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Pressure volume curve and alveolar recruitment/de-recruitment. A morphometric model of the respiratory cycle

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Summary. *Hypothesis:* The changes in pulmonary volume taking place during respiration are accompanied by the opening and closing of the alveoli, with the number of alveoli open, at the same transpulmonary pressure (TPP) differing, depending on whether the lung is insufflated or deflated.
Material and methods: Seventy 344 Fischer rats divided into five groups. Group 1 lungs were fixed by instilling 10% formalin through the trachea to a pressure of 25 cm H_2O. The lungs of the next four groups were air-filled and fixed via the pulmonary artery: group 2 lungs were fixed in inflation at 10 cm H_2O TPP; group 3 lungs were fixed in inflation at 20 cm. H_2O TPP; the lungs of groups 4 and 5 were fixed in deflation and, therefore, were inflated with air up to 27 cm. H_2O to drop to 20 cm in group 4 and to 10 cm in group 5. The lungs were processed for light microscopy, carrying out a morphometric study. The results were statistically processed.
Results: The lungs insufflated with liquid fixative at 25 cm of TPP reached higher values in the variables Pulmonary Volume, Internal Alveolar Surface (IAS) and Number of Alveoli, being statistically significant ($p<0.05$) in comparison with the other four groups. In the lungs fixed in deflation, the pulmonary volume, IAS and number of alveoli were greater than in those fixed in inflation. The lungs fixed to 20 cm in deflation displayed significant statistical differences compared with those fixed to 20 cm in inflation. The IAS and number of alveoli gave good rates in relation with the pulmonary volume ($r \geq 0.65$). Three variables were used to measure the size of the alveoli, alveolar cord, alveolar surface and Lm, but none showed significant modifications.
Conclusion: This study supports the hypothesis that changes in lung volume are related to the increase/decrease in the number of alveoli that are open/closed and not to the modification in the size of the alveoli. Alveolar recruitment is the microscopic expression of pulmonary hysteresis, since the number of alveoli open in deflation is greater than the number open during inflation.

Key words: Morphometry, alveolar recruitment, hysteresis, TLC, transpulmonary pressure

Introduction

It has been proposed that mechanical ventilation strategies that minimize lung stress and avoid the ventilator-induced lung injury that can occur during respiration should be used so that a greater gaseous exchange takes place (Amato et al., 1998; Lichtwarck-Aschoff et al., 2000; Dreyfuss and Saumon, 2001). These strategies are decided upon in relation with the pressure-volume curve (p-v) (Rimensberger et al., 1999; Hickling, 2001). On the basis of the hypothesis of alveolar recruitment/de-recruitment, it has been proposed that each point on the PV-curve (Fig. 1) corresponds to a morphology of the microscopic pulmonary architecture itself (Cheng et al., 1995). Therefore, the decision in favor of a ventilatory strategy is made on the basis of a hypothesis of alveolar recruitment. The hypothesis of recruitment is based on the fact that when transpulmonary pressure (TPP) increases during respiration, the lung dilates due to the increase in the number of alveoli (Dreyfuss and Saumon, 2001). Nonetheless, it appears that among certain authors there is not full consensus regarding the hypothesis of alveolar recruitment, since they ask “Does the change in lung volume as manifested in the PV-curve reflect what occurs at the level of 300 million alveoli?” (Lichtwarck-Aschoff et al., 2000).

This question has been implied in many morphometric studies that have measured the size of the peripheral airspaces (PAS) during different phases of the respiratory cycle. For some authors, the modifications in pulmonary volume are related to the size of the alveoli

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(Forrest, 1970; D'Angelo, 1972; Mercer et al., 1987). Gil and Weibel (1972), in a morphometric study, were among the first authors to propose that lung volume increased by septal unpleating of and alveolar recruitment, and that the opening and closing of the alveoli was related to pulmonary hysteresis. The differences existing in the PV-curve between insufflation and deflation (Fig. 1) are known as pulmonary hysteresis. Smaldone et al. (1983), in a functional work, proposed that pulmonary hysteresis was due to changes in the population of the alveoli.

Recently, Carney et al. (1999) considered that the concept of alveolar recruitment did not receive sufficient consensus, and measured subpleural alveoli "in vivo" during the respiratory cycle. The results of these experiments support the idea of alveolar recruitment. Carney et al. (1999) indicate that studies that were made on fixed lungs did not support the theory of alveolar recruitment and, therefore, propose that fixation techniques produce artifactual distortion during preservation and limit the ability to differentiate alveolar ducts (Carney et al., 1999). Should this proposal be correct, morphometric descriptions made on fixed lungs would not be accurate. To us there seem to be two reasons for considering the proposal of Carney et al. (1999) to be incorrect: 1) morphometric studies have been performed on fixed tissue supporting the hypothesis of recruitment (Klingele and Staub, 1970; Gil and Weibel, 1972; Gil et al., 1979); and 2) in an "in vivo" study carried out with a methodology similar to that used by Carney et al. (1999) the alveoli increased in size with the TPP (D'Angelo, 1972). We propose to demonstrate the following hypothesis with fixed tissue: the changes in pulmonary volume taking place during the respiratory cycle are accompanied by the opening and closing of the alveoli, with the number of alveoli open at a given TPP varying, depending on whether the lung is in insufflation or deflation. The term PAS is used instead of alveoli due to the difficulty in differentiating the alveoli ducts in the histological cut (Hansen and Ampaya, 1974; Gil et al., 1979).

Materials and methods

Seventy Fischer 344 rats aged five months have been used (Pinkerton et al., 1982), with 50% male and 50% female in all the groups. The animals were supplied by Iffa Credo®. They were classified into five groups with 14 animals in each:

25-cm group: After being anaesthetized with pentothal (0.1 mg per gram of body weight), a middle thoracotomy was carried out on these animals, permitting the excision of the lungs from the chest and enabling them to be fixed by instilling 10% formalin through the trachea at a TPP of 25 cm H_20, for forty-eight hours (Fig. 2).

10-cmI group (inflation): The procedure was similar to the previous case up to the point at which the lungs, together with the heart, were excised from the chest. Subsequently, the trachea was connected to an air source (Safam® REM+), which enabled the TPP to be maintained at 10 cm. pressure H_2O for forty-eight hours; with this TPP it was hoped to attain 40% total lung capacity (TLC) (Gil et al., 1979). Fixation was carried out through the pulmonary artery with 10% formalin (Fig. 3).

