Membranous bronchioles and connective tissue network of normal and emphysematous lungs

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IN THE CONVENTIONAL DESCRIPTION of the morphology of the lung, the latter is divided into successively smaller and smaller cone-shaped units surrounded by a septum and centered around a bronchovascular bundle. In this design, the smallest pulmonary unit completely surrounded by a septum is the secondary lobule (27). It contains three to five acini, which are the basic units participating in gas exchange. In contrast to the lobules, individual acini cannot be recognized by gross examination. In a previous study (26), we prepared serial histological sections of blocks of lung tissue from normal, senile, and emphysematous lungs to reconstruct the peripheral airway tree in three dimensions. Unexpectedly, these reconstructions showed that bronchi and bronchioles coursed within a septum and entered the supplied pulmonary unit at its side.

To understand the relationship between classic interlobular septa and the septa containing an airway, which we observed, a reconstruction of the total septal system in three dimensions was attempted and projected onto the corresponding airway tree. First, normal lungs were examined. In a second step, reconstructions were performed from lungs with centrilobular emphysema, in an attempt to relate the loss of the orderly appearance of the parenchyma in the latter condition to the construction principle of the normal lung.

VERBEKEN, E. K., M. CAUBERGHS, and K. P. VAN DE WOESTIJNE. Membranous bronchioles and connective tissue network of normal and emphysematous lungs. J. Appl. Physiol. 81(6): 2468–2480, 1996.—Three-dimensional reconstructions of the septal system of normal human lungs revealed that airways course within the interlobular septa, i.e., between the two blades formed by the peripheral boundaries of adjacent lobuli of whatever order, and enter the supplied pulmonary unit at its side. This is not in keeping with the classic view of a peripheral airway in the center of a lung unit and submitted to radial traction by attached alveolar septa. The basic design of the lung fibrous scaffold appears to be in conformity with the laws of fractal geometry. Similar reconstructions in centrilobular emphysema disclosed tortuosities of both intra-acinar and interlobular septa, with consequent distortions of the corresponding intraseptal bronchioles and collapse of lung units of different sizes. It is suggested that in centrilobular emphysema competition for space, besides intrinsic airways narrowing because of inflammation and loss of elastic recoil, is a cause of flow limitation.

AIRWAY OBSTRUCTION; EMPHYSEMA; FIBROUS SEPTA; FRACTALS; COMPETITION FOR SPACE

MATERIALS AND METHODS

The study was performed on excised human lungs obtained at autopsy: two normal lungs and four lungs with centrilobular emphysema were investigated. The lungs were perfused intrabronchially for 72 h with a 10% Formalin solution at a pressure of 2.5–3.0 kPa. This type of slow fixation was found to fix the lung at a volume close to total lung capacity (26).

Because lobular septa are barely visible by light microscopy in normal lungs, we developed a technique to better visualize these septa. A barium gelatine suspension was prepared by suspending 1.5 g of gelatine powder in 3 ml of water and heating it to 40°C. To this gel, 37 ml of barium sulfate, also at 40°C (Micropaque, Guerbet, Brussels, Belgium), were added. With a 22-gauge needle, 5–10 ml of this suspension were injected in the subpleural parenchyma at five or more locations chosen randomly on both lateral and medial sides of each normal lung. The gelatine stiffens when coming in contact with the Formalin-filled tissue at room temperature, thus allowing further processing for histology. The barium suspension mostly demarcated a pulmonary unit of variable size at the pleural surface, although spilling over of the contrast medium in an adjacent area was frequently observed. Three injected peripheral tissue blocks contiguous to the pleura were resected from the two normal lungs. In the emphysematous lungs, the injection procedure was not used. Five tissue blocks contiguous to the pleura were selected at random from the four lungs. Zones of marked destruction (bullae) were avoided, however.

