MORPHOLOGICAL CHANGES IN THE LUNGS OF RATS LIVING UNDER COMPRESSED AIR CONDITIONS*

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Plates 2 to 5

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Previous papers (1, 2) have described the apparatus and technique used by the authors in prolonged experiments upon the effects of high oxygen tensions on rats. The structural changes produced in the lungs of animals living in an environment having a high oxygen tension, particularly those accompanying long exposure, have never been thoroughly observed, nor has the effect of age received any appreciable comment. Our observations have shown that rats under 1 month of age do not give clinical evidence of suffering the acute pulmonary effects of 83.6 per cent oxygen; that these effects appear with advancing age; that older animals surviving acute oxygen poisoning are immune on second exposure. In this paper we describe structural differences in the lungs of young and old rats; differences in the effects of 80 per cent oxygen on animals of various ages; and finally the structural changes found in the lungs of rats which have become resistant to the acute effects of toxic concentrations of oxygen.

Autopsies and Pathological Technique

244 albino rats were observed during this study. 137 autopsies were performed, 21 of which were upon normal animals to serve as controls. All dead and dying animals were removed for autopsy as soon as possible. In addition to these, live animals were removed every day during the first 8 days of exposure, and at weekly or biweekly intervals after that. Live rats were killed with an intraperitoneal injection of 0.5 to 1.0 cc. of 1 per cent potassium cyanide. The lungs were removed and fixed in Zenker's fluid for 24 hours. Hematoxylin and eosin stain was used

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routinely. In addition to this, Mallory's aniline blue stain for connective tissue, Mallory's phosphotungstic acid hematoxylin stain, and Foot's reticulum stain were made on the lungs of representative animals after varying periods of exposure.

Histology of the Normal Rat Lung

Grossly the lungs of old and young rats are a diffuse, uniform pink color, and no distinction exists other than the difference in size. Microscopically a striking difference in the cellularity of the alveolar walls is found.

From birth to roughly 4 months of age the alveolar walls are much more cellular and thicker than in adult animals. The individual alveolar cells in young animals are larger and more distinct than in adults, and the nuclei are more vesicular. This cannot be explained on the basis of incomplete expansion of the lungs, because the alveolar walls in areas having equivalent distention in young and old still show this prominent difference in cellularity (Figs. 1 to 4).

During the 5th and 6th months of life the size and number of the alveolar lining cells gradually diminish. After 6 months of age the microscopic appearance is constant. The alveolar walls are thin and contain relatively few lining cells, the nuclei of which stain more intensely than in young rats. The difference between the lungs of rats under 100 days of age and those in the 6th or 7th month of life is so distinct that one can invariably distinguish young from old by study of lung sections (Figs. 1 to 4).

Pathology of Acute Oxygen Poisoning at Different Ages

A. Adult Rats (over 5 Months Old).—The pathological changes in acute oxygen poisoning are essentially those of an intense pulmonary edema, as first described by Lorrain Smith (3, 4) and confirmed by numerous authors, with right-sided dilatation of the heart as described by Karsner (5). The other organs of the body are normal except for congestion of the viscera and a slight amount of tubular damage in the kidneys. This injury was manifested by areas of degeneration and the presence of occasional mitotic figures in the tubular epithelium.

Gross Pathology.—The first evidence of the toxicity of 80 per cent oxygen tensions for rats appears during the 3rd day of exposure and becomes most intense on the 4th day. A description of the clinical picture has been given in a previous paper (2). The normal, uniform pink coloration of the lungs is replaced by a diffuse mottling with dark beefy red, and purple patches due to the intense congestion. Occasionally small white spots of peripheral emphysema are found. The lungs are greatly distended with edema and occupy almost the entire thoracic cavity. When the latter is opened, they fail to collapse. There is a pleural

effusion of several cubic centimeters of clear, pale yellow, serous fluid which clots on standing. The quantity averages about 5 cc. on the 3rd day, but on the 4th day it is increased to approximately 10 cc. This effusion in itself is probably one of the greatest causative factors for the respiratory embarrassment observed.

Microscopic Findings.—Most of the alveoli are filled with an eosinophilic, finely granular, serous exudate, containing a number of desquamated alveolar cells, polymorphonuclear neutrophiles, red blood cells, and fibrin (Figs. 6 and 7). Occasionally there is a slight to moderate amount of patchy hemorrhage into the alveoli. Many desquamated alveolar cells are present.

Considerable emphysema is found in the alveolar ducts and alveoli toward the periphery of the lungs, and is most marked in rats showing the greatest amount of pulmonary edema.

