Pathogenesis of Lesions Induced in Rat Lung by Chronic Tobacco Smoke Inhalation^{1, 2, 3}

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ABSTRACT—Lesions were induced in the lungs of specific-pathogen-free F344 rats by chronic tobacco smoke exposure. Animals exposed to 7 cigarettes/day were killed after 1, 1.5, or 2 years of exposure. Parallel lifetime exposures induced pulmonary tumors in 9% of the animals. In serially killed animals, four types of lesions were found: 1) perivascular or peribronchiolar accumulation of lymphoreticular cells, 2) fibrotic and cellular enlargement of peribronchiolar septa, 3) type II cell hyperplasia with septal fibrosis, and 4) air-space enlargement (emphysema). However, emphysema occurred only in animals exposed to a higher (10 cigarettes) dose of tobacco smoke. Ultrastructural studies showed all of the focal lesions to be infiltrated by cells typical of the inflammatory response. The type II hyperplastic and peribronchiolar alveolar lesions involved larger portions of the parenchyma in fibrotic changes but differed in structure, location, and frequency. The incidence of the peribronchiolar alveolar lesions was temporally related to tumor incidence.—JNCI 1982; 69:117–129.

Numerous previous reports have described the effects of tobacco smoke exposure on lung structure. In studies by Roe's group (1, 2), the major lesions induced in the rat lung by lifetime exposure were columnar, cuboidal, and squamous metaplasias of the alveolar epithelium. All three types of lesions were significantly more frequent in smoke-exposed than in control animals (1) and could be induced by intratracheal instillation of smoke condensate (2). However, neoplasms could be induced only by intratracheal instillation of the polycyclic aromatic hydrocarbon-containing fraction of smoke condensate (2). No statistically significant increase in pulmonary neoplasm incidence was found in animals exposed to smoke by inhalation, presumably because the animals died prematurely from chronic respiratory disease (1).

Short-term studies conducted independently by Walker et al. (3) on rats exposed to tobacco smoke for 6 weeks showed characteristic lesions at the level of the respiratory bronchiole, including peribronchiolar and perivascular infiltration by lymphocytes, focal pneumonitis, and alveolar cuboidal or columnar metaplasia. The metaplastic changes, along with increases in macrophage size and number, were thought to be dose-dependent. The other lesions were considered early manifestations of chronic pulmonary infection (3). Morphologic changes have also been induced in the lungs of beagle dogs by 2–5 years of tobacco smoke exposure (4). Marked fibrosis and emphysema were found, as were type II cell hyperplasia and squamous metaplasia of the alveolar epithelium. At the epithelial-mesenchymal interface, projections of the epithelial cells into the stroma and thickening and reduplication of the basal lamina (5) were also seen.

Despite the substantial number of experiments with chronic tobacco smoke inhalation, only two laboratories have reported significant induction of pulmonary tumors in exposed rodents. One report showed a twofold higher inci-

dence of adenocarcinomas in smoke-exposed mice than in control mice. The gas phase of the smoke was found to induce an even higher tumor incidence than the whole smoke (6). More recently, a lifetime exposure of rats to tobacco smoke produced a 9% incidence of respiratory tract tumors, including adenomas, adenocarcinomas, and squamous cell carcinomas (7). In this experiment, animals exposed in parallel with those for the lifetime study were killed at earlier intervals for assessment of morphologic lesions induced by smoke inhalation. The present report characterizes those lesions. Many of the alterations appeared to be similar to those described above. However, 1 lesion was found that had not been found in previous experiments, i.e., fibrotic thickening of alveolar septa in peribronchiolar locations.

MATERIALS AND METHODS

Exposure conditions.—SPF female F344 rats were exposed to tobacco smoke in the Maddox/ORNL smoking machine as previously described (7). Beginning at 12–14 weeks of age, the animals were gradually exposed to increasing dosages of tobacco smoke from standard 85-mm, nonfiltered experimental cigarettes (National Cancer Institute code 16). Two final dose levels were used, 7 or 10 cigarettes/day, and the animals were killed at time intervals from 1 to 2 years after exposure began. The exact number of animals killed at various times in each dose group is shown in table 1. Since mortality was high in the 10-cigarette-per-day group, all the remaining animals in this group were killed at 1.5 years. Both untreated and sham-exposed groups were killed in parallel with the exposed animals. Additional data were

ABBREVIATION USED: SPF=specific-pathogen-free.

