Ultrastructural Morphometry of the Blood-Air Barrier in Pulmonary Sarcoidosis*

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Stereologic techniques were utilized in an electron microscopic study of biopsy samples obtained from the lungs of seven patients with pulmonary sarcoidosis. Relative fractional volumes of alveolar septal components and the arithmetic mean thickness and harmonic mean thickness of alveolocapillary membranes (blood-air barrier) were compared with values for normal lungs. Based on morphometric analysis, increases in the arithmetic mean thickness and the harmonic mean thickness of the alveolocapillary membranes appeared too small to account for the reduction in gas transfer present; however, there was a quantitative relative increase in interstitial tissue in alveolar septa, which does not take part in gas exchange, at the expense of the capillary bed, which is critical to this function.

Sarcoidosis is one of several interstitial pulmonary diseases in which diffusion abnormalities were originally thought to be due to an increase in the membrane component of diffusion as a result of anatomic thickening of the blood-air barrier (alveolocapillary membrane)1. More recent physiologic studies have provided evidence that abnormalities of gas transfer may be the result of other pathologic alterations accompanying the disease.2,3 Reduction of capillary bed, loss of total diffusing surface, and associated abnormalities of ventilation and perfusion have been considered significant in the disturbed physiology of these patients. An accurate quantitative determination of the interstitial tissue changes present in sarcoidosis would be valuable in clarifying the individual contribution of these factors to abnormal blood-air gas exchange in these and similar diffuse pulmonary diseases.

The DeLesse principle4 has been used by geologists for stereologic measurement of samples for over 100 years and has been applied more recently to pulmonary tissue by Weibel and associates.5,6 It is based on the fact that structures randomly dispersed in a volume are quantitatively represented on sections of that volume. Application of the principle to extremely thin sections of homogeneous respiratory tissue permits quantitative measurement of randomly distributed alveoli in the pulmonary parenchyma. Recently, we have described application of this technique to biopsy specimens of normal and diseased human lungs7,8 and have used it in the present study to compare the results of morphometric analysis in biopsy specimens of pulmonary sarcoidosis with those previously described for normal human lungs.

METHODS

In seven patients with pulmonary sarcoidosis, lung tissue was obtained at the time of open-lung biopsy performed in each case for the purpose of histologic diagnosis (Fig 1). Criteria for this diagnosis were those commonly accepted,9,10 but the disease process was sufficiently advanced that few granulomas were seen. The results of pulmonary function studies performed by standard techniques11-18 were compatible with reduced lung volumes and impaired steady-state carbon-monoxide diffusing capacity.
There was no evidence of airway obstruction, as shown by the mean values (ranges within parentheses) in the following tabulation:

| Age, yr | 40 (24-57) |
| Duration of disease, yr | 0.7 (0.2-2.3) |
| TLC, percent of predicted normal | 65 (31-84) |
| FEF 25-75% | 130 (98-148) |

where TLC is the total lung capacity, and FEF 25-75% is the mean forced expiratory flow during the middle half of the forced vital capacity (formerly called the maximal midexpiratory flow rate).

Normal pulmonary tissue, as previously described and included here for comparative purposes, was obtained from a further nine patients at the time of resection of solitary circumscribed pulmonary tumors and was taken from parenchyma remote from the lesion itself. Specimens were obtained from the randomly arranged respiratory portion of the lung, excluding the immediately subpleural zone.