The capillaries of the alveolar walls are engorged with red blood cells. The arteries and veins are surrounded by a large, loose meshed, clear zone of perivascular edema into which there are varying degrees of cellular infiltration. This gives a very striking picture under low power magnification (Figs. 5 and 7). The cellular infiltration consists mostly of large mononuclear phagocytes. Polymorphonuclear eosinophiles and neutrophiles are present in lesser numbers, the former being more numerous. Cells of the lymphocytic series are occasionally seen. Numerous perivascular lymphatics are found throughout the lungs. They are dilated and contain precipitated serum. These are increased above normal in size and numbers (Fig. 7). The alveolar capillaries and walls contain a relative increase in the number of polymorphonuclear leucocytes (Fig. 6). The trachea, bronchi, and bronchioles are uninjured.

B. Young Rats (under 100 Days of Age). Gross Pathology.—In rats from 9 to 40 days of age there are no abnormal findings. Rats 100 days old at entry show definite signs of congestion on the 4th day, although the edema is very slight as compared with older animals. In some cases there is a slight pleural effusion.

Microscopic Findings.—Rats under 40 days of age show no evidence of oxygen poisoning other than an early perivascular edema and slight cellular infiltration with some dilatation of lymphatics (Fig. 5). Animals approximately 100 days of age show marked perivascular edema and cellular infiltration, dilated lymphatics, some desquamation of alveolar cells, but only small patchy areas of alveolar exudate as compared with older animals. The hyperplasia and hypertrophy of the alveolar lining cells is marked.

Mortality Statistics for Different Ages and Sexes

An analysis of the mortality data has already been published (2). It suffices to say here that all deaths from acute oxygen poisoning

occurred in rats on the 4th day of exposure. There were no deaths in animals under 120 days of age, while in the older animals the mortality was directly proportional to the age. There was no significant difference in the mortality rate for males and females.

Changes in the Lungs of Surviving Animals during Continued Exposure

Findings from 4th through 7th Days.—During the 5th and 6th days of exposure there is a rapid removal of the serous exudate from the alveoli. After the 5th day no animal showed any pleural effusion. By the end of the 1st week of exposure all evidence of the acute edema which began on the 3rd day had disappeared, and the lungs resumed a more normal gross appearance. The dark purple color previously seen, changed gradually to a dark red, and then to mottled pink about the 7th day. These color changes paralleled the disappearance of the generalized congestion such as was seen microscopically. The lungs did not reacquire a diffuse, pink coloration but became finely mottled with small, pink areas on a white or greyish white background, some of this latter being due to small blebs of emphysema. The mottling persisted throughout the experiment and even after the animals had been returned to normal air for as long as 214 days. Numerous areas of decreased blood supply were found microscopically, in which it was difficult to distinguish the capillaries because of the few red blood cells present. This gross mottling of the lungs is explicable on the basis of the irregular distribution of blood through the capillary bed.

Microscopically the lungs were in fair condition. The alveolar exudate had disappeared, the perivascular edema and cellular infiltration were decreased and in some instances gone. The alveolar cells, on the other hand, were enlarged and increased in number causing a marked thickening of the alveolar walls. A slight amount of desquamation persisted. Areas of emphysema were present, but more striking were the small patches of atelectasis, involving only a few alveoli irregularly throughout the lungs.

Animals Developing Secondary Respiratory Infection

At the end of the 3rd week of exposure a few of the older animals began to die at sporadic intervals. At autopsy, all of these showed varying degrees of purulent bronchitis, bronchiectasis, and bronchopneumonia. Such rats had a poor appetite, lost weight rapidly, and became dyspneic, apathetic, inactive, and weak. Grossly the lungs showed congestion, consolidation, and frequently lobar atelectasis, while microscopically the characteristic findings of bronchopneumonia were present.

Animals Remaining Free of Secondary Respiratory Infection, 2nd Week to Termination of Experiment

A. Gross Appearance.—Beginning with the 2nd week of exposure the gross appearance of the lungs became constant, and the pink and white mottling persisted. There was no consolidation, pleural effusion, or edema.

B. Microscopic Changes.—The hyperplasia and hypertrophy of alveolar cells continued and numerous mitotic figures were found throughout the experimental period. The alveolar walls were thickened by the increased alveolar cells, and there was a diminution in the number of blood-containing capillaries in the walls (Figs. 8 to 11). This increase in cellularity occurred in both young and old rats, and after the first 2 weeks of exposure no estimation of age could be made from the histological picture.

After 1 month of exposure the small arterioles of the lungs became prominent and apparently more numerous (Fig. 15). Their walls were thickened and the lumina narrowed. Later, hyalinization of the walls occurred and occasionally thrombosis (Figs. 13 and 15). These changes in the walls made the small vessels stand out prominently, which probably accounted for the apparent increase in number. Frequently vessels showed thrombosis which appeared to be due to a proliferation of the endothelial cells without any evidence of hyalinization. In vessels showing the most extensive involvement, the appearance was identical to that of the small renal arterioles undergoing hyalinization in chronic vascular nephritis. It is the type of lesion which is usually associated with hypertension. Measurements of the hearts of a representative group of rats failed to show any conclusive evidence of hypertrophy in either ventricle, although the right ventricle tended to be a fraction of a millimeter thicker in exposed animals in some instances. If true hypertension in the pulmonary circuit were responsible for these changes, one would have expected definite right-sided hypertrophy. It is fair to say, however, that this point was not adequately settled by studying these small animals.

