains included a modified Bielschowsky silver stain, Congo red, thioflavin-S, periodic acid methenamine silver (PAM), and periodic-acid-Schiff (PAS). Apoptosis was evaluated through analysis of nuclear and cellular morphology (LM and EM) and DNA fragmentation. The terminal deoxynucleotidyl transferase (TdT) labeling assay (TUNEL, ApopTag, Intergen Company, New York, NY) was used to assess cells with DNA strand breaks (72). Immunohistochemistry (IHC) was performed using the following antibodies at the indicated dilutions: GFAP, 1:100 (Boehringer Mannheim, Indianapolis, IN, USA); apolipoprotein E (apoE), 1:500 (Bioscience, Saco, Maine, USA); tau AT8 phosphorylated Ser-202 and Thr-205, 1:300 (Innogenetics, Alpharetta, GA, USA); NF- κB p65 transcription factor, 1:100 (Transduction Laboratories, Lexington, KY, USA); anti-macNOS (macrophage inducible iNOS) mAb N32030, 1:500 (Transduction Laboratories, Lexington, KY); and CD68 KP1, 1:100 (Novocastra Laboratories, Newcastle upon Tyne, UK). Briefly, 5 μm paraffin sections were dried overnight, deparaffinized in xylene, and rehydrated through graded alcohols. Slides were immersed in 10 mM citrate buffer (pH 6.0) and heated in a microwave oven, 4 cycles of 5 minutes each, to unmask antigenic sites. Thereafter, slides were removed and cooled for 15 minutes at room temperature before washing in PBS. Endogenous peroxidase activity was inhibited by rinsing slides in 0.1% hydrogen peroxide for 10 minutes. Following washing in PBS, sections were incubated overnight at 4°C with antiserum. Immunohistochemical localization was performed

using an avidin-biotinylated peroxidase complex method (Vectastain ABC kit, Vector Laboratories, Burlingame, CA). Negative controls included utilization of nonspecific IgG instead of the primary antibodies and preabsorption of primary antibodies with the respective cognate peptides. The histopathologic severity of the brain findings was assessed semiquantitatively using grades from 0–3 [0) no pathological change, 2) moderate change to 3) most severe]. The parameters evaluated included the presence of histological elements characteristic of neuronal, glial necrosis or apoptosis; the distribution and characteristics of astrocytes (H&E, GFAP, and NF- κ B p65), microglia (H&E), and oligodendroglia; and vascular changes (H&E and 1- μ m toluidine-blue sections). The olfactory epithelium was evaluated using the terminology suggested by Hardisty et al (54) for rat olfactory tissues. Pathological findings were divided into 5 categories: degeneration, regeneration, postdegenerative atrophy, inflammation, and respiratory metaplasia/basal cell hyperplasia. The grading system scored pathology as follows: 0) within normal limits, 1) minimal (apparent loss of a few sensory cells), 2) mild (focal loss of sensory cells with some sustentacular or other cellular damage involving up to about 25% of the olfactory mucosa in the section), 3) moderate (more extensive epithelial damage or lesions extending into the lamina propria to involve Bowman's glands or other structures, with up to 50% of the olfactory mucosa in the section involved); 4) marked (up to 75% of the olfactory mucosa in the section involved; other tissue changes occur, such as inflammation or edema, in addition to sensory cell loss, sustentacular cellular and/or Bowman's gland acinar ablation); and 5) severe (complete loss of olfactory mucosa in the affected area along with changes that might inhibit repair, such as loss of basal cells in association with epithelial erosion or ulceration). Sections were read blindly by experienced observers with no access to the codes regarding the geographical source of the animals. Light and electron microscopic images were transmitted to Adobe Photoshop 4.0 using a Nikon scanner.

Statistics was performed using the InStat program (Graph Pad, San Diego, CA). Differences in the hematological parameters between control and exposed dogs were tested with the unpaired *t*-test. Significance was assumed at $p < 0.05$. Data are expressed as mean values \pm SD.

RESULTS

Air Quality Data

People and animals in Mexico City are chronically exposed to a complex mixture of air pollutants. The numbers of hours per year that SWMMC residents have been exposed to ozone above the USNAAQS for the years 1984–1998 are: 40, 30, 740, 959, 1,224, 1,403, 1,561, 1,395, 1,146, 1,061, 1,249, 1,080, 1,123, 1,203, and 1,342, respectively. The marked increase in the number of daily ozone excesses in Mexico City initially started in the fall of 1986 when the atmospheric air deteriorated from highly reductive to oxidative, coinciding with the introduction of a new gasoline with a tetraethyl lead concentration of 0.64 ml/gal (47). In 1989, a new change in reactivity of the emitted VOCs occurred following the introduction of methyl-*t*-butyl ether (MTBE) to Mexico City gasolines (18). Figure 1 illustrates the average daily concentrations of ozone (ppm), PM₁₀ (ug/m³), NO (ppb), NO₂ (ppb),

SO₂ (ppb), CO (ppm), relative humidity (%) and temperature (°C) for the year 2000. PM₁₀ and PM_{2.5} exceeded their respective NAAQS (annual PM₁₀ 78 ug/m³ and PM_{2.5} 21.6 ug/m³ vs standards of 50 ug/m³ and 15 ug/m³), respectively (31, 41).

Clinical and Gross Pathological Observations

The two older dogs from our SWMMC animal facility, males ages 6 and 7 years, exhibited decrements of attention and activity and disturbances of the sleep/wake cycle during the year preceding the study; one of them already had pre-dementia scores (Table 2). The caretakers reported transient episodes during which the dogs failed to recognize their habitual caretakers and would sporadically show short episodes of decreased body language. No changes in food intake were noted, and their weights remained stable. Using the Uchino et al criteria for evaluation in dogs (105), all Tlaxcala control animals showed scores of 10 corresponding to normal. SWMMC animals, except the 7-year-old with a score of 29, had scores still within the normal range (10–21); however, caretakers were aware of alterations of sleep patterns and barking.

All of the animals in this study were healthy without evidence of overt neurological, respiratory, or cardiovascular disease. There were no overweight dogs. For 2 of the SWMMC dogs, ages 3 and 8 yr, owners reported occasional epistaxis that did not require veterinary attention. The results of the CBCs are shown in Table 3. There was a significant difference ($p < 0.0001$) in the numbers of monocytes between controls (3.02 ± 1.4) and exposed (10.36 ± 3.28).

Lungs from MC dogs displayed patchy whitish pleural areas with scattered anthracotic macules. These pleural changes were seen in every lobe and had no apparent relationship with the ribs or lung apices. The changes were particularly prominent in older dogs (>5 years). Enlarged, congested, anthracotic pulmonary hilar lymph nodes were observed in all MC dogs. No evidence of intercurrent lung disease was seen in any of the animals. The hearts showed no gross abnormalities. No other gross abnormal findings were seen, with the exception of small calculi in the pyelocalyceal system of the SWMMC 12-year-old dog.

