Am J Physiol Lung Cell Mol Physiol 305: L405–L408, 2013;
doi:10.1152/ajplung.00169.2013.

Invited Editorial

A retrospective of lung morphometry: from 1963 to present

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THE PUBLICATION, fifty years ago, of my little monograph "Morphometry of the Human Lung" (30) summarized the results of a most unusual four years of work by an anatomist in the fascinating environment of the Cardiopulmonary Laboratory headed by the Nobel laureates André F. Cournand and Dickinson W. Richards at Columbia University Division of Bellevue Hospital in New York, the Mecca of cardiopulmonary physiology of the time. While I was on a postdoc fellowship in pathology at Yale University Cournand had invited me to join his group with the mandate to "do anything on the structure of the lung that is of interest to physiology," an extraordinary challenge for a young Swiss anatomist. I cut short my fellowship and moved to New York, setting up a microscopy lab in a room next door to the installations where cardiac catheters were shoved to obtain physiological information on the pulmonary circulation.

Morphometry Is of Interest to Physiology

My first challenge was to find out what on the structure of the lung would be of interest to physiologists. The answer soon came forward by my discussions with Domingo M. Gomez, a Cuban cardiologist who had fled Fidel Castro's reign of terror and was given refuge by Cournand. At heart, Gomez was a biomathematician with the grand goal of modeling the lung to predict its functional performance and limitations at all levels, from the airways to the gas exchanger. For that he needed to know the size of things in the lung. His first question was: how many alveoli are there in a human lung? There was no reliable information in the literature. But neither was there a method for counting such structures by microscopy on very thin sections (Fig. 1). So we had to invent our own method, which was an exercise in mathematics considering the geometric probability of cutting alveoli with a random section. With some assumptions on alveolar shape we could calculate the number of alveoli in the unit volume of lung from counts of alveolar profiles (Fig. 1*A*) and an estimate of alveolar volume. We decided to test the method by slicing pea aspic with a defined number of embedded peas; Cournand was not at all amused by this project since he did not see how pea aspic could be of interest for physiology. But the paper got published in the *Journal of Applied Physiology*, including a picture of the sliced aspic, presenting our first estimates that a human lung comprised about 300 million alveoli (35). This was my first exercise in what would, a couple of years later, be called stereology: developing methods for estimating 3D information from measurements on 2D sections.

Gomez was not really interested in the number of alveoli — it is indeed a parameter of some developmental but limited physiological interest — but he wanted to use it to calculate the alveolar surface area, a fundamental parameter of pulmonary diffusing capacity. But it then occurred that this surface could be estimated directly, as proposed by Tomkeieff and Campbell (28), by randomly placing a test line of known length L on the section and counting its intersections I with the alveolar surface trace (Fig. 1); the surface per unit volume (S_V) then is $S_V = 2 \cdot I/L$, the coefficient 2 resulting from geometric probability considerations. And then we had also found that material scientists estimated volume fractions on sections by simple differential point counts (11). With that we had the main tools at hand to quantitatively assess the structure of lung parenchyma in of terms volumes, surface areas, and numbers.

These new methods now allowed us to describe the quantitative properties of lung parenchyma by systematic measurements on five suitably prepared human lungs. Besides alveolar number we measured alveolar surface area to amount to 80 m^2, the volume of capillaries at 140 ml, and the capillary surface to be about 10% lower than alveolar surface. But Gomez was not satisfied with that: he wanted "a comprehensive quantitative survey of the entire lung as an organized and integrated structure" (36). So I had to add a morphometric study of the airway tree by a systematic quantitative assessment of a plastic cast of the human bronchial tree; but here stereological methods failed because the measurements did not concern bulk parameters but had to be recorded in respect to the airway hierarchy as a dichotomous tree. This broad study on lung morphometry resulted in a paper called "Architecture of the Human Lung" published in *Science* in 1962 (36).

When working on the measurements of the capillary network it became clear that the light microscopic study was inadequate to resolve the critical structures that establish the direct exposure of capillary blood to alveolar air; the resolving power of the electron microscope would be required. I was very fortunate that I could join the laboratory of George E. Palade at Rockefeller Institute, the pioneers in the new field of electron microscopic cell biology, to extend our studies to the cellular level. These electron microscopic studies of the gas exchanger were done on rat lungs, which allowed us to develop the methodology including the stereological measurement of the effective thickness of the gas exchange barrier (30, 38).

