NUCLEIC ACID SYNTHESIS AND MITOTIC ACTIVITY DURING DEVELOPMENT OF COMPENSATORY HYPERTROPHY OF THE LUNG IN RATS

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In the process of regeneration of the liver and compensatory hypertrophy of the kidney, together with a considerable increase in weight and stimulation of proliferation in the remaining part of the organ, the nucleic acid content is increased when calculated for the whole organ [4, 5, 7, 13]. This is evidences of a true restoration of the mass of the organ.

The more detailed study of the changes in nucleic acid synthesis during regeneration of the internal organs of mammals has been carried out mainly on the regenerating liver as experimental model [6, 8-12]. However, so that the general principles governing the biochemical changes during compensatory hypertrophy and regeneration can be established, it is imprtant to study this problem in relation to other organs.

The object of the present investigation was to study the character of the changes in nucleic acid synthesis and in the morphological indices (weight, mitotic activity) during compensatory hypertrophy of the lung in the early stages after pneumonectomy.

Investigations of this type not only provide a more detailed picture of the completeness of recovery of the lung, but also shed light on the special features of the course of compensatory hypertrophy in the particular organ. This enables the experimental conditions to be planned for future studies of the mechanisms of regeneration and compensatory hypertrophy of the lungs.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 160-250 g. The left lung, approximately 37% of the total lung tissue, was removed from the experimental animals. A mock operation was carried out on the control rats, in which thorax was opened and the wound then closed in layers. A group of intact animals served as first control.

Two series of experiments were undertaken. In series I the changes in weight of the residual lung were determined at various times after removal of the left lung. For this purpose rats of the same stock underwent operation at the same time and were sacrificed by decapitation, in groups of 6-7 animals at a time, 24, 36, and 48 h and 3, 5, 7, and 10 days after the operation.

In the experiments of series II the course of synthesis of nucleic acids and the character of the change in the dry weight and mitotic activity of the lung cells were studied during the development of compensatory hypertrophy. In these experiments animals of different stock underwent the operation, and the experimental material was accumulated gradually. The periods of observation in this series were 20, 24, and 48 h and 3, 4, 5, and 7 days after the operation. At each time 4-11 animals were sacrificed. Since the largest number of dividing cells in rats' lungs is observed in the mornings (7-10 a.m.) [1], as a rule the animals were sacrificed at 9-11 a.m.

Measurement of the dry weight of the lung and determination of the concentration of RNA in $\mu g/100$ mg dry weight of tissue by the method of Schmidt and Thannhauser [14], followed by spectrophotometry by A. S. Spirin's method [3], were carried out on the experimental and control rats at the time of operation in the left lung, and at various times after its removal, in the right lung.

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TABLE 1. Change in Weight of the Lungs of Experimentatl and Control Rats at Various Times after Operation.

Time after operation	Group of animals	Number of animals	Weight of lungs: Right, In mg	Weight of lungs: Both, In mg	Weight of lungs: Both, In % of body weight	Dry weight of 100 mg tissues (in mg): Left lung at time of operation	Dry weight of 100 mg tissues (in mg): Right lung at time of sacrifice
	Control (without mock operation)	9	902,0	1392,0	0,56	—	—
20 h	Experiment.	8	1236,0	—	0,55	21,6	18,8
24 h	Control (with mock operation)	4	740,0	1147,0	0,62	—	—
	Experiment.	6	1220,0	—	0,61	22,4	19,2
36 h	Experiment	8	932,0	—	0,51	—	—
48 h	Control (with mock operation)	4	1350,0	1955,0	0,68	—	22,0
	Experiment	6	986,0	—	0,52	22;0	21,8
3 Days	Control (with mock operation)	12	854,0	1360,0	0,67	—	21,8
	Experiment	7	960,0	—	0,51	22,1	22,0
4 Days	Control (with mock operation)	6	888,0	1440,0	0,73	—	21,9
	Experiment	9	1103,0	—	0,60	22,0	22,4
5 Days	Control (with mock operation)	9	977,0	1494,0	0,64	—	22,2
	Experiment	7	1207,0	—	0,68	22,0	22,0
7 Days	Experiment	7	1071,0	—	0,63	21,7	12,6
10 Days	Experiment	7	1192,0	—	0,59	—	—