Non-Central Nervous System (CNS) Findings

Pulmonary Histopathology: Dogs from Tlaxcala exhibited scattered minute anthracotic pleural areas and discrete areas of pleural thickening. SWMMC dogs showed respiratory bronchiolar epithelial hyperplasia; smooth muscle hyperplasia was salient in terminal and respiratory bronchioles (Figure 2A). Chronic mononuclear cellular infiltrates along with macrophages loaded with PM commonly surrounded the bronchiolar walls and extended into adjacent vascular structures (Figure 2A). NF- κ B was seen in the nuclei of alveolar type II cells and cytoplasm of alveolar macrophages in all SWMMC animals starting at age 4 weeks. In lung-associated lymph nodes, a distortion of the normal architecture with dilatation of peritubercular and subcapsular sinuses occurred, and a mixture of coal-like particles and brown-yellow, birefringent particles was present in macrophages. Tlaxcala dogs exhibited mild bronchiolar epithelial hyperplasia along with focal smooth muscle cellular hyperplasia. Alveolar macrophages with PM were present in small

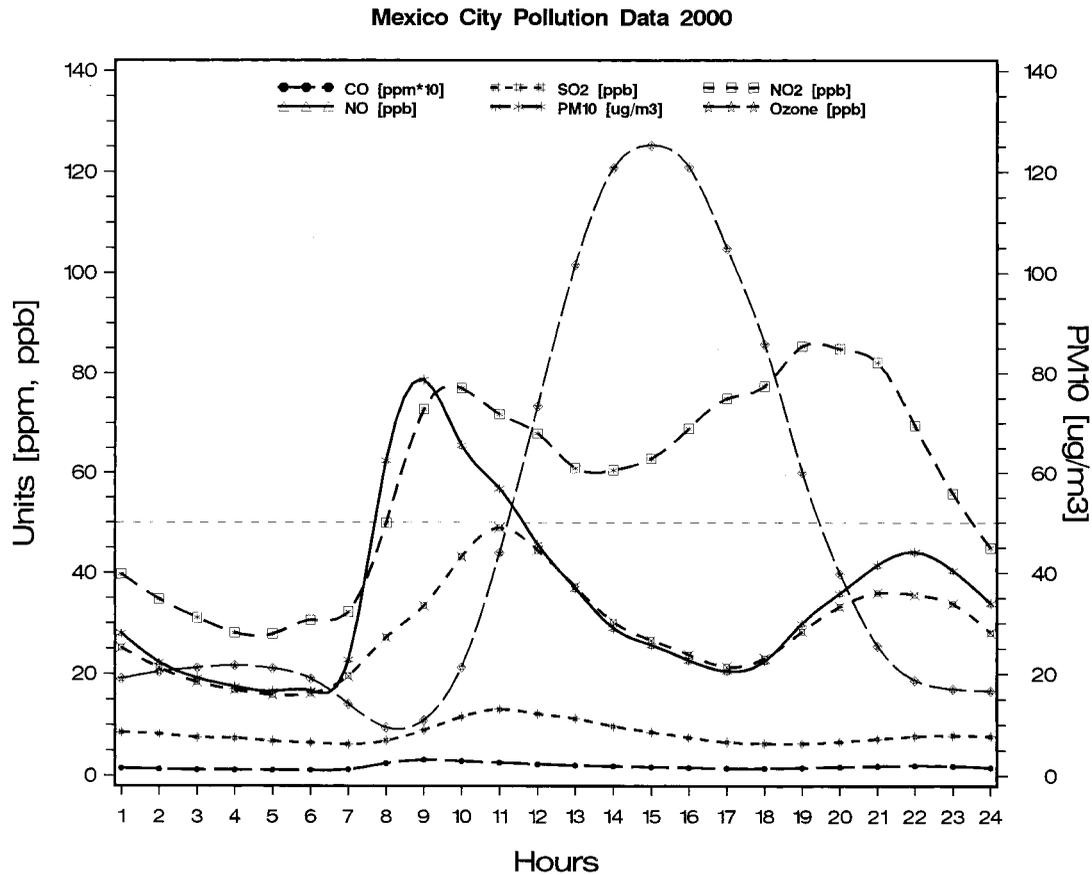


FIGURE 1.—SWMMC air pollutant data, daily averages, year 2000; main pollutants included. Notice that animals and people exposed to the SWMMC environment are sequentially exposed to significant concentrations of ozone, PM₁₀, and nitrogen oxides (NO). The dashed horizontal line marks the PM₁₀ annual standard (50 ug/m³). The two main pollutants above the current standards are ozone and PM₁₀.

numbers, and rare foci of inflammatory cells were seen in association with either terminal bronchioles or pulmonary blood vessels.

Nasal Histopathology Respiratory Epithelium: Young Tlaxcala dogs showed unremarkable pseudostratified ciliated columnar epithelium with goblet cells, whereas older dogs had focal areas of squamous metaplasia and mild-to-moderate thickening of the basement membrane. SWMMC dogs younger than 3 months displayed unremarkable respiratory nasal epithelium. Changes in SWMMC dogs became progressively worse with age and were characterized by extensive replacement of the mucociliary epithelium by focal goblet cell hyperplasia in younger dogs, followed by replacement of the ciliated goblet cell epithelium by squamous metaplastic epithelium with scattered intraepithelial

polymorphonuclear leucocytes (PMNs) (Figure 2B, C). iNOS granular positivity in ciliated epithelial cells was observed in all dogs (controls and SWMMC) with intact respiratory epithelium. In dogs with squamous metaplastic changes, however, the iNOS positivity extended to include the full thickness of the epithelium (Figure 2C). The submucosal glands exhibited dilatation and atrophy in a few cases, and there were numerous glands with strong iNOS positivity in SWMMC dogs (Figure 2C). In addition, marked thickening of the basement membrane and a predominantly chronic, discrete, inflammatory submucosal infiltrate were observed.

Nasal Histopathology Olfactory Epithelium: Sections from the endoturbinates regions III and IV representative of olfactory epithelium (82) were identified and compared in the different dogs. Grossly, the olfactory region showed a distinct yellowish-brown color in Tlaxcala and the younger SWMMC dogs. Older dogs in Tlaxcala and SWMMC exhibited a gray-whitish discoloration of the olfactory area. Microscopically, the neurosensory epithelium was intact and well defined in all control dogs and SWMMC dogs younger than 3 months. Microscopic changes were detected in SWMMC young dogs, the earliest in the 8-month-old. The changes were focal and characterized by disruption of the orientation of the sensory olfactory and sustentacular cells as well as degeneration with loss of both of these cell types, resulting in a decrease in the

TABLE 2.—Total scores for the Dementia Index for control and SWMMC dogs.*

Age	Controls (average score)	SWMMC
< 3 yr	10	10
4-5 yr	10	15
>6-8 yr	10	16
11 and 12 yr	10	28

*Criteria of Uchino (105) for evaluation of dementia in dogs: 10-21 normal, 21-29 predementia, >29 dementia.

TABLE 3.—CBC hematological data for control and SWMMC canines >1 year.

Groups	Hb	Ht	WBC	Lymph	Monos*	PMN	Platelt	MPV
Control	15.4 ± 2.7	44.8 ± 7.6	14.5 ± 3.1	45.9 ± 38.5	3.0 ± 1.4	29.6 ± 30.5	291,000 ± 17900	9.2 ± 1.1
SWMC	15.7 ± 2.4	48.4 ± 7.4	12.4 ± 4.7	78.5 ± 10.9	10.3 ± 3.2	7.1 ± 5.0	248,000 ± 72000	10.4 ± 1.4

**p* < 0.0001.

thickness of the neuroepithelium (Figure 2D). Production of pseudoglandular cyst-like structures was seen sporadically; however, proliferation of undifferentiated cells originating from the basal layer was rare. The changes became progres-

sively worse in older animals (>5 years) where large patches of olfactory mucosa were thin and devoid of any architectural orientation. Pyknotic nuclei and scattered PMNs and mononuclear cells were observed at all levels of the altered

