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ENVIRONMENTAL FACTORS IN EMPHYSEMA AND A MODEL SYSTEM WITH NO_2¶

Since Leannec's classic description of pulmonary emphysema,[1] hazards have persisted in talking about emphysema. Approaches to the understanding of emphysema have been much like those of the fabled three blind men, each of whom described the elephant according to the part most accessible to him.

Some investigators have used clinical and radiological criteria. Others have insisted that there be a measurable degree of obstruction to airflow.[2] A third group knows the disease best morphologically at a point in time when measures to reverse the faltering function and structure of the lungs have failed and the injured tissue can be examined post-mortem.[3,4]

"Emphysema" is a word that represents several concepts. It has been defined in 1959[5] according to stages, with and without destructive features. On the other hand, its definition was limited in 1962 to a stage in which destruction of tissue could be guaranteed.[6] In the latter sense, destruction would have to be either congenital or the result of an acute event in middle life, due to the omission of a developmental phase. The current dictionary definition does not require alveolar destruction,[7] but all agree on distension of the air spaces peripheral to the terminal bronchioles.

Although physiologists have been impressed with chronic obstruction to airflow,[8] some pathologists have stressed the association between large air spaces and destroyed alveolar septums.[9] As a result, experimental design has often been aimed at creating a destructive lesion with necrotizing agents that give rise to scars and distortion. These have been either high concentrations of real pollutants,[10-13] or unlikely agents such as war gases,[13] directly applied enzymes,[14] or intravenously delivered physical or chemical agents[15,16] that would be relevant only under most extraordinary conditions.

In this dilemma, our laboratory sought guidance from epidemiology. Through the years it has indicted coal mines,[17] asbestos pits,[18] fermenting

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silage,[19] and other especially confined, contaminated working spaces as contributing to chronic lung disease.[20] Also, superimposed infections are more likely to thrive in such lungs than in the well-defended normal lung,[8,21] and add to local emphysematous changes. In addition, the proclivity of the person with repeated attacks of obstructive disease to develop emphysema is well known. Even lung proteins released from injured pulmonary tissues, it has been suggested, may act as autoantigens.[22] However, the widespread incidence of emphysema is not explained by such selected conditions.

More recently, epidemiologists have uncovered highly significant correlations between emphysema and the amount of smoking individuals do. Anderson and Ferris[23] and Hammond[24] have unequivocal relevant data, and this is supported by correlations between smoking and post-mortem findings of chronic pulmonary disease reported by Auerbach, *et al.*[25] In addition, positive correlations have been made between high levels of air pollution in communities and the incidence of chronic respiratory disease among their inhabitants.[26]

Therefore, between cigarette smoking and breathing polluted air or smog, a large part of the population inhales "tars," oxidants, carbon monoxide, sulfur oxides, oxides of nitrogen, and lesser amounts of a variety of metallic compounds and less well-defined products of photochemical reaction.[27] Yet, not everybody who smokes heavily becomes disabled with emphysema, while the disease may occur in rural persons who have not been known to smoke.[24] As in all disease, vulnerability may be weighted heavily by genetic predisposition.[28]

From an array of likely etiologic models from our daily atmosphere, we chose nitrogen dioxide, because it is ubiquitous and is a toxic oxide of nitrogen. It is found in tobacco smoke[29] in relatively high concentrations, and is an important effluent of the automobile engine.[30] It is relatively insoluble and so is able to reach the periphery of the lung.[30] Finally, in high concentration NO_2 causes severe, acute disease of the lungs.[19]

In a proper model for emphysema, we have sought to avoid acute effects that produce edema, inflammatory cells, or visibly injured cells. (At 20 ppm, no tissue response was detected for four to six days, and then it was hypertrophic rather than inflammatory.[31]) A second requirement was the stimulation of a very slow, covert type of response by tissue elements that are normally present in the lung so that one could follow the earliest changes with a reasonable degree of specificity for both the inciting agent and the histologic response. (The victim of emphysema is probably conscious of a slowly developing disorder for several weeks or months without being able to identify it with any particular episode in his life.) A third feature was the achievement of some obstruction to airflow and generalized

distension of peripheral airspaces due to a lesion that could lead to, or result in, loss of alveolar septums.

In our laboratory, therefore, we moved rapidly from acutely toxic concentrations of NO_2 to levels at which tissues no longer responded angrily, but were affected so that disability and death ensued slowly during the natural lifetime of the rat.[32,33] Ultimately, a low enough range of concentrations was used so that experimental animals survived their natural lifespans with minimal evidence of exposure to nitrogen dioxide.[34,35] Such levels approximated but did not quite reach down to the realistic ambient concentrations of well below 1 ppm reported by public health authorities in smoggy environments. We have recently shifted from exclusive use of the rat to the use of the stump-tailed macaque, a species with a much longer life expectancy than 2-3 years, so that the influence of environmental factors might be prolonged, as it most likely is in man.

Now, we can see how the rat responds to continuously administered nitrogen dioxide. This is not unique with the rat because similar responses have been observed also in the rabbit by Wasserman's group,[36] in the dog in Coffin's laboratory[37] in Cincinnati and in the mouse by us.[31]

Figure 1 shows the rat and his lungs in the advanced stage of the disease. The dorsal kyphosis, one aspect of the enlarged thoracic cavity, is obvious. The lungs were removed without attempting to contain air or blood. The normal pair collapsed under atmospheric pressure whereas the experimental one retained its highly inflated state, was dry but distinctly heavier than the normal control (left pairs). The pairs to the right are the same after being filled with Zenker's fixing fluid to end-pressures of about 25 cm H_2O. Such large lungs in the rat resulted from higher than ambient levels of NO_2,[32,38] as we shall see later.

In the interest of logic, I shall present our model system starting with the lowest concentration of NO_2 used. This concentration was 0.8 ± 0.2 ppm of NO_2, a level already achieved in smog, with 21 rats, one month of age.[34] They and an equivalent control group lived out their natural lives of two to three years and died of similar commonplace diseases of old age, apparently unrelated to NO_2.

The only difference between the groups was a sustained tachypnoea of about 20 per cent above normal in rats exposed to NO_2.[33,34] This was the same as with 2 ppm. Microscopically, lungs were essentially without blemish except for occasional evidence of bronchiolar epithelial changes of the type to be described in the experiment with 2 ppm NO_2. Microscopy was done on Zenker's filled and fixed, paraffin-embedded, whole lung sections, prepared always along the axis of greatest dimension. The filling

end-pressure was 25 cm H_2O. The 6 μ sections were stained with hematoxylin and eosin.

A similar experiment with $2\pm$ 1 ppm of NO_2 also lasted for the natural lifetimes of the animals and death was due to similar diseases in both groups, as in the 0.8 ppm experiment.[34] Again, respiratory infection was found only rarely. All rats grew normally and the exposed ones had elevated respiratory rates, as in the former experiment. Grossly, experimental lungs were not appreciably different in appearance from controls.