20-cmI group (inflation): The procedure was similar to that of the previous group, with the exception of the fact that the TPP applied to the excised lung was 20 cm. H_2O (Fig. 3), 80% TLC (Gil et al., 1979).

20-cmD group: (deflation): The procedure was similar to that of the previous group with the difference that the TPP rose to 27 cm H_2O, for one minute, and was then dropped to 20 cm H_2O (Fig. 3) with the aim of attaining 90% TLC (Gil et al., 1979).

10-cmD group (deflation): this differed from the previous group in that the TPP, after rising to 27 cm H_2O, dropped to 10 cm (Fig. 3) 80% TLC (Gil et al., 1979).

Before inserting the fixative into the lung, the vascular bed was washed with saline solution. All the excised lungs collapsed spontaneously before the TPP was applied. During fixation, which lasted for forty-eight hours in all cases, the excised lungs were submerged in 10% formalin. The post-fixation was carried out keeping the lungs submerged in the same fixative for fifteen days.

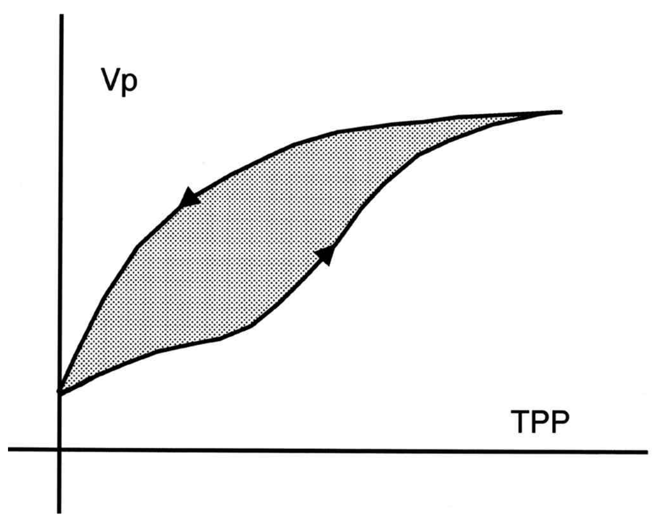

Fig. 1. Pressure volume curve. The ascending line corresponds to inflation and the descending line to deflation. At the same transpulmonary pressure the volume is smaller in inflation than in deflation. The dotted area is the hysteresis.

Handling of the tissue

Once fixed, the lungs were cut transversally into 0.5 cm-thick sections. Three slices per lung were chosen at random. They were dehydrated and placed in paraffin. 7 µm cuts were made and stained with methylene blue.

Morphometric study

This was systematized into three phases (Escolar et al., 1994):

Image capture: An Olympus® BX50 microscope, a Sony® XC-57CE video camera, a Data Translation® DT 3155-PM digitalization card, a Power Macintosh® 7200/90 computer and a capturing program, Grabber®, were used. The images were captured in 256 gray tones with a size of 551/400 pixels; they were captured at x100 y x20 (Figs. 4, 5). The image capture at x100 was performed in the following manner: from each lung slice a section was chosen and divided into 13 zones (Escolar et al., 1997) from which seven were chosen at random. Within each of these, a histological field was then chosen at random. The images captured at x20 were chosen at random from each of the three lung sections; a total of seven fields per section.

Processing of the captured images: A Power Macintosh® 7200/90 computer was used with the NIH Image® 1.60b7 program. The x100 images were transformed by means of the threshold option into two colors (Fig. 6). With the images captured at x20 the looped option was used to select the area colored in black to be quantified (Fig. 7).

Quantification of the graphs: A Power Macintosh® 7200/90 computer was used together with the NIH Image® 1.60b7 processing and quantification program, together with a further program designed by ourselves (Escolar et al., 1997). The variables studied were classified into planimetric and volumetric categories.

Planimetric variables

Alveolar cord: this is the distance, taken at random, that exists between two walls of a single PAS (Fig. 6). It is expressed in µm.

Wall thickness: this is the thickness of the alveolar septum (Fig. 6). It is expressed in µm. For quantification of these first two variables the computer drew 551 straight vertical lines and 400 horizontal lines on the x100 images. On the white area it measured the line segments that corresponded to the alveolar cord and on the black area those that corresponded to the alveolar wall (Fig. 6).

The area of the PAS: this is the surface area of a terminal

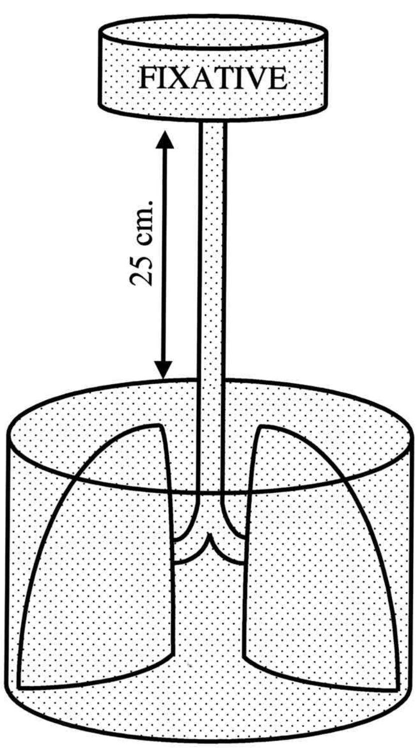

Fig. 2. Model of pulmonary fixation of the 25-cm. H_2O group. The liquid fixative is introduced via the trachea into the lungs with a transpulmonary pressure of 25 cm of H_2O.

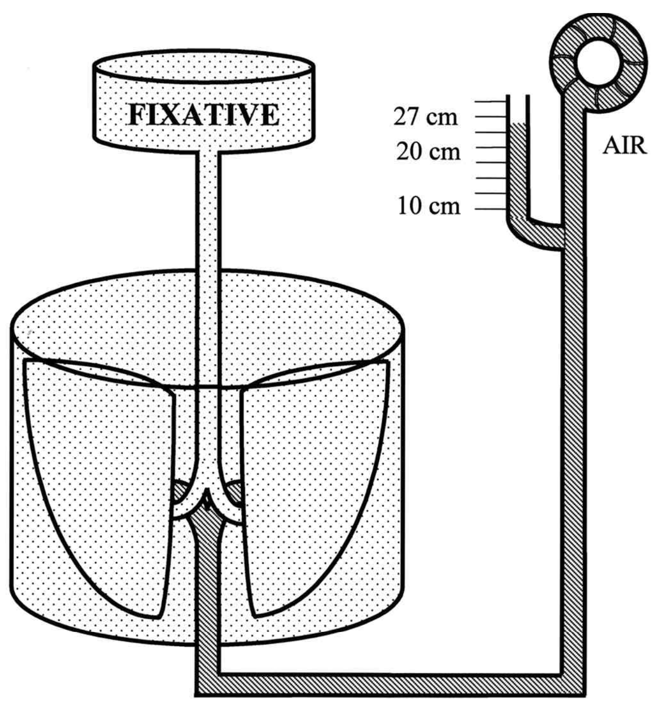

Fig. 3. Model of lung fixations 10 cmI, 20 cmI, 20 cmD and 10 cmD groups. The fixative is inserted through the pulmonary artery, at the same time as the lungs are inflated with air through the trachea. The transpulmonary pressure is maintained automatically by the air source. A pressure gauge was placed in the air circuit to control any possible pressure variations. The lung was kept submerged in fixative.

