MORPHOMETRIC STUDY OF THE LUNGS OF EXPERIMENTAL ANIMALS

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A morphometric method of studying the structures of the lungs is suggested and has been tested on 400 albino rats. The necessary formulas for the volume and diameter of the alveoli, the number of alveoli in the lung, the total area of the alveolar surface, the volume of air in the whole lung, and so on, are given. The proposed method of mathematical analysis is simple and can be recommended for the study of age changes and pathological changes in the lungs of experimental animals.

The introduction of morphometric methods into scientific research widens the scope of analysis based on the quantitative assessment of information regarding the state of the structures of an organ under normal and pathological conditions. In particular, there is considerable interest in the morphometric study of the lungs, the object most frequently used in experimental research and attracting the attention of the pathologist.

The method of mathematical analysis of changes in the lung structures of experimental animals tested by the writers on the results of morphometric studies of 400 noninbred albino rats incorporates a number of histometric approaches published previously [1, 2, 4, 7-9, 11]. Unlike methods of fixation of the lungs suggested earlier [5, 6, 10], the method of fixation of the lungs with 10% formalin used by the writers is simple and reliable. "Correction factors for fixation shrinkage" given by Weibel [12] for the human lungs differ negligibly from the corresponding factors obtained in the present investigations. Preparation of the organ for histometry includes determination of the volume of the fixed lung, weighing, processing of the pieces of lung tissue and staining the histological sections. The histometric method of study of the lungs, based partly on Weibel's recommendations [11], and adapted for practical purposes, is described below.

The ratio between the volumes of the air passages and of the respiratory part of the lung is measured in histological sections with the light microscope by means of Avtandilov's ocular measuring grid [3]. This stage includes photography of a known area on randomly chosen histological sections and also of random areas of the respiratory part of the lung, in accordance with Strukov's concept of "biological area" [8]. Histometric analysis of the structures of the "biological areas" of the lung consists of accurate counting of the number of alveoli found, with allowance for their diameter, and determination of the area that they occupy on several photographic plates (up to 10). On the basis of the results obtained the introductory parameters of histometry of the lung can be obtained: K_a is the total number of alveoli and S_a is the total area of the alveoli. Close attention is required when these parameters of the lung are determined for the informativeness of the work as a whole depends on this step.

The next stage in the histometric investigations is determination of the number of alveoli and the total area of the alveolar surface in the lung of the experimental animal, for which the following data are found.

1. The number of alveoli per mm^2 of histological section (n) by the equation $n = K_a/S_{nx}$, where S_{nx} is the area of cross section of the lung tissue photographed on the plates.

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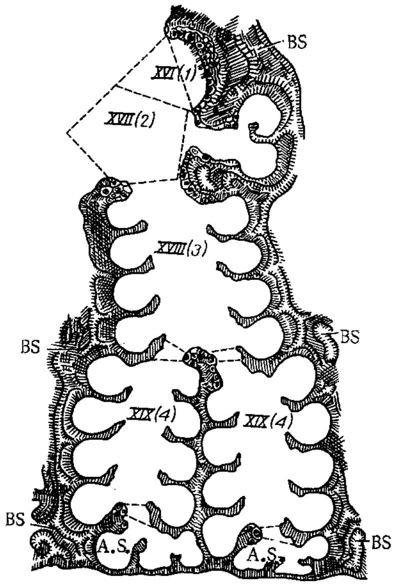

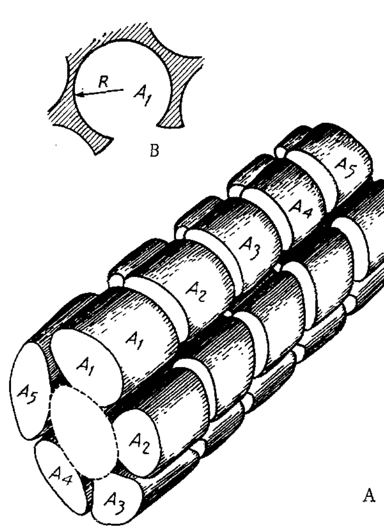

Fig. 1. Fig. 2.