A number of large pulmonary arteries began to show marked pathological changes about the 45th day of exposure. The walls of these vessels were thickened in an irregular manner. The media first assumed a loose meshed appearance with clear areas interspersed between the tissue elements. In places the media was hyalinized, and definite hyaline cartilage formation was frequently found (Fig. 14). In two instances calcification had occurred with these changes.

All rats autopsied during the last month of exposure showed the changes described in the above paragraphs.

Removal to Normal Air after 72 Days Exposure

On the 72nd day the first exposure was discontinued. The chamber was decompressed over a 2 hour period according to the stage method formulated by Boycott, Damant, and Haldane (6). The animals remained in the experimental chamber for the next 40 days

under normal atmospheric conditions. During decompression 28 per cent of the rats died, all with symptoms of acute asphyxia. These showed considerable fibrosis of the lungs, complicated frequently with bronchopneumonia.

The surviving animals appeared to be in excellent health during the interval in normal air and made rapid gains in weight (2). Autopsies were performed at frequent intervals. The cellularity of the alveolar walls and the vascular lesions continued to be present. Slight amounts of fibrosis and emphysema were seen, together with some increase in interstitial tissue. The lungs, on the whole, were in the same condition as they had been since the 2nd week of the first exposure.

Reexposure to 83.6 Per Cent Oxygen Tension after 40 Days in Normal Air

After 40 days in normal air, the remaining 35 rats were reexposed to the same conditions that existed during the first exposure. This second exposure lasted 10 days. During this time no changes occurred.

The important finding during this exposure was that none of the rats developed acute oxygen poisoning. They remained in excellent health, irrespective of the fact that all were now adults. On the 4th day, when the acute pulmonary edema was most intense during the first exposure, no alveolar exudate or congestion was found. Control rats developed classical oxygen poisoning beginning with the 3rd day of exposure.

On the 10th day of the second exposure the chamber was again decompressed and the animals returned to normal atmospheric conditions. Whereas 28 per cent of the survivors died during decompression after the first exposure, no deaths occurred this time, and there were no signs of temporary illness. The results of the second exposure may be summarized by saying that complete adaptation to the toxic action of an environment having an 83.6 per cent oxygen tension was produced by the first exposure, so that on reexposure no changes occurred either clinically or pathologically.

At the present writing, 214 days after conclusion of the experiment, all of the surviving animals are apparently in good health and are being retained for future study. 33 days after the end of the second

exposure three rats were autopsied. The lungs showed the same picture as during exposure, although the hypertrophy of alveolar cells was somewhat less marked than formerly.

DISCUSSION

An explanation for the adaptation of rats to toxic oxygen tensions probably involves both physiological and morphological changes. The alveolar walls of young rats are much more cellular and thicker than those of normal old rats. Young rats do not develop the edema of acute oxygen poisoning, whereas old rats show the classical pulmonary edema. After prolonged exposure to a toxic oxygen tension the alveolar cells of both young and old rats become hyperplastic and hypertrophied, causing a great increase in the thickness of the alveolar walls, and the two can no longer be distinguished histologically. These changes persist even after removal to normal oxygen tensions. On reexposure to toxic tensions no signs of oxygen poisoning are found in either the young or old. In other words, the lungs of old rats were "made young" during the first exposure, so that on reexposure old rats behaved as young ones did on first exposure.

Although the morphological difference between the lungs of rats under 3 months of age and those over 6 months old can be correlated with the increased resistance of young rats and the adaptation of old rats to 83.6 per cent oxygen tensions, this cannot fully explain the differences between the reaction of young, old, and adapted rats. No obvious difference between the morphology of the lungs of 1 month old rats and 3 months old rats can be detected; nevertheless, a definite increase in susceptibility occurs gradually with advancing age, as manifested by the increasing severity of the clinical symptoms. Therefore, this change in resistance with advancing age can only be explained by some physiological alteration in the body. The fact that marked histological changes are produced in the lungs of rats by prolonged exposure indicates that the defensive mechanism responsible for this increased resistance is at least in part in the lungs. We believe that an alteration in the physiology of the alveolar cells occurs, and that this is the primary factor in the adaptation which results, and in the difference in the susceptibility of various age groups.