lung unit (Fig. 6). It is expressed in μm^2. The PAS area was measured directly by the computer.

Mean linear intercept index (Lm): this was obtained by adding together the wall thickness and the alveolar cord. It is expressed in µm

Parenchymatous tissue proportion: this is the percentage of black with respect to white (Fig. 6). It was calculated directly by the computer. The results were only used to calculate other variables and are therefore not presented.

Internal alveolar perimeter: this is the intermediate line that exists between the white and black colors of the two-colored image (Fig. 6). The results were only used to calculate other variables and are therefore not

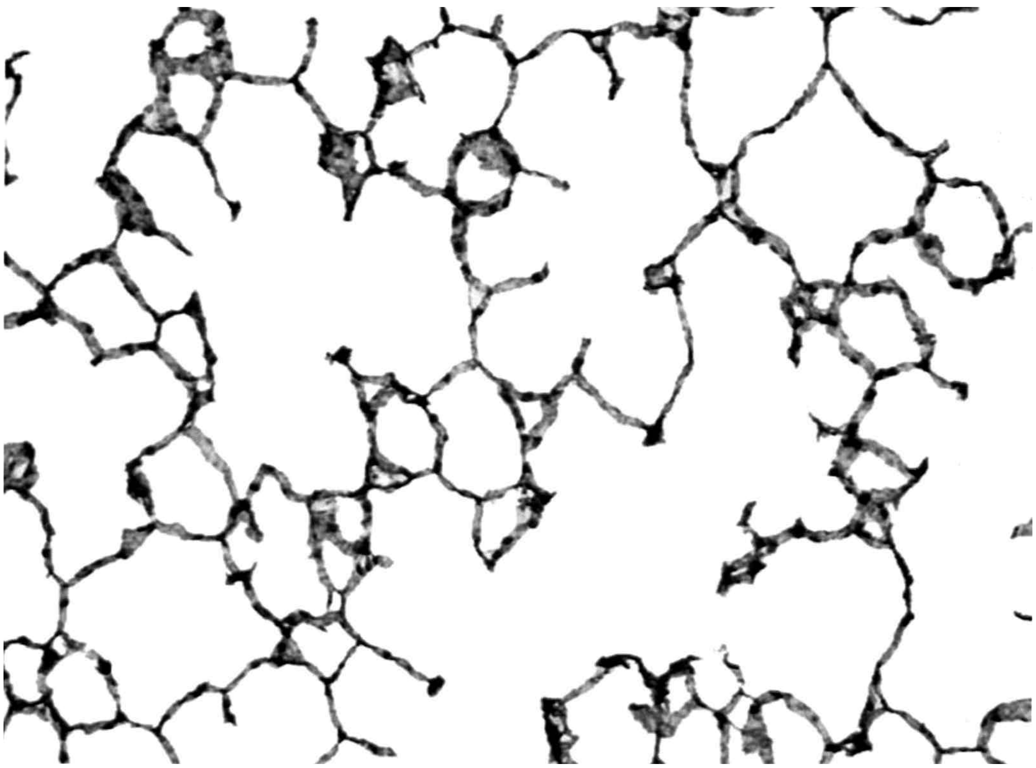

Fig. 4. Microscopic image of a lung field captured at x100 and 256 gray tones.

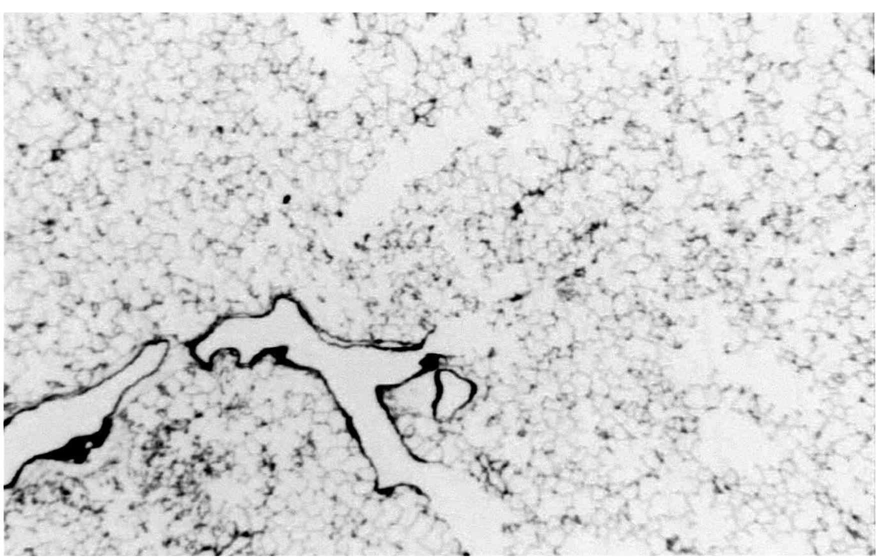

Fig. 5. Microscopic image of a lung field captured at x20 and 256 gray tones.

presented.

Volumetric variable

The pulmonary volume (Pv) was measured by liquid displacement, by submerging the lungs in liquid with the trachea clamped. This is expressed in cm^3.