Whole-body plethysmography, essentially by the method of Amdur and Mead,[38] was used to study transthoracic resistance to airflow and dynamic compliance in lightly anesthetized (Nembutal®) rats. At either high or low respiratory rates, measurement of the two functions varied in the same way and within the same ranges for both experimental and control rats.[81]

Because experience with higher concentrations indicated that the structure most vulnerable to NO_2 was the bronchiolar epithelium,[33] special attention was given to its microscopic morphology. However, in similarly fixed lungs the impression was gained that NO_2-exposed lungs had a greater variability in alveolar dimensions than normal. Alveolar chords, therefore, were measured along the orthogonal X and Y axes of grids

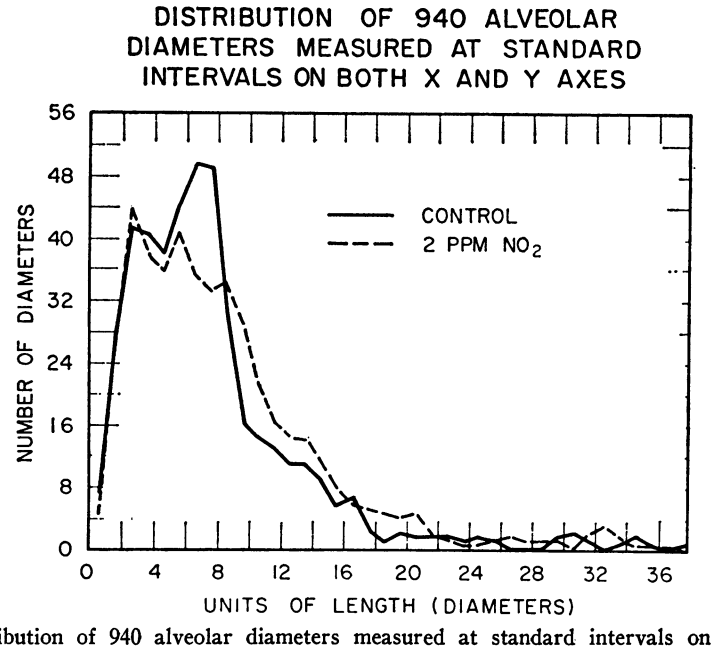


FIG. 2. Distribution of 940 alveolar diameters measured at standard intervals on both X and Y axes.

superimposed on enlarged photomicrographs. The distributions of over 900 chords are shown graphically in Figure 2. The median chord length is longer in the selected experimental animal than in its control, and the distribution is broader, as represented by the crossing-over of the descending limb of the curve. This has not been done with enough specimens and is shown only to confirm the appearance of the lung under the microscope.

Regular hematoxylin and eosin (H and E) sections revealed some differences in the bronchiolar epithelium, essentially in the respiratory bronchioles, between control and experimental animals. Epithelial cells exposed to NO_2 were more uniform in size and the lumenal surfaces were smoother than in controls. However, the precise nature of the morphological differences was too obscure to describe accurately.

In order to improve the histology of the epithelium, lungs of sacrificed rats were filled immediately with osmium tetroxide and immersed in the same fixing fluid. Areas containing terminal bronchioles were cut out and embedded in Maraglas. From such blocks, thin sections were cut, both for light microscopy (1 μ thick) and for electron microscopy. Sections for light microscopy were stained with toluidine blue. Figures 3-6 reveal the effects of 2 ppm of NO_2 after about two years of continuous exposure, in comparison with the normal.

The normal epithelium, shown in Figures 3a and 4, has a somewhat irregular monolayer with nuclei at various distances from the basement membrane. Most cells are well covered with a brush of long cilia but the surfaces of several protruding cells lack cilia. Some of these may be Clara cells, presumably having special functions.[39] Others seem to be in the process of exfoliating as though an active replacement of cells were going on. Along the ciliated surface are found two types of "bleb-like" features. One is made up of sections of exfoliated cells and the other, more numerous type represents cytoplasmic protrusions, some of which are pinched off. These account for the seeming irregularity of normal epithelium seen in paraffin-embedded, 6 μ sections.

In contrast, Figures 3b, 5, and 6 show the wider, more uniform cells from exposed animals. These cells average about one-third larger in area, per unit length of basement membrane, than normal, as determined by planimetry. Few cilia remain and the cells lack most of the exfoliative activity and blebbing of cytoplasm. Altogether, it is a picture of relative dormancy or restrained activity. Figure 6 demonstrates an additional feature in the experimental material, rod-like cytoplasmic inclusion bodies. The pattern of toluidine blue staining in some sections suggests also that connective tissue elements may be somewhat more abundant. This will require further quantitative study.

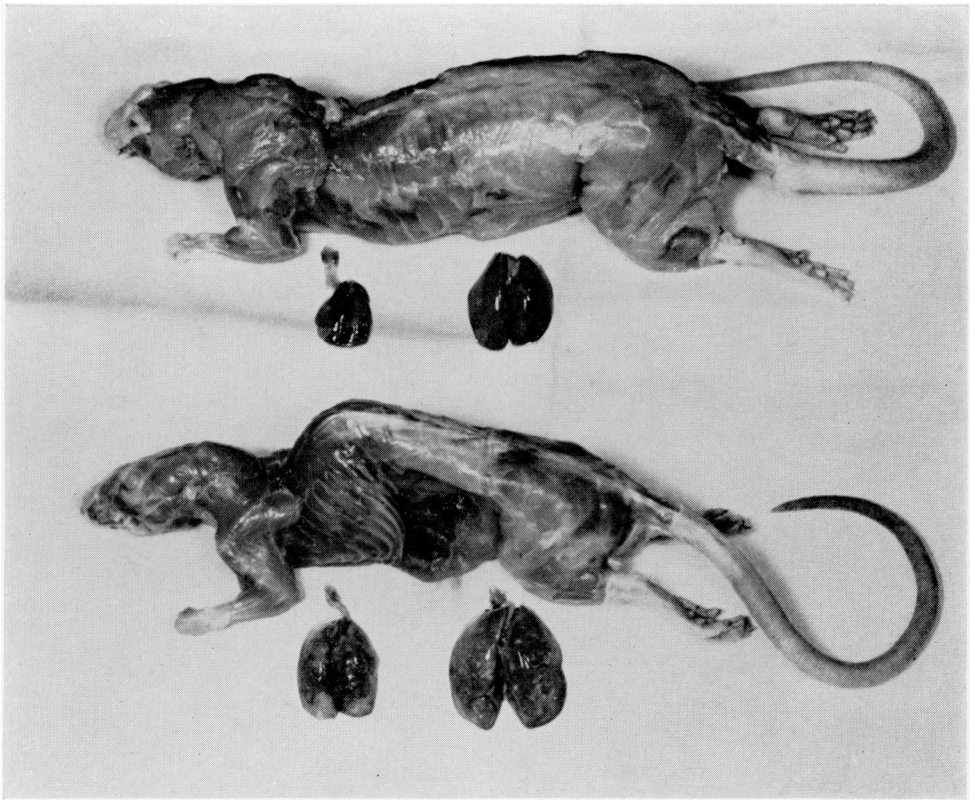
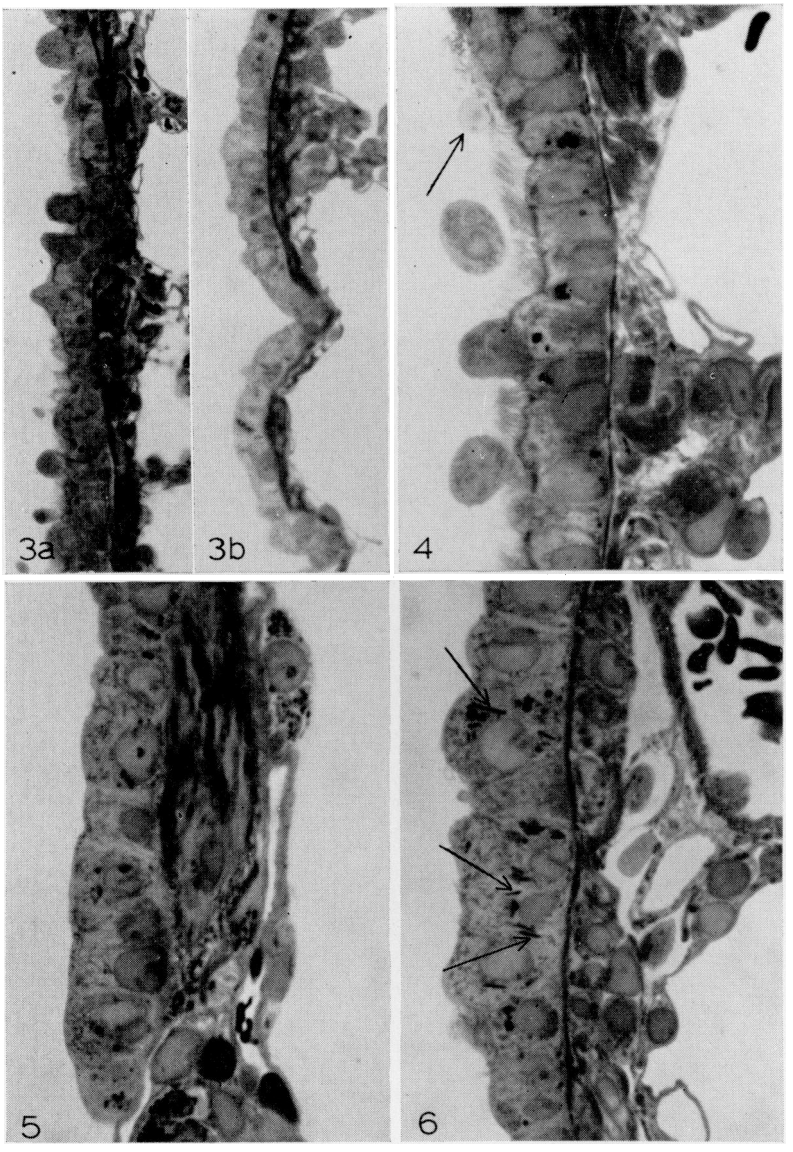


