REGENERATION AND COMPENSATORY HYPERTROPHY OF THE LUNGS IN TADPOLES

L. D. Liozner, A. G. Babaeva, L. K. Romanova, and G. V. Kharlova

Laboaratory of Growth and Development (Head, Professor L. D. Liozner), Institute of Experimental Biology (Director, Professor I. N. Maiskii) of the AMN SSSR, Moscow
(Presented by Active Member AMN SSSR N. A. Kraevskii)
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The lung of the tadpole possesses a high degree of regenerative power [6]. It was decided to use this convenient object for the study of the effect of removal of different amounts of tissue on the course of regeneration. We were interested in both the changes in the injured lung and in the reaction of the intact lung to the removal of lung tissue, i.e., not only in regeneration but also in compensatory hypertrophy. Our principal aim was, therefore, to investigate the relationship between the regeneration of the lungs, considered as a single system of organs, and the character of the injury.

EXPERIMENTAL METHOD

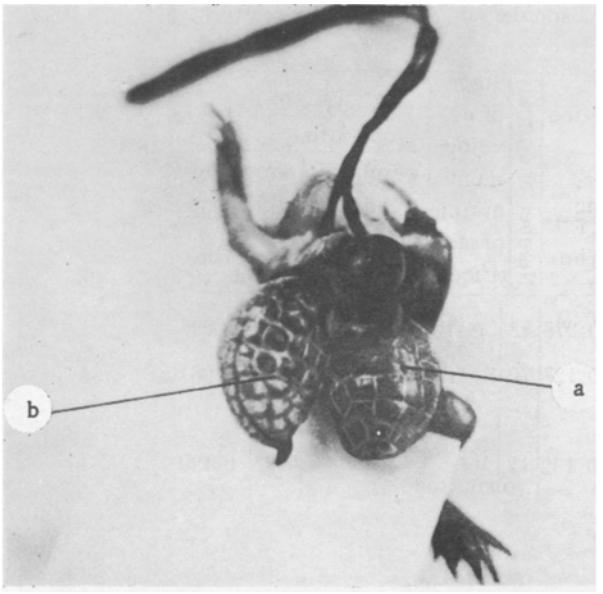
The first series of experiments was carried out on tadpoles of Pelobates fuscus and Rana ridibunda, in the first and second stages of development according to L. Ya. Blyakher [1]. A puncture wound was made with a dissecting needle in the skin a short distance lateral to the vertebral column, and the lung was exteriorized by exerting slight pressure on the tadpole's side. The tadpole's lung consists of a proximal cylindrical and a distal alveolar division. A hair ligature was applied to the distal part of the cylindrical division, after which the lung situated distally to the ligature was removed (i.e., the whole alveolar portion). The object of this experiment was to discover what part the cylindrical portion plays in the regeneration of the lung.

The remaining series of experiments were conducted on tadpoles of Rana temporaria in the first and second stages of development. The following were removed: 1) half of one lung; 2) the whole of one lung; 3) half of both lungs; 4) the whole of one lung and half of the other. During pneumonectomy the lung was resected at the border between the alveolar and cylindrical divisions, and when half the lung was resected this was done in the center of the alveolar portion. No ligatures were applied. After operation, the animals were kept for 24 h in a room at a temperature of not more than 10°.

The sacrificed tadpoles were weighed on pharmaceutical scales and the lungs on torsion scales. The lungs were fixed in Bouin's fluid. In total preparations (7-10 animals in control and experimental series), stained with hematoxylin and couterstained with picrofuchsin, the mean number of mitoses was counted in 4000 cells of the lung epithelium, and the number of mitoses in the blood cells was also recorded. The numerical results were analyzed statistically by the Fisher-Student method.

EXPERIMENTAL RESULTS

Thirty days after the operation 7 tadpoles of Pelobates fuscus (out of 26) and 6 of Rana ridibunda (out of 40) survived. In all the former, the lung consisted entirely of clearly differentiated alveolae, filled with air. The degree of regeneration varied. In two animals the regenerating lung was almost equal in size to the contralateral lung, not undergoing operation (Fig. 1), while in 5 animals it was slightly smaller (Fig. 2). The intact lung was larger than the lungs of the control tadpoles. The regenerating lung differed from the intact lung in its shape: its distal end was rounded, a scar was present at the site of healing of the wound edges, and in some cases the ligature remained at the end of the lung. In the tadpoles of Rana ridibunda, the degree of regeneration of the lungs was less marked. In all the experimental animals the lung consisted of a cylindrical part and an alveolar part, containing from 2 to 10 alveoli, whereas the alveolar part of the lung in the control animals contained from 25 to 30 alveoli.