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TABLE 1.—Number of animals used for morphogenesis studies

Duration of smoke exposure, yr	No. of animals			
	Treatment group: Untreated	Sham treated	7 cigarettes/day	10 cigarettes/day
1.0	10	10	10	8
1.5	9	9	9	14
2.0	10	12	11	0
2.5	2	4	6	0

obtained from animals in the lifetime study; these rats were killed after exposure for 2.5 years and maintenance for an additional 7 months without exposure. One animal from each treatment group was tested for infective organisms. Blood samples were tested by Microbiological Associates, Bethesda, Md., for the presence of serum antibodies to several viruses: PVM (pneumonia virus of mice), Reo-3 (reovirus type 3), GDVII (Theiler's mouse encephalomyelitis GDVII), k (newborn mouse pneumonitis, k-virus), polyomavirus, MVM (minute virus of mice), MHV (mouse hepatitis virus), Sendai virus, and RCV (rat corona virus). Cultures prepared from the postcaval lung lobe were tested for mycoplasma (8). All test results were negative.

Tissue preparation.—Before they were killed, the animals were anesthetized with 30 mg pentobarbital/kg body weight. For tissue fixation the trachea was cannalized; the airways and lungs were removed and fixed with buffered formaldehyde under 20 cm of pressure for 24–48 hours. The lung lobes were cut along the bronchus, except for the left lobe, which was cut in cross section just anterior to the entry of the bronchus. The tissues were embedded in paraffin. Sections from the right lobes were used to determine the number of fibrotic lesions in the lungs of smoke-exposed animals. All of the sections were examined, and the cumulative number of lesions, normalized to the number of animals killed, was used as an estimate of the frequency of each type of lesion. Sections from the left lobes, stained with hematoxylin and eosin, were used for morphometric analysis. For each sample, 10 fields were selected at random and viewed at 100X magnification through a Weibel ocular. Fields comprised of more than one-third conducting airways or vasculature were excluded. The mean linear intercept was determined, and point counts (420 points/lobe) were made to obtain the volume percent of alveolar versus non-alveolar air space and respiratory versus nonrespiratory tissue. Alveoli were defined as enclosed circles or as open semicircles with a radius greater than half the distance across the opening. Additional sections of the left lobes were stained with Snook's silver stain for reticulum, aldehyde fuchsin, and Lushbaugh's stain.

Each time rats were killed, tissues were also processed for electron microscopy by airway perfusion of a 2% glutaraldehyde solution containing 4.6% Tyrode's solution and 0.05 M collidine buffer (pH 7.3). The tissues were fixed for 2 hours under 15-cm pressure and retained in collidine buffer until further processing. After exposure to 2% osmium tetroxide in 0.1 M cacodylate for 3 hours, selected blocks of tissue were dehydrated in acetone and embedded in a standard epoxy mixture (9). Sections 2 μ m in thickness were cut on the Sorvall JB-4 microtome and stained with 0.05%

toluidine blue in 2.5% sodium carbonate. Selected areas of the blocks were thin sectioned on the Sorvall MT-2 ultramicrotome, mounted on Formvar-coated grids, and stained with uranyl acetate and lead citrate (10). Thin sections were examined with the Siemens 101 or Hitachi HU-11B electron microscope. For each pathologic change studied, at least four examples were examined ultrastructurally.

RESULTS

Some changes in the lung parenchyma following smoke exposure were readily apparent on a gross level. Lungs from animals exposed to 7 cigarettes/day for 1 year had dark foci about 1 mm in diameter scattered over their surfaces. With longer exposures or with exposure to 10 cigarettes/day, there were increasing numbers of larger white nodules about 1–4 mm in diameter. Four types of lesions could be distinguished microscopically in the pulmonary parenchyma: 1) perivascular or peribronchiolar accumulation of lymphoreticular cells, 2) fibrotic and cellular enlargement of peribronchiolar septa, 3) type II cell hyperplasia with septal fibrosis, and 4) air-space enlargement (emphysema). The first type of lesion was most common in the lungs of animals exposed to 7 cigarettes/day for 2 years. The last lesion type was seen only in animals exposed to 10 cigarettes/day.