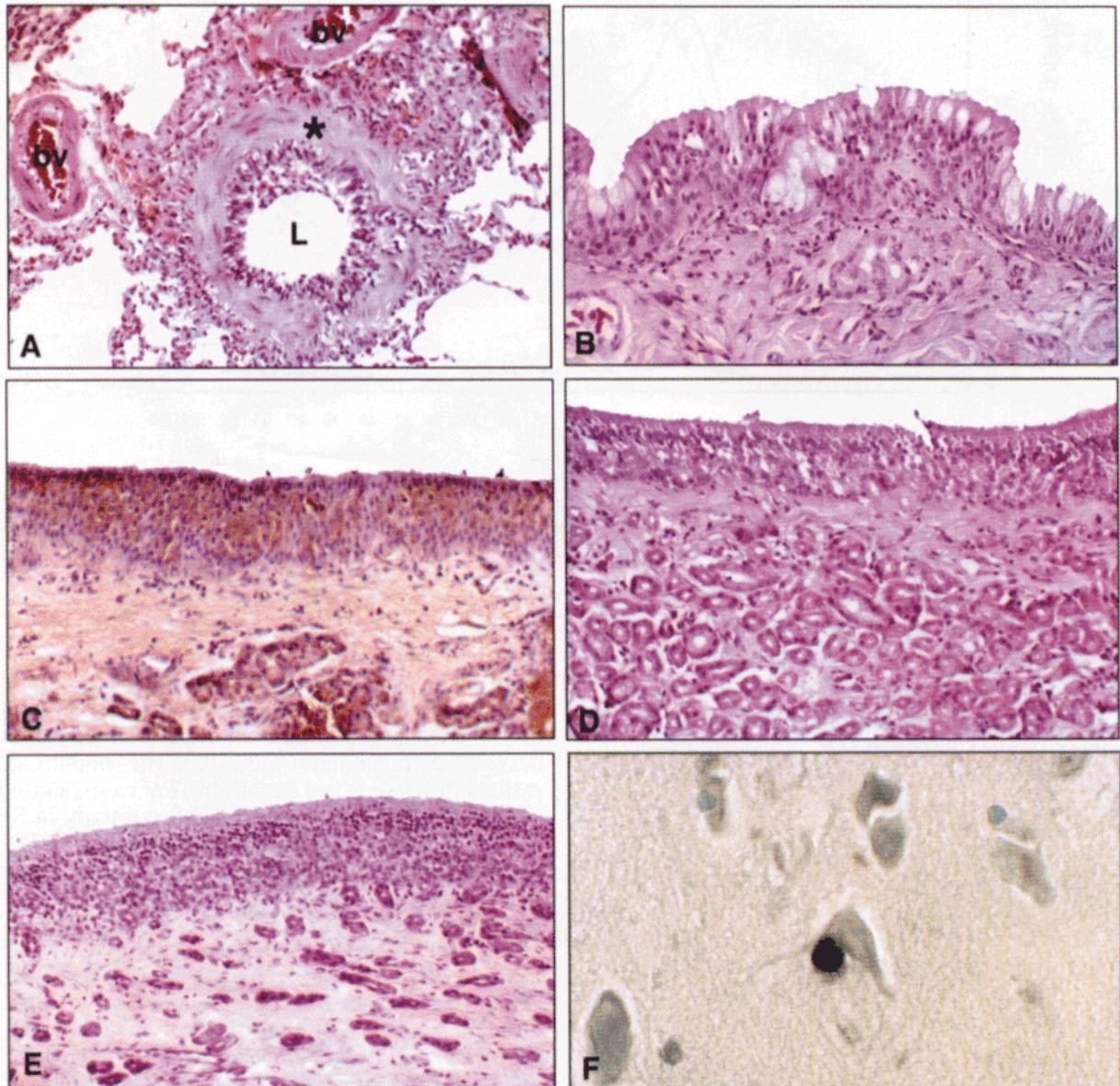


FIGURE 2.—SWMMC dogs. 2A) Respiratory bronchiole, 3-year-old. Note the striking smooth muscle cell hyperplasia (black*) and desquamated bronchiolar cells in the lumen. A chronic mononuclear cell infiltrate with numerous macrophages loaded with particulate matter (white*) is seen in the peribronchiolar region and extends to adjacent blood vessels (bv). H&E. ×107. 2B) Nasal respiratory epithelium, 1-year-old. The epithelium is variably thickened and contains abundant goblet cells and a paucity of ciliated cells. Inflammatory cells have infiltrated the epithelium and submucosa. H&E. ×214. 2C) Nasal respiratory epithelium, 3-year-old. The normal mucociliary epithelium has been replaced by a metaplastic squamous epithelium with strong iNOS expression. ×214. 2D) Olfactory epithelium, same dog as 2B. There is focal loss of sensory and sustentacular cells without evidence of regeneration. The basement membrane is thickened, and a few chronic inflammatory cells are present in the submucosa. H&E. ×214. 2E) Olfactory epithelium, same dog as 2C. There are epithelial disorganization and paucity of Bowman's glands. H&E. ×214. 2F) Olfactory bulb. A glial cell apoptotic nucleus is seen in close contact with a neuron. TUNEL. Lightly counterstained with methyl green. ×420.

TABLE 4.—Olfactory epithelial histopathology in control and SWMMC canines.

Histopathology	Groups	0	1	2	3	4
Olfactory epithelium*	Control					
	SWMMC					
Degeneration	Control	5	3	0	0	0
	SWMMC	9	17	4	2	0
Regeneration	Control	7	1	0	0	0
	SWMMC	14	12	5	1	0
Postdegenerative atrophy	Control	7	1	0	0	0
	SWMMC	29	2	1	0	0
Inflammation	Control	6	2	0	0	0
	SWMMC	10	20	2	0	0
Respiratory metaplasia	Control	5	3	0	0	0
	SWMMC	15	12	1	3	1
Basal cell hyperplasia	Control	8	0	0	0	0
	SWMMC	32	0	0	0	0

*Descriptive terminology based on Hardisty et al (54) for rat olfactory tissues.

epithelium and in the submucosa (Figure 2D). The basement membrane was thickened, and an increase in loose connective tissue was noticed in older animals (Figure 2D). In some areas there was focal replacement of the degenerative neurosensory epithelium by squamous metaplastic epithelium. Tubuloacinar olfactory glands were intact in younger SWMMC and control animals; however, SWMMC dogs older than 1 year showed scattered hyperchromatic nuclei and deposition of abundant gold-brown pigment in the cytoplasm. Areas with atrophic Bowman's glands surrounded by loose connective tissue were focally prominent (Figure 2E). Bundles of unmyelinated olfactory nerve fibers and myelinated nerve fibers (trigeminal nerve) were unremarkable in Tlaxcala dogs ≤ 3 years and the younger SWMMC canines; however, in SWMMC dogs older than 5 years, the degeneration of the sensory and sustentacular cells was accompanied by an apparent loss of nerve bundles in the lamina propria. Strongly positive iNOS staining was seen in the slender apical portion of the neurosensory neurons projecting to the surface of the epithelium, as well as in the ducts of the olfactory glands coursing through the propia and epithelium. A summary of the olfactory epithelial findings is presented in Table 4.

CNS Findings

All dogs were mesaticephalic except one—a mixed collie breed that was dolichocephalic. Brains were cut on the coronal plane, and there was no evidence of ventricular dilatation or cortical atrophy in any animal. The leptomeninges were thickened in SWMMC animals older than 8 years; however, the change was minimal. A summary of neuropathological findings is seen in Table 5.

Brain Histopathology Olfactory Bulbs: Apoptotic cells, seen in the different layers in all control and exposed animals, were rare in controls and few to moderate in exposed dogs. They corresponded mainly to glial cells (Figure 2F). Reactive

astrocytosis present in all layers (including external and internal plexiform layers, mitral cell layer, and the olfactory glomeruli) varied in intensity but was seen in every SWMMC dog starting at age 8 months. Senile plaques and NFTs were not seen by tau and modified Bielschowsky silver stain. Vascular changes characterized by foamy yellowish structures in the walls of small arterioles and capillaries were better defined in 1-um-thick, toluidine blue, plastic-embedded sections, and displayed the same features as the vascular changes described in cortical areas. Except for a few white matter reactive astrocytes in the older control dogs, the olfactory bulbs were unremarkable.

Brain Histopathology Cortical Sections: Astrocytes with a small amount of eosinophilic cytoplasm were seen around blood vessels and neurons in young SWMMC dogs. These astrocytes were predominantly cortical and did not stain with GFAP. Reactive astrocytes were focally prominent in subpial areas of all SWMMC animals starting at age 8 to 12 months; positive GFAP processes could be seen along penetrating cortical blood vessels. Very few GFAP-positive astrocytes were seen in the gray matter regardless of the age of the dog; however, the white matter showed progressively increased numbers of reactive astrocytes. In the younger animals the positive processes were restricted to perivascular areas (Figure 3A); in the older animals (>5 years) the reactive astrocytes were abundant in the neuropil without any obvious relationship with blood vessels (Figure 3B). A few multinucleated astrocytes were scattered in the white matter of older dogs, and some showed hyperchromatic nuclei. Foci of satellitosis and chromatolysis were observed in the cortical gray and appeared more prominent in frontal and entorhinal cortices. Neurons in layers III-VI showed dark, shrunken cytoplasmic bodies surrounded by numerous glial nuclei; up to 8 nuclei were seen (Figure 3C). Large pyramidal neurons were particularly affected. Neuronal changes with satellitosis were seen in the 8- and 12-month-old dogs.