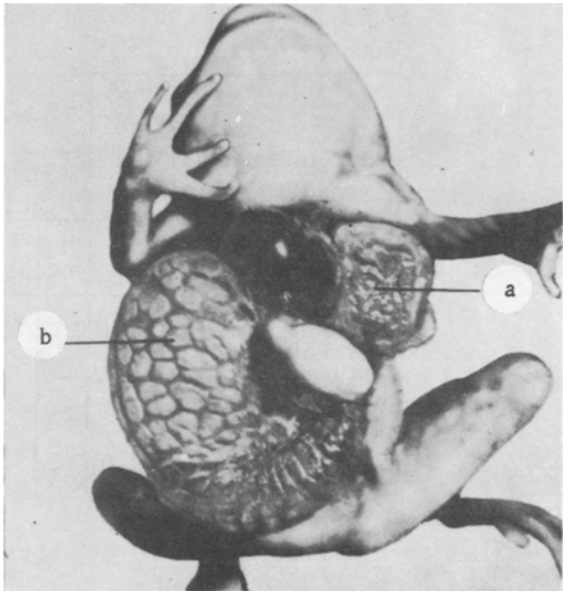


Fig. 1. Lung of a young frog Pelobates fuscus 30 days after subtotal resection of the left lung. a) Regenerating lung; b) contralateral intact lung.

Fig. 2. Lung of a young frog Pelobates fuscus 30 days after subtotal resection of the left lung. a) Regenerating lung; b) contralateral intact lung.

The results showed that regeneration of the lungs in tadpoles in which only the cylindrical portion was left took place by transformation of the cylindrical portion into alveoli and not by the budding off of tissues from the wound surface.

Since the characteristic feature of the natural metamorphosis of the lungs of tadpoles is the formation of alveoli from the cylindrical portion, it can be suggested that regeneration of the lung takes place by a combination of two processes: regenerative hypertrophy of the residual portion of the organ and age differentiation.

Some animals of the 2nd-5th series of experiments were sacrificed 10 days after operation (summer of 1960), when they were in stages of development ranging from the first to the fifth. It will be seen in Table 1 that regeneration of the lungs took place both after removal of one half of both lungs and after the simultaneous removal of the whole of one lung and half of the second. The weight of the injured organ did not differ substantially from the weight of the lung in the control tadpoles. In the series of experiments in which only one half of one lung was removed regeneration also was observed, but the weight of the organ did not attain the weight of the whole lung of the control tadpoles. We did not observe budding off of tissues from the wound surface: regeneration of the lungs took place by regenerative hypertrophy. In the series of experiments in which the whole of one lung was removed compensatory

TABLE 1. Change in Weight of the Lungs and Number of Alveoli in the Lungs of Tadpoles 10 Days after Operation

Experimental conditions	No. of animals	Stage of development at time of sacrifice	Right lung weight		No. of alveoli	Left lung weight		No. of alveoli
			in mg	as % of body weight		in mg	as % of body weight	
Control	29	IIIa—V	0,78	0,108	27	0,55	0,126	26
Removal of left lung	27	IIIa—V	0,95	0,183	25	—	—	—
Simultaneous removal of left and half of right lung	8	IIIb—V	0,57	0,127	14	—	—	—
Simultaneous removal of one half of both lungs	10	IIIa—V	0,53	0,114	11	0,55	0,118	10
Removal of half of the left lung	28	IIIa—V	0,60	0,142		0,44	0,105	14

TABLE 2. Changes in Weight of the Lungs of Tadpoles at Various Periods after Operation

Experimental conditions	Number of animals	Stage of development at time of sacrifice	10 days after operation: weight of right lung in mg	10 days after operation: weight of right lung as % of body wt	10 days after operation: weight of right lung in mg	10 days after operation: weight of right lung as % of body wt	Number of animals	Stage of development at time of sacrifice	21 days after operation: weight of right lung in mg	21 days after operation: weight of right lung as % of body wt	21 days after operation: weight of right lung in mg	21 days after operation: weight of right lung as % of body wt
Control	29	II—III	0,32	0,091	0,27	0,078	38	I—II	0,22	0,100	0,21	0,095
Removal of right lung	39	I—V	—	—	0,37	0,127	70	I—III	—	—	0,24	0,084
Simultaneous removal of right and half of left lung	40	I—III	—	—	0,40	0,132	12	II—young frog	—		0,26	0,120

TABLE 3. Mitotic Activity of the Lung Epithelium in Tadpoles (in %)

Experimental conditions	Intervals after operation (days) 3	7	10	21
Control	0,35	0,45	0,25	0,25
Removal of one lung	1,59	0,56	0,60	—
Removal of half of both lungs	2,70	1,25	0,53	0,55
Simultaneous removal of a whole lung and half of the other lung	3,00	1,44	2,10	3,04
Removal of half a lung:				
intact	0,85	0,21	—	—
injured	2,36	1,12	0,38	—

hypertrophy of the remaining part of the organ took place. In the series in which one half of one lung was removed the mean weight of the intact organ was rather greater than in the controls; this excess weight was statistically significant ($P = 0.005$).