Our findings permit three possible explanations for the higher re-

sistance of young or of adapted older animals. First, the increase in the thickness of the alveolar walls may mechanically decrease the rate of diffusion of the alveolar gases, so that at increased oxygen tensions the total amount of oxygen reaching the capillaries is only sufficient to approximate the oxygen tension of the blood under normal atmospheric conditions.

Second, the fact that definite vascular changes have been demonstrated after prolonged exposure points toward the possibility that generalized alterations in the physiological structure of the capillary bed in the lungs may be responsible for this adaptation.

Third, the marked increase in number and size, as well as the changed appearance of the alveolar cells, forces one to consider the possibility of some change in their physiological properties. Although the available evidence has been accepted by the majority of physiologists to mean that the gaseous pulmonary exchange is a process of simple diffusion, the results of these experiments compel one to consider the possibility of the alveolar cells engaging in a process of physiological activity under certain environmental conditions. The strongest evidence for this theory is the fact that adapted animals remained in perfect health and showed no clinical symptoms of dyspnea or anoxemia on sudden removal to normal oxygen tensions. If the mechanical theory of impaired diffusion were the sole factor, one would expect to find definite signs of respiratory distress at least temporarily after removal to lowered oxygen tensions. On the other hand, this does not eliminate the rôle that might be played by an increase in the resistance of the capillary endothelium.

CONCLUSIONS

1. The alveolar cells in the lungs of young rats are greater in number and size than in old rats, a gradual transition to the state in the latter occurring from about the 4th to the 6th months of life.

2. On prolonged exposure to an environment having an 83.6 per cent oxygen tension, the cellularity of the alveoli is increased in both young and old animals, so that after 2 to 3 weeks it is impossible to distinguish them by morphological differences. Numerous mitotic figures are present in the alveolar cells. This hyperplasia and hypertrophy is a change which persists for months after the rats return to normal air.

3. Young rats do not develop the symptoms of acute oxygen poisoning, although some perivascular edema and dilatation of the lymphatics results on the 4th day of exposure, when intense acute pulmonary edema is present in old rats.

4. The mortality of acute oxygen poisoning is directly proportional to the age of the animals, although the majority of rats under 6 months of age survive this state and continue in apparent good health for as long as 72 days. All deaths during the acute stage in an 83.6 per cent oxygen tension occur on the 4th day of exposure.

5. After 1 month of exposure lesions are to be seen in the small arterioles of the lungs, consisting of a thickening and hyalinization of the walls with ultimate thrombosis of many. These vascular changes are identical with those seen in the arterioles of the kidney in chronic vascular nephritis.

6. Around the 45th day of exposure the large pulmonary arteries contain lesions in the media. The walls become loose meshed, thickened, and hyalinized, and hyaline cartilage formation is associated with these changes.

7. Reexposure of animals following an interval of 40 days in normal air subsequent to the first exposure of 72 days, does not produce any clinical or pathological changes. An adaptation to this toxic oxygen tension is produced during the first exposure, so that oxygen poisoning does not occur on second exposure. The increased cellularity of the alveolar walls persists.

8. The similarity in the morphological structure of the alveoli in young rats and in previously exposed old rats has a definite relationship to the adaptation that occurs to an oxygen tension of 83.6 per cent, preventing the development of acute oxygen poisoning on reexposure.

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EXPLANATION OF PLATES

The stain used for all the microscopic preparations was hematoxylin and eosin.

PLATE 2

FIG. 1. This field shows the architecture of the lung of an adolescent rat. The cellularity and thickness of the alveolar walls are well represented. Normal, young rat, 48 days old, male. Camera lucida drawing. ×80.

FIG. 2. The histology of the normal adult rat lung is illustrated in this drawing. The characteristic thinness and diminished cellularity of the alveolar walls are easily seen. Several small blood vessels are present showing the normal relation of the perivascular tissues. A comparison of this drawing with Fig. 1 shows the relative difference in the thickness and cellularity of the alveolar walls in young and adult rats normally. Normal rat, 277 to 288 days old, male. Camera lucida drawing. ×80.

FIG. 3. Magnification of the field outlined in Fig. 1. The cellularity and thickness of the alveolar walls in normal young rats is seen more clearly. The relatively large size of the alveolar cells is also well represented. Camera lucida drawing. ×340.

FIG. 4. Magnification of the field outlined in Fig. 2. A comparison of this drawing with Fig. 3 shows the differences in the detailed histology of the lungs of normal old and young rats. The alveolar cells are more numerous and larger in young rats than in old, causing the alveolar walls to be thicker in the former. Camera lucida drawing. ×340.

PLATE 3

FIG. 5. Zones of perivascular edema are prominent, and these are the only changes found in young rats on the day when acute oxygen poisoning is most intense in old animals. The alveoli contain no exudate. Female rat, 37 days old. Removed from 83.6 per cent oxygen tension in excellent health after 4 days of continuous exposure, and killed for autopsy. Camera lucida drawing. ×155.