The paucity of frontal neurons was remarkable in ≥ 4 -year-old dogs (Figure 3D). TUNEL revealed positive astrocytic and oligodendroglial nuclei in close relationship with dark neurons. In younger dogs apoptotic cells were restricted to perivascular areas, predominantly in the white matter. No neurons showed positive TUNEL nuclei. Older animals (>5 years) exhibited numerous apoptotic nuclei both in the white matter and, to a lesser degree, in the cortex. Control animals exhibited a rare apoptotic nucleus in the white matter. Microglial cells with ameboid profiles could be seen in the cortex and white matter. Scattered cortical ameboid microglia could be seen as early as 8 months; however, their number increased with age, particularly in the gray matter. Endothelial cells exhibited a granular cytoplasmic staining with the CD68 antibody, and a few pyramidal neurons also exhibited

TABLE 5.—Summary of neuropathological findings in SWMMC canines.

Age groups	NF- κ B nuclear	NF- κ B glial	iNOS	Neuronal changes	LM vascular pathology	Apoptosis	GAFF	NFT*	Nonneuritic plaques
0-3 mo (n = 9)	+	0	0	0	0	0	0	0	0
8 mo to 3 yr (n = 6)	+	+	+	+	+	+	+ perivascular	0	0
4-8 yr (n = 15)	+	++	++	++	++	++	++	+	+
11-12 yr (n = 2)	+	++	+++	+++	+++	++	++	+	+

*Tau and modified Bielschowsky silver stain.

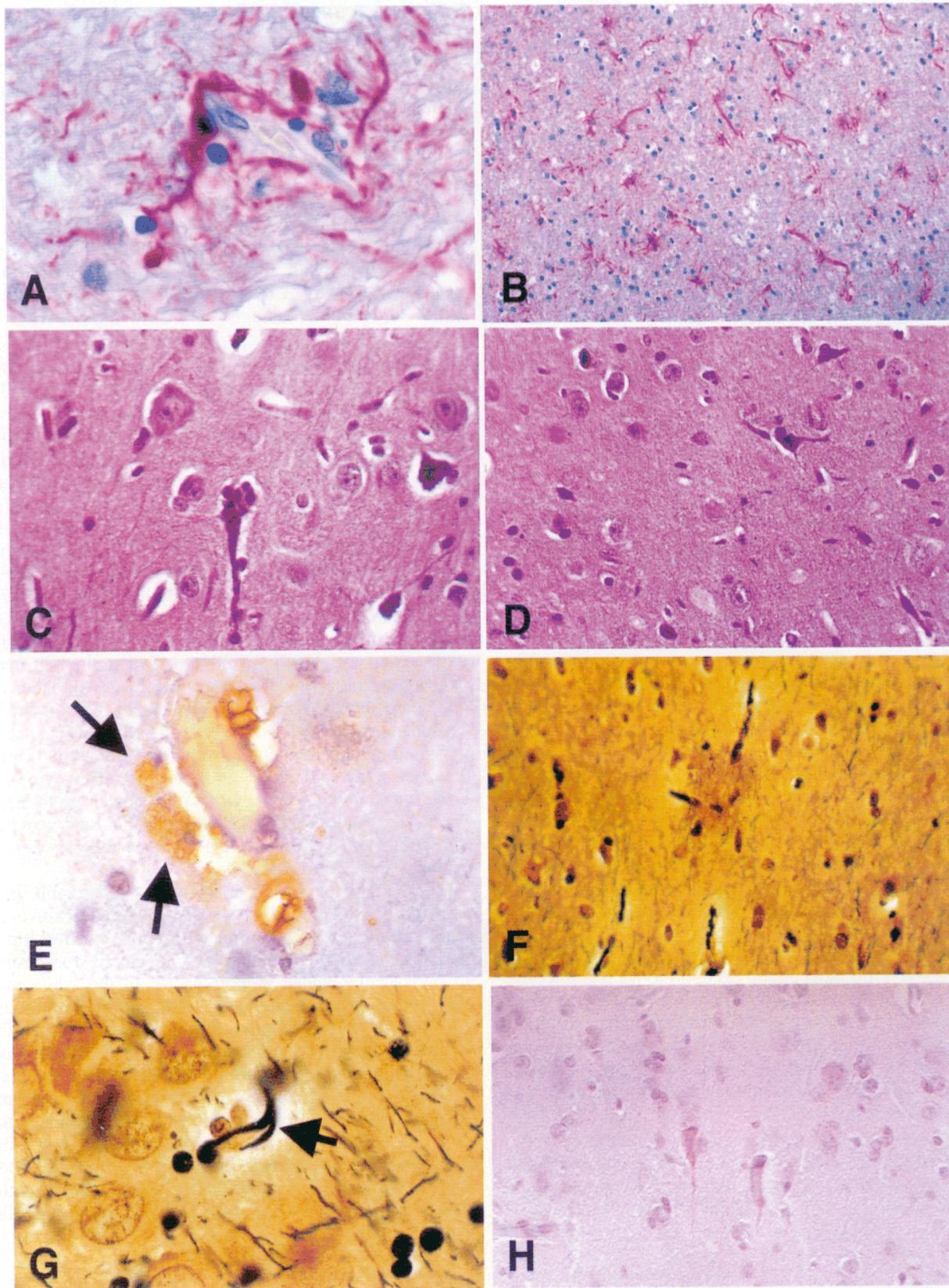


FIGURE 3.—SWMMC dogs. 3A) Frontal white matter, 3-year-old. GFAP-positive astrocytic processes are seen primarily around blood vessels. Light counterstaining with hematoxylin. $\times 420$. 3B) Frontal white matter, 12-year-old male with predementia scores. There is a moderately reactive astrocytosis. GFAP. $\times 42$. 3C) Frontal cortex, 4-year-old. Dying neurons exhibit condensation of both cytoplasm and nuclei, are surrounded by numerous glial cells, and do not develop apoptotic bodies. H&E. $\times 420$. 3D) Frontal cortex, 4-year-old. Nerve cell loss and numerous microglia-like ameboid cells are apparent. H&E. $\times 214$. 3E) Frontal cortex, 6-year-old. Strong positive apoE staining is present in lipid deposits located in blood vessel walls; two macrophages (arrows) exhibit abundant granular positive staining. $\times 1072$. 3F) Frontal cortex, 7-year-old. An argyrophilic non-neuritic plaque is present in the center of the photomicrograph. Modified Bielschowsky silver stain. $\times 214$. 3G) Parahippocampal gyrus, 8-year-old. A tangle (arrow) appears as dark bands curving through the cytoplasm of the neuron. Modified Bielschowsky silver stain. $\times 420$. 3H) Frontal cortex, 4-year-old. Pyramidal neurons show weak tau AT8—positivity. No counterstain. $\times 107$.

a weak granular positive cytoplasmic staining. ApoE was particularly prominent in relation to the lipid vacuoles in cortical and subcortical blood vessels and could be seen in neurons, astrocytes, and perivascular macrophages (Figure 3E). Rare nonneuritic plaques and NFTs were seen with the modified Bielschowsky silver stain in 3 SWMMC dogs ages 7, 8, and 12 (Figures 3F, G). NFT-bearing cells were frontal pyramidal and parahippocampal neurons. In 2 additional dogs of age 4, tau-positive neurons were seen in the frontal cortex (Figure 3H). In these 2 dogs, weak tau hippocampal neuronal cytoplasmic staining was seen, although the modified Bielschowsky silver stain was negative. Nuclear immunoreactivity to the p65 subunit of NF- κ B was present in olfactory bulb neurons at age 2 wk and abundant in cortical neurons and Purkinje, cerebellar granular, and perivascular cortical cells by age 8 months (Figure 4A-C).

