REGENERATIVE HYPERTROPHY OF THE LUNGS IN RATS AFTER ONE-STAGE REMOVAL OF THE ENTIRE LEFT LUNG AND THE DIAPHRAGMATIC LOBE OF THE RIGHT LUNG

COMMUNICATION I. CHANGES IN THE NUMBER AND DIMENSIONS OF THE ALVEOLI AND IN THE THICKNESS OF THE INTERALVEOLAR SEPTA

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During the last 10-15 years both abroad [14, 19] and in the Soviet Union [1, 10, 13] one-stage bilateral operations for removal of up to 50-60% of functioning lung tissue have been successfully performed. It is, therefore, particularly important to investigate the compensatory powers both of the body as a whole and of the residual lung [2, 3, 5, 7, 11, 12, 16]. Insufficient attention has been paid, however, to the study of the compensatory mechanisms brought into play during exclusion of the greater part of the respiratory surface. For instance, contradictory conclusions have been reached regarding the character of the changes in the residual lung.

In the opinion of a number of authorities [9, 17, 20], after extensive resections emphysema develops in the lung, and this gradually leads to functional atrophy of the organ.

On the other hand, investigations by B. G. Gol'dina [6], A. A. Birkun [4] and I. K. Esipova and E. V. Ryzhkov [8] have shown that the compensatory, adaptive changes in the residual lung after resection are expressed morphologically by the opening up of physiological atelectases, the enlargement of the alveoli and hypertrophy of the interalveolar septa, the regeneration of the elastic framework of the alveoli and the formation of new capillaries etc.

Tiemann [21], Cohn [15] and Hilber [18] cite data showing that the formation of new alveoli may occur, and stress the importance of this process in the restoration of the removed lung tissue.

The aim of the present investigation was to investigate the changes in the size of the alveoli and in the functional capacity of the hypertrophied alveoli, i.e., to obtain numerical data relating to the morphological characteristics of the process of regeneration after extensive resections.

METHODS

The experiments were carried out on sexually mature male rats weighing 197-324 g, kept on the usual briquette diet of the institutions of the AMN SSSR. Under ether anesthesia, and observing the rules of asepsis and antisepsis, the diaphragmatic lobe of the lung was exteriorized through an incision in the sixth intercostal space by means of fenestrated ophthalmic forceps. A silk ligature was applied to the hilum of this lobe, and the lobe was excised by cutting through the parenchyma of the lung with scissors. After the muscles and skin had been

TABLE 1

Number of Animals Used at Various Periods after Operation

Group of animals	Period after operation				Total number of animals
	7 days	1 month	3 months	6 months	
	Number of animals used				
Experimental	8	13	10	11	42
Control	9	7	10	11	37

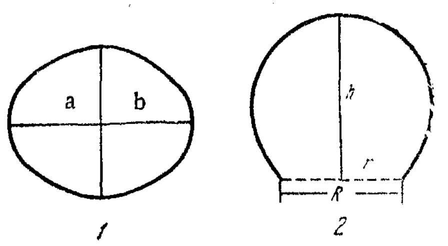

Fig. 1. Types of alveoli measured and their parameters. 1) Alveolus of closed type: a) maximum diameter; b) minimum diameter – a line drawn at right angles to the maximum diameter; 2) alveolus of open type: R) width of inlet to alveolus – a line joining the ends of the open outline; h) depth of the alveolus – a line perpendicularly bisecting R.

closed in layers with silk sutures, an incision was made in the fifth intercostal space on the left, and the left lung excised by the method described above. Disulfan powder was sprinkled inside the chest. Since the left lung accounts for 36.8% by weight, and the diaphragmatic lobe of the right lung 25.7% by weight of the whole lung tissue, in our operations of one-stage removal of the left lung and the diaphragmatic lobe of the right lung, in practice we removed about 63% of the lung tissue. In order to evaluate the results obtained, 79 rats were investigated. The periods of investigation were: 7 days, 1, 3, and 6 months (Table 1).