The methods used for tissue fixation and examination by electron microscopy and the applications of morphometric principles to the analysis of tissue specimens were based on the work of others and have been described by us previously. For each patient, 20 electron micrographs were prepared at a magnification of 2,200 times by randomly selecting tissue sections and photographic fields, as described by Weibel (Fig 2). Each micrograph was enlarged to match the translucent test pattern of 168 points and 84 lines on which point-counting volumetric analysis was applied to air spaces, capillaries, and the components of alveolar septal tissue. Fractional air space and fractional capillary space were determined by this method, as previously described elsewhere. Septal tissue was divided into intercapillary tissue and alveolocapillary tissue by lines drawn perpendicular to the alveolar epithelial surface at the lateral extremities of the capillaries. Intercapillary tissue is composed of interstitial cells and connective tissue, while alveolocapillary tissue is made up of capillaries and their overlying alveolar epithelium. This arbitrary division of tissue components was used to separate that portion of alveolar septal tissue directly related to gas exchange (alveolocapillary tissue) from that which was not (intercapillary tissue) and, thereby, allows assignment of a numerical value to each. Such a value provides a basis for comparison of normal with diseased states in which an impairment of diffusion is known to be present. The relationship of alveolocapillary tissue to intercapillary tissue not only affords an index of the proportions of each in the septal tissue studied, but its value is independent of the degree of lung inflation present, a factor of importance in noninflated specimens such as those used in this study.

The arithmetic mean thickness of the blood-air barrier was calculated by relating tissue volume to capillary surface area, as recommended by Weibel for consideration of diffusion problems. The harmonic mean thickness of the blood-air barrier was derived by a method independent of relative tissue volume, using calculation from harmonic mean intercept lengths. Correction factors for fixation and processing and actual degree of lung inflation were not considered pertinent for comparative purposes because of the manner in which the pulmonary tissue was obtained and because tissues obtained from normal and abnormal lungs were identical and processed. Mean values and standard deviations were obtained for all measurements.

**Results**

In normal lungs the tissue volume of alveolar septa was composed of 21.2 ± 1.1 percent alveolocapillary tissue and 15.9 ± 1.9 percent intercapillary tissue, as previously defined. Since they do not contain air, the relative proportion of the contents of alveolar septa remains independent of the degree of lung inflation.

In the lungs of patients with sarcoidosis, alveolocapillary tissue volume was reduced by one-third to 13.9 ± 2.6 percent, but intercapillary tissue volume increased to 57.6 ± 6.2 percent, or 3 times the normal values, indicating that the bulk of additional tissue was deposited preferentially between septal capillaries and not in the alveolocapillary membranes. This distribution of abnormal tissue in intercapillary sites was substantiated by a marked reduction in the ratio of alveolocapillary tissue to intercapillary tissue, which fell from 1.33 ± 0.19 in normal lungs to 0.24 ± 0.08 in diseased lungs, a value which is also uninfluenced by the degree of lung inflation.

The arithmetic mean thickness of the alveolocapillary membranes had an average value of 1.665± 0.128µ in normal lungs, which closely approximates previously estimated values. This was increased to 3.299µ ± 0.346µ in pulmonary sarcoidosis, or about twice normal. The harmonic mean thickness, which has been shown to relate more directly to gas diffusion, had a mean value in normal lungs of 0.829µ ± 0.670µ. In the diseased lungs studied, this was increased by a factor of 1.6 to 1.321µ ± 0.057µ.
DISCUSSION

Sarcoidosis is a systemic granulomatous disease of unknown etiology that is most commonly encountered in the thorax. In about 20 percent of cases, there is progression to interstitial pulmonary fibrosis, with distortion and replacement of normal pulmonary architecture. This is responsible for abnormalities of pulmonary function characterized by small lung volumes and abnormal carbon-monoxide diffusing capacity. In a small number of patients, there may be evidence of airway obstruction. Occasionally, diffusing capacity may be impaired in the absence of roentgenographic evidence of parenchymal involvement.

General acceptance of the value of diagnostic lung biopsy in pulmonary sarcoidosis implies that, as with other diffuse pulmonary diseases, the abnormalities present in the tissue sample obtained are representative of widespread alterations throughout the lung. While their distribution cannot be determined from a biopsy specimen, it is clear that there must be major derangement of structures involved in gas transfer when diffusing capacity and other aspects of pulmonary function are significantly reduced. This was true in the present series, in which small lung volumes and reduced diffusing capacity were found without evidence of airway obstruction.