The NF- κ B protein complex seemed localized in the cytosol of reactive astrocytes in dogs ≥ 1 year, as indicated by the morphological similarity of NF- κ B-positive cells and reactive GFAP-positive astrocytes (Figure 4C). NF- κ B nuclear positivity was not seen in control dogs. iNOS-positive endothelial cells were first seen at age 4 weeks in SWMMC dogs. Older dogs showed strong positivity in endothelial, glial, and subpial astrocytes, cortical neurons in layers III-V, and the choroid plexus epithelium (Figure 4D, E, G). Strong staining was also observed in hypothalamic, thalamic, and midbrain (substantia nigra) regions, and in pons neurons and blood vessels. In dogs older than 5 years, substantia nigra neurons showed variable degranulation and strong cytoplasmic iNOS staining (Figure 4H). Control dogs older than 6 years showed weak iNOS positivity in endothelial and white matter glial cells.

Electron Microscopy: Frontal sections from control and exposed animals matched by age and gender were examined by transmission electron microscopy. Three early vascular alterations seen clearly in the 1- μ m, toluidine blue sections in dogs ages 8 months to 3 years included capillary endothelial cells with short anemone-like processes extending into the lumen of the vessels, focal absence of astrocytic foot processes around small capillaries and arterioles, and intracytoplasmic accumulation of electron-lucent and medium-density lipid droplets in smooth muscle and pericyte cells (Figures 5A-D, 6A-C). Lipid accumulation in the smooth muscle cells of the cortical small arterioles started as small, discrete globules, which fused and became more prominent with the age of the animal (Figure 6D, E). Only scattered cortical arterioles and capillaries could be seen with these lipid structures starting with the 8-month-old SWMMC dog; the oldest 12-year-old dog showed extensive vascular changes with the majority of cortical vessels involved. Similar, more discrete vascular findings were seen in the thalamus, hypothalamus, basal ganglia, midbrain, and cerebellum.

Ultrastructural changes in capillaries showed endothelial cells with increased luminal and abluminal pinocytotic vesicles, scanty mitochondria, rough endoplasmic reticulum (RER), and glycogen. Vessels displayed irregular luminal surfaces (Figure 6A). Starting at age 1, astrocytic foot processes were focally absent around the basement membrane (BM) of capillaries and in some areas retracted, leaving a greatly distended perivascular space (Figure 5A, 6A). The absence of focal

astrocytic foot processes was common in dogs older than 3 years. Two dogs ages 7 and 12 years displayed partially degranulated intraluminal PMN leucocytes in white matter blood vessels (Figure 6B). Young dogs showed reduplication of the BMs in cortical capillaries, with focal thickening. This change was usually associated with absent, retracted, or disintegrating foot astrocytes. Older dogs (>5 years) displayed isolated red blood cells (RBC) lying in the neuropil around abnormal capillaries (Figure 6C) and increased amounts of lipofuscin in glial cytoplasm (Figure 6F).

DISCUSSION

Healthy canines in Mexico City exhibited early activation of the NF- κ B transcription factor and iNOS expression. Canines ages ≤ 1 year showed signs in the olfactory bulbs and frontal capillaries of BBB dysfunction and neuronal chromatinolysis and satellitosis in layers III to VI. Reactive astrocytosis, white matter glial cell apoptosis, and strong apoE vascular immunoreactivity were seen in animals older than 1 year. Deposition of lipid droplets in smooth muscle cells and pericytes was observed in dogs ≥ 3 years, and nonneuritic plaques and NFTs were apparent in 4- to 12-year-olds. These dogs also exhibited nasal respiratory and olfactory pathology with a histopathological picture that has been described in experimental animals and humans upon exposure to air pollutants and inhaled toxic substances (20, 54, 85). The breakdown of both the nasal respiratory and the nose-brain olfactory barriers increases the likelihood of diminishing their protective functions and enabling the delivery of potentially toxic inhaled substances (39, 88). The olfactory bulb, a limbic paleocortex that receives monosynaptic sensory efferents from the olfactory mucosa, was involved early in all SWMMC dogs. Capillary pathology was a major finding in the olfactory bulb and frontal cortex. Strong expression of iNOS in endothelial cells and astrocytes, 2 major components of the defensive BBB, was a crucial finding in these dogs. iNOS is transcriptionally regulated by cytokines and redox-sensitive transcriptional factors (94, 104). Nitric oxide can lead to an opening in the BBB (104); in the acute phase of experimental bacterial meningitis, iNOS-derived NO contributes to peroxynitrate formation and BBB breakdown (113). The BBB breaching in these animals was illustrated by the extravasated RBC. Grammas (53) has shown that brain microvessels in AD produce high, potentially toxic levels of nitric oxide that in turn kills neurons.

Brain endothelial cells are important regulators of CNS homeostasis, and EM studies of the BBB in AD patients have demonstrated decreased mitochondrial content, increased pinocytosis, accumulation of collagen, and focal necrotic changes (32). Endothelial cells participate in inflammatory responses and are powerful producers of soluble inflammatory mediators (12, 68, 78). Elevated pinocytosis may increase transcytotic activity of the endothelium, which in turn translates into an increased influx of plasma components, such as low density lipoprotein (LDL), into the subendothelial space (30). Interestingly, nitric oxide (NO) concentrates in lipophilic cell compartments and regulates oxidant-induced lipid oxidation. ApoE plays a central role in the brain response to injury and neurodegeneration (93), and the progressive deposition in smooth muscle cells and pericytes of