Perivascular or Peribronchiolar Cell Accumulation

Perivascular lesions occurred frequently in the lung parenchyma after 2 years of smoke exposure, averaging 3 lesions/section of approximately 0.5 cm². The frequency after a 2-year exposure was approximately double that after a 1-year exposure. The lesions were characteristically associated with venules and arterioles in the parenchyma. They were most common in branches of the pulmonary vein located at or below the level of the terminal bronchiole (figs. 1A, 1B) and were also found in bronchial veins, but they were never seen in the adventitia of major arteries. Perivascular accumulations of cells were also found rarely in unexposed rats and consisted of large lymphocytes and macrophages (fig. 1C). The lesions of smoke-exposed lung tissues contained predominantly particulate-laden macrophages (figs. 1A, 1B). An ultrastructural analysis of several lesions associated with venules showed that lymphocytes, plasma cells, and, occasionally, fibroblasts were closely apposed to macrophages in the adventitia (fig. 2). While macrophages and lymphocytes were usually seen adjacent to the basal lamina of the epithelium, the cell mass was separated from the epithelium by a basement membrane in some lesions. In addition to the adventitial lesions, a separate lesion was sometimes found around major veins, consisting of lymphocytes within the lumen of lymphatic vessels. The lymphatic endothelium in these lesions frequently contained smoke particulates (figs. 3A, 3B). However, lesions with characteristics intermediate between those of the lymphatic and adventitial lesions were not found, so a common etiology seemed unlikely.

Lesions similar to the other adventitial perivascular lesions were also found near the bronchiolar-alveolar junction, most frequently between the bronchiole and the bronchial vein and extending only part of the way around the

bronchiole. Therefore, most of these lesions were also perivascular in location.

Fibrotic and Cellular Enlargement of Peribronchiolar Septa

Septal enlargement was characteristic in peribronchiolar locations, but it was also found in type II hyperplasias. The enlarged septa in both lesions had similar ultrastructural features. Generally, both histologic types of lesions were larger than perivascular lesions and could be discerned readily in paraffin-embedded lung tissue. Neither type of lesion was found in the lungs of control animals, and the frequency of both types increased with the number of cigarettes smoked and the cumulative time of smoke exposure (table 2). The type II hyperplastic lesions always constituted more than 90% of the total number of large lesions. However, the total number of peribronchiolar lesions increased markedly in the later stages of exposure at a dose of 7 cigarettes/day. In animals exposed at the higher dose rate of 10 cigarettes/day for 1.5 years, the peribronchiolar lesions constituted only 2% of the large lesions, even though the cumulative dose of tobacco smoke was comparable to that delivered in the 2-year exposure at the lower dose rate. The peribronchiolar lesions constituted 7% of the large lesions under the latter conditions. In paraffin-embedded sections treated with Lushbaugh's stain for collagen, the amount of collagen seen in lesions showing septal enlargement appeared to increase in relation to the dose of tobacco smoke. No obvious increases in reticular fibers (Snook's silver stain) or elastic fibers (aldehyde fuchsin) were observed.

Although the two types of lesions exhibiting septal enlargement differed in location, frequency, and the type of lining epithelium present, the septal constituents were similar when examined ultrastructurally. Peribronchiolar lesions were characterized by an attenuated epithelium (fig. 4A) and by distortion of the normal semicircular profile of the alveoli (fig. 4B). In extreme cases, the septa were fused into a single mass. About half of the enlargement lesions in peribronchiolar locations also showed alveolar bronchiolization (fig. 4C), and a new columnar epithelium frequently appeared to extend into the alveolar duct (fig. 4A). Although

septal thickening may also have occurred in loci separate from larger lesions, this was evidently rare; a few areas where enlargement was seen in isolation from neighboring lesions were found by serial sectioning to be adjacent to lesions. Type II hyperplastic lesions were observed most frequently at the pleura (figs. 5A-5C) but were also in the lung parenchyma adjacent to the distal airways (fig. 1B). In animals exposed to a dose of 7 cigarettes/day for 1 or 2 years, about 60% of the lesions were located on the pleura. When pleural lesions embedded in epoxy resins were serially sectioned, nearly all of them were found to be adjacent to sites where one or more alveolar ducts terminated on the pleura; about half were also distal to or surrounding perivascular lesions (fig. 5B). Fibrosis was obvious at the light microscopic level in the alveolar septa of all type II cell lesions.

The septal enlargements studied by electron microscopy contained numerous cells of the lymphoreticular system, i.e., plasma cells, lymphocytes, polymorphonuclear leukocytes, macrophages, and mast cells. Fibroblasts were also seen in all of the lesions and frequently appeared hypertrophic relative to those in unaffected septa (fig. 6). There were also extensive bundles of collagen fibers within the interstitium. Capillaries within the septa had swollen or hypertrophic endothelial cells that frequently occluded the lumen. In other areas the basal lamina showed extensive thickening, infolding, or reduplication (fig. 6).

