THE REGENERATIVE POWER OF THE HYPERTROPHIED LUNG OF THE CRESTED TRITON

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A result of the successful development of the surgery of the lungs during the last 50 years was the wide use of various operative procedures on these vitally important organs. In a number of cases on clinical grounds it is necessary to operate on a lung which has already suffered damage, or on a lung in which compensatory hypertrophy has taken place because of absence of the other organ of the pair. Since we were unable to find in the accessible literature any mention of the subject of the regenerative power of the hypertrophied lung, we deemed it of interest to find out whether the hypertrophied lung was capable of regeneration. Moreover the hypertrophied lung might be expected to show changes after injury representing the combined effect of compensatory hypertrophy and regeneration. For this reason our investigation had also to include the delineation of these two processes and the study of their special individual features. However, the investigation of this problem in mammals is attended by great difficulties both of performance of the experiments and of analysis of the results. This led us to seek a new experimental model on which to study the laws of regeneration of hypertrophied lungs, using for this purpose animals readily accessible for experimental procedures and permitting a comprehensive study of the processes of regeneration.

The caudate amphibia satisfy these requirements.

EXPERIMENTAL METHOD

Two main series of experiments were carried out on the crested triton (Triturus cristatus), of both sexes and weighing 4500-7000 mg. In the first series of experiments compensatory hypertrophy was first induced in the right lung of the animals by removal of the left lung, and 2 months after this operation one half of the hypertrophied lung was resected (operations performed on 23 tritons of which 17 survived).

So that the character of the restorative process taking place in the injured, hypertrophied lung could be compared with the process of regeneration of normal lung, we carried out a second series of experiments in which the left lung was removed completely and at the same time one half of the right lung was resected (operations performed on 49 tritons of which 27 survived).

As a main control we used tritons on which no operation was performed, and which were kept under the same conditions as the animals undergoing operation.

Furthermore, in order to discover whether there were any differences in the course of the continuing compensatory hypertrophy, on the one hand, and the regeneration of the injured, hypertrophied lung on the other, a supplementary control was set up. Compensatory hypertrophy ran its course in the right lung of the animals of this control group for 4 months after removal of the left lung (operation performed on 30 tritons of which 20 survived).

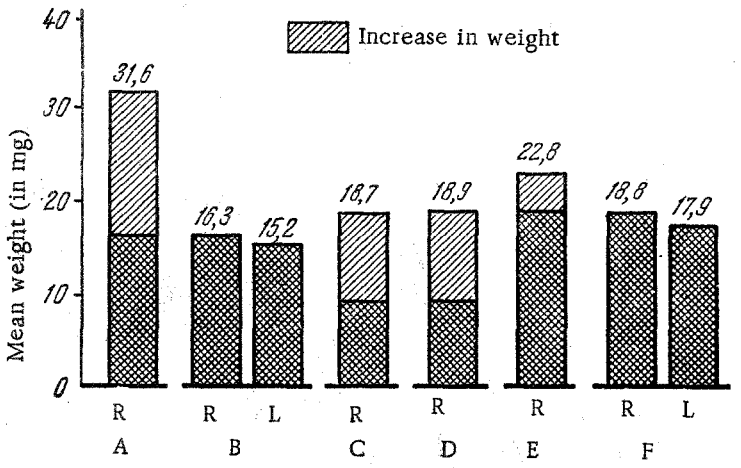

Fig. 1. Mean weight of the lungs of tritons 2 months after operation R) Right lung; L) left lung; A) removal of the left lung B) control of A; C) series I (removal of half the right lung 2 months after left-sided pneumonectomy) D) series II (simultaneous total removal of the left and half the right lung); E) removal of the left lung. Period of observation four months. F) controls of series I and II and of E.

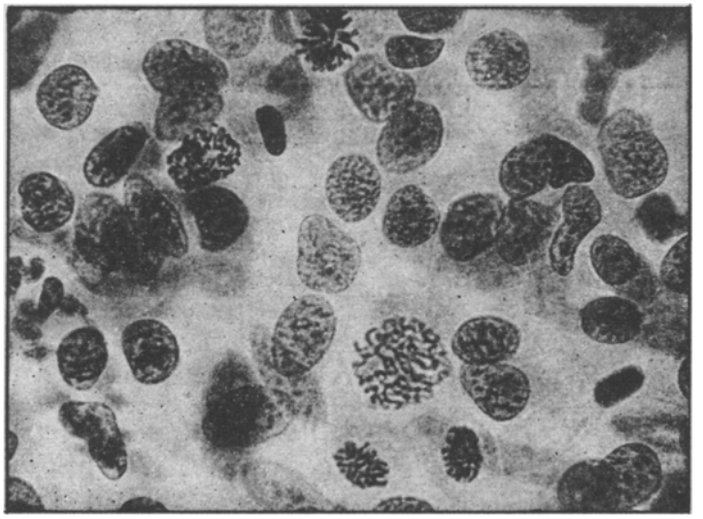

Fig. 2. Mitoses in the cells of the pulmonary epithelium of the regenerating lungs. Total preparation. Magnification: ocular 5×, objective 100 ×.

When the entire lung was removed, the resection was made at the point of its separation from the laryngotracheal division; when half the lung was removed, this was done at the level of the commissure joining the lung to the sex gland.

Two months after operation (in the first series 2 months after the second operation) the tritons were killed.

The lungs were fixed in a state of inflation in Bouin's fluid. Total lung preparations were stained with hematoxylin-eosin and with hemotoxylin and counterstained with picrofuchsin, and mounted in balsam so that the internal surface of the lung, lined with pulmonary epithelium, was turned toward the cover glass. In the films (4-5 cases from each series and from the controls were taken) we measured the width of the lungs, counted the cells of the pulmonary epithelium in one field of vision and estimated the average number of mitoses per

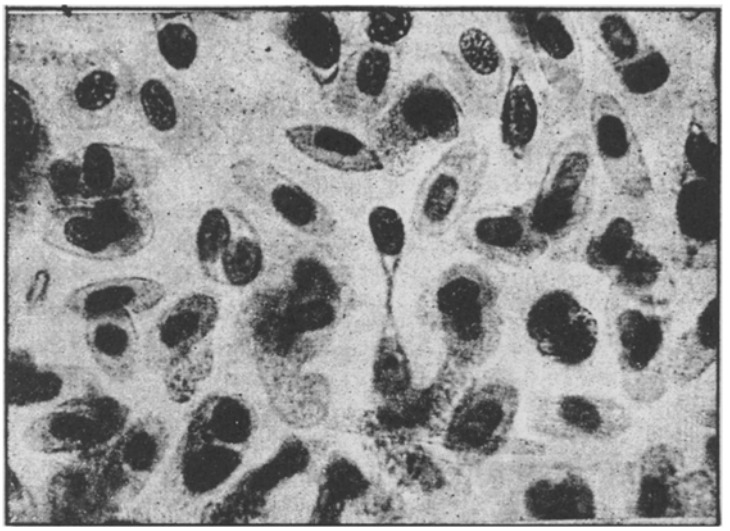

Fig. 3. Amitosis in a cell of the pulmonary epithelium of the regenerating lung