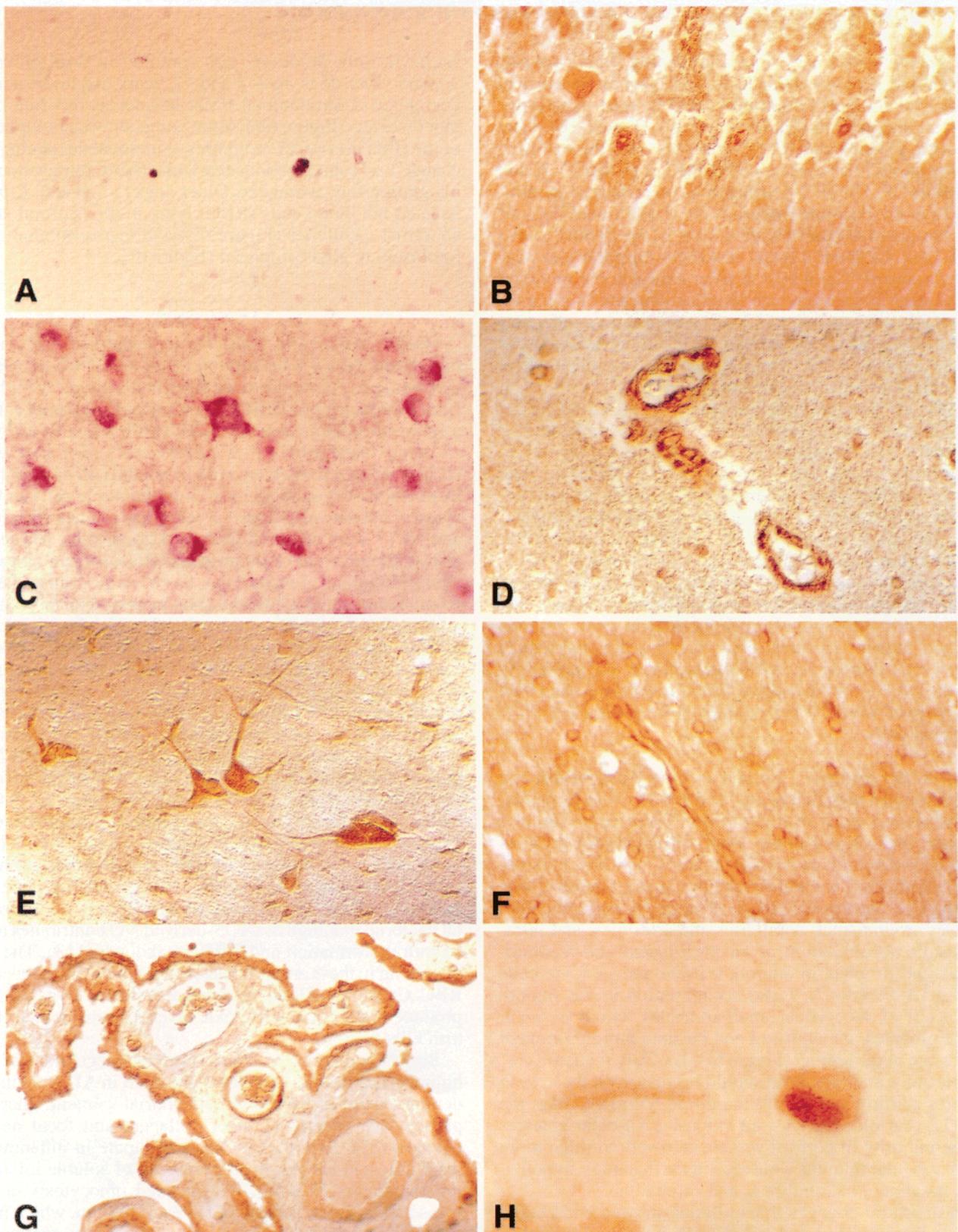


FIGURE 4.—SWMMC dogs. 4A) Frontal cortex, 8-month-old. Nuclear immunoreactivity to the p65 subunit of NF- κ B is seen in a neuron and a glial cell. NF- κ B with a red substrate. $\times 214$. 4B) Cerebellum, 4-year-old. Nuclear immunoreactivity to the p65 subunit of NF- κ B is present in Purkinje cells; nucleoli are negative. Nomarski optics. NF- κ B with a brown substrate. $\times 214$. 4C) Frontal white matter, 7-year-old. Cytoplasmic immunoreactivity to the p65 subunit of NF- κ B is seen in glial cells, both astrocytes and oligodendrocytes. NF- κ B with a red substrate. $\times 420$. 4D) Frontal white matter, 3-month-old. iNOS-positive endothelial cells are seen; note the negativity of the nucleus. Nomarski optics. No counterstain. $\times 214$. 4E) Hypothalamic neurons, 8-month-old. Strong iNOS staining in the cytoplasm. Nomarski optics. No counterstain. $\times 420$. 4F) Frontal white matter, 6-year-old. Immunoreactivity for iNOS is present in the cytoplasm of glial and endothelial cells. No counterstain. $\times 214$. 4G) Choroid plexus, 8-year-old. Strong iNOS immunoreactivity is present in the cytoplasm of the epithelial and endothelial cells. No counterstain, $\times 107$. 4H) Pigmented substantia nigra cell, 6-year-old. A well-granulated cell shows moderate iNOS staining. No counterstain. $\times 1072$.

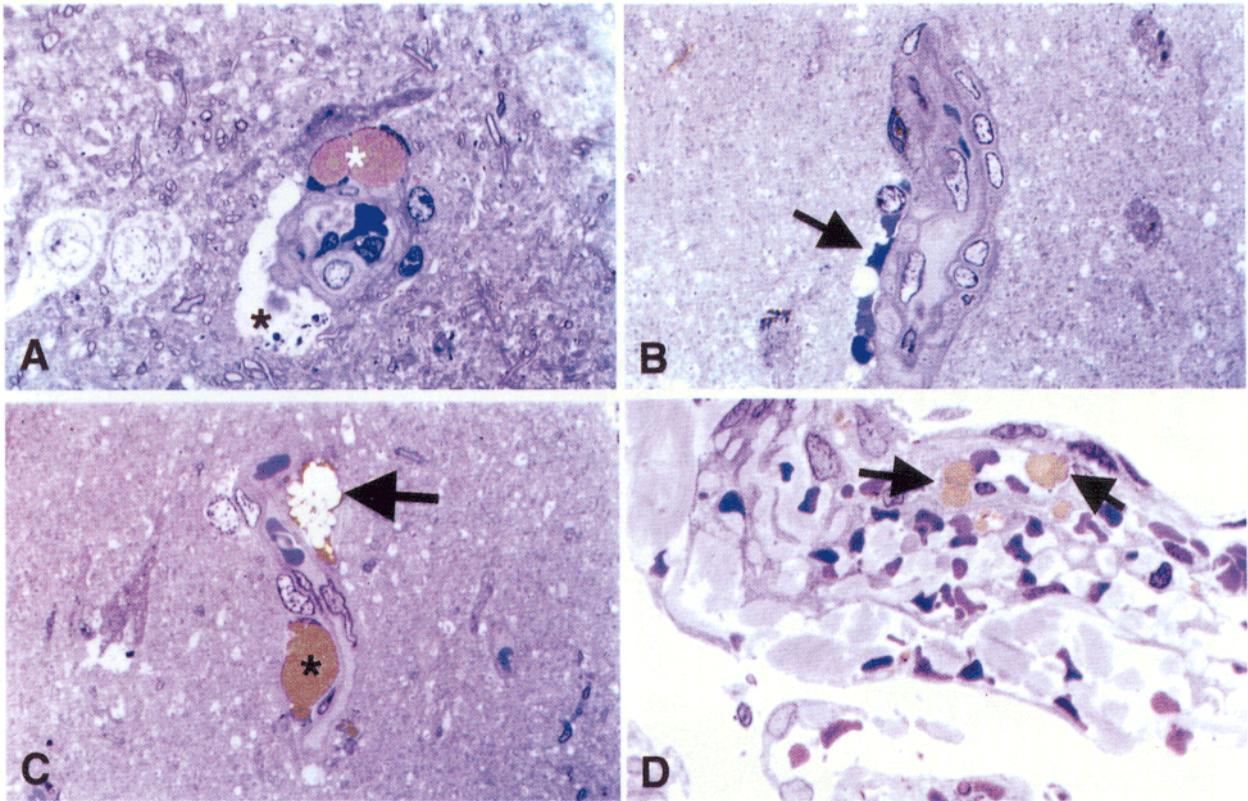


FIGURE 5.—SWMMC dogs. Frontal cortex, 1- μ m-thick epoxy resin-embedded, toluidine blue-stained sections. 5A) 2-year-old. Small cortical blood vessel shows 2 lipid deposits (white*) in the blood vessel wall. Red blood cells are present in the lumen. Focal absence of astrocytic foot processes and unidentified debris are present around the vessel (black*). $\times 420$. 5B) 6-year-old. Several red blood cells are seen outside the vessel wall (arrow). The nucleus above the arrow shows clumping of the chromatin. Neurons contain lipofuscin pigment. $\times 420$. 5C) 12-year-old. Blood vessel is compressed by large lipid deposit (*). In focal areas the lipid has been partially removed (arrow). An adjacent neuron is shrunken. $\times 420$. 5D) 3-year-old. Frontal leptomeninges. Lipid deposits are present in leptomeningeal blood vessels (arrows) $\times 420$.

immunoreactive apoE in these dogs could be indicative of a dysfunction in lipid transport as seen in AD (93).