Pulmonary parenchyma volume: this is the volume of the lung that does not have vessels or airways. In histological fields captured at x20 (Fig. 5), the surface of the bronchi and vessels was measured (Fig. 7). In this way, in a histological field (S'p) with an area of 220.400 pixels it was possible to find out what proportion was occupied by the conduction airway surface (S'cw) and what proportion was occupied by pulmonary parenchyma (S'pp).

$$S'p = S'cw + S'pp$$

The surface of a histological field (S'p), the conduction airway surface (S'cw) and the pulmonary parenchyma surface (S'pp) were multiplied by one pixel in height. In this way, the conduction airway volume fraction (V'cw) was obtained, together with the pulmonary parenchyma volume (V'pp) fraction, which corresponded to a lung volume of 220.400 pixels (V'p)

$$V'p = V'cw + V'pp$$

In order to obtain the pulmonary parenchyma volume (Vpp) the following formula was applied:

$$Vpp = \frac{Vp \,.\, V'pp}{V'p}$$

Parenchymatous tissue volume (Vpt): Once the pulmonary parenchyma volume and the proportion of the parenchymatous tissue (pt) are known, it is possible to obtain the parenchymatous tissue volume, which is the volume of the pulmonary parenchyma without gas. This is expressed in cm^3.

$$Vpt = \frac{Vpp \,.\, pt}{100}$$

The distal airspace volume is obtained by subtracting the pulmonary parenchyma volume from the parenchymatous tissue volume. This is expressed in cm^3.

Number of PAS: This was obtained considering that the shape of the PAS is close to that of a sphere (Gil et al. 1979), the alveolar cord being its radius. After calculating the volume of the distal air units, the air volume is divided by the volume of the distal airspace.

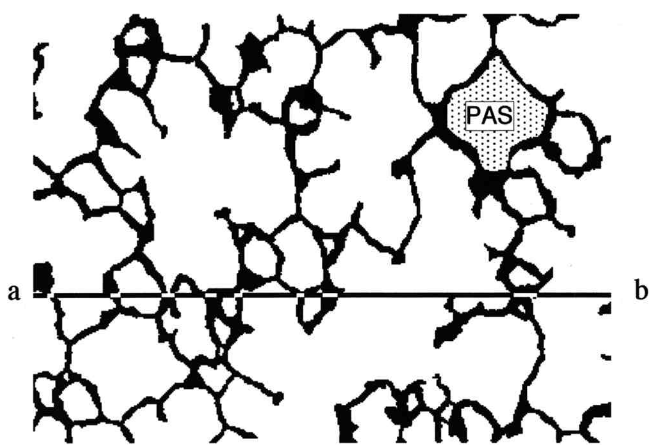

Fig. 6. Fig. 4 converted into a two-color image: white for air and black for tissue. PAS: a peripheral airspace. On the line "ab" the wall thickness measurements are represented in white and the alveolar cord measurements in black.

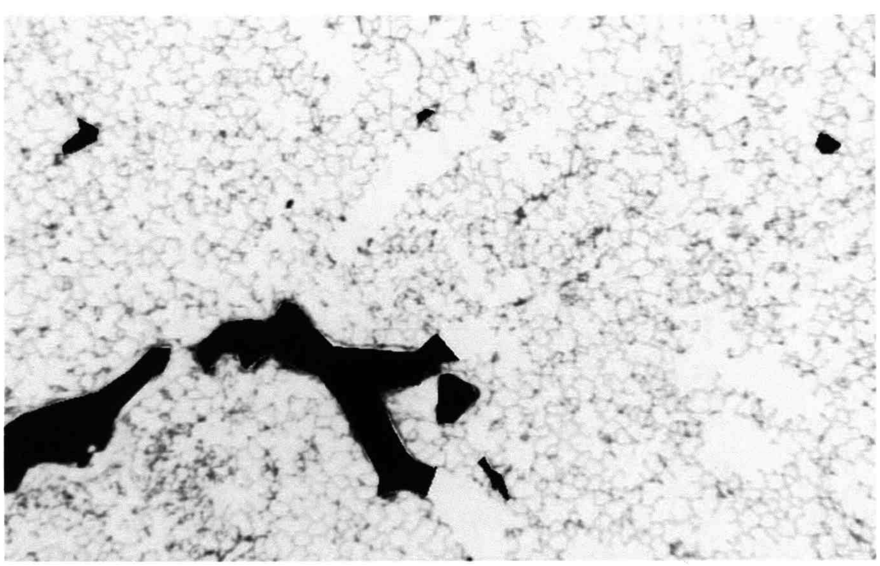

Fig. 7. Fig. 5, in which the quantified vessel and airway surface areas have been highlighted in black.

This is expressed as multiplied by 10^{-6}.

Internal alveolar surface (IAS): It is calculated from the volume of the pulmonary parenchyma and the internal alveolar perimeter (Escolar et al., 1996). This is expressed in cm^3 times 10^{-7}.

Statistical study

The values are presented as mean ± a standard deviation. When the values came close to normal distribution (Kurtosis and Skewness rates) they were compared with the ANOVA test. If they did not follow normal distribution, non-parametric tests were applied, first the Kruskal-Wallis test, followed by Mann-Whitney's U test. All the variables were related to the pulmonary volume, using the correlation test, considering $r = 0.6$ to be good coefficients. The statistics program used was StatView® 5.0.

Results

The results are shown in figures 8 to 17 and in Table 1 and from them we wish to emphasize: It has been assumed that the pulmonary volume reached in the lungs filled with liquid fixative at 25 cm corresponded with 100% of TLC. Thus, 45% TLC corresponded to the lungs from the 10-cmI group, 51% to those of the 20-cmI group, 66% to the 20-cmD group, and 55% to the 10-cmD group, (Fig. 8). The pulmonary volume (Fig. 8), the pulmonary parenchyma volume (Fig. 9), and the distal airspace volume (Fig. 10) increased/decreased upon the raising/lowering of the TPP. The results of these variables obtained in deflation were superior to those obtained in deflation; there were significant differences between lungs fixed at 20 cm of TPP. The highest value was obtained in lungs filled with liquid fixative at 25 cm; this was significant in comparison with the other four groups in which lungs were fixed by airway. The behavior of the volume of the parenchymatous tissue (Fig. 11) was not linear with respect to the modifications of the TPP and no group displayed significant differences when compared to the others.

The behavior of the variables related with the size of the PAS, alveolar cord (Fig. 12), the area of the PAS (Fig. 13), and the Lm (Fig. 14), was very similar in the five groups and did not show any significant differences.

The wall thickness (Fig. 15) decreased/increased when the TPP increased/decreased, reaching significant values in the lungs fixed through the trachea to 25 cm,

Table 1. Results from the correlation of the pulmonary volume variable with the rest of the variables.