The largest peribronchiolar lesions exhibited an aberrant morphology in which lengths of the basal lamina were completely surrounded by lymphoreticular cells, indicating areas of probable fusion of adjacent septa. Although the cellular constituents were similar to those in smaller lesions, they included fragments of cells embedded in an amorphous extracellular material resembling fibrin (fig. 7). These were thought to be derived from capillaries that were no longer patent. In densely fibrotic and cellular areas, there were relatively few capillaries of normal appearance. Both the squamous and type II epithelial cells in these areas sometimes contained an osmiophilic cytoplasm with an elaborate endoplasmic reticulum. Both epithelial cells and fibroblasts in these areas frequently contained osmiophilic granules resembling lipid droplets (fig. 7).

TABLE 2.—Number and percent incidence of large lesions observed in lung after chronic tobacco smoke exposure^a

Duration of smoke exposure, yr	No. of large lesions and percent incidence			
	7 cigarettes/day		10 cigarettes/day	
	Peribronchiolar ^b (%)	Type II ^b (%)	Peribronchiolar ^b (%)	Type II ^b (%)
1.0	0.3±0.1 (33) ^c	9.1±2.6 (89) ^{d,e}	0.1±0.1 (14)	11.6±3.0 (86) ^f
1.5	0.7±0.4 (43) ^g	17.4±3.4 (86) ^{e,h}	0.5±0.2 (42)	25.5±2.5 (100) ^f
2.0	1.9±0.4 (90) ^{c,g}	25.3±4.5 (100) ^{d,h}	—	—

^a *P*-values for the peribronchiolar comparisons were derived by a conditional exact test. Corresponding values for the type II lesion comparisons were derived by *t*-tests.

^b Values are means ± SEM.

^c *P*≤0.02.

^d *P*≤0.001.

^e *P*≤0.05.

^f *P*≤0.01.

^g *P*≤0.06.

^h *P*≤0.07.

Type II Cell Hyperplasia

The cellular and extracellular constituents involved in enlargement of the interstitium in type II hyperplastic lesions were similar to those described in the preceding section. All of the type II lesions studied showed some degeneration of basal lamina structure; in areas where marked degeneration occurred, the type I epithelium was separated from the underlying connective tissue (fig. 8). Degenerative changes were frequently, though not always, found in areas where luminal macrophages were in close contact with the type I epithelium. The presence of phagocytes (i.e., macrophages and polymorphonuclear leukocytes) in the interstitium had little relationship to the position of basal lamina degeneration. The lining epithelium in hyperplastic lesions consisted of cells with an undifferentiated appearance, which could be flattened, distorted in shape, or hypertrophic. Some of the cells showed long microvilli on their luminal surfaces. In addition, most lesions contained cuboidal, type III cells, with an extensive endoplasmic reticulum rather than the lamellar bodies typical of type II cells (fig. 9). Other type III cells had a dense cytoplasm, abundant profiles of endoplasmic reticulum, and osmiophilic inclusions (fig. 10). Lymphocytes were frequently found as cellular constituents of the epithelium. Binucleate cells and evidence of stratification in the epithelium were seen in about half of the lesions. A few lesions also contained large cells with an exceptionally clear cytoplasm (fig. 8).

Air-Space Enlargement

In addition to the focal lesions described above, we detected a general tendency for enlargement of the air space in animals exposed to a high dose of tobacco smoke for 1 year or more. This trend was indicated by an increase in the apparent nonalveolar air space. Although a similar trend was seen in the surviving animals of the low-dose group, it fell short of statistical significance. Exposure of animals to 7 cigarettes/day for up to 2.5 years did not significantly influence the mean linear intercept (table 3). However,

exposure to 10 cigarettes/day had a more pronounced effect on lung architecture: The mean linear intercept was increased significantly above control levels at both 1 and 1.5 years of exposure (table 3).