FIG. 1. Control and experimental rats of the same age (4 months). The rat at the bottom received 25 ppm NO_2 for eight weeks. Lungs to the left were removed without attempting to retain blood. They were allowed to contract under atmospheric pressure. Note the difference in lung volumes and the severe kyphosis in the experimental animal. To the right, the same lungs are shown after having been filled to end-pressures of 25 cm H_2O with Zenker's fixing fluid. The relatively voluminous lungs of the experimental animal are clearly seen.



FIGS. 3a and b. Thin sections (1 μ) of the epithelium of the terminal bronchiole from a control animal (3a) and from an animal exposed to 2 ppm NO_2 for two years (3b), at the same magnification. Note the more active appearance of the epithelium of the control animal with "blebs" and cilia, whereas they are absent from the tissue of the exposed animal. Toluidine blue stain. $\times$ 600.

FIG. 4. Thin section (1 μ) of Maraglas-embedded tissue. The micrograph is taken from a longitudinal section of a terminal bronchiole of a two-year-old control rat. Cells extending to the basement membrane possess numerous long cilia, whereas those protruding from the surface are unciliated. Both free cells and small spherical bodies (arrow) are seen on the surface of the cilia. Round, densely staining inclusions can be seen in the cytoplasm of the epithelial cells. Toluidine blue stain. $\times$ 1500.

FIGS. 5 and 6. Micrographs of tissue, prepared in the same manner as that in Figure 4, from an animal exposed to 2 ppm NO_2 for two years. Note the lack of cilia on the surface of the epithelium of the terminal bronchiole. The surface is smooth, lacking both exfoliated cells and "blebs." Round, densely stained inclusions are seen in the cytoplasm but, in addition, elongated structures that also stain intensely are present (6, arrows). These were not present in the control tissue. Touidine blue stain. $\times$ 1500.