Pul. Par. Vol.	.99
Par. Tiss. Vol.	.95
Dist. Air Vol	.99
Alv. Cord	.16
Lm.	-.06
PAS Area	.35
Wall Thick.	-.25
IAS	.98
No. PAS	.65

Pul. Par. Vol.: Pulmonary parenchyma volume. Par. Tiss. Vol.: Parenchymatous tissue volume. Dist. Air Vol.: Distal airspace volume. Alv. Cord: Alveolar cord. Lm: Mean linear intercept index. PAS Area: Peripheral airspace area. Wall Thick: Wall thickness. IAS: Internal alveolar surface. No. PAS: Number of PAS. The correlation coefficients (r) are considered to be good when they are equal to or greater than 0.6.

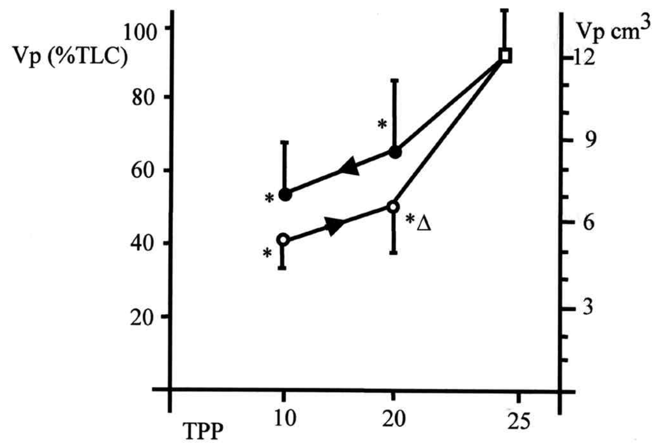

Fig. 8. Representation of the values obtained in the pulmonary volume (Vp) variable. They are represented as a percentage fraction of the TLC at left and in cm^3 at right. *: $p < 0.05$ with respect to the control group; Δ: $p<0.05$ with respect to the 20 cm D group.

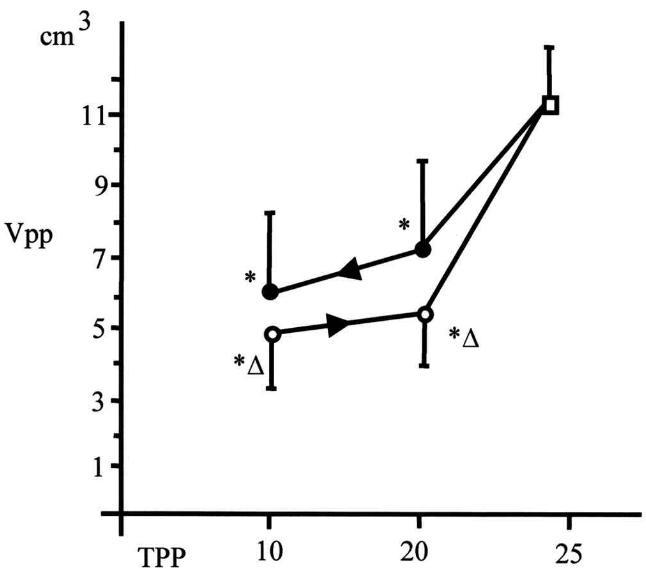

Fig. 9. Representation of the values obtained in the pulmonary parenchyma volume (Vpp) variable. *: $p<0.05$ with respect to the 25 cm group; Δ: $p<0.05$ with respect to the 20 cm D group.

compared to the four groups insufflated with air. The values obtained in deflation were very similar to those obtained during inflation.

The behavior of the IAS and the number of PAS was very similar to that of the pulmonary volumes; they increased/decreased upon rising/lowering of TPP (Fig. 16). The IAS values were higher in deflation than in deflation, reaching significant differences among the lungs fixed to 20 cm. The highest value was reached by lungs fixed to 25 cm through the trachea and was found to be significant compared to the rest of the groups.

The pulmonary volume gave good rates when related with the distal pulmonary volume, the volume of parenchymatous tissue, the IAS and the number of PAS. The correlation rates obtained with the rest of the variables were low (Table 1).

Discussion

In order to show that the increase/decrease of the

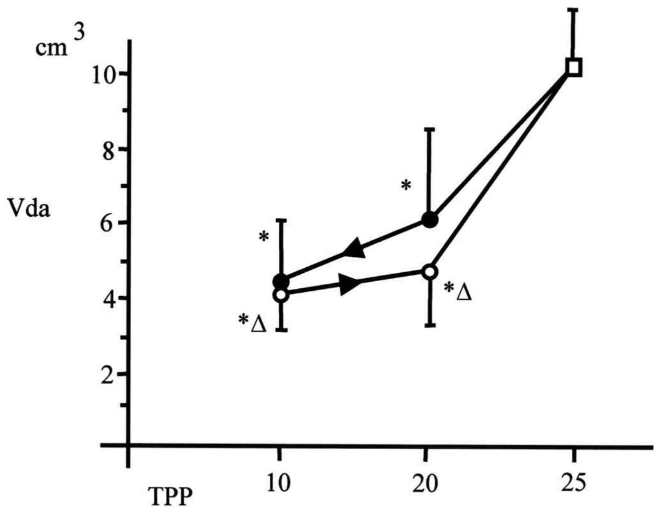

Fig. 10. Representation of the values obtained in the distal air volume (Vda) variable. *: p<0.05 with respect to the 25 cm group; Δ: p<0.05 with respect to the 20 cm D group.

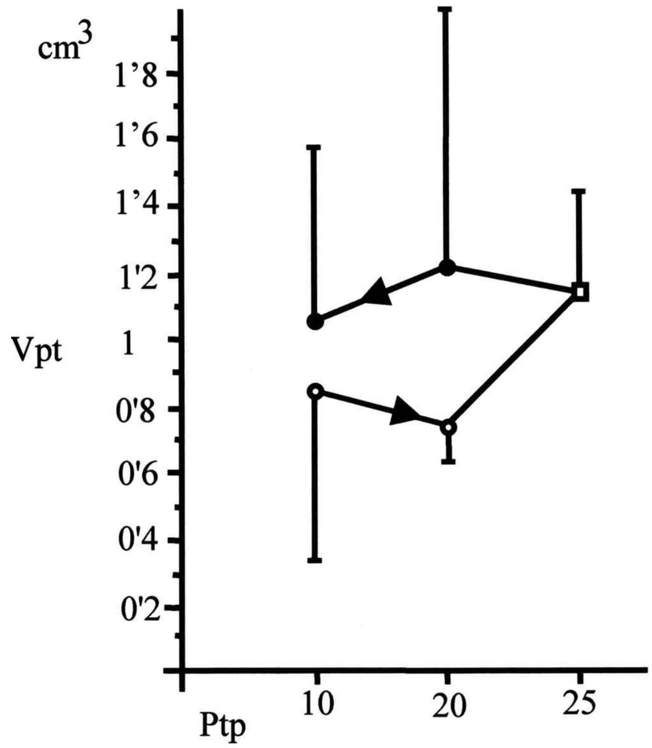

Fig. 11. Representation of the values obtained for the parenchymatous tissue volume (Vpt) variable.