The number of alveoli in the remaining lung or in its residual part did not change. Hence, during regeneration and compensatory hypertrophy marked hypertrophy of the structural units of the lung took place, but no new units were formed.

Experiments carried out in the summer of 1961 confirmed the earlier findings and also provided information on the trend of development of regeneration and compensatory hypertrophy, because observations were made at two periods (Table 2).

The results in Table 2 show that 10 days after operation regeneration of the residual half of the lung and compensatory hypertrophy of the whole lung were observed. After 21 days, however, the compensatory hypertrophy was much less marked. The weight of the regenerating lung, like that of the control, was slightly reduced, although still greater than the weight of the control lung.

Changes in the mitotic activity of the lung epithelium were studied in a special series of experiments. Animals were sacrificed 3, 7, 10, and 21 days after operation. The results of these series were generally parallel to the changes in the weight indices (Table 3).

Three days after operation, the mitotic coefficient was increased 5-9 times in all the experimental series. The increase was smaller in cases of compensatory hypertrophy, i.e., after removal of the whole lung (1.59%) and, in particular, of half a lung (0.85%). In every case in which regeneration of an injured lung took place, the mitotic activity

was considerably increased. The number of mitoses in the blood cells also was largest at this period. Since high mitotic activity in the lung epithelium and blood cells was observed in the series of experiments in which a relatively small amount of tissue was removed (half of one organ), it may be suggested that the increase in mitotic activity was largely due to the presence of a wound surface and to the processes takeing place in connection with the wound.

This suggestion is strongly supported by the fact that a more prolonged increase in mitotic activity took place in the series of experiments in which one half of both lungs was removed simultaneously than in the series in which one whole lung was removed. Nevertheless, the amount of lung tissue removed was also of considerable importance: after the simultaneous removal of one whole lung and half the other the mitotic activity remained high for a longer time and on a higher level.

Seven days after the operation, the character of the change in mitotic activity of the lung was different. The level of the mitotic activity in the lungs undergoing compensatory hypertrophy did not differ significantly from that in the lungs of the control tadpoles. The proliferation leading to an increase in the size of the intact lung was now apparently complete, having produced its effect. Meanwhile the cells of the pulmonary epithelium in the injured and regenerating lungs continued to divided intensively: their mitotic coefficient was from 2 to 2.5 times greater than in the lungs of the control animals. The mitotic activity in the injured lungs, however, was lower than in the preceding period.

Ten days after the operation, the increased mitotic activity persisted only in the regenerating half of the lung (simultaneous removal of one whole lung and half the second). In the remaining series of experiments the mitotic activity of the lung epithelium was not significantly different from that in the control tadpoles. The increased mitotic activity of the lung epithelium in the series in which only half of one lung was left was still present 21 days after operation. This shows that the regeneration process was not yet complete, and cell proliferation was continuing. It is interesting that the mitotic activity showed no tendency to subside.

The results showed that the lungs of tadpoles are capable both of regeneration and of compensatory hypertrophy, although the process was not permanent in our experiments. Regeneration took place, not as a result of the budding off of tissues from the wound surface, but by regenerative hypertrophy, the principal method of regeneration of most internal organs of vertebrates [2-5]. Regenerative hypertrophy was supplemented by the formation of alveoli from the cylindrical part of the lung. In the course of regeneration, hypertrophy of the alveoli took place. The regeneration of the half lung was a long, drawn-out process. Further research is required in order to discover the extent to which regeneration of the lung takes place at long intervals after operation, i.e., to discover whether complete compensation for the lost tissue takes place.

SUMMARY

The following was excised in Rana temporaria tadpoles: 1) half of the lung, 2) a whole lung, 3) simultaneously a whole lung and half of the second one, 4) half of the both lungs. Ten days after the operation, the authors observed regeneration in the injured lungs reaching the weight of a whole lung in control tadpoles. Besides, compensatory hypertrophy of the uninjured lung was revealed. Mitotic activity was markedly augmented in all the experimental series 3 days after the operation. In 7 days the mitotic activity of the lung which had undergone compensatory hypertrophy did not differ from the control; in 10 and 21 days increased mitotic activity was noted only in the third series of experiments. Restoration of the lung occurred by means of the process of regenerative hypertrophy.

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