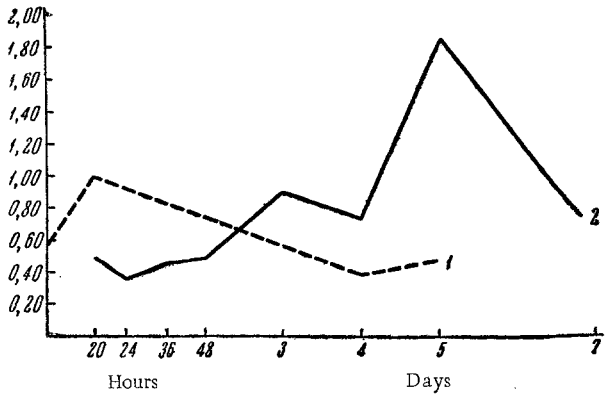
In sections of the lung 7 μ in thickness, stained with hematoxylin-eosin, the number of mitoses to 4000 cells of the interalveolar septa not undergoing division was counted. The mitotic activity was expressed in promille.

The numerical results were analyzed by the statistical method of Fisher and Student. The differences were regarded as significant if $P \leq 0.02$.

EXPERIMENTAL RESULTS

Analysis of the results of the experiments of series I, reflecting the trend of the changes in the relative weight of the remaining lung, showed that two peaks occurred during development of compensatory hypertrophy of the organ: the first considerable increase in the weight of the lung was observed 24 h after the operation, after which the weight of the lung fell significantly (by 18-20%). The second marked increase in wight took place on the 5th day, and from this moment stable hypertrophy of the organ was observed: on the 7th and 10th days the weight of the lung in the experimental animals was equal to the weight of two lungs of the intact rats (Table 1).

In the experiments of series II the relative weight of the lung showed changes of a somewhat different pattern during compensatory hypertrophy: the residual lung increased considerably in size 20 h after removal of the opposite organ (by 30% compared with the right lung in the control), but after 24 h its weight decreased, although this decrease was not significant ($P = 0.11$). On the 3rd day after the operation the weight of the lung of the experimental rats was 41% greater than the weight of the right lung, and amounted to 80% of the weight of both lungs of the control animals. In the later periods after the operation (4th, 5th and 7th days) the compensatory hypertrophy varied within the same limits as on the 3rd day after the



Changes in mitotic activity in the lungs of the control and experimental rats at various times after operation. 1) Control; 2) Experiment. Along the axis of abscissas—time after operation; along the axis of ordinates—MC (in %).

operation, demonstrating stabilization of the process. The absence of an accurate reproduction of the change in the curve of relative weight of the hypertrophied lung in the experiments of series II, as occurred characteristically in the animals of series I, was evidently due both to the different experimental conditions in these series and to the individual reaction of the lung to removal of the paired organ.

In the rats undergoing the mock operation, the relative weight of the lungs was slightly greater than in the intact rats (Table 1). However, this increase was not significant 2, 3, and 5 days after the operation ($P = 0.035$, 0.623, and 0.694). On the 4th day after the mock operation the difference became significant ($P = 0.001$). This suggest that the pneumothorax and the phenomena associated with healing of the operation incision in the chest wall (partial necrosis of the tissues, inflammation, etc.) were reflected in the morpho-physiological state of the lung, and in the volume of blood filling it, manifested by an increase in the weight of the organ. It has been shown [2], in fact, that in pneumothorax the conditions of circulation of the blood in the pulmonary system are disturbed, causing congestion of the blood vessels of the lung with blood on the side of the pneumothorax.