FIG. 6. This area shows an extensive alveolar exudate consisting of fibrin, desquamated alveolar cells, and polymorphonuclear leucocytes. The capillaries of the alveolar walls are studded with polymorphonuclear leucocytes. Female rat, 437 to 444 days old. Removed from 83.6 per cent oxygen tension when acutely ill after 4 days of continuous exposure, but died during the decompression to normal oxygen tension. Camera lucida drawing. ×735.

FIG. 7. This shows well the diffuse serous exudate in the alveoli, with some fibrin and cellular debris. Also, one sees the striking zones of perivascular edema and the dilated lymphatics. Male rat, 122 to 130 days old. Found dead after 4 days of continuous exposure to 83.6 per cent oxygen tension. Camera lucida drawing. ×145.

PLATE 4

FIG. 8. This slide illustrates the increased cellularity and thickness of the alveolar walls which occur after prolonged exposure. Also, one sees small, patchy areas of atelectasis interspersed between expanded alveoli. Note that the alveoli contain no exudate, and there is no perivascular edema. Female rat, 87 days old. Removed from 83.6 per cent oxygen tension in good health after 49 days of continuous exposure, and killed for autopsy. Camera lucida drawing. ×80.

FIG. 9. A comparison of this slide with Fig. 8 shows the similarity of the histological structure of the lungs of old and young rats after prolonged exposure. They can no longer be differentiated by morphological differences. Contrasting this drawing with Fig. 2, the increase in the cellularity of the alveolar walls after exposure is readily seen. Patchy areas of atelectasis are present. The alveoli contain no exudate. Male rat, 170 to 180 days old. Removed from 83 per cent oxygen tension in good health after 44 days of continuous exposure, and killed for autopsy. Camera lucida drawing. ×80.

FIG. 10. Magnification of the field outlined in Fig. 8. This drawing is to be compared with Fig. 3 in order to emphasize the increased cellularity of the lungs of young rats after prolonged exposure. The increase in the size of the alveolar cells and the nuclei is also clearly seen. Camera lucida drawing. ×340.

FIG. 11. Magnification of the field outlined in Fig. 9. A comparison of this drawing with Fig. 4 shows the marked increase in cellularity of the lungs of adult rats after prolonged exposure. A comparison with Fig. 10 shows the morphological similarity of the lungs of old and young rats after exposure, in contrast to the difference in the structure of the lungs of normal old and young rats. Camera lucida drawing. ×340.