All tissue blocks were serially sectioned. Sections were cut parallel to the pleural surface and stained with Masson's trichrome; 1,500–2,000 serial sections, 4 µm thick, corresponding to 7.7–10.3 mm in depth of peripheral lung tissue [taking into account the shrinkage during processing: 0.78 for each dimension (24)] were prepared. To reach the level of the cartilaginous bronchi, one block from an emphysematous lung was cut into 20-µm-thick sections; 1,700 serial sections were examined, corresponding to 43.5 mm of peripheral lung tissue. The serial sections represent, after correction for shrinkage, an average tissue block volume of 2.40 cm³ for normal lungs and 6.55 cm³ for emphysematous lungs. In the latter, larger tissue blocks were taken, with the purpose of examining more lobuli. From these serial sections, both the peripheral airway tree and the parenchymal septa were reconstructed. An order number was attributed to each airway by using Horsfield's counting method (12), with order 1 corresponding to the terminal bronchioles (TB). Two TB meet to form an order 2 membranous bronchiole (MB₂), and so on. If two branches of differing order meet, the numbering is continued from the highest order of the two meeting branches. Bifurcations were found to be dichotomous (26). The most distal cartilage in the airway tree was located at the bifurcations. Accordingly, in keeping with Von Hayek (27), we called MB the daughter branches originating at the level of a cartilaginous bifurcation, with the mother branch being a bronchus.
An additional tissue block was resected from a normal lung at the level of the lobar bronchi and sectioned serially: 1,500 sections were prepared.

RESULTS

Reconstruction of the septa from a normal lung. In Fig. 1, a subpleural pentagonal area is shown, corresponding to a secondary lobule (27). After injection, the barium sulfate filled only one-half of the pentagon because of the presence of a thin septum between the injected and the noninjected areas. A histological section of the corresponding area, ~1 mm under the pleura, is presented in Fig. 2. It shows that the noninjected one-half of the pentagon is divided into two areas of equal size, A and B, by a septum perpendicular to the one separating the injected from the noninjected area. Similarly, the injected area is divided by a septum into two parts, C and D. These septa are recognized because of their continuity rather than because of their thickness. At deeper levels, these thin septa are less regular, apparently because of alveolar outpouchings, and thus difficult to follow. Also larger parts of the septum delineating the pentagon at the pleural surface are lost in the depth of the lung. In fact, we were unable to reconstruct the septa starting from the pleura, even when guided by the barium sulfate. We were more successful in reconstructing the septa starting from the opposite side, namely from the depth of the lung. It turned out that each airway bifurcation appeared to generate systematically a septum, the reconstruction of the septa thus necessitating a three-dimensional reconstruction of the airway tree. This reconstruction is presented schematically in Fig. 3, A and B. Figure 3A schematizes the last generations of bronchi, the MB, and the first generation of respiratory bronchioles (RB) as well as the relationship between the airway bifurcation...
sections and the origin of septa. Figure 3B shows the same airway tree in relation to the supplied parenchymal volumes. The drawing emphasizes that airways course within septa corresponding to the outer walls of two adjacent parenchymal units. Figures 4, 5, 6, and 7 group a selection of the serial sections between the last generations of bifurcating bronchi (section 1400) and the peripheral parenchyma (level 338). At level 1400, the outline of the pentagon is only partly seen. A bronchus is bifurcating into a branch that leaves the tissue block (B1?) and a bronchus, B1 (Fig. 3A). Cartilage is found at the bifurcation. At level 1300, B1 branches into an airway, the order of which is not defined (MB3?), and a MB of the third order (MB1). It is MB3 that supplies the pentagon. Remarkably, MB3 enters the corresponding lung unit not at its center but at its side. At level 1250 (not shown), a side branch originates, classified as a second-order MB (MB2E), which will supply area E (Fig. 3B). MB2E penetrates deeper into the parenchyma, separating area E from areas A and B by a septum parallel to the pleural surface and, hence, parallel to the serial sections. Such a septum, because it is not perfectly flat, will not be visualized in one single histological section.

After its bifurcation at level 1250, the second daughter branch of MB3 proceeds as MB2. It can be seen that the bifurcation of the airways traces a septum, which we therefore called a “branching” septum. This septum is projected on the pleural surface (as part of the pentagon, at the level of A and between A and D).