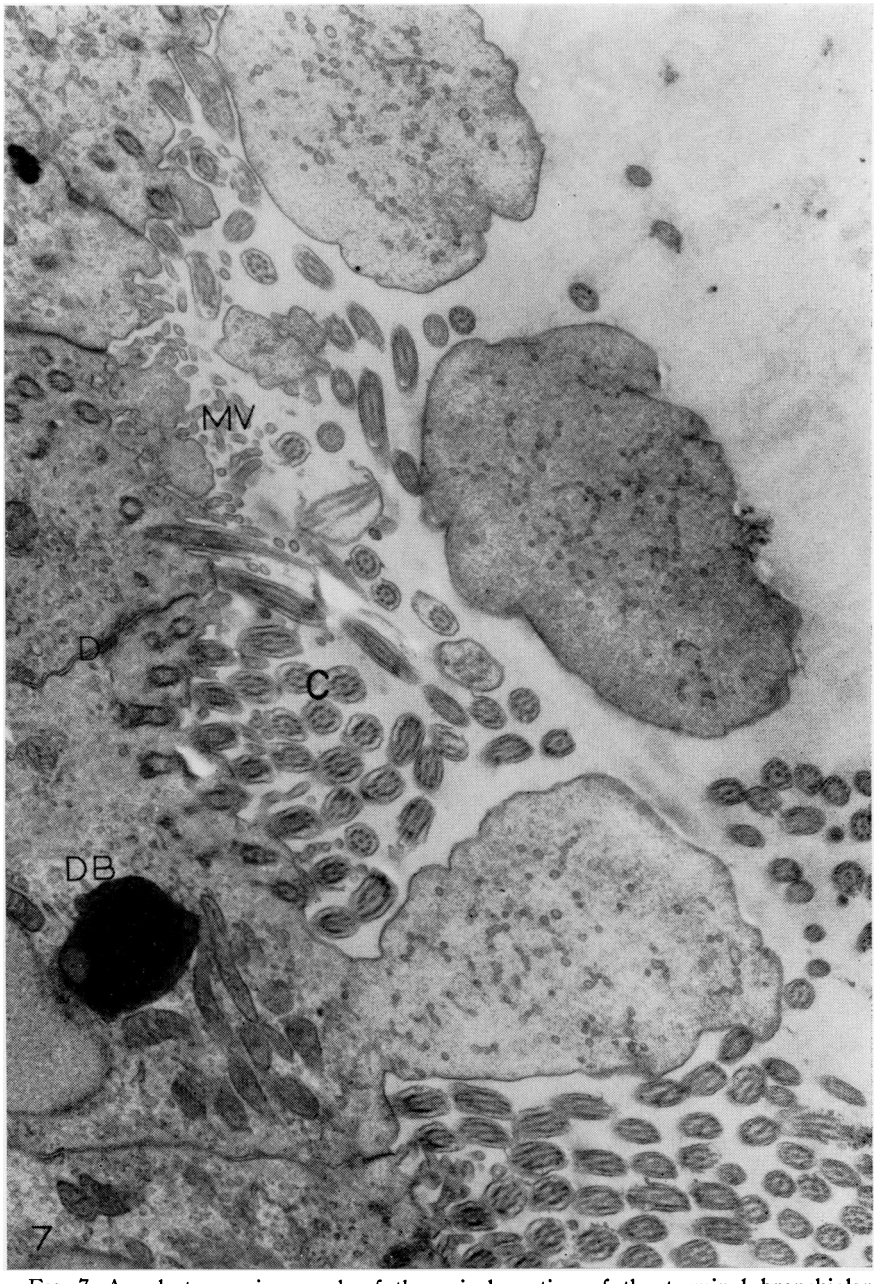


FIG. 7. An electron micrograph of the apical portion of the terminal bronchiolar epithelial cells from the same tissue of the control animal seen in Figure 5. Profuse cilia (C) are seen sweeping along cellular elements. They contain small vesicles and flocculent material. These structures are derived from cytoplasmic blebbing of the surface of the epithelial cells. The attachment of a cytoplasmic bleb to the apical cytoplasm of a ciliated epithelial cell is seen. Only small, smooth-membraned vesicles are present in these blebs. Microvilli (MV), desmosomes (D), dense body (DB). × 18,000.

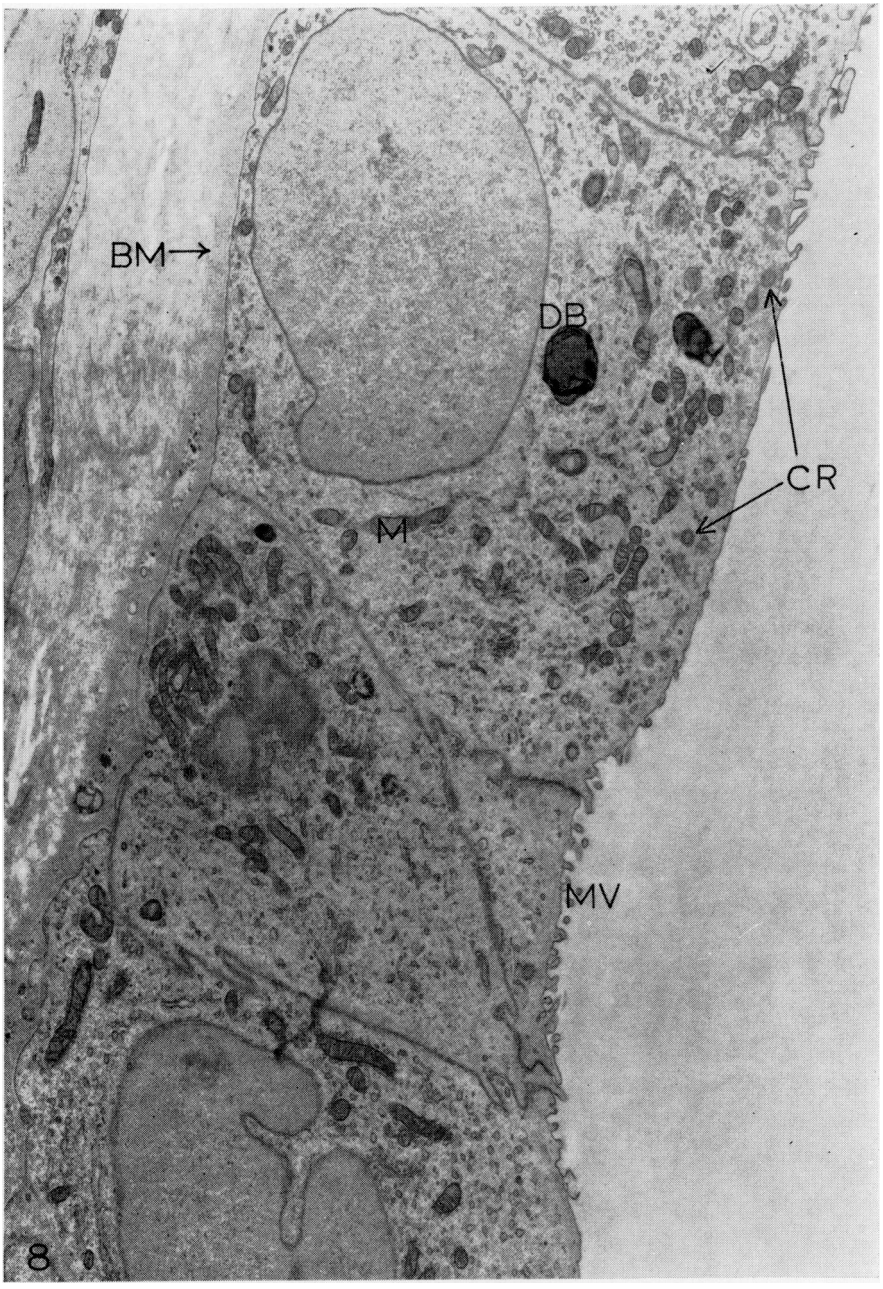


FIG. 8. Epithelial cells from the terminal bronchiole of a rat exposed for two years to 2 ppm NO_2. The cilia have been removed from these cells but the "roots" (CR) remain in the apical cytoplasm. Microvilli (MV) remain on the luminal surface of the cells. Dense body (DB), mitochondria (M), and basement membrane (BM). × 12,000.