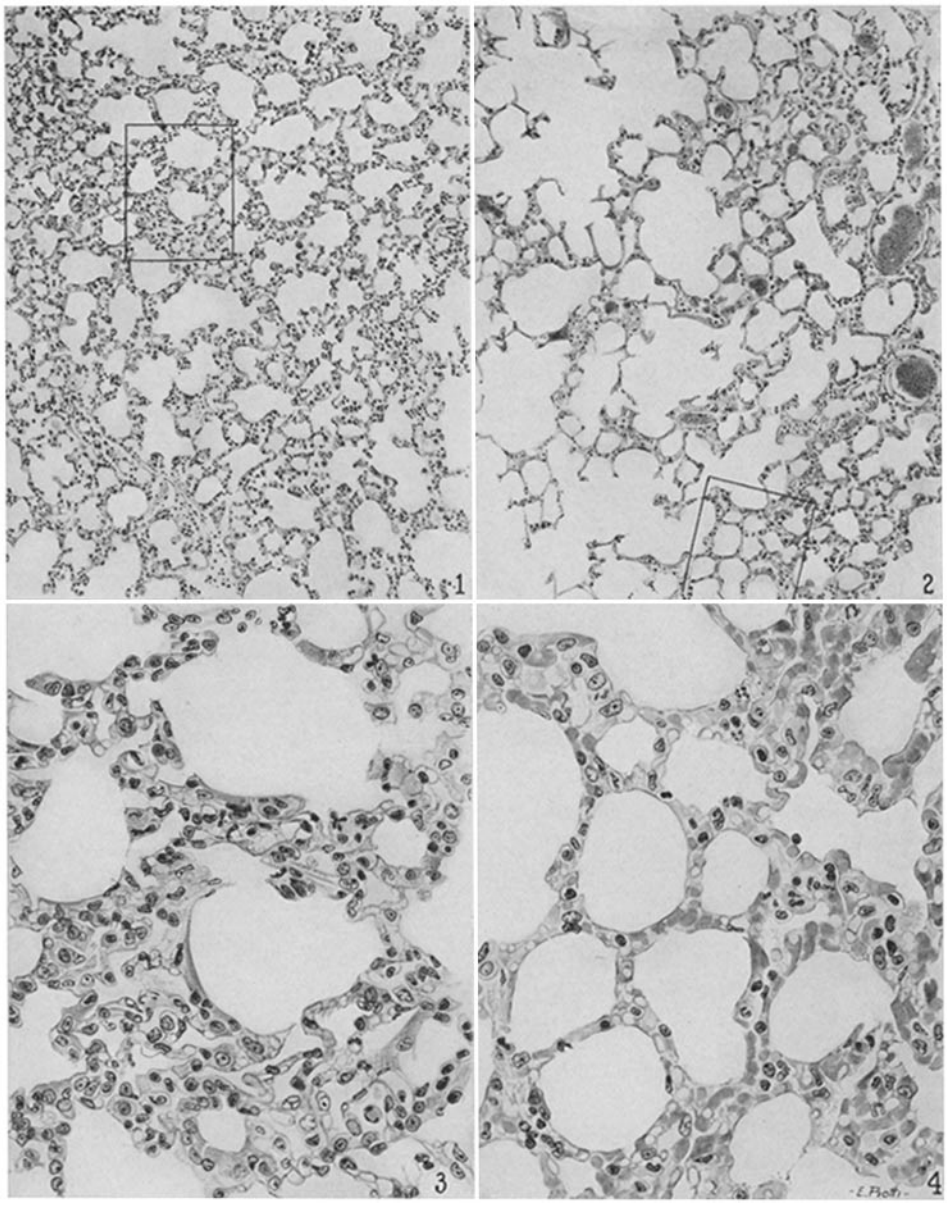
PLATE 5

FIG. 12. Mitotic figure in an enlarged alveolar cell from Fig. 9. Camera lucida drawing. ×1260.

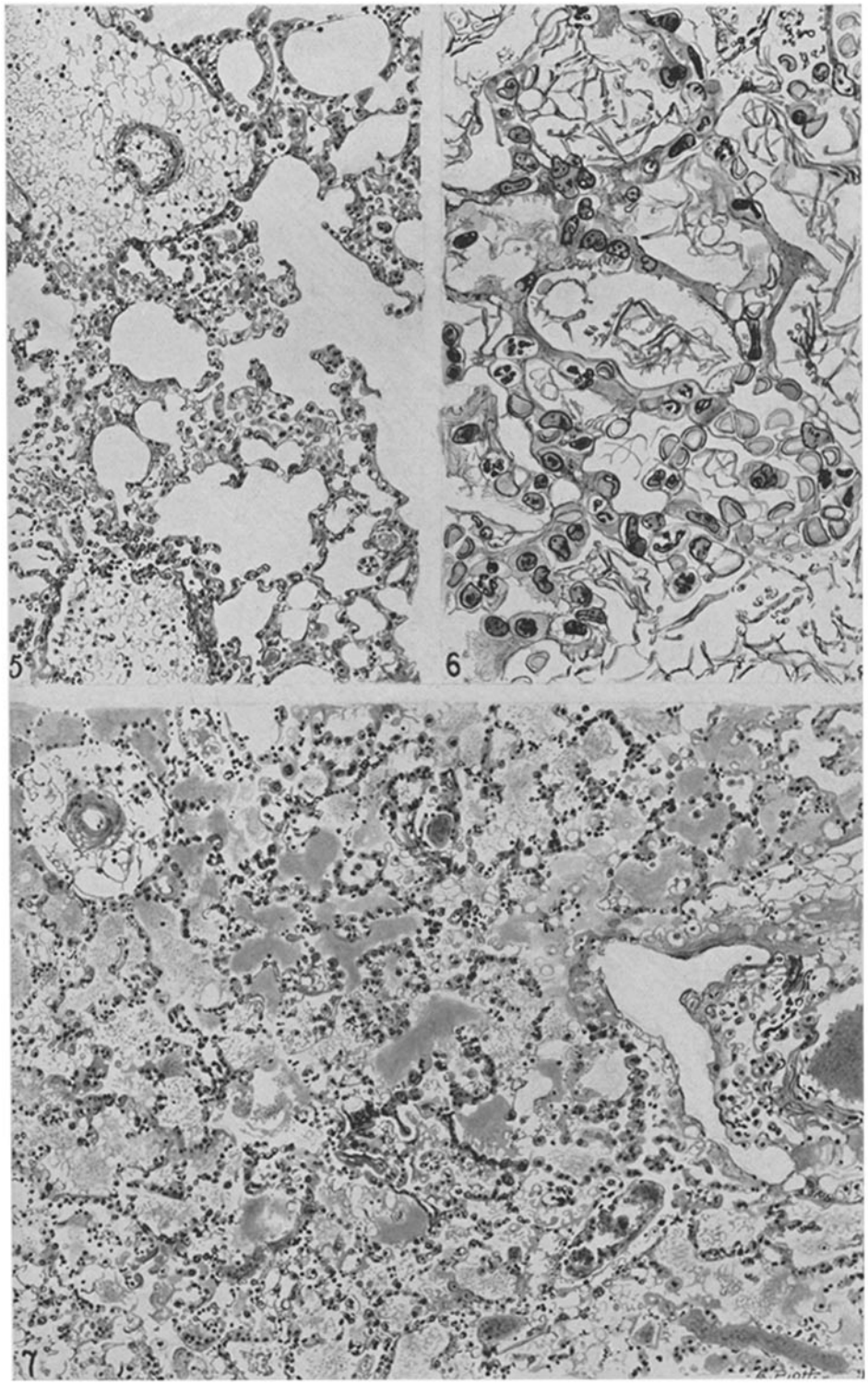
FIG. 13. Thickened, hyalinized wall of a small pulmonary arteriole, with endothelial proliferation. Male rat, 178 to 188 days old. Found dead in 83.6 per cent oxygen tension after 52 days continuous exposure. Camera lucida drawing. ×390.