DISCUSSION

We obtained the present results on tobacco smoke inhalation in parallel with a lifetime study that demonstrated the induction of several types of lung tumors by tobacco smoke (7). The animals were maintained under SPF conditions; thus the histopathologic interpretation of pulmonary lesions was not complicated by chronic lung disease, and survival was not compromised by infection. In the lifetime experiment, the observation of tobacco smoke-induced tumors clearly was dependent on the long-term survival of the experimental animals. The mean survival time ($\pm$ SD) was 2.3 ± 0.6 years for rats exposed to 7 cigarettes/day, 2.4 ± 0.4 years for untreated controls, and 2.6 ± 0.4 years for sham-exposed controls. The survival curves for these 3 groups were not significantly different. The earliest of the 5 adenomas found among the treated animals appeared at 2.0 years, and the average age at death of the animals with these tumors was 2.6 years. For the 5 other lung and nasal tumors, which were adenocarcinomas and a squamous cell carcinoma, the average age at death was 3.1 years. Only 1 alveologenic carcinoma was observed in the control animals over their life-span. The final incidences of respiratory tract tumors were 9 and 1% in the experimental and control groups, respectively.

The present studies have shown that the most common type of focal lesions to develop in the lungs of tobacco smoke-exposed animals consisted of accumulated lymphocytes and macrophages in the vascular adventitia. Similar accumulations were also found in the lamina propria of terminal bronchioles, where some of them were continuous with lesions surrounding the bronchial veins or branches of the pulmonary veins. While all of the focal lesions were found to include cellular infiltrates typical of the inflam-

TABLE 3.—Morphometric analysis of lungs after exposure to cigarette smoke^a

Duration of exposure	Mean linear intercept, μ m	Percent of volume			
		Air space		Tissue	
		Alveolar	Nonalveolar	Respiratory	Nonrespiratory
12 mo					
Age controls	74.8 $\pm$ 3.7	50.6 $\pm$ 2.8	26.8 $\pm$ 3.5	21.0 $\pm$ 4.7	1.6 $\pm$ 0.6
10 cigarettes/day	82.6 $\pm$ 7.0 ^b	46.6 $\pm$ 1.8 ^b	32.1 $\pm$ 4.4 ^b	19.7 $\pm$ 3.1	1.6 $\pm$ 0.6
18 mo					
Age controls	70.0 $\pm$ 5.2	49.8 $\pm$ 1.6	25.9 $\pm$ 3.7	22.9 $\pm$ 4.2	1.5 $\pm$ 0.9
10 cigarettes/day	86.8 $\pm$ 4.6 ^b	42.4 $\pm$ 1.8 ^b	36.1 $\pm$ 2.6 ^b	19.9 $\pm$ 2.5	1.5 $\pm$ 0.7
24 mo					
Age controls	71.5 $\pm$ 3.6	45.8 $\pm$ 3.9	31.4 $\pm$ 3.7	21.3 $\pm$ 3.2	1.4 $\pm$ 0.9
7 cigarettes/day	67.6 $\pm$ 3.8	43.8 $\pm$ 2.7	30.9 $\pm$ 3.1	23.7 $\pm$ 2.9	1.5 $\pm$ 0.6
30 mo ^c					
Age controls	67.8 $\pm$ 1.9	43.7 $\pm$ 2.8	33.5 $\pm$ 3.2	20.7 $\pm$ 4.0	2.0 $\pm$ 1.2
7 cigarettes/day	69.7 $\pm$ 9.2	34.0 $\pm$ 2.5	39.9 $\pm$ 6.5	24.4 $\pm$ 7.0	0.6 $\pm$ 0.4

^aValues are means $\pm$ SD.

^bSignificantly different from untreated controls ($P < 0.05$).

^cAnimals were exposed for 30 mo, followed by 7 mo without treatment.

matory response, the cell types found in the vascular adventitia were typically restricted to lymphocytes and macrophages. Similar accumulations of lymphoreticular cells in perivascular locations have been noted in the normal course of aging in the rat (11) and in cases of multiple sclerosis in humans (12).

Although perivascular lesions were often contained within some of the more extensive lesions, it is not clear whether they played an essential role in the pathogenesis of the larger lesions. The larger lesions could have been induced by infiltration of lymphoreticular cells into the surrounding parenchyma or could have been initiated independently. Since these locations were probably subjected to tobacco smoke exposure under similar local conditions, inflammatory responses could have resulted from the same, presumably chemotactic, biochemical intermediaries in both cases. However, the larger lesions showed the full spectrum of inflammatory cells, including polymorphonuclear leukocytes and mast cells. Another feature common to the more extensive lesions was degradation of the basal lamina, particularly under the type I or squamous epithelial cells. We considered this alteration a possible contributing factor in fibrogenesis and/or epithelial hyperplasia. The proximity of phagocytes, particularly macrophages, to these sites suggested that enzymes released from the cells during phagocytosis may affect the structure of the lamina. The presence of lymphocytes, monocytes, and macrophages in the interstitium has also been reported in human diffuse interstitial lung diseases (13).