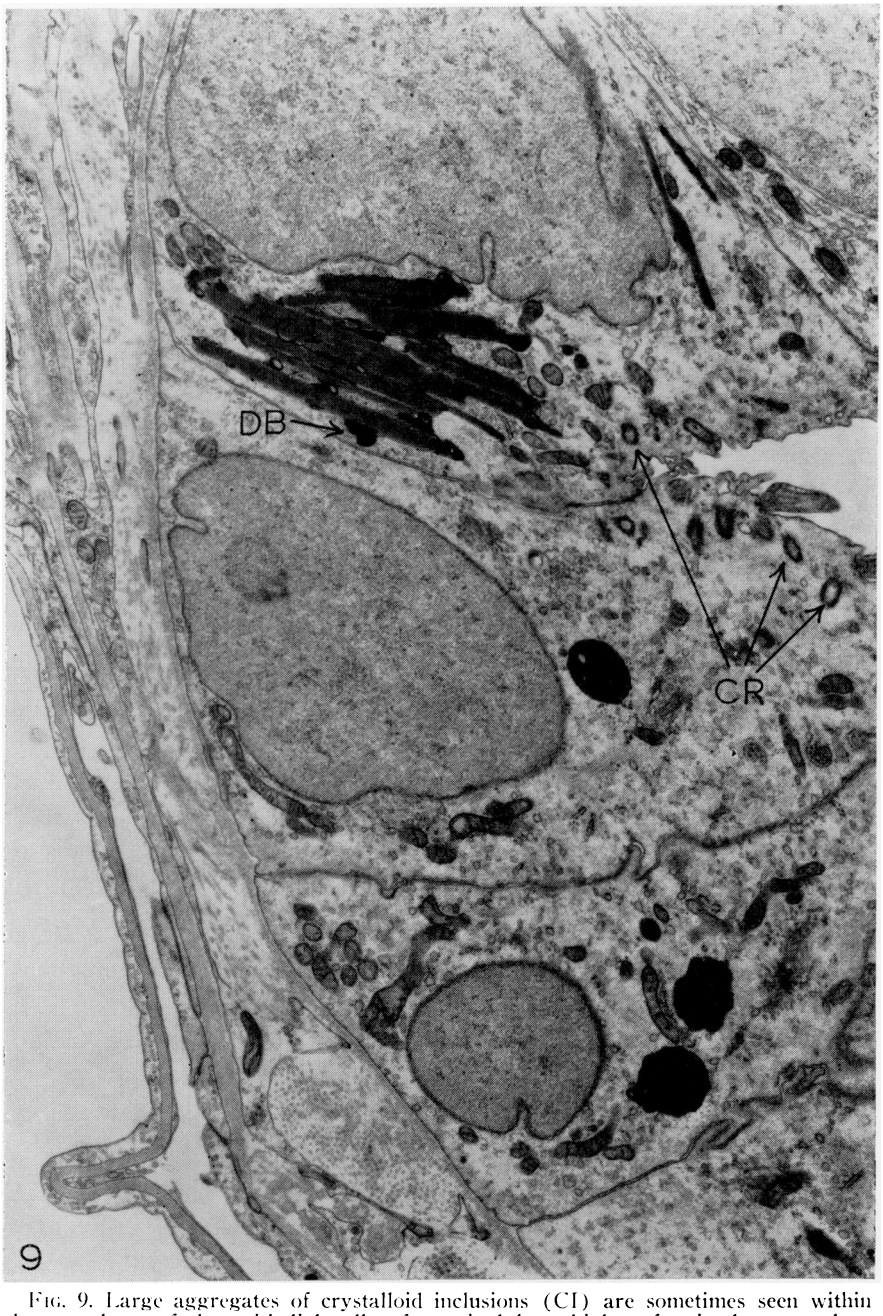


FIG. 9. Large aggregates of crystalloid inclusions (CI) are sometimes seen within the cytoplasm of the epithelial cells of terminal bronchioles of animals exposed to 2 ppm NO_2 for two years. Dense bodies (DB) are seen along the surface of the crystalloids as well as in the cytoplasm of other epithelial cells. Roots of cilia (CR) can be seen in the apical cytoplasm. × 15,000.

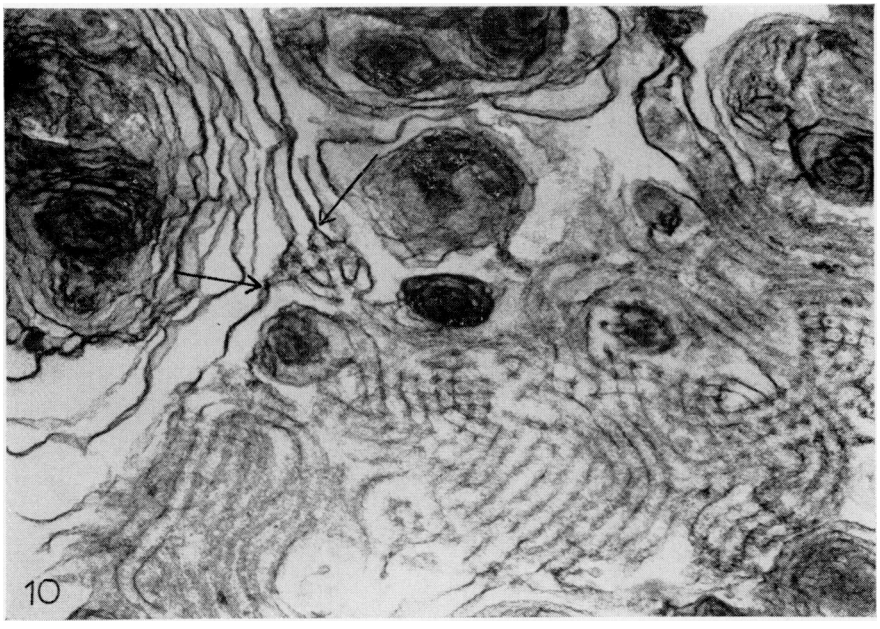


FIG. 10. Concentrically swirled membranes apparently involved in the formation of the grid-like material by a process of splitting and intersecting (arrows). ×67,000.