A comparative appraisal of the state of the lung as a whole (its volume and dimensions) and, in particular, of its morphological structure (dimensions of the alveoli, thickness of the interalveolar septa, etc.) may be obtained only if certain rules of fixation are observed. For this reason the sacrifice of the animals and fixation of the lungs were carried out as follows. Under ether anesthesia the trachea was dissected out and, at the moment of inspiration corresponding to the maximum volume of the lungs, a ligature was applied to it. The chest was then opened with care, in order not to injure the lungs. A 7% glucose solution was then injected under a pressure of 30-35 mm Hg through a cannula introduced through the incision into the right ventricle of the heart and the pulmonary artery, until the bulk of the lung tissue had become pale pink in color, indicating that the lung capillaries were free from blood. The drainage was effected through an incision in the left ventricle. Under the same pressure, Susa's fixing solution was injected into the pulmonary vessels until the tissues had become pale, indicating that the solution had penetrated into the capillaries. The heart-lung tissue-complex was extracted from the chest and immersed in the fixing solution for 24 hours, after which the lungs were treated in the usual way. Before the volume of the lobes was determined the lungs were placed in 70° alcohol until they sank to the bottom of the vessel, indicating that the air had been expelled from most of the alveoli by the alcohol. The volume of the lungs was determined by the volume of 70° alcohol expelled into a graduated cylinder. The upper lobe of the right lung, facing in the same direction in every case, was embedded in paraffin wax and sections of it were cut to a thickness of 7.15μ and 25μ. The sections were stained with hematoxylineosin by Mallory's method and with fuchsin by Weigert's method. To define the histological characteristics of the lungs, ten animals were studied (5 experimental and 5 control) at each period. By means of an Abbe drawing apparatus, under magnification of 400 times, from the sections cut to a thickness of 25μ the outline of 100 alveoli of closed type and 100 alveoli of open type were traced on paper (Fig. 1). By using such a large number of alveoli it is possible to smooth out the differences in their dimensions resulting from differences in the degree of stretching of the alveoli in different areas of the lobe. The area of the closed alveoli was calculated from the fomula for the area of an ellipse ($S = \pi ab$), having first measured the maximum diameter of the alveolus and the line perpendicularly bisecting this diameter. In order to describe the alveoli of open type, two measurements were taken: R – the width of the inlet to the alveolus and h – the depth of the alveolus, i.e., a line perpendicularly bisecting the line closing the inlet to the alveolus (R). The values of these measurements were used to determine the volume of the alveoli from the formula for the volume of a segment

TABLE 2

Changes in the Volume of the Residual Lobes of the Right Lung at Different Periods after Operation

Group of animals	Time of observation	Volume of the left lung (in cc)	Vol. of right lung (in cc): diaphragmatic lobe	upper lobe	middle lobe	accessory lobe	total	Vol. inc. related to rt. lung of control animals without diaphragmatic Lobe (in %)	Vol. inc. related to both lungs of control animals (%)
Experimental	7 days	—	—	1,40	1,48	1,32	4,20	124,5	—
Control		1,70	1,35	0,56	0,71	0,60	3,22		
Experimental	1 month	—	—	2,25	2,36	2,32	6,93	219,3	15,5
Control		2,00	1,83	0,65	0,78	0,74	4,00		
Experimental	3months	—	—	2,37	2,35	2,21	6,93	228,4	25,5
Control		1,90	1,51	0,67	0,74	0,70	3,62		
Experimental	6 months	—	—	2,36	2,43	2,52	7,31	293,0	37,6
Control		1,88	1,57	0,55	0,70	0,61	3,43		

TABLE 3

Changes in the Area and Volume of the Alveoli and the Thickness of the Interalveolar Septa and in the Number of Alveoli in the Upper Lobe at Different Periods after Operation