This was the state of my studies on lung morphometry in 1963 on my return to Switzerland. The exposure to powerful environments in respiratory physiology and cell biology during my four years in New York determined my future research program. Besides applying morphometric methods to the study of lung growth (6) and lung mechanics (2), I tried to achieve what Gomez had intended: *1*) to develop a model and approach to estimate the structural foundations of the pulmonary diffusing capacity $D_{L_{O_2}}$ (31, 34) based on the model of Roughton and Forster (24), and *2*) to set this in relation to physiology. After considerable efforts to obtain human lung specimens suitably prepared we could obtain estimates of $D_{L_{O_2}}$ in the human lung (10). The value estimated at 150 $ml \cdot min^{-1} \cdot mmHg^{-1}$ was larger than physiological estimates of 100 in exercising humans (13). To understand and elucidate this difference we resorted to comparative physiological studies on

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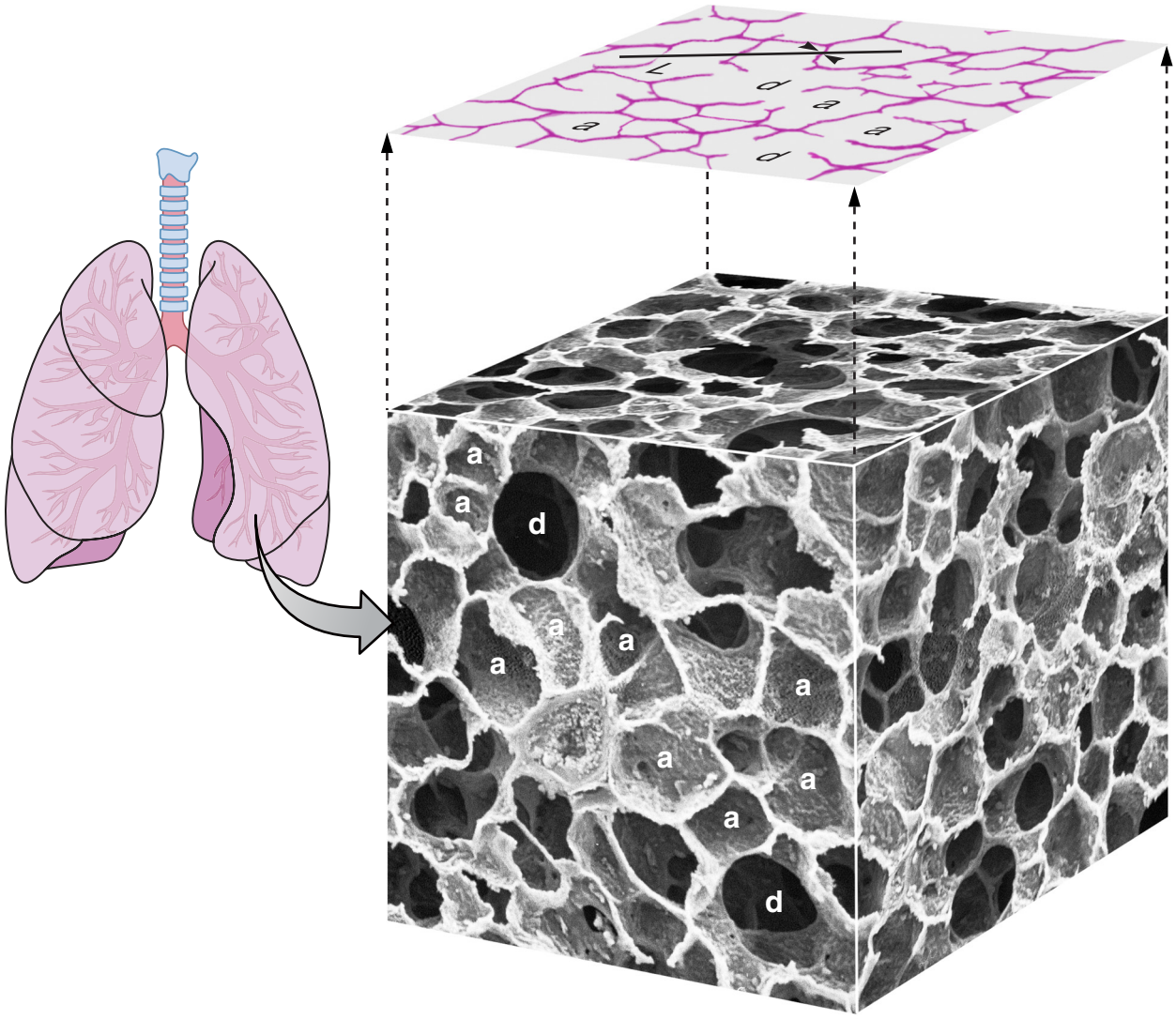