The hypertrophied lung of the animals sacrificed in the early periods after the operation (20 and 24 h) was edematous, its capillaries were dilated and filled with blood, and a homogeneous, pink effusion was present in the lumen of certain alveoli. The dry weight of 100 mg tissue of the lung in this state after 20 h averaged 18.8 mg, and after 24 h—19.2 mg, whereas the dry weight of 100 mg of tissue of the left lung, removed from these animals at operation, was 21.6 and 22.4 mg respectively (Table 1). The differences between the experimental and control values were significant ($P = 0.001$) 20 h after the operation and close to significant ($P = 0.03$) 24 h after the operation. These results demonstrate that the rapid increase in weight of the residual lung immediately after removal of the left lung was due mainly to edema and to the increased degree of filling of the organ with blood.

The DNA concentration remained unchanged at this period, while the RNA concnetration fell on the average by 8-10%, the difference between the experimental and control values being statistically significant ($P = 0.001$). The mitotic activity of the cells of the interalveolar septa remained at the lower limit of normal (MC = 0.37-0.5%) (Fig. 1).

TABLE 2. Concentration of Phosphorous of RNA and DNA per 100 mg Dry Weight of Rats' Lung Tissue at Different Times after Operation

Time after operation	Number of animals	Experiment (removal of left lung)				Control (mock operation)	
		Phosphorous of RNA (in μg)		Phosphorous of DNA (in μg)		Phosphorous (in μg)	Phosphorous (in μg)
		Removed left lung	Right lung	Removed left lung	Right lung	Right lung	
20 h	4	146.9	126.5	287.6	282.9	—	—
24 h	4	141.4	128.7	273.0	272.7	—	—
48 h	4	141.9	135.2	277.6	282.8	140.0	280.5
3 days	6	143.7	144.8	279.5	272.5	147.8	286.1
4 days	6	145.7	220.3	279.5	323.4	141.5	274.6
5 days	12	142.0	174.4	270.0	270.8	135.8	272.6
8 days	4	144.8	149.9	277.7	273.5	—	—

By the 3rd day of development of compensatory hypertrophy of the lung, synthesis was taking place in it normally: the concentration of RNA and DNA was almost the same as in the lungs of the control animals (Table 2).

The significant increase in the RNA concentration by a mean value of 52%, and of the DNA by 16%, on the 4th day was followed by a stable increase in the relative weight of the hypertrophied lung starting with the 5th day after pneumonectomy.

This period of intensification of nucleic acid synthesis was followed by a period of intensified proliferation of the lung cells (see Figure). On the 5th day after operation the mean value of MC was 1.9%), i.e., two or three times higher than in the lungs of the control rats undergoing the mock operation. Starting with the 5th day the concentration of DNA and RNA fell, to reach normal by the 7th day after the operation. The mitotic activity of the cells of the hypertrophied lung at this period was almost the same as in the control.

Hence, three periods can be distinguished in the development of compensatory hypertrophy of the lung after left-sided pneumonectomy: a period of pseudohypertrophy, when the sequelae of the operation – edema and hemorrhages – still persisted in the organ, RNA synthesis was depressed: a period of intensified synthesis of RNA and DNA, followed by increased proliferation of the cells and an increase in the mass of the organ; a period of stabilization of the process, marked by restoration of normal nucleic acid synthesis and mitotic activity, and stabilization of the weight of the organ.

In the process of development of compensatory hypertrophy of the lung, as during regeneration of the liver, a close relationship is found between the level of nucleic acid synthesis and the degree of proliferation of the cells of the organ: the intensified onset of mitosis in the cells is preceded by an increase in the DNA concentration in the tissue of the organ.

Meanwhile, no sign was found of the intensification of RNA synthesis which should have preceded the intensification of DNA synthesis, because the conentration of both types of nucleic acids in the present experiments rose simultaneously on the 4th day after the operation. Clearly shorter intervals between the times of investigation are necessary in order to detect this feature observed regularly by other authors during regeneration of the liver [7].

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