The septum “actively” traced by MB3? and MB3 (prolonged by MB2) continues into a septum (between B and C) that completes the separation between the noninjected and the injected areas, AB and CD, respectively. This is a second type of septum that is not positioned between two bifurcating airways but, on the contrary, between an airway and a vein and separates the volumes B and C. We called this an “extended” septum. At level 640, MB2 branches into two first-order MB (MB1), again tracing a branching septum between the two airways (part of the septum separating A from B and D from C) and prolonged by extended septa.

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**Fig. 3.** A: schematic reconstruction of membranous (MB) and respiratory bronchioles (RB) and corresponding septa of pentagon. Septa originating at airway bifurcations are shaded. No distinction is made here between branching and extended septa (see further). MB1, MB2, MB3, 1st, 2nd, and 3rd order of MB, respectively; B1, bronchus of 1st order; ?, unknown order; RB1, RB2, RB3, RB4, RB supply areas A and B, C and D, respectively. Bifurcation of bronchi B1-B1? generates a septum (not shaded) partially seen on Fig. 2, not related to pentagon. Bifurcation of MB3?-MB3 (continued by MB2) generates a septum projected on pleural surface as a septum demarcating a small part of pentagon and as a second septum between A and D. Separation MB3-MB3 is at origin of a septum partly separating areas B-C from A-D. B: volume units of pentagon and corresponding MB and RB. Lung units A, B, C and D are supplied by 1st-order RB: RB1, RB2, RB3, and RB4, respectively. Figure emphasizes intraseptal course of airways. x-Axis, no. of sections starting from pleura. Asterisks point to presence of cartilage.

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**Fig. 4.** Selected serial sections are projected onto a schematic drawing of peripheral airway tree. Level closest to pleural surface corresponds to level 338 of Fig. 7. See Figs. 5–7 for photographs and corresponding drawings of sections.
Fig. 5. Selected photographs and corresponding drawings of serial sections. At level 1400, a bronchus is branching into B1 and B1?. Contrast material is filling lumen of B1 and of B1?; there is also some material inside parenchyma. V, veins within septa, limiting (partly) pentagon (drawing: thin continuous lines). a, Artery. At level 1300, B1 branches into MB1E and MB1E and MB2E into MB1E and MB1E (supplying volume E). Arrow indicates onset of a branching septum (thick line on drawing) generated by bifurcation B1? and B1. Level 1100 shows location of MB2 between a branching septum (thick line on drawing) and extended septum (dotted line on drawing). Arrows point at persistent branching septa, generated at previous bifurcations. Periphery of pentagon (thin lines on drawing) and separation AB-CD are now completely demarcated.
Fig. 6. Selected photographs and corresponding drawings of serial sections. At level 640, MB₂ bifurcates into two MB₁, creating a septum (arrowhead) clearly shown at level 550. At level 500 (not shown), MB₁ divides into RBₐ and RBₐ, supplying volumes A and B, respectively. A new branching septum perpendicular to septum separating A and B (not indicated in Fig. 3) appears, clearly shown at level 438 (arrowhead) (extended part of it is seen to left of RBₐ). Septum traced by MB₁ persists (double arrowhead), as does its extended septum (thick arrow). Latter is blurred because of alveolar outpouchings. (Masson’s trichrome.)
completing the separation between A and B and D and C, respectively.

The volumes A, B, C, and D are supplied by further dichotomous branching of intra-acinar airways. The number of alveolar interdigitations increases progressively. The principle by which branching and extended septa are generated is maintained, however. Further generations of intra-acinar air passages systematically generate a branching septum, down to alveolar ducts. As shown in Fig. 7, at level 338, the latter air passages are separated by septa that are not stretched anymore but still persist. They become manifest at the places where small venules are coursing within them (extended septa). The serial sections show that pulmonary arteries very closely accompany their airways. Veins, on the contrary, are found never to meet the bronchoarterial axis. They are situated in the extended septa.

Figure 8 shows the construction principle of the lung. Each airway bifurcation generates a “septal unit” consisting of branching and extended septa. Because no septa are lost, the complexity of the septal meshwork increases progressively until the very periphery of the parenchyma is reached. The septal meshwork observed at the pleural surface represents then a projection of the septa generated by bifurcating airways (branching septa) and by extended septa. Both may be regarded as remnants of the original mesenchymal bed (see Discussion). The latter includes also the pleura. Because the extended septa contain the veins, it follows, according to the construction principle, that the lobar veins necessarily meet the bronchoarterial axis in between the first bifurcating airways. Hence, the branching septum originating at the first intralobular airway bifurcation contains also the lobar vein. The tip of the intrapulmonary system of extended septa is thus found at the level of the second airway bifurcation. This is illustrated in Figs. 9 and 10.