FIG. 14. A large branch of the pulmonary artery is shown, illustrating the thickening of the media with hyaline cartilage formation. Female rat, 217 to 220 days old. Exposure was 30 days in 83.6 per cent oxygen tension, 53 days in normal air, than 44 days reexposure to 83.6 per cent oxygen tension. Camera lucida drawing. ×115.

FIG. 15. The same slide as Fig. 13. Five small blood vessels stand out prominently because of the hyalinization and thickening of the walls. Endothelial proliferation with thrombosis is seen in one. The alveolar epithelial cells show some desquamation due to postmortem changes. Camera lucida drawing. ×155.



(Smith *et al.*: Lung changes under compressed air)



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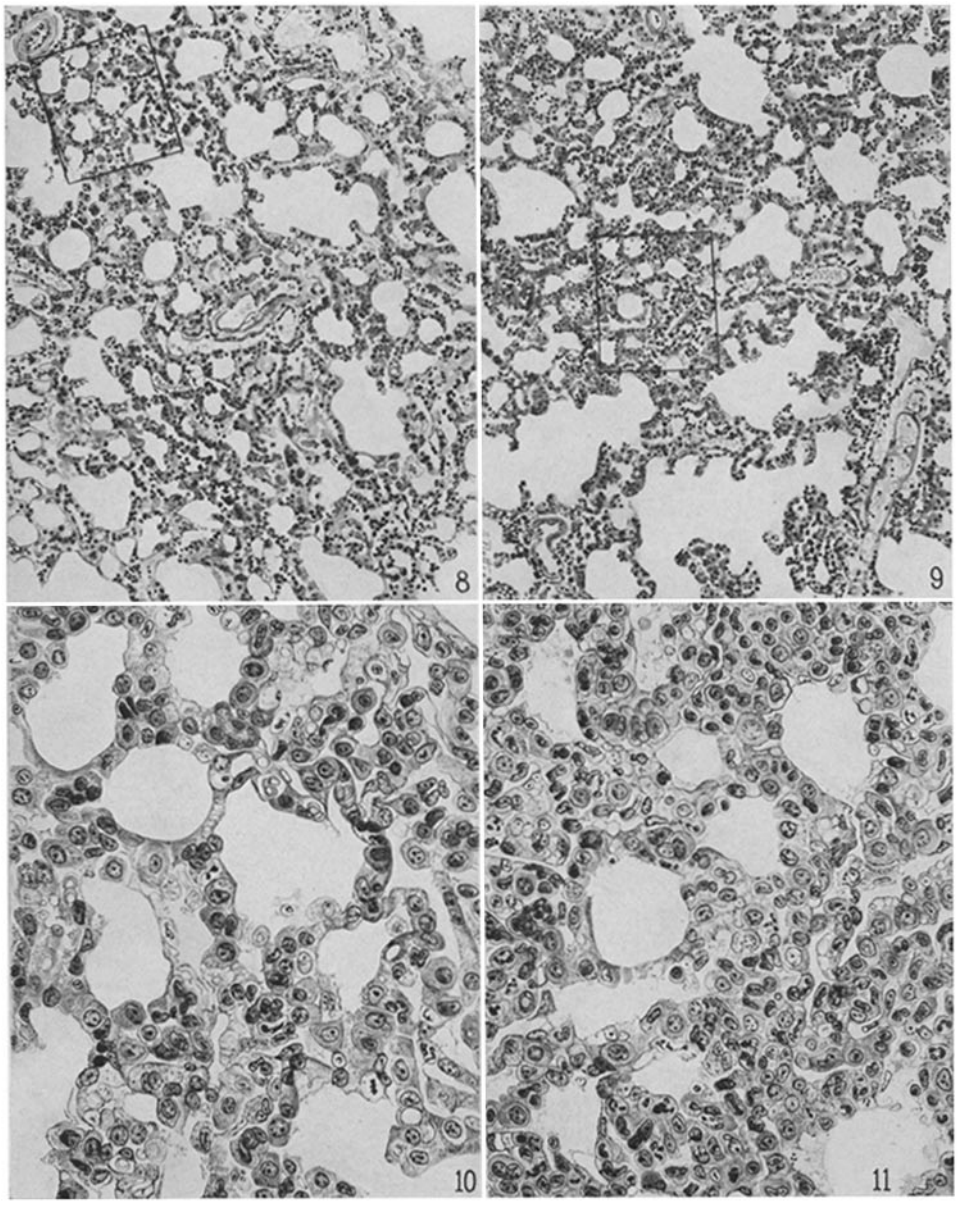