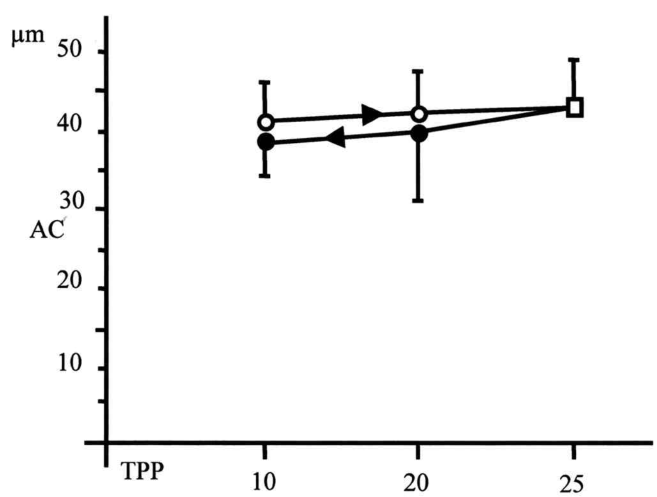

Fig. 12. Representation of the values obtained for the alveolar cord (AC) variable.

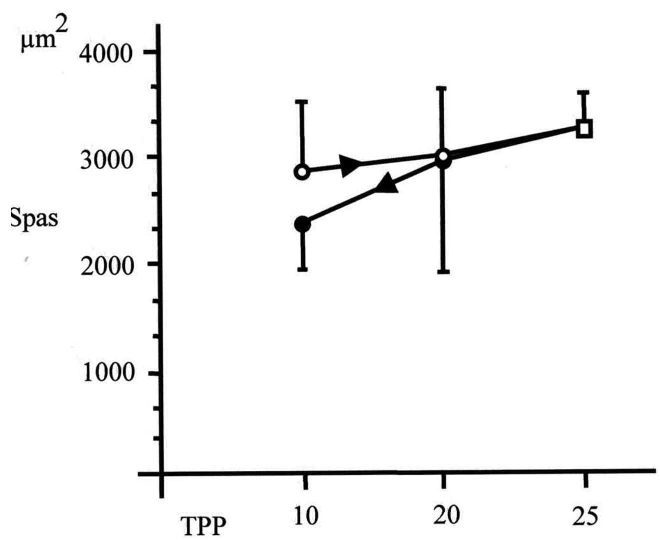

Fig. 13. Representation of the values obtained for the peripheral airspace area (PAS) variable.

pulmonary volume is produced by an alveolar recruitment/decrease, a model of the respiratory cycle has been developed on the basis of the proposal of Gil et al. (1979). We have made tissue measurements at points of the PV-curve that should cover between 40% and 90% of the TLC. Nonetheless, the few statistically significant differences found when comparing the groups among themselves have led us to reconsider what fraction of TLC is reached in the different groups. The liquid-filled lung requires less TPP to reach TLC than the gas-filled lung (Bachofen et al., 1970; Gil et al., 1979). This is not an obstacle for assuming that the TLC is the same in air-filled as in liquid-filled lungs. We propose, as a reference of 100% of TLC, lungs filled with liquid at 25 cm of TPP; in our laboratory, 25 cm of TPP is the minimum accuracy necessary for reaching TLC (Escolar et al., 2000). With this procedure it was possible to deduce that the volumes reached are within a narrower range (Fig 8). The differences found between our results and those of Gil et al. (1979) did not surprise us since there exists no unanimity on the TPP necessary for reaching TLC in lungs filled with gas (Klingele and Staub, 1970; Gil et al., 1979; Rimensberger et al., 1999).

When the TPP rose or fell, the lung expanded or retracted to the degree that the distal airspace volume increased and decreased. The fact that the pulmonary

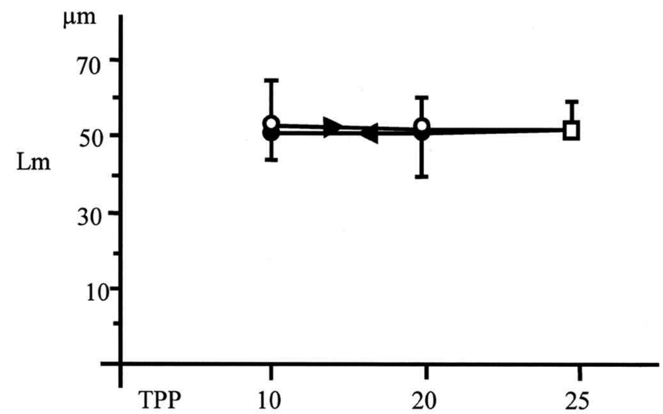

Fig. 14. Representation of the values obtained for the Lm variable.

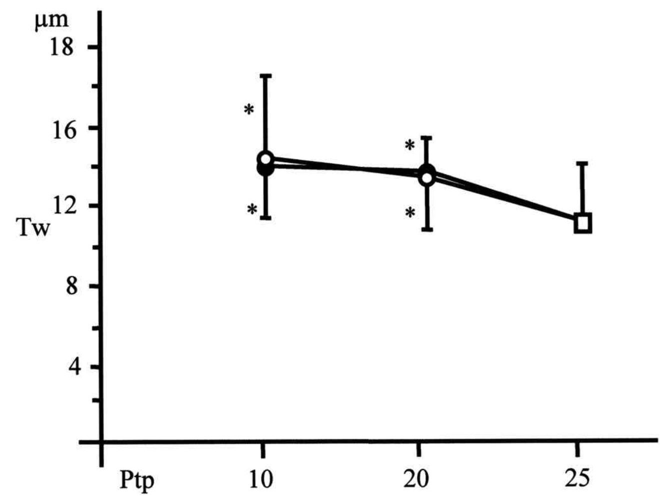

Fig. 15. Representation of the values obtained for the wall thickness (Tw) variable. *: p< 0.05 with respect to the 25 cm group.