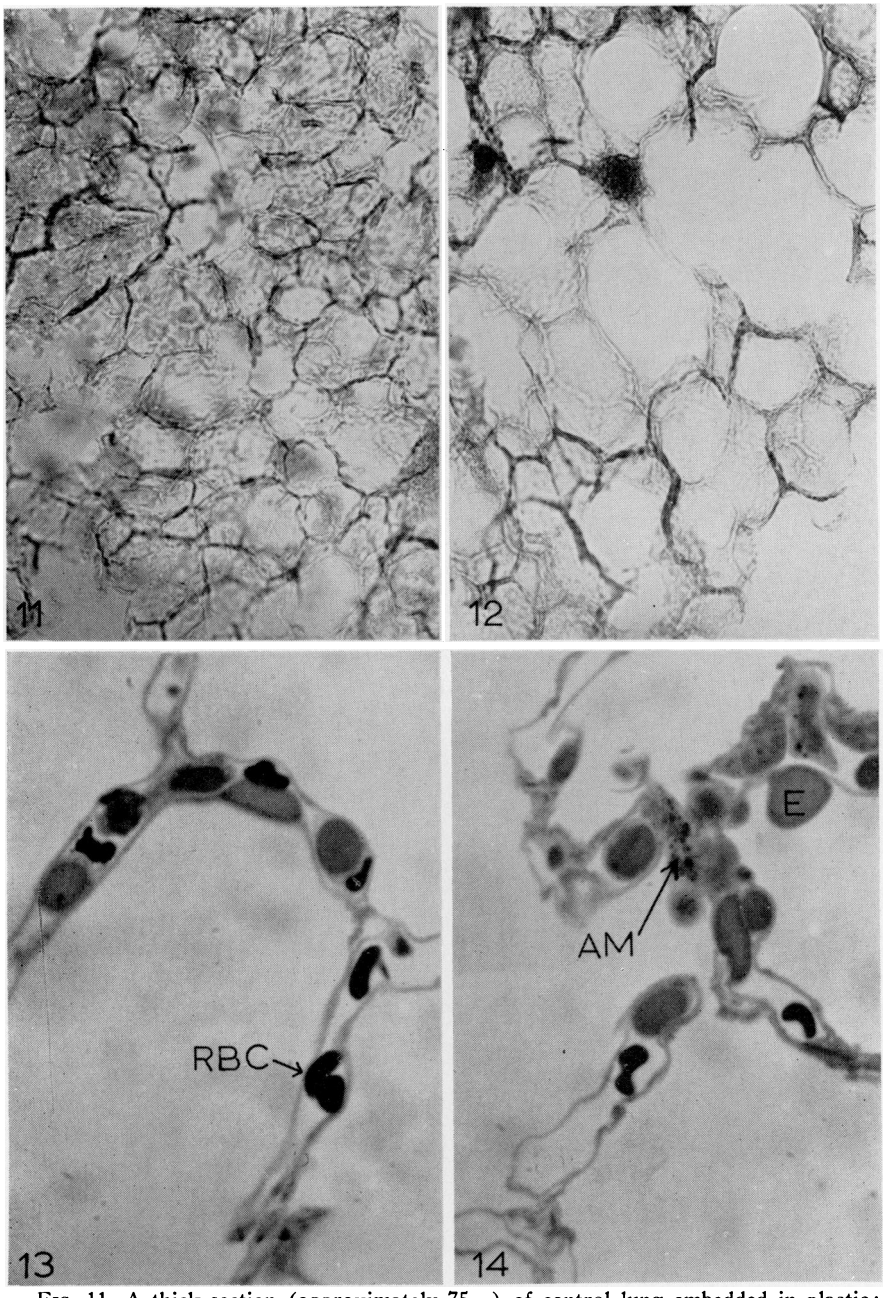
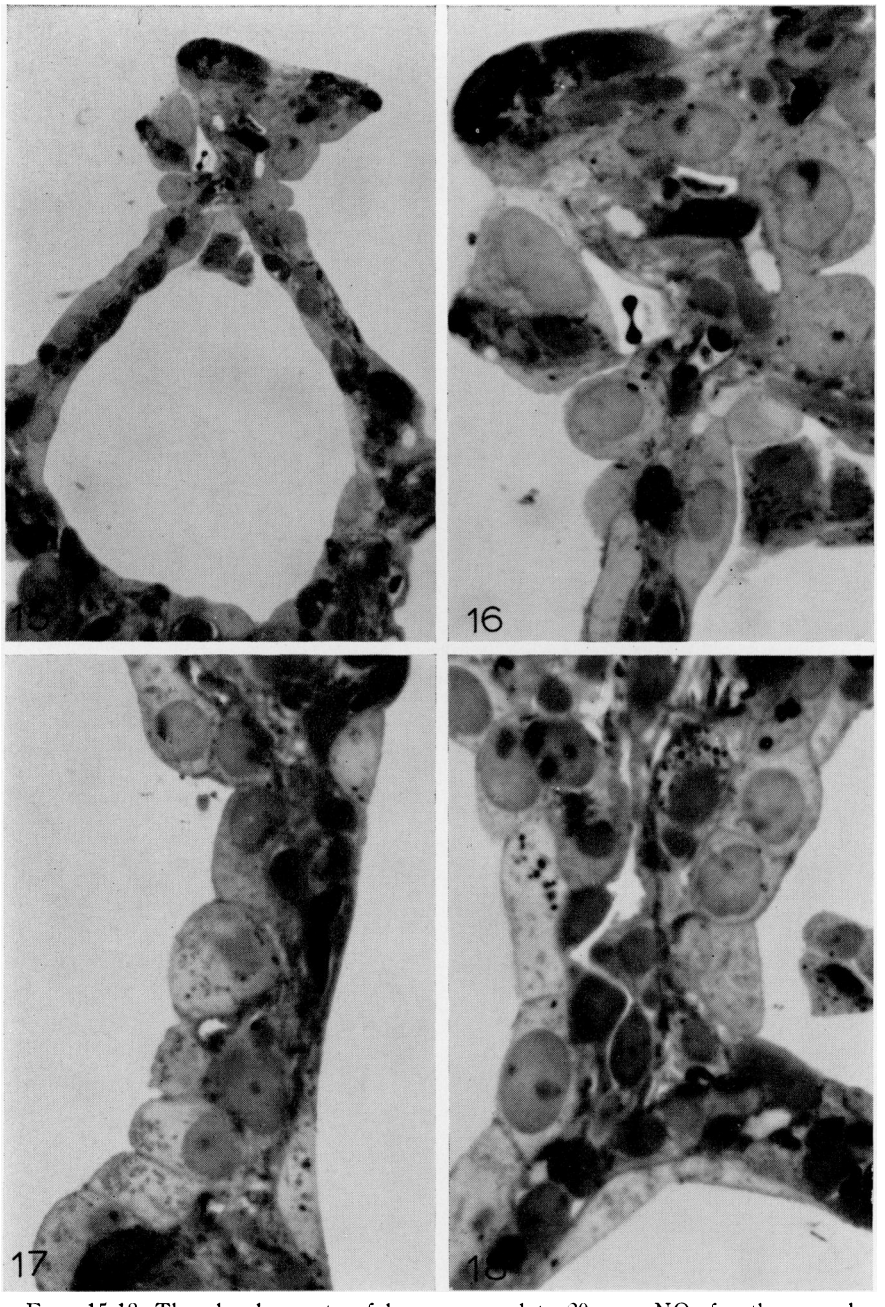


FIG. 11. A thick section (approximately 75 μ) of control lung embedded in plastic; note the small size of the alveoli. Tissue was fixed in OsO_4 but unstained. × 150.

FIG. 12. The size of the alveoli in this lung from a rat treated with 20 ppm NO_2 for 4 weeks are considerably larger than those of the control animal. The alveolar septa are also much coarser in the experimental animal. Tissue was fixed in OsO_4 but unstained. × 150.

FIGS. 13 and 14. Typical sections of control rat lung. Capillaries containing red blood cells (RBC) comprise the major portion of the alveolar septa. Note the attenuated air-blood barrier. Endotheliai nuclei are observed most frequently but other cells are also present. Epithelial lining cell (E) and alveolar macrophages (AM). × 1,500.



FIGS. 15-18. The alveolar septa of lungs exposed to 20 ppm NO_2 for three weeks are presented in these four Figures. Note the increased cellularity, most of which is due to an increase in alveolar epithelial tissue. Capillaries are not as prominent as in the control (see Figs. 13 and 14). The hypertrophy of the alveolar lining epithelium is marked. The nuclei as well as the cytoplasms show an increase in size. The thickness of the air-blood barrier has been effectively increased. Figure 16 is an enlargement of the upper portion of Figure 15; an RBC in the center of Figure 16 shows that the magnification of Figures 16-18 is the same as that of Figures 13 and 14. × 600; 1,500; 1,500; 1,500.

Electron micrographs of the same material confirm and extend these observations. The normal ciliary pattern extends to the most distal cells of the respiratory bronchiole and is shown together with emerging and pinched-off cytoplasmic blebs in Figure 7. The following Figures (Figs. 8, 9) reveal the residual intracytoplasmic roots of the missing cilia near the smooth cellular surface, together with remaining microvillae and crystalloid, cytoplasmic inclusions not yet seen in normal rats of the same age. Alveoli contain concentric membranes and grid-like material thought by other workers to be related to surfactant.[40] It is found here in both experimental and control tissues. There has been a question as to whether the grid material is derived from the concentrically arranged membranes, and if it is, how it is formed. Figure 10 clearly shows that the grid-like structure is formed by a splitting and intersecting of the concentric membranes.