Observations in emphysema. Emphysematous destruction was evident in all five tissue blocks, and its severity differed from one lobule to another, even within the same tissue block, with completely destroyed lobuli being found next to less affected tissue. This is illustrated both by a three-dimensional drawing of the reconstructed peripheral airway tree and the lobular septa (Fig. 11) and by photographs (Fig. 12). Although emphysema is evident, not all units are involved to the same extent. Unit A appears almost normal by visual inspection. The overall architecture of alveolar ducts is well preserved, with smoothly stretched walls. Most of the alveolar outpouchings are also readily discernible. Units E–K are characterized by marked emphysema. No normal alveoli are present. Air-space walls are thin or remarkably thickened and then usually closely associated with small air spaces that appear collapsed. In general, the very large air spaces are found in the center and the smaller and collapsed air spaces at the periphery of the unit. This is
Fig. 9. Model of tip of intrapulmonary system of extended septa. White areas are branching septa, shaded area represents an extended septum. Levels a, b, and c are shown in corresponding photographs of Fig. 10. Veins (V) and pulmonary arteries (A) meet at level of lobar bronchi (B).

Fig. 10. Levels a, b, and c are shown in the corresponding photographs of Fig. 9. Veins (V) and pulmonary arteries (A) meet at level of lobar bronchi (B).
Although the construction principle is maintained, its anatomic expression is hardly visible, thus reducing the model rather to a conceptual framework in normal lungs. This explains why it has been stated that the smallest lung unit defined anatomically is a lung lobe, because a lobe is enwrapped more or less completely by visceral pleura (30); and, furthermore, why the definition of smaller pulmonary units, such as an acinus or a lobulus, is determined more by functional considerations than by the anatomy (8). Earlier, Von Hayek (27) pointed out that lobules were not always demarcated by septa at the surface of a lung lobe or in the