Lesions of comparable structure to the type II hyperplastic and peribronchiolar fibrotic lesions have been induced in many previous studies of lung pathology, but authors have tended to classify the cuboidal and columnar metaplastic lesions together. The exceptions that particularly note "bronchiolization" of the peribronchiolar alveoli have been reports on mice and hamsters infected with influenza virus (14), mice exposed to synthetic smog or calcium chromate dust (15), and hamsters treated with chemical carcinogens (16). In experiments on rats treated with various agents, particularly intratracheal instillation of tobacco smoke condensate (2) or of casein and carbon particulates (17), or a single exposure to benzo[*a*]pyrene followed by lifetime tobacco smoke inhalation (1), bronchiolization appeared to have occurred but to have been classified with other types of metaplasia. Our studies, based on the improved structural detail obtained by the examination of tissues embedded in epoxy resins, suggest that the bronchiolar lesions differ in several respects from the more common cuboidal metaplasias, which were identified here as type II hyperplasias. Since only about half of the peribronchiolar lesions showed epithelial metaplasia, our studies are also the first performed on rodents to suggest that fibrotic and cellular enlargement of the peribronchiolar septa precedes the replacement of the squamous, type I epithelium with a columnar epithelium. However, prominent areas of peribronchiolar fibrosis without columnar metaplasia have also been noted in the lungs of human smokers and of nonsmokers exposed to lung irritants (18).

In the present studies, hyperplasia of the type II epithelium appeared concurrently with changes in the intersti-

tium. Alveolar bronchiolization was rather infrequent and appeared to occur well after inflammatory and fibrotic changes in the interstitium. In addition, the two types of larger lesions differed in other respects: Their locations were typically dissimilar, the type II lesions being mainly at the pleura. More importantly, the frequency of the lesions was related to the duration of tobacco smoke exposure. Increasing the time of exposure at the lower dose of tobacco smoke from 1 to 2 years led to approximately a tripling in the frequency of type II hyperplasias but to a sixfold increase in the frequency of peribronchiolar lesions. While exposure to 10 cigarettes/day for 1.5 years induced as many type II hyperplasias as the 2-year exposure to 7 cigarettes/day, it induced far fewer peribronchiolar lesions. Thus the peribronchiolar lesions were dependent on the duration of exposure and/or on the age of the exposed animals.

In summary, the rat inhalation model offers a prototype of human syndromes induced by tobacco smoke exposure. However, the exposure level required for induction of emphysematous changes also leads to high mortality. The ultrastructure of the lesions appears to implicate the inflammatory response in their pathogenesis. The least common lesion seen, fibrosis of the peribronchiolar alveoli, is temporally related to tumor incidence and can be considered a precursor lesion.

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FIGURE 1.—Perivascular lesions. 1A) Particulate-laden macrophages forming a perivascular “cuff” in the parenchyma below the terminal bronchiole (T). Animal was exposed to 10 cigarettes/day for 1.5 yr. Bar = 200 μ m. 1B) Perivascular lesion (arrow) associated with a small venule near the alveolar duct (AD). Animal was exposed to 7 cigarettes/day for 2 yr. Bar = 200 μ m. $\times 70$. 1C) Perivascular cuff of lymphocytes (arrows), untreated control animal at 1.5 yr. Toluidine blue. Bar = 20 μ m. $\times 570$

FIGURE 2.—Fine structure of a perivascular lesion. The air space (A) is bordered by type II epithelial cells (II). Capillary endothelial cells (E), lymphocytes (L), and plasma cells (P) are also present in the adventitia of a venule (V). The cells containing dense inclusions are macrophages. Macrophages (M) are also present in the air space. Slight degradation of the basal lamina underlying the epithelium is also apparent (arrow). Bar = 10 μ m. $\times$ 1,800. Inset: Light micrograph of the same lesion. Animal was exposed to 7 cigarettes/day for 2 yr. Bar = 50 μ m. $\times$ 190