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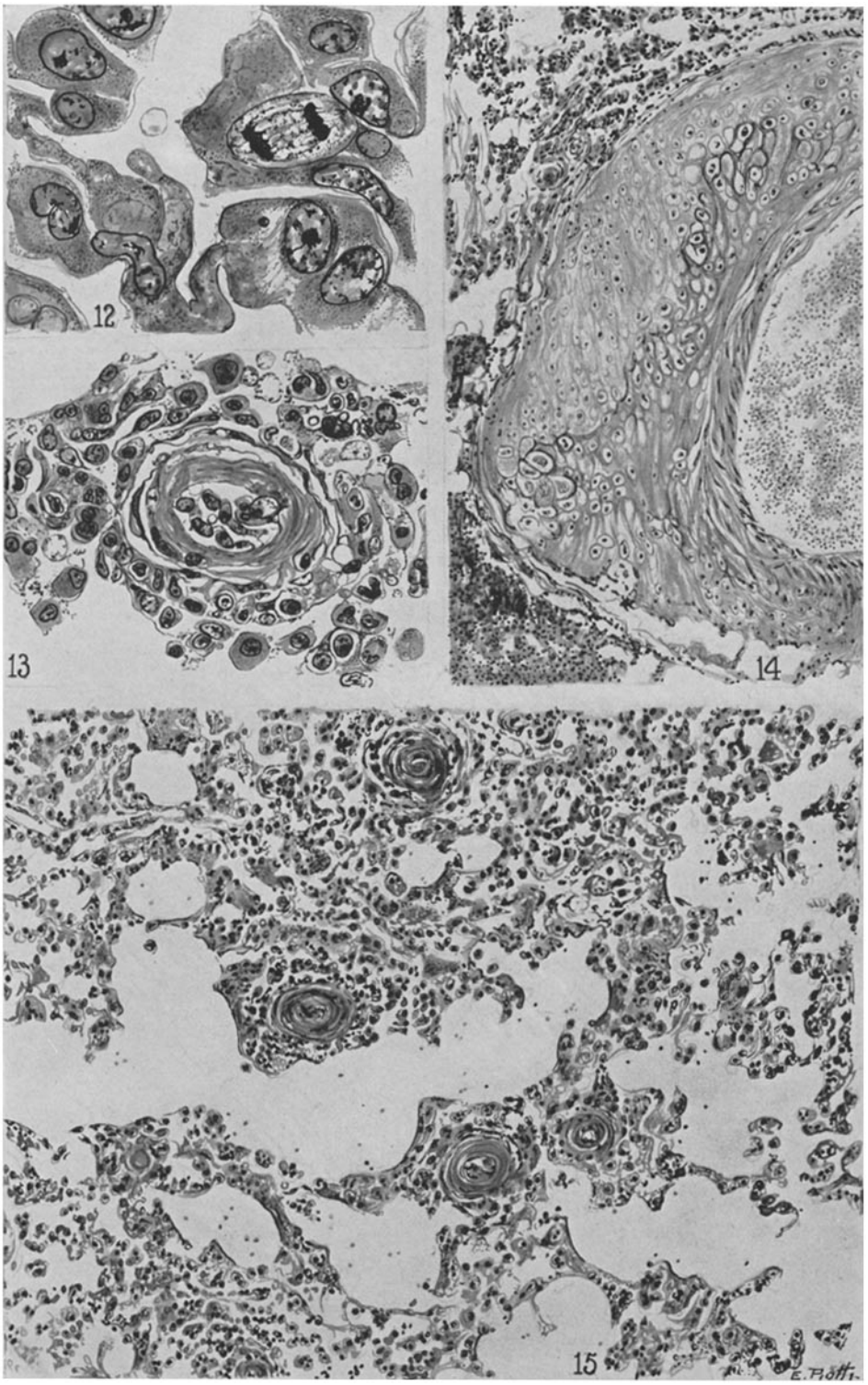

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