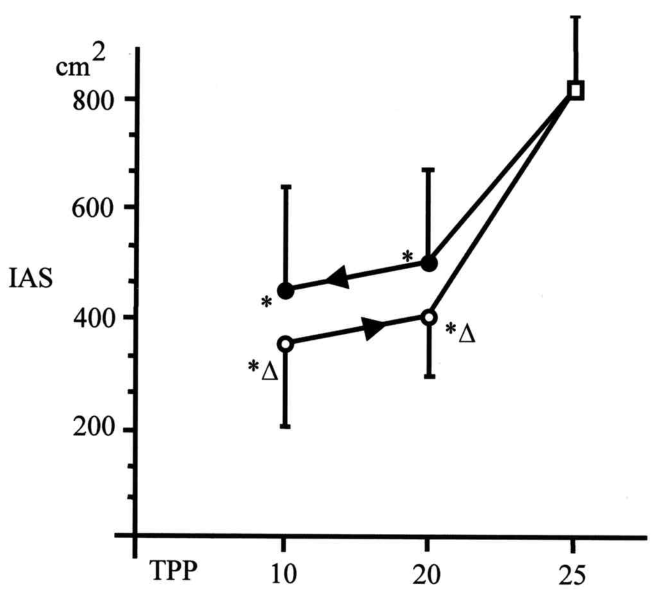

Fig. 16. Representation of the values obtained for the internal alveolar surface (IAS) variable. *: p<0.05 with respect to the 25 cm group; Δ: p<0.05 with respect to the 20 cm D group.

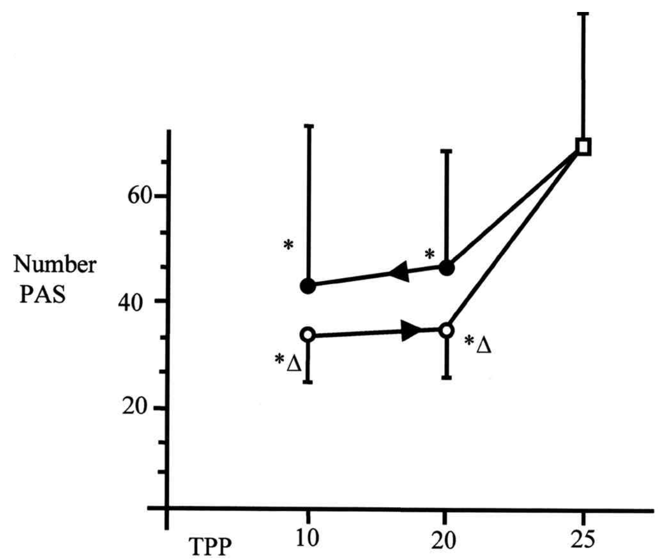

Fig. 17. Representation of the values obtained for the number of peripheral airspaces (Nº PAS) variable. *: p<0.05 with respect to the 25 cm group; Δ: p<0.05 with respect to the 20 cm D group.

parenchymatous tissue did not undergo substantial quantitative modification considerably reduces the possibility of an artifact (Mercer et al., 1987; Miserocchi et al., 1993). This is important if our results are compared with those for unfixed tissues (Carney et al,. 1999).

The origin of the discrepancies in the size of the PAS among authors who are in favour of or against the hypothesis of recruitment may lie in the different methodologies used: conservation of the tissue, freezing (Storey and Staub, 1962; Forrest, 1970; Klingele and Staub, 1970); vascular fixation (Gil and Weibel, 1972); in vivo study (D'Angelo, 1972; Carney et al., 1999); quantified variables such as the radius of the alveolus (Forrest, 1970), Lm (Gil and Weibel, 1972), point count (Carney et al., 1999), alveolus mouth diameter and maximum alveolus depth (Klingele and Staub, 1970) and the pulmonary insufflation degree, since some authors present this as a fraction of the TLC (Forrest, 1970; Klingele and Staub, 1970) and others as a fraction the TPP (Storey and Staub, 1962; D'Angelo, 1972; Gil and Weibel, 1972; Mercer et al., 1987). We could in fact add many more possible causes of possible discrepancy, such as the apparatus used for insufflating the lungs, insufflation/deflation time, number of respiratory cycles preceding pulmonary incision, etc. All this makes it difficult to reach a conclusion regarding the exact causes of the discrepancies in the results. Our results coincide with those of Carney et al. (1999), who suggest that at the beginning of insufflation the lung is distended by the increase of the PAS up to a determined volume, 20% of TLC, after which the lung increases in volume due to the aperture of the PAS and not due to any modification in the size of the PAS. One cannot discard the possibility of the size of a small number of PAS being modified during the respiratory cycle (Gil et al., 1979). However, we do not consider this number to influence the normal functioning of the lung. For Bachofen et al. (1970) the PAS are larger in saline-filled lungs than in air-filled lungs. The size of the PAS in lungs filled with liquid fixative was somewhat larger than in lungs filled with gas. We consider these size differences to be minimal, since the size of the PAS of the lungs used showed no substantial modifications.

The IAS is a parameter that always increases with insufflation (Dunnill, 1967; Klingele and Staub, 1970; D'Angelo, 1972). Gil and Weibel (1972) describe this as the consequence of an unfolding of the walls of the PAS. Despite the fact that wall thickness has been considered an unreliable variable (Gil et al., 1979), there have been descriptions of air-filled and liquid-filled in which the wall thickness decreased during inflation (Gil and Weibel, 1972; Tsunoda et al., 1974). We suggest two possible reasons why wall thickness may decrease during inflation: The alveolus dilates and its walls stretch like elastic, becoming thinner. At the beginning of inflation the walls are folded forming a bulge and upon unfolding the walls thin. However, we consider that the interpretation of the results of the variable wall thickness involves certain difficulties (Escolar et al., 1997).