Group of animals	Period of observation	Volume of upper lobe (in cc)	Area of alveolus (in μ^2)	Volume of alveolus (in μ^3)	Number of alveoli in upper lobe	Thickness of of interalveolar septa (in μ)
Experimental	7 days	1,40	3576	140×10^3	10×10^3	7,56
Control		0,56	2435	81×10^3	$6,9\times10^6$	5,89
Experimental	1 month	2,25	6763	322×10^3	$7,1\times10^3$	6,47
Control		0,65	3187	128×10^3	$5,0\times10^6$	5,91
Experimental	3 months	2,37	6373	256×10^3	$9,2\times10^3$	7,41
Control		0,67	2713	80×10^3	$8,3\times10^3$	6,60
Experimental	6 months	2,36	6763	329×10^3	$7,1\times10^3$	7,31
Control		0,55	2303	69×10^3	$7,9\times10^6$	6,08

of the sphere:

$$V = \frac{1}{6}\pi h(h^2 + 3r^2),\ \text{where } r = \frac{R}{2}\ .$$

In order to solve the problem of the number of alveoli, the volume of the lobe was divided by the mean volume of one alveolus. In each case we measured the thickness of 50 interalveolar septa throughout the section from the upper lobe by means of an ocular micrometer.

RESULTS

One week after the operation the residual lobes (upper, middle and accessory) of the right lung had already increased in volume by comparison with the analogous lobes of the lungs of control rats. The degree of enlargement of the lobes varied at different times (Table 2).

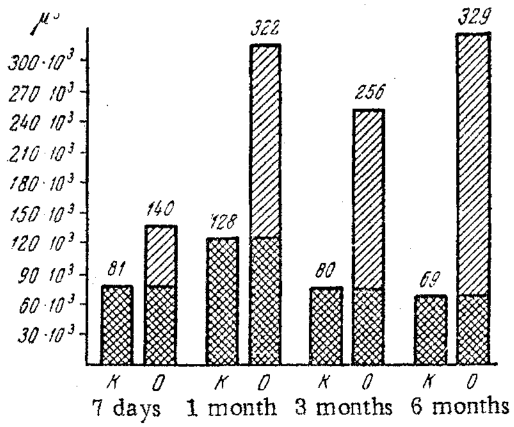

Fig. 2. Changes in the volume of the individual alveoli of the right lung at various periods after operation. K) controls; O) experimental. The more lightly shaded part of the column represents the increase in volume over that of the controls.

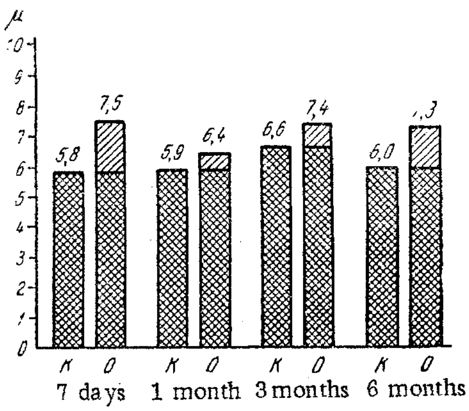

Fig. 3. Thickness of the interalveolar septa of the right lung at various periods after operation. K) controls; O) experimental. The more lightly shaded part of the column represents the increase in thickness over that of the controls.

Seven days later the enlargement of the upper lobe amounted to 150%, the middle lobe 108.4% and the accessory lobe 120% over the volume of the corresponding lobes of the lungs of the control rats. It it is remembered that the total volume of the lobes left behind at operation amounted on the average to 1.87 cc, then the increase in their volume was 2.33 cc (4.20-1.87 cc), i.e., 2.2 times. The volume of the operated lung, however, still had not reached the volume of both lungs of the control animals (Table 2). One month later, the remaining lobes of the right lung were so enlarged that they occupied the whole thoracic cavity, displacing the heart to the left. The operated lung not only had reached the volume of the right lung of the control animals, but was actually 15.5% larger in volume than both lungs together of the control animals. Subsequently (3 and 6 months later) a further increase in volume took place, but this was relatively slight.