Functionally, it is suggested that inhaled particles, infectious or not, might be retained, or at least detained, due to deficient ciliary cleansing of alveoli and bronchioles.[41,42] Free or unwanted cellular and extracellular elements would tend to accumulate and, possibly, to interfere with airflow and also exaggerate the deleterious influence of foreign materials on local tissues.

The next higher concentration, 4 ppm, was terminated after 16 weeks due to lack of chamber space. Grossly, the lungs were not clearly different from controls but the terminal bronchiolar epithelium was hypertrophic, characterized by increased height and uniformity of the cells.[38] We do not know whether or not such lungs would have become grossly emphysematous on further exposure.

In all cases, continuous breathing of 10 ± 1 or more ppm gave rise eventually to large, air-containing lungs that did not collapse under atmospheric pressure. Animals exposed to 10 ± 1 ppm began to die of respiratory failure after 16 months. They grew less well and developed thoraces with increased anterior-posterior diameters, as represented by the dorsal kyphosis in Figure 1.[32,33]

H and E and special stains for connective tissue elements and periodic acid-Schiff positive materials revealed features in addition to the bronchiolar epithelial hypertrophy. Bronchial epithelium showed striking activation of goblet cells and layering of the mucus over the surface of the bronchial epithelium.[32] At the junctions of respiratory bronchioles and alveolar ducts, the patency of airways was often embarrassed by the combination of hypertrophic bronchiolar epithelium and small aggregates of macrophages, bound together by fibrinous strands together with bits of amorphous, proteinaceous debris.[32] Behind such barriers, alveolar ducts were distended and alveoli were larger and more variable in size than

normal. Although alveolar septa were missing at various points, as seen in thick sections (Figures 11 and 12), destruction of parenchyma was not an outstanding feature at this stage of disease. Also, elastic tissue staining seemed slightly more prominent in affected lungs than in controls. Lungs of rats dying of exposures to 12 or 25 ppm looked alike, except that the longer survival of 16 to 30 months of the former allowed changes in them to become more advanced than in rats that died after 5-6 months from 25 ppm. This inverse relationship to concentration may be critical to pathogenesis. In recovering animals, the hypertrophic epithelium receded, leaving the inhomogeneity of peripheral air spaces as a residual part of the disease.

The increased volume of the emphysematous lungs is readily explained by the enlarged air spaces of the parenchyma but the increase in weight, which may become more than twofold, is not easily accounted for.[32,33] Such lungs were compared with those of normal controls of the same age by wet-to-dry weight ratios in a search for inapparent edema. There proved to be no difference in free, extracellular fluid, the averages for ratios being 4.1 and 4.2 for normal and emphysematous lungs, respectively.[35]

Large blood vessels were tied-off at the heart in some animals and their lung weights compared with those of lungs having open-ended vessels. About eight per cent of the total weight of all lungs could be accounted for by blood in tied vessels. The difference in such blood between normal and emphysematous lungs was too little to explain the excessive weight of the experimental lungs.[43] The total blood volume, therefore, was determined by measuring the iron content of incinerated lungs. Iron determinations were made also on the circulating heart blood of each animal. Then, from the total lung weight and the iron values for blood, the total amounts of pulmonary blood and of blood-free tissue were calculated. The absolute amount of blood proved to be rather stable, regardless of the total weight of the lungs, emphysematous or normal. On this basis, emphysematous lungs proved to have less blood per gram of lung than did controls, and about 40 per cent more tissue in this particular experiment.[43] See Table 1.

In order to see the structure of the heavy, emphysematous lungs in greater detail than is possible in 6 μ sections, the technique used in the case of the "2 ppm experiment" was applied and 1 μ sections were made from Maraglas-embedded blocks incorporating terminal bronchioles. In order to orient the structure, thick sections ($\sim$ 75 μ) were examined first with a dissecting microscope. The bronchiolar walls were deeply stained with osmium, the dilated alveolar ducts were easily located and confluence of alveoli was seen.

TABLE 1. BLOOD AND TISSUE WEIGHTS OF CONTROL AND EMPHYSEMATOUS LUNGS

Type of animal	*Preparation*	*Weight (g)*		
		Tissue & blood	*Blood*	*Tissue*
Controls	Untied	2.11	0.36	1.75
		2.10	0.30	1.80
		2.77	0.59	2.18
	Means	2.33	0.42	1.91
	Tied	2.58	1.01	1.57
		3.02	0.28	2.74
		2.55	0.72	1.83
		2.08	0.81	1.27
		2.70	0.99	1.71
	Means	2.59	0.76	1.82
Emphysematous	Untied	3.24	0.51	2.73
		3.42	0.44	2.98
	Means	3.33	0.48	2.85
	Tied	3.16	1.13	2.03
		4.72	0.93	3.79
		3.39	0.67	2.72
		2.90	0.70	2.20
	Means	3.54	0.86	2.68

The 1 μ sections of alveolar septa (Figs. 13-18) confirm the data to the effect that the excessive weight of the nonedematous, emphysematous lungs resides in tissue, mainly in areas of hypertrophic epithelial lining of the alveoli.[45] This is consistent with the hypertrophy of the bronchiolar epithelium previously recognized in 6 μ sections. What are normally thin, tenuous membranes, seen in Figures 13 and 14, separating the alveolar spaces from the wide capillary channel of a septum, now consist of greatly hypertrophied, contiguous cells that tend to compress the enclosed capillaries, often to the point of obliteration, as seen in Figures 15-18. Thus, although hypertrophic bronchiolar epithelium and some macrophages may account for some of the increase in weight, septal walls are apparently responsible for most of the excess. They could also account for the observed functional changes in respiration.[44]

Clearly, gas exchange across such a barrier would be embarrassed. Therefore, if NO_2 were reduced or removed periodically to prevent pre-

mature death from central anoxia, longer survival in a chronic state of hypoxia would be promoted and further pulmonary changes might take place. Thus, compromised capillaries might lead to slow, piecemeal destruction of alveolar tissue due to pulmonary anoxia.

Perhaps, then, by selective timing and dosage of NO_2, lesions of the lung may be achieved that resemble more closely the generalized destructive, bullous-forming stage of disease recognized by many pathologists as the *sine qua non* of terminal emphysema in man.[6,9]

In summary, we have described a solitary model of an emphysema-like disease, based on the covert effects of a noxious gas that is ubiquitous in our environment and causes changes in lungs slowly. The model suggests how some of the features that define emphysema in man may occur.