Rhodin's inflammatory animal model of vascular inflammation (95) illustrates the effects of a single injection of TNF α , and IL-1 β upon the endothelium. IL-1 β -induced damage includes vesiculation of the endothelial cytoplasm, accumulation of plasma lifting smooth muscle cells away from the endothelium, and their association with gaps and discontinuities of the endothelial lining. Rhodin suggested that disruption of the BBB by pro-inflammatory cytokines initiates compensatory responses that alter the normal homeostatic properties of the endothelium. Indeed specific combinations of cytokines (IL1 + TNF α /interferon γ) induced a significant neuronal cell injury in mixed neuron/glia cultures (61). Cytokines in the systemic circulation presumably could stimulate hematopoietic bone marrow cells capable of crossing the BBB to undergo differentiation into brain microglia (42). Microglia become activated and secrete a myriad of inflammatory cascade mediators, including nitric oxide, proteases, arachidonic acid derivatives, reactive oxygen species, and cytokines (4, 8, 14, 29, 49, 80, 103). Moreover, amyloid precursor protein (APP) is a microglial acute phase protein (8, 9). Microglia are present in significant numbers in normal brains, but their distribution is not uniform (70, 89). More microglia are found in the cortex than in the white matter, and

densely populated areas include the olfactory telencephalon, hippocampus, basal ganglia, and substantia nigra (70); such distribution may explain the "vulnerability" of these areas (76). Thus, a sustained inflammatory stimulus, such as the one encountered in the lower respiratory tract upon exposure to air pollutants, produces lung endothelial and epithelial injury and cytokine release from both circulating inflammatory and resident lung cells (25). Cytokines present in the systemic circulation have the ability to trigger brain events in cascades leading to different activators of transcription transduction pathways, including kinases/NF- κ B in vascular cells of the CNS (97). The brain blood vessels have both constitutive and induced expression of receptors for different proinflammatory ligands (97).

We observed nuclear staining of NF- κ B first in olfactory bulb neurons and the frontal cortex, then other cortical regions, Purkinje cells, and perivascular cells. Cytoplasmic staining of cortical and white matter glial cells was also apparent. NF- κ B plays a crucial role in regulating cytokine cascades, and functional NF- κ B complexes are present in neurons, astrocytes, oligodendroglia, and microglia; NF- κ B may be involved in iNOS induction stimulated by cytokines and/or lipopolysaccharide (LPS) in various cell types and tissues (79). Genes encoding injury-responsive cytokines are induced by NF- κ B in neurons and glial cells; these include

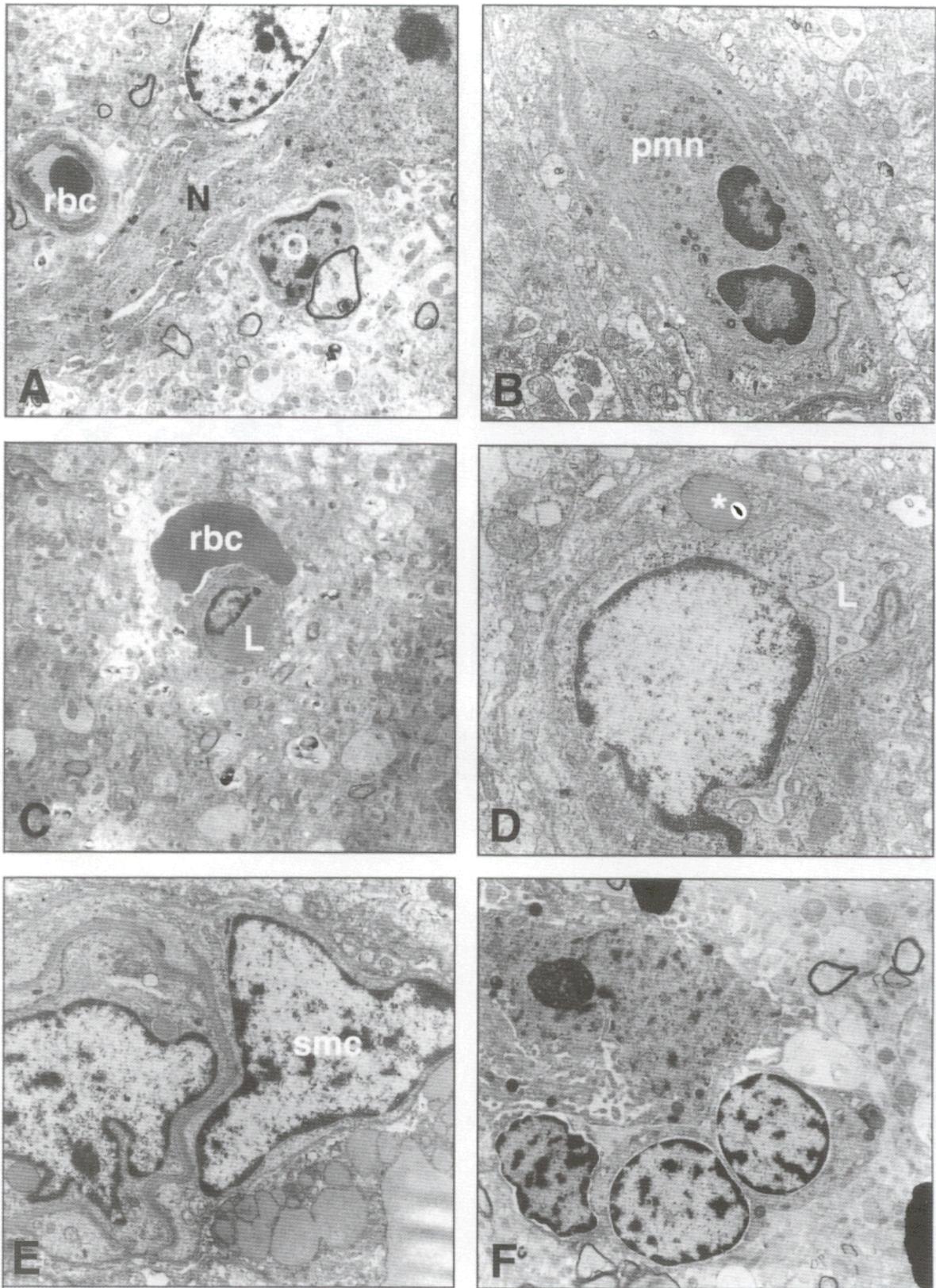


FIGURE 6.—Electron microscopy of frontal sections; SWMMC dogs. 6A) 1-year-old. A large pyramidal neuron (N) is adjacent an oligodendrocyte (O) and an astrocyte (top of picture). Capillary shows luminal RBC and endothelial cell's processes projecting into the lumen. There is focal absence of astrocytic foot processes in a segment of the capillary (*). The oligodendrocyte exhibits lipofuscin and a degenerating axon. $\times 3000$. 6B) 12-year-old male. White matter capillary. A partially degranulated polymorphonuclear leucocyte (pmn) is in close contact with the endothelial surface. $\times 7000$. 6C) 8-year-old female. White matter capillary. RBC lies outside a capillary, its lumen marked (L). Notice the disrupted adjacent neuropil. $\times 3000$. 6D) 3-year-old male. White matter capillary. Note presence of lipid deposit in cytoplasm of a pericyte (*). Capillary lumen is marked L. $\times 12,000$. 6E) 12-year-old male (same as 6B). White matter capillary. Abundant deposits of lipid in cytoplasm of smooth muscle cells (smc). $\times 7000$. 6F) 7-year-old male. Cortex. Three glial cells are seen adjacent to a pyramidal neuron. This neuron shows enhanced affinity for toluidine blue in the $1\text{-}\mu\text{m}$ -thick section. Lipofuscin is seen in glial cytoplasm. $\times 7000$.

TNF α , IL6, and APP (79). High-level activation of NF- κ B may promote sustained production of neurotoxins; activation of NF- κ B in astrocytes results in increased expression of nitric oxide synthase and increased nitric oxide production—an observation clearly demonstrated in these animals. The enzyme nitric oxide synthase 2 (inducible NOS) is strongly present in glial and endothelial cells, microglia, scattered pyramidal neurons, apoptotic bodies in the white matter, and the choroid plexus epithelium. Both NF- κ B activation and iNOS expression are concomitant with white matter glial apoptosis and neuronal satellitosis but precede extensive glial reactivity—evidenced by GFAP upregulation. Acarin et al (1) described activation of NF- κ B in severely damage immature rat brain affected by neuronal degeneration and BBB disruption; moreover, this activation preceded astrocytic hypertrophy and GFAP upregulation in a sequence similar to ours. Blockage of persistent NF- κ B activation with anti-oxidants (34) is an avenue that can be explored in experimental animals exposed in controlled chambers to air pollutants.