It may be postulated that the increase in the volume of the residual part of the organ is the result: 1) of an increase in the volume of the remaining alveoli and of hypertrophy of the interalveolar septa, following cellular hyperplasia and an increase in the number of elastic fibers and capillaries; 2) of the opening up of physiological atelectases and subsequent hypertrophy of both the newly opened and the previously functioning alveoli, with preservation of the normal morphological structure of the interalveolar septa; 3) of mechanical stretching of the alveoli with thinning of the interalveolar septa and emptying of the capillaries, leading to the development of emphysema. Finally, the possibility is not excluded 4) of the formation of new alveoli, the functioning of which may also influence the volume of the operated lung.

Since the increase in the volume of the individual lobes of theremnant of the lung was roughly the same, we shall subsequently use only the results obtained from the study of the upper lobes, assuming that analogous changes took place in the other lobes.

Analysis of the results obtained by measurement of the alveoli showed that at early periods after operation a considerable increase was already taking place in the area and volume of the alveoli (Table 3).

On the basis of these findings and of measurement of the volume of the upper lobe, we provisionally calculated the number of alveoli in the upper lobe.

Seven days later the mean volume of one alveolus was increased 1.7 times. As the postoperative period advanced a further increase in volume was observed: after one month the mean volume of one alveolus was 2.5 times, after 3 months 3.2 times and after 6 months 4.7 times greater than the mean volume of one alveolus of the lungs of the control animals, as is shown graphically in Fig. 2.

Besides the increase in the volume of the alveoli, mention must be made of the hypertrophy, admittedly very slight, of the interalveolar septa (which is illustrated in Fig. 3), which were rich in cells and capillaries.

On examination of the entire lobe areas of emphysematous change in the parenchyma of the lung could be seen, with grossly dilated alveoli, the walls of which were thinned and lacking in capillaries. These areas were mainly found at the periphery of the lobe and occupied a comparatively small space.

The increase in the volume of the residual part of the organ evidently took place not only on account of the true hypertrophy and emphysema of previously functioning alveoli, but also as a result of the development of "new" alveoli. For instance, after seven days, the number of alveoli in the upper lobe of the lung of the experimental animals, according to our calculations, was greater by 3.1×10^6 on the average than the number in the upper lobe of the lung of the control rats. Since the mean volume of the alveolus at this period was 140×10^3, it may be considered that the addition of a number of alveoli in the early periods after operation takes place as a result of the opening of physiological atelectases, with the subsequent hypertrophy of the alveoli, and not on account of the formation of new alveoli, for the dimensions of such newly developing alveoli would be smaller. We cannot categorically deny that the formation of new alveoli is possible.

If this process does take place, however, it cannot be the principal mechanism of regeneration of the injured organ.

It may be concluded from these results that a leading part in the process of recovery after resection of a considerable proportion of the lung tissue is played by the reconstruction of the parenchyma of the residual part of the organ, accompanied by the opening up of physiological atelectases, by an increase in the volume of both the previously functioning alveoli and of those newly opened up, by an increase in the thickness of the interalveolar septa, by cell hyperplasia and by the formation of capillaries. The appearance of foci of emphysema in the operated lung is not a dominant sign of the process of morphological reconstruction.

Because the increase in the volume of the residual lung, when caused by injury, is mainly the result of hypertrophy of the structural units of the organ – the alveoli – and also of proliferation of the cells of the interalveolar septa and of an increase in the number of capillaries, and because it is regenerative in character this process may be described as regenerative hypertrophy, a characteristic form of regeneration of many internal organs.

SUMMARY

In 42 white rats the left lung and the diaphragmatic lobe of the right lung, which represented 63% of the total lung tissue, were removed. Measurement of the thickness of the interalveolar septa of the volume of the alveoli and their number at different periods after the operation showed that in the process of compensatory readjustment of the pulmonary parenchyma the main role belongs to "regenerative hypertrophy," which is characterized by a considerable increase of volume of the remaining part of the lung. The latter is a result of an increase of volume of the already functioning and of the newly opened alveoli, of thickening of the interalveolar septa of hyperplasia of the cellular elements and of the formation of new capillary vessels.

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