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REFERENCES

1. Laennec, R. T. H.: *Treatise on Diseases of Chest and an Mediate Auscultation.* Translated by J. Forbes. London, Longmans, Rees & Co., 1834 ed. 4 p. 141-162.
2. Fenn, W. and Rahn, H.: *Handbook of Physiology,* Vol. II, Section 3: *Respiration.* Washington, D.C., American Physiological Society, 1965, pp. 1354, 1401, 1408, 1409.
3. Gough, J.: The pathology of emphysema. *Postgrad. med. J.,* 1965, *41,* 392-400.
4. Strawbridge, H. T. G.: Chronic pulmonary emphysema (an experiment study). I. Historical Review. *Amer. J. Path.,* 1961, *37,* 161-173.
5. Ciba Guest Symposium: Terminology, definitions and classification of chronic pulmonary emphysema and related conditions. *Thorax,* 1959, *14,* 286-299.
6. American Thoracic Society: Definitions and classification of chronic bronchitis, asthma and pulmonary emphysema. *Amer. Rev. resp. Dis.,* 1962, *85,* 762.
7. *Dorland's Medical Dictionary,* 24th ed. Philadelphia and London, W. B. Saunders Company, 1965, p. 481.
8. Richards, D. W.: Pulmonary emphysema: etiological factors and clinical forms. *Ann. intern. Med.,* 1960, *53,* 1105-1120.
9. Boren, H. G.: Experimental emphysema. *Amer. Rev. resp. Dis.,* 1965, *92,* 1-15.
10. Gray, E. Leb., MacNamee, J. K., and Goldberg, S. B.: Toxicity of NO_2 and vapors at very low levels. *Arch. industr. Hyg.,* 1952, *6,* 20-21.
11. Kleinerman, J. and Wright, G. W.: The reparative capacity of animal lungs after exposure to various and single and multiple doses of nitrite. *Amer. Rev. resp. Dis.,* 1961, *83,* 423-424.
12. Hernandez, J. A., Anderson, A. E., Jr., Holmes, W. L., and Foraker, G. A.: Pulmonary parenchymal defects in dogs following prolonged cigarette smoke exposure. *Amer. Rev. resp. Dis.,* 1966, *93,* 78-83.
13. Rossing, R. G.: Physiological effects of chronic exposure to phosgene in dogs. *Amer. J. Physiol.,* 1964, *207,* 265-272.
14. Gross, P., Babyak, M. A., Talker, E., and Kaschok, M.: Enzymatically produced pulmonary emphysema. *J. occup. Med.,* 1964, *6,* 481-484.
15. Strawbridge, H. T. G.: Chronic pulmonary emphysema: An experimental study: III. Experimental pulmonary emphysema. *Amer. J. Path.,* 1960, *37,* 391-407.
16. McLaughlin, R. F., Jr., Tyler, W. S., and Edwards, D. W.: Chlorpromazine induced emphysema. Results from initial study on horse. *Amer. Rev. resp. Dis.,* 1965, *92,* 597-608.

17. Cummins, S. L. and Sladder, A. F.: Coal miner's lung: An investigation into the anthracotic lungs of coal miners in South Wales. *J. Path. Bact.,* 1930, *33,* 1095-1132.
18. Biological Effects of Asbestos, Conference. *Ann. N.Y. Acad. Sci.,* 1965, *132,* 1-766.
19. Lowery, T. and Schuman, L. M.: Silo-Filler's Disease—A syndrome caused by nitrogen dioxide. *J. Amer. Med. Ass.,* 1956, *1962,* 153-160.
20. Heimann, H.: Status of air pollution health research. *Arch. environm. Hlth,* 1967, *14,* 488-503.
21. Ehrlich, R.: Effect of air pollutants on respiratory infection. *Arch. environm. Hlth,* 1963, *6,* 638-642.
22. Balchum, O. J. and Van Dyke, J.: Antibodies to lung in pulmonary disease in man. *Arch. environm. Hlth,* 1964, *8,* 139-142.
23. Anderson, D. and Ferris, B. G.: Air pollution levels and chronic respiratory disease. *Arch. Environm. Hlth,* 1965, *10,* 307-311.
24. Hammond, E. C.: Annual meeting of Air Pollution Control Association, Cleveland, 1967 (abstract).
25. Auerbach, O., Stout, A. P., Hammond, E. C., and Garfinkel, L.: Smoking habits and age in relation to pulmonary changes. *New Engl. J. Med.,* 1963, *269,* 1045-1054.
26. Dohan, F. C., Events, G. S., and Smith, R.: Variations in air pollution and the incidence of respiratory disease. *J. Air Pollut. Control Ass.,* 1962, *12,* 418-422.
27. Stern, A. C.: *Air Pollution.* New York and London, Academic Press, 1962, pp. 3-193.
28. Talamo, R. C., Blennerhossett, J. B., and Austen, K. F.: Familial emphysema with alpha 1-antitrypsin deficiency. 10th Aspen Conference on Research in Emphysema, June 7-10, 1967.
29. Haagen-Smit, A. J., Brunelle, M. F., and Hara, J.: Nitrogen oxide content of smoke from different types of tobacco. *Arch. industr. Hlth,* 1959, *20,* 399-400.
30. Murphy, S. D., Leng, J. K., and Davis, H. V.: Effects on animals of exposure to auto exhaust. *Arch. Environm. Hlth,* 1963, *7,* 66-76.
31. Freeman, G., Crane, S. C., and Furiosi, N. J.: Unpublished data.
32. Freeman, G. and Haydon, G. B.: Emphysema after low level exposure to NO_2. *Arch. Environm. Hlth,* 1964, *8,* 125-128.
33. Haydon, G. B., Freeman, G., and Furiosi, N. J.: Covert pathogenesis of NO_2 induced emphysema in the rat. *Arch. Environm. Hlth,* 1965, *11,* 776-783.
34. Freeman, G., Furiosi, N. J., and Haydon, G. B.: Effects of continuous exposure of 0.8 ppm NO_2 on respiration of rats. *Arch. Environm. Hlth,* 1966, *13,* 454-456.
35. Freeman, G., Stephens, R. J., Crane, S. C., and Furiosi, N. J.: Lesion of the lung in rats breathing 2 ppm of NO_2. Submitted for publication.
36. Haydon, G. B., Davidson, J. T., Lillington, G. A., and Wasserman, K.: Nitrogen dioxide induced emphysema in rabbits. *Amer. Rev. resp. Dis.,* 1967, *95,* 797-805.
37. Riddick, J. H., Jr., Campbell, K. I., and Coffin, D. L.: Histopathologic changes secondary to nitrogen dioxide exposure in dog. Personal communication.
38. Amdur, M. O. and Mead, Jere: Mechanisms of respiration in unanesthetized guinea pigs. *Amer. J. Physiol.,* 1958, *192,* 364-368.
39. Nidea, A. H. and Yamada, E.: Some observations on the fine structure and function of the non-ciliated bronchiolar cells. Sixth Inter. Congress for Electron Microscopy (1966), Vol. II Biology, pp. 599-600.
40. Leeson, T. S. and Leeson, C. R.: Osmiophilic lamellated bodies and associated material in lung alveolar spaces. *J. Cell Biol.,* 1966, *28,* 577-580.
41. Kilburn, K. H.: Mucociliary clearance studies. 10th Aspen Conference on Research in Emphysema, Aspen, Colorado, June 7-10, 1967.
42. Battigelli, M. C., Hengstenberg, F. R., Monnella, R. J., and Thomas, A. P.: Mucociliary activity. *Arch. Environm. Hlth,* 1966, *12,* 460-466.
43. Freeman, G., Crane, S. C., Stephens, R. J., and Furiosi, N. J.: Pathogenesis of NO_2-induced emphysema in the rat. Submitted for publication.
44. Freeman, G., Crane, S. C., Furiosi, N. J., and Haydon, G. B.: Covert induction of emphysema in rats by NO_2. *Clin. Res.,* 1966, *14,* 150 (abstract).