The answer to the question of whether the change in lung volume as manifested in the PV-curve reflects what occurs at the level of 300 million alveoli is far from straightforward. Perhaps this question has been raised because the interpretation of what occurs at the level of alveolar architecture during respiration has been made on the basis of functional studies (Cheng et al., 1995). The ascending part of the PV-curve (Fig. 1) is inferior to the descending part because at the outset of insufflation the pulmonary volume increases more slowly than at the end. During deflation, the situation is similar: at the outset the lung de-insufflates more slowly than at the end. The existing surface between the ascending and descending part of the PV-curve is known as pulmonary hysteresis (Cheng et al., 1995). The hypothesis of recruitment assumes that the opening of the PAS is greater at the end of insufflation than at the beginning and that de-recruitment increases at the end of the deflation. If the number of PAS is related with the pulmonary volume, according to the PV-curve, there would be more PAS open during deflation than in inflation. Our results support the hypothesis of recruitment, since the number of alveoli has increased/decreased whenever the pulmonary volume has done so. We can propose that the pulmonary volume increases/decreases due to recruitment/de-recruitment of the PAS. At the same TPP the pulmonary volume and the number of PAS were greater in deflation than in insufflation, which supports the proposal of Smaldone et al. (1983) that pulmonary hysteresis is related to changes in the population of the alveoli.

In conclusion, through measurements of fixed tissue we have been able to demonstrate that the increase/decrease in pulmonary volume is mainly due to recruitment/de-recruitment of the PAS and that in a single TPP the number of PAS open is greater in deflation than in inflation.

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References

Amato M.B.P., Barbas C.S.V., Medeiros D.M., Magaldi R.B., Schettino G.P.P., Lorenzi-Filho G., Kairalla R.A.A., Deheinzelin D., Munoz C., Oliveira R., Takagaki T.Y., and Carvalho C.R.R. (1998). Effect of a protective-ventilation strategy on mortality in the acute respiratory distress syndrome. N. Engl. J. Med. 338, 347-354.

Bachofen H., Hildebrandt J. and Bachofen M. (1970). Pressure-volume curves of air- and liquid-filled excised lung-surface tension in situ. J. Appl. Physiol. 29, 422-431.

Carney D.E., Brendenberg C.E., Schiller H.J., Picone A.L., McCann II U.G., Gatto L.A., Bailey G., Fillimger M. and Nieman G.F. (1999). The mechanism of lung volume change during mechanical ventilation. Am. J. Respir. Crit. Care Med. 160, 1697-1702.

Cheng W., DeLong D.S., Franz G.N., Petsonk E.L. and Frazer D.G. (1995). Contribution of opening and closing of units to lung hysteresis. Respir. Physiol. 102, 205-215.

D'Angelo E. (1972). Local alveolar size and transpulmonary pressure in situ and in isolated lungs. Respir. Physiol. 14, 251-266.

Dreyfuss D. and Saumon. G. (2001). Pressure-volume curves. Searching for the grail or laying patients with adult respiratory distress syndrome on Procrustes' bed? Am. J. Respir. Crit. Care Med. 163, 2-3

Dunnill M.S. (1967). Effect of lung inflation on alveolar surface area in the dog. Nature 214, 1013-1014.

Escolar J.D., Gallego B., Tejero C. and Escolar M.A. (1994). Changes occurring with increasing age in the rat lung: Morphometrical study. Anat. Rec. 239, 287-296.

Escolar J.D., Martínez M.N., Escolar M.A., Arranz M., Gallego B. and Roche P.A. (1996). Tobacco smoke and age a risk factor in emphysema. Morphometrical study on the rat. Histol. Histopathol. 11, 7-16.

Escolar J.D., Tejero C., Escolar M.A., Montalvo F. and Garisa R. (1997). Methodological contribution for the morphometric study of the lung: approximation to the ideal sample size and quantification. Anat. Rec. 247, 501-511.

Escolar J.D., Tejero C., Escolar M.A., Garisa R. and Roques M. (2000). Ideal transpulmonary pressure for excised lung. Morphometric study of the rat. Eur. J. Anat. 4, 53-60.

Forrest J.B. (1970). The effect of changes in lung volume on the size and shape of alveoli. J. Physiol. 210, 533-547.

Gil J. and Weibel E.R. (1972). Morphological study of pressure-volume hysteresis in rat lungs fixed by vascular perfusion. Respir. Physiol. 15, 190-213.

Gil J., Bachofen H., Gehr P. and Weibel E.R. (1979). Alveolar volume-surface area relation in air- and saline-filled lungs fixed by vascular perfusion. J. Appl. Physiol. 47, 990-1001.

Hansen J.E. and Ampaya E.P. (1974). Lung morphometry: a fallacy in the use of the counting principle. J. Appl. Physiol. 37, 951-954.

Hickling K.G. (2001). Best compliance during a decremental, but not incremental, positive end-expiratory pressure trial is related to open-lung positive end -expiratory pressure. A mathematical model of acute respiratory distress syndrome lungs. Am. J. Respir. Crit. Care Med. 163, 69-78.

Klingele T.G. and Staub N.C. (1970) Alveolar shape changes with volume in isolated air-filled lobes of cat lung. J. Appl. Physiol. 28, 411-414.

Lichtwarck-Aschoff M., Mols G., Hedlund A.J., Kessler V., Markström A.M., Guttmann J., Hedenstierna G. and Sjöstrand U.H. (2000). Compliance is nonlinear over tidal volume irrespective of positive end-expiratory pressure level in surfactant-depleted piglets. Am. J. Respir. Crit. Care Med. 162, 2125-2133.

Mercer R.R., Laco J.M. and Crapo J.D. (1987). Three-dimensional reconstruction of alveoli in the rat lung for pressure-volume relationships. J. Appl. Physiol. 62, 1480-1487.

Miserocchi G., Negrini D., del Fabbro M. and Venturoli D. (1993). Pulmonary interstitial pressure in intact in situ lung: transition to intertitial edema. J. Appl. Physiol. 74, 1171-1177.

Pinkerton K.E., Barry B.E., O´Neil J.J., Raub J.A., Pratt P.C. and Crapo J.D. (1982). Morphologic changes in the lung during the lifespan of fischer 344 rats. Am. J. Anat. 164, 155-174.

Rimensberger P.C., Cox P.N., Frndova H. and Bryan A.C. (1999). The open lung during small tidal volume ventilation: Concepts of recruitment and "optimal" positive end-expiratory pressure. Crit. Care. Med. 27, 1946-52.

Smaldone G.C., Mitzner W. and Itoh H. (1983). Role of alveolar recruitment in lung inflation: influence on pressure-volume hysteresis. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 55, 1321-1332.

Storey W.F. and Staub A.C. (1962). Ventilation of terminal air units. J. Appl. Physiol. 17, 391-397.

Tsunoda S., Fukaya H., Sugihara T., Martin C.J. and Hildebrandt J. (1974). Lung volume, thickness of alveolar walls, and microscopic anisotropy of expansion. Respir. Physiol. 22, 285-296.

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