Fig. 1. Quantitative microscopy must be done on thin sections cut from small blocks of tissue suitably sampled from the lung. Such sections do not show the 3D structure of alveoli (a) and alveolar ducts (d) in lung parenchyma as revealed by scanning electron microscopy; their profiles are flat. The application of stereology allows quantitative information on the 3D structure to be assessed on such thin 2D sections: e.g., randomly dropping a line of length L on the section allows to estimate the alveolar surface density by counting intersections between test line and surface trace and dividing by L; the arrowheads point to 2 of the 6 intersections on this example. (Illustrator: Patrick Lane; ScEYEnce Studios)

mammals from the Etruscan shrew, the smallest mammal with the highest O_2 consumption, to the cow and horse, and comparing athletic and sedentary species. This was done in the framework of an integrative study of the entire pathway for oxygen, from the lung to the muscle mitochondria, in an international collaboration with C. Richard Taylor at Harvard and Hans Hoppeler, the expert on muscle structure and function (26, 40, 41). Important insights were furthermore gained in the elegant pneumonectomy experiments of Connie Hsia at Dallas, which gave strong support to the $D_{L_{O_2}}$ model as predictor of the real gas exchange capacity (15).

There Is More that Is of Interest for Physiology

The description of the gas exchanger was only one aspect of morphometric characterization of the lung. The quantification of the lung cell population was equally important (7), as was the study of how the cell population reacted to serious damages, as in acute respiratory distress syndrome (ARDS), to restore a functional gas exchanger (3) or how the development of the lung is determined by various agents (1). A most important aspect is the role of the secretory alveolar epithelial cell, the type II cell, in the production and processing of surfactant constituents through its cytoplasmic organelles (17). This can be studied with the same stereological methods applied to problems of cell biology (38), but this potential has not been fully exploited. Many of these problems become important in lung pathology, such as the loss of surface in emphysema (27) or the formation and distribution of edema (4, 5). Some of these aspects are dealt with in some detail in the

two companion papers by Ochs and Mühlfeld recently published in this journal (20, 21).

The Importance of Stereology

In all this morphometric work stereological methods played the central role. So they needed to be continually developed to improve their accuracy and efficiency. This was greatly helped by the interdisciplinary collaborations that arose from the International Society of Stereology founded in 1961 by Hans Elias (32, 33). Our contributions arose chiefly from the intense collaboration with the mathematicians Roger Miles (19) and Luis Cruz-Orive (8). From the 1980s onward very significant progress came from the Danish group led by Hans-Jørgen Gundersen (12) that developed new approaches to unbiased stereology so that the arsenal of stereological methods was significantly enlarged (9, 14, 23). Most influential was the formulation of the disector principle for counting particles of arbitrary shape (25) that indeed made my shape-dependent counting method (35) obsolete; a variant of the disector method was developed to count alveoli, and that caused the number of alveoli to be updated to about 400 million in the human lung (22). These new methods augment but do not supplant the "classic" stereological methods for estimating volumes and surfaces, etc., with points and lines as geometric sampling probes, which, if properly used, are unbiased methods that allow accurate estimates of basic morphometric parameters "of interest to physiology."

Stereological methods are used to an increasing extent in studies of experimental pathology or in characterizing morphological lung changes related to genetically modified organisms. In the course of time the tendency to use shortcuts or simple approaches ignored some of the essential pitfalls of stereology, raising questions about the reliability of such estimates since inaccuracies are difficult to detect a posteriori (37). For example, the mean linear intercept is often used as an estimator of alveolar size which is problematic: *1*) it does not measure the size of alveoli, but rather the extent of the entire acinar air space, alveoli and ducts together, and *2*) it is very sensitive to the volume at which the lung was fixed (18). Realizing the problems that arise from an uneducated use of stereological methods, the American Thoracic Society and European Respiratory Society have commissioned a group of experts to formulate Standards for the Quantitative Assessment of Lung Structure that were published in the *American Journal of Respiratory and Critical Care Medicine* as an "official research policy statement" of the two societies (16). These Standards are a comprehensive and concise statement of rules, but it may not be easy to design specific study protocols from these rules.

It is therefore fortunate that Matthias Ochs and Christian Mühlfeld, two highly experienced users of stereology, have recently published in this journal two companion papers discussing examples of a problem-based approach to quantitative microscopy of the lung (20, 21). After presenting details of basic principles of lung stereology they discuss their application in the assessment of pathological changes of lung structure, such as in acute lung injury, pulmonary fibrosis, or emphysema, elaborating detailed protocols on the approach and results on several cases of special interest. This may help newcomers to avoid some of the clandestine pitfalls of this sophisticated methodology that may cause the results to be not only inaccurate but even misleading. The papers emphasize the study of morphological changes in the fine structure and cellular constitution of the lung parenchyma, which is certainly the main field of application of these methods, particularly in view of new possibilities of controlled experiments, e.g., with transgenic animals. The interesting prospect of using stereology with newer, possibly in vivo imaging modalities such as high-resolution CT (29) is also mentioned. This may warrant some special attention in the nearer future because it contains a great potential.

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