Fig. 1. Model of mathematical analogue of $^1/_4$ acinus of the lung of a noninbred albino rat aged 210 days: XVI (1) terminal bronchiole of the XVI order of bronchi; XVII (2) respiratory bronchiole of the XVII order of bronchi; XVIII (3) respiratory bronchiole of the XVIII order of bronchi, alveolar passage of the first order; XIX (4) respiratory bronchiole of the XIX order of bronchi, alveolar passage of the second order. A. S.) alveolar sac formed by terminal alveoli of the respiratory bronchiole of the XIX order. Scale: 1 mm to 5 μ.

Fig. 2. Model of cylinder of respiratory bronchiole with alveoli: A) alveolar passage (cylinder of passage distinguished by broken line). $A_{1,\,2,\,3,\,4,\,5}$) Alveoli. B) The simplest model of an alveolus with a smooth surface consisting of $^5/_6$ of a sphere. It is characterized by one measurement – the mean diameter of the alveolus.

2. The "mean area of cross section" of an alveolus ($\bar{S}$), obtained by random section in any direction, using the equation $\bar{S} = S_a/K_a$.

3. The apparent volume of the alveolus (V_a) is found by the equation $V_a = \bar{\beta} \cdot S^{2/3}$, where β is a dimensionless coefficient depending on the configuration, and for alveoli is taken to be 1.55. To obtain the volume of an alveolus of the lung (V_a', V_a'') of a typical group of animals, a mathematical analogue of an acinus (Fig. 1) with alveoli having different degrees of truncation of their inlet must be created. For this purpose the following equations are used:

a) $V_a' = \pi h^2\,[R-(1/3)\,h]$, where h is the depth of the alveolus, or

b) $V_a = (4/3)\pi h^3 (5/6)$, where the volume of a true alveolus is 5/6 of the volume of a sphere.

4. The diameter of a mean alveolus by the equation: $D_{m\cdot a} = \sqrt[3]{(V_a \cdot 6)/\pi}$.

5. The volumetric fraction (ρ) of the given tissue component and, in particular, of alveoli in histological sections of the lung which give a two-dimensional image of the three-dimensional system of randomly distributed tissue structures, by the equation $\rho = n \cdot \bar{S}/S_n$.

6. The number of alveoli in 1 mm^3 lung tissue (N_a) by the equation $N_a = n^{3/2}/\beta\sqrt{\rho}$.

7. The number of alveoli in the whole lung (N_{AT}) of a particular experimental animal is found by the equation $N_{AT} = N_a \cdot \varphi V_{LS}$, where φV_{LS} is 90% of the total volume of the histological preparation of the lung.

8. The total area of the alveolar surface (S_{AT}) through the tissue structures of whose air-blood barrier the process of gas exchange takes place is found by the equation

$$S_{AT} = N_{AT} \cdot \delta_a \left(\frac{\rho_a \cdot \varphi V_L}{N_{AT}} \right)^{2/3},$$

where δ_a is the mean coefficient of configuration, with a value of 4.87, determined by the use of models considering only one geometrical property of the alveolus, namely, the ratio surface/volume. The parameter φ V_L is 90% of the total volume of the native lung. The results of determination of the total area of the alveolar surface by the geometric method and by the method of the "mean chord length" and by linear measurements on a model of the alveolus were found to agree satisfactorily in every case.

To determine the volume of air in the lung of the experimental animal the volumes of the suggested model of a mathematical analogue of the cylinders of a bronchus (Fig. 2) must be calculated separately for each segment and for the whole order of bronchi in the lung as a whole by the equation $V_W = R^2h$. The total volume of air in all branches of the bronchi and bronchioles forming the "conductive zone," in the alveolar passages forming the "transitory zone," and also in the alveoli of the respiratory zone correspond to the volume of air found in the whole lung.

Differences in the structure of the lung associated with age or with pathological changes can be differentiated precisely by means of this morphometric method when used in practical research.

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