FIGURE 3.—Lymphocyte accumulation within lymphatic vessels located in vascular adventitia (A). A) Vessel containing lymphocytes (L) also has smoke particulates in lymphatic endothelium (arrow). Bar = 10 μ m. B) Vessel occluded by large lymphocytes (L). Note that several of the lymphatic endothelial cells contain aggregated smoke particulates (arrows). Animal was exposed to 7 cigarettes/day for 2 yr. Bar = 10 μ m. $\times$ 1,500

FIGURE 4.—Peribronchiolar septal lesions. 4A) Minimal fibrotic and cellular enlargement of septa, with an atypical airway epithelium in the distal part of terminal bronchiole (arrows). Bar = 100 μ m. $\times$ 110. 4B) More extensive lesion showing peribronchiolar lymphoreticular cell accumulations (arrows) and distortion and fusion of alveolar septa adjacent to terminal bronchiole. Bar = 100 μ m. $\times$ 50. 4C) Lesion showing a mucociliary bronchiolar epithelium on free surfaces of enlarged septa (arrows). Animals were exposed to 7 cigarettes/day for 2 yr. Bar = 100 μ m. $\times$ 170

FIGURE 5.—Small pleural type II hyperplastic lesion. 5A) Type II hyperplasia distal to terminal bronchiole. Bar = 500 μ m. $\times$ 25. 5B) Pulmonary vein with lymphoreticular cell accumulations (arrows) apical to type II hyperplastic lesion. Bar = 500 μ m. $\times$ 25. 5C) High-magnification view of the epithelium, showing type II cells (arrows). Animal was exposed to 7 cigarettes/day for 2 yr. Toluidine blue. Bar = 10 μ m. $\times$ 860

FIGURE 6.—Minimal septal enlargement at the periphery of a peribronchiolar fibrotic lesion. Plasma cells (P), lymphocytes (L), and hypertrophic fibroblasts (F) are present in interstitium. Collagenous thickenings (C) are also found at irregular intervals within alveolar septa. Thickening (arrows) and reduplication (double arrows) of basal lamina underlying the epithelium were also found. Most capillaries contained a hypertrophied endothelium (E), which frequently occluded the lumen completely. A few hypertrophic type II cells (II) were present. Bar = 10 μ m. $\times$ 1,600. Inset: Higher magnification view of occluded capillary at top of micrograph (E) with an adjacent plasma cell (P). Capillary lumen is marked by arrowheads. Note extensive infolding of basal lamina (double arrows). Animal was exposed to 7 cigarettes/day for 2 yr. Bar = 3 μ m. $\times$ 3,400

FIGURE 7.—Portion of a peribronchiolar fibrotic lesion that contains fibrotic septa showing collagenous thickenings (C), a fibroblast containing osmiophilic granules (F), and capillaries in which the endothelium appears to be degenerating (D). Animal was exposed to 7 cigarettes/day for 2 yr. Bar = 10 μ m. $\times$ 1,700

FIGURE 8.—Portion of a type II hyperplastic lesion showing cuboidal epithelial cells with exceptionally clear cytoplasm and secretory inclusions (Se) and collagenous areas in the interstitium (C). Most of the cells comprising the cuboidal population are type II cells (II). Degradation of the basal lamina was extensive, leading to detachment of type I epithelial cells from the septum in some areas (*arrows*). Animal was exposed to 7 cigarettes/day for 2 yr. Bar = 10 μ m. $\times$ 1,600

FIGURE 9.—Type II hyperplastic lesion showing cuboidal and squamous (S) epithelial cells. A columnar cell with extensive endoplasmic reticulum represented a third type of epithelial cell (III). Basal lamina underlying the epithelium is degraded in several areas (arrows). Lymphocytes (L) are present in both epithelium and interstitium. Interstitium also contains plasma cells (P), mast cells (M), macrophages with ingested particulate matter, and extensive areas filled with collagen fibers (C). Hypertrophic fibroblasts (F), were present. Nearly all capillary endothelial cells (E) were hypertrophied and occluded the capillary lumen. Profiles of linearly oriented acellular structures adjoined macrophages in the air space (A). Animal was exposed to 10 cigarettes/day for 1.5 yr. Bar = 10 μ m. $\times$ 1,800

FIGURE 10.—Portion of a type II hyperplastic lesion containing cuboidal cells (III) that showed numerous profiles of endoplasmic reticulum and densely osmiophilic granules in the cytoplasm. Basal lamina also was reduplicated (arrows). Animal was exposed to 7 cigarettes/day for 2 yr. Bar = 5 μ m. $\times$ 2,300