Inflammation clearly occurs in the Alzheimer's brain (3), and damage microcirculation is crucial (38, 52, 53). Based on our findings of inflammation and endothelial damage in these highly exposed dogs, we hypothesize that the initial and perpetuating inflammatory source is primarily the respiratory tract. Events occur so that circulating cytokines have the ability to trigger expression of numerous cytokine receptors in the endothelium of cerebral capillaries; endothelial cells are damaged; the BBB is broken; microglia are activated; neurons and glial cells are damaged; and the brain perpetuates the inflammatory mechanisms. The appearances of nonneuritic plaques and NFTs are late events in these dogs.

Consistent with our findings in this work are medical evaluations of clinically healthy, well-nourished SWMMC children who exhibit nasal mucosal abnormalities, hyperinflation, and interstitial markings by chest X-ray, spirometric abnormalities, and peripheral blood smears suggestive of bone marrow involvement (23). Moreover, SWMMC children show an imbalance of serum cytokines, and significant positive correlations are seen between TNF α and IL8, IL8 and endothelin, and TNF α and endothelin (23). The radiological abnormalities and spirometric changes suggest the development of small airway disease with the accompanying inflammatory picture and bone marrow effect (23). Sherwin has shown centriacinar inflammatory disease in young individuals residing in Los Angeles and demonstrated significant differences in lung inflammation scores between Miami and Los Angeles residents (102). Thus, chronic lung parenchymal inflammation seems widespread in Mexico City and occurs in at least some young urban residents of the United States.

We observed a significant difference in the number of monocytes between MC and Tlaxcala dogs. Canine monocytosis is associated with infections, trauma, hematologic disorders, stress, autoimmunity, or any disease with significant tissue destruction (100). Monocytopenia in the model of thermal injury and sepsis in mice results from an imbalance in myelopoiesis driven by the increased expression of macrophage colony-stimulating factor receptor (116); clearly this avenue of research merits exploration in these canines.

Exposure to complex mixtures of pollutants, predominantly PM and ozone, causes structural changes in the canine

lung induced by a sustained inflammatory process and resulting in airway and vascular remodeling and altered repair (22). Ultrafine PM is seen in alveolar type I and II cells, endothelial cells, interstitial macrophages, and intravascular macrophage-like cells. In lung histopathology, PM plays a crucial role, because there is a significant transport of ultrafine PM from the epithelium to the interstitium, and to the endothelial and intravascular compartments in these exposed dogs. Ultrafine particles are associated with stronger inflammatory responses, prolonging of their lung retention, induction of procollagen expression, and increases in airway fibrosis (6, 44, 87). Ultrafine particles less than 0.1 μ m possess unique physicochemical features (64), are produced by the burning of fossil fuels or photochemical reactions, can easily penetrate into deep lung regions, and are deposited in the lung by diffusion; deposition spreads over large areas of the lung. Enhanced proximal airway deposition for women, both for ultrafine and coarse particles, has been attributed to smaller dimensions of the upper airways, which could result in an increase in inertial impaction (64). Kim and Jaques (64) suggested that, because of complex upper airway geometry and enhanced turbulence, the end result could be an increase in diffusive deposition of ultrafine particles in women. Deposition of PM in head regions, although small (<3%), could become significant if both the respiratory and olfactory barriers are impaired. Further, total lung deposition is greater (5–15%) in women than men. Women receive a greater dose of ultrafine particles in the head and tracheobronchial regions (64).

Particulate matter concentrations in Mexico City are above the US current standards for most of the metropolitan area, and a persistent haze blankets the city, especially in the winter. Heavy metals in the atmosphere are present in aerosol form, primarily as metal oxide particulates. A cluster typically identified with fuel oil includes Cr, Ni, V, and S, and high correlations are found between Zn, Cu, and Mn elements associated with industry or traffic (83). The traffic contribution of metals is present only in the fine PM fraction (83). Carbon-containing aerosols account for 20 to 35% of the PM₁₀ and 25 to 50% of the PM_{2.5}. The additional chemical layering of a carbon core by nitrates, sulfates, and other organic materials and metals such as iron causes greater local oxidative damage than the vaporized state (17). Another aspect of pollution in MC that could be relevant to nasal/brain pathology is the presence of organic dusts, lipopolysaccharides (LPS), and endotoxins in PM₁₀ (15); a large contributor is the 500 tons of canine fecal material deposited daily on the streets.

Injection of the bacterial endotoxin LPS into the hippocampus, cortex, or substantia nigra of adult rats produces substantia nigra degeneration (65); in mixed astrocytic and microglial cell-culture systems, the addition of LPS and cytokines causes upregulation of iNOS in microglia cells (94), and LPS increases nuclear translocation of NF- κ B in microglia (56). Intraperitoneal injection of LPS in rats induces endothelial and astrocytic iNOS formation (60).

Regulation and assessment of pollution have focused on ambient concentrations; however, indoor pollution is significant and represents an important contribution to overall PM exposure (62, 109, 112). High indoor PM_{2.5} concentrations are related to tobacco exposure, cooking activities, the use

of air fresheners, and contaminated particle board materials (63, 106, 109, 112). A comparison of worldwide epidemiological studies shows that both the incidence and prevalence figures for dementia increase with age and that women have a higher risk for developing AD according to every report available in the literature (7, 46, 74, 101). There are also significant differences in incidence and prevalence around the world (74, 101, 110). The increased risk of AD, as well as PD, with lower literacy (50, 90) could be related to exposure to toxins and PM in the environment, starting in childhood, and occupational history involving exposure to iron, copper, manganese, mercury, zinc, and lead (51). The higher risk for women could be related to the greater dose of ultrafine particles in their heads, total lung deposition, nasal anatomy with anterior distribution in the olfactory epithelium (71), household exposures (cooking habits), and decreased levels of neuroprotective steroidal structures after menopause (10).

Microglial activation is a central element in CNS demyelination (115). The prevalence of multiple sclerosis has doubled in Mexico City in the last 10 years, and 70% of patients are females (35). Data on the incidence and prevalence of AD and PD are not available from reliable sources in Mexico City; however, empirical observations point toward an increase in their incidence in the last decade (personal communication, Patricia Mendoza MD, Mexico City, May, 2001).

Nasal findings in this work emphasize the potential impact of upper respiratory physiology and metabolic xenobiotic detoxification (11, 19, 37, 62, 69) upon inhaled gases and materials in the nose when the barriers are no longer intact. SWMMC children and canines show indirect evidence of attempts to restore and protect the nasal epithelium against the effects of continuous exposure to inhaled pollutants (27). Ultrafine PM deposition in nasal epithelial cells and intercellular spaces, the transudate between epithelial cells in nasal biopsies from healthy children in SWMMC (27), and the significant damage to the respiratory and olfactory epithelia in canines highlight the importance of nasal tissues in the study of neurodegenerative diseases. Elucidating the relationship(s) between nasal and brain pathology remains an important task. The chronic inflammatory process elicited within the respiratory tract upon exposure to outdoor and indoor air pollutants (2, 33, 40, 45, 48, 73) could serve as the trigger for a chain of events involving the brain.

In summary, we have described canines chronically exposed to significant concentrations of ozone, PM, and a myriad of other air pollutants. These animals exhibited early and persistent activation of NF- κ B, strong expression of iNOS, and alterations in the BBB in cortical capillaries. Degenerating cortical dark, shrunken neurons exhibited prominent satellitosis and were surrounded by apoptotic glial cells, although no apoptosis was identified in the neurons themselves. The paucity of frontal neurons even in young dogs and the evidence of dysfunctional lipid transport in vascular smooth muscle cells and pericytes were striking. The histopathology we observed in these urban dogs is of sufficient magnitude to warrant concern that similar histopathology may be occurring in humans residing in large polluted metropolitan areas. If so, these alterations could provide insight into the underlying early pathophysiological mechanisms responsible for neurodegenerative diseases such as Alzheimer's and Parkinson's.

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