The structures examined require the magnifications of electron microscopy for their measurement. It is not necessary to determine lung volume to measure the thickness of the blood-air barrier or to compare the relative proportions of the structures in the alveolar septa that are involved in blood-air gas transfer with those that do not function in this way. These relationships are independent of the degree of lung compression or inflation.

When the relative proportions of septal structures were compared, it was found that the portion involved in the blood-air gas-transfer process (alveolocapillary tissue) was reduced to two-thirds of the anticipated normal amount. On the other hand, intercapillary tissue, which is made up of interstitial cells and connective tissue that are not active in gas exchange, increased to between three and four times the normal value. This relative increase in intercapillary tissue volume, therefore, was at the expense of structures related to gas diffusion and was due principally to loss of vascular bed. The reduction in the ratio of alveolocapillary tissue to intercapillary tissue from 1.33:1 in normal septa to 0.24:1 in patients with sarcoidosis substantiated this preferential distribution of cells and connective tissue in these nonrespiratory sites.

Reduction in alveolar and capillary surface areas in the lungs of patients with this disease has been noted previously, but neither a quantitative factor nor the distribution of diseased tissue in alveolar septa could be determined without the use of electron microscopy, since these structures cannot be adequately visualized at lower magnifications. Loss of pulmonary capillary bed results in a diminished diffusing surface for gas transfer but also may be associated with a more rapid transit time of capillary blood through it, so that oxygen uptake is impaired, particularly during exercise. Although noninflated lung tissue was used for this study, lung inflation does not affect quantitation of tissue contents by the methods employed; however, calculation of total surface areas of alveoli and capillaries is distorted by these methods, and although each was reduced to about one-third of anticipated normal values based on sex and height, we believed it was better to regard this as a supportive observation only, although the trend was clear.

It has been calculated that the membrane component of the diffusing capacity requires increases of five times normal to produce a measurable increment in the normal alveolar-arterial oxygen tension gradient. In the lungs of these seven patients with pulmonary sarcoidosis, the arithmetic mean thickness of the blood-air barrier was derived by relating tissue volume to capillary surface only, since this method is believed to be more accurate in evaluating problems of gas diffusion. The tissues of this blood-air barrier appeared morphologically normal, and the measurements obtained were similar to those previously determined for human lung free from disease.

The method used to determine harmonic mean thickness of the blood-air barrier, which has been shown to relate more directly to gas diffusion than arithmetic mean thickness, does not depend on subdivision of septal tissue and point-counting volumetric analysis for its determination but depends directly on harmonic mean intercept lengths. This measurement was within normal limits, and the ratio of the arithmetic mean thickness to the harmonic mean thickness was 2.5:1, which is close to the ratio of 2:1 reported in the normal lungs of a number of mammalian species. It reflects an optimal functional relationship between structural integrity and gas permeability in the blood-air barrier, which appears to be independent of the level of lung inflation.

Based on the calculations of others, the degree of thickening of the blood-air barrier demonstrated in this study should not interfere significantly with diffusion of oxygen. The excess accumulation of cellular and fibrous tissue in alveolar septa is associated with reduction of capillary bed and may be expected to result in reduced and uneven lung compliance.
and nonuniform regional ventilation. Such minor thickening as was found in the diseased blood-air barrier of these patients might conceivably become significant if ventilation-perfusion relationships became sufficiently abnormal and erythrocytic transit time was markedly reduced. Gas transfer might then depend on a combination of interrelated variables; however, increase in pulmonary extravascular water volume from any cause may produce entirely different problems, and neither this nor the influence of biochemical changes in the cells and interstitium can be assessed from the present study.

Because of the technical impossibility of examining each of the alveolocapillary membranes in an entire lung by electron microscopy, the assumption has been made in this study that the tissue examined is representative of major areas of abnormality in diseased lungs. This is based on commonly accepted principles underlying the validity of lung biopsy in diseased lungs. This is based on commonly accepted principles underlying the validity of lung biopsy in diseased lungs. This is based on commonly accepted principles underlying the validity of lung biopsy in diseased lungs. This is based on commonly accepted

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