Fig. 10. Selected photographs and corresponding drawings at levels a, b, and c in Fig. 9. Branching septa (thick continuous lines on drawing) are arrowheaded. Extended septum (arrow, dotted line on drawing) in which vein courses is partially blurred because of alveolar outpouchings (arrow on drawing). Vein V is meeting A₁ at level c branching septum, thus merging into extended septum (shaded area on drawing).
Implications for peripheral airways. The classic representation of the fibrous tissue of the lung as a network attached at the periphery of the airway, anchored between two more rigid frameworks, and thus submitting the peripheral airway to radial traction appears to be an incomplete picture of the lung anatomy. According to the present results, the airways enter the unit that they supply at its side, with the alveoli of the unit being generated by the daughter branches of the supplying airway. The alveolar septa, seen as alveolar attachments (AA) of the MB, are attached to the outer side of the blades formed by the interlobular septa, rather than to the airway wall itself. Accordingly, the AA do not necessarily correspond to the amount of support provided directly by the connective tissue network to the MB. This may have consequences for the permeability of the peripheral airways in emphysema. As early as in 1957, Leopold and Gough (14) observed marked airway narrowing of the bronchioles supplying the emphysematous spaces. These authors suggested that, besides an anatomic narrowing of the bronchioles caused by inflammation in their walls, these airways got kinked or compressed during expiration “because they pass unsupported into the emphysematous tissue.” As an expression of this support, Anderson and Foraker (1) counted the number of AA on MB; that number was reduced in emphysema and proportional to bronchiolar lumen size. This observation was confirmed by others in emphysematous patients and in smokers (13, 15, 16, 21, 22). AA were inversely related to the emphysema score (15, 19, 21) and to the elastic recoil pressure of the lungs (21, 22). Functional airway obstruction detected clinically or expressed by a reduction of forced expiratory volume in 1 s (FEV₁) was correlated with the severity of emphysema (9, 13, 18, 19) and a reduction of the diameter of the MB (d̅) (9, 20, 26) or of AA (13, 19, 21). The separate contribution of these factors to airflow obstruction was evaluated by means of multiple-regression analysis. Berend and Thurlbeck (2) observed on excised human lungs a joint relationship between maximal expiratory flow at a given elastic recoil pressure (5 cmH₂O) and both inflammation score of the small airways and emphysema score. This suggests that emphysema, apart from its effect on elastic recoil, also influences expiratory flow by another mechanism. Similarly, when FEV₁ was related to pathological (including scores of small-airway inflammation and fibrosis) and morphometric indexes of small airways (d̅, AA), the severity of emphysema turned out to be the most important factor for obstruction, with none of the bronchiolar variables having a significant value in predicting the reduction of ventilatory function (9, 19). Apparently, an inflammatory thickening of bronchiolar walls or a loss of alveolar attachments or even a decrease of small-airway diameter was related to airflow obstruction only because of its correlation with emphysema. This conclusion was not confirmed by recent structure-function studies. Hogg et al. (11) and Gelb et al. (6, 7) observed only very weak or no correlations between airflow limitation measured in vivo and both severity of emphysema and inflammatory scores of small airways in resection specimens (11) or on computerized tomography lung scans [completed by pathological examinations (7)]. Accordingly, the relationship between increase in peripheral airway resistance or airflow limitation and small-airway diameter observed in excised emphysema-
Fig. 12. Photographs of 2 serial sections (top: section 1200, deepest level of drawing; bottom: section 1060, most peripheral level of drawing) used for reconstruction of Fig. 11. Area A is close to normal. Intra-acinar septa are stretched as in normal lungs. Emphysema is worst in areas E, F, G, J, and K. Unit B is almost totally collapsed. Intra-acinar septa are tortuous, especially in areas B and C, and focally in area D. Unit C is collapsed to a lesser extent. Collapse of distal intra-acinar units such as alveolar ducts is marked in severely affected regions (arrows).
tous lungs (25, 26) does not appear to be a direct consequence of emphysema itself or of airway wall inflammation. Apparently, another mechanism responsible for peripheral airway narrowing is operating.

In this respect, we observed collapse of units of different sizes in the emphysematous lungs, resulting in tortuous septa of corresponding order. This is manifest both at the level of intra-acinar air passages and of MB...
(Fig. 12, area B). Bends in the septa are accompanied by corresponding deformations of the airways coursing in those septa. Accordingly, local airway stenosis might result from a competition for space between enlarged emphysematous air spaces and the surrounding structures, to the extent that all structures of a lung unit are constrained to a fairly fixed volume because the expansion of the lung is limited by its own fibrous scaffold including the pleura and, finally, by the chest wall. In such a system, expansion of one structure may result in compression and deformation of adjacent structures, depending on the relative compressibility of these structures.

Besides the inflammatory narrowing of nonrespiratory bronchioles described in smokers and in pulmonary emphysema (3, 4, 9, 11, 32), a concurring noninflammatory stenosis may be found, related to compression by the enlarged air spaces. Air-space enlargement will be the cause of airway narrowing only in the presence of competition for space. The relationship between the severity of emphysema and the degree of flow limitation is thus indirect. Thus the observed correlations may be weak (6, 7, 11).

Two other observations can be explained by the mechanism of competition for space. 1) In keeping with Linhartova et al. (16), we observed that focal airway narrowing in emphysema is marked mainly in the MB, is less pronounced in the bronchi with discontinuous intramural cartilagenous support, and virtually absent in the larger bronchi (26). 2) Wilson et al. (31) documented a smaller percent change with lung deflation of the diameter of small airways (ID <1 mm) in emphysematous lungs compared with normal lungs and with larger airways in the same emphysematous lungs. Conversely, we measured during inflation an increase in peripheral airway resistance at recoil pressures exceeding 0.8 kPa in emphysematous, but not in normal, lungs (26). In the presence of compression of the peripheral airways by enlarged air spaces, the narrowing will be proportional to the compressibility of the airway walls and will increase with further inflation of the air spaces (or further stiffening of the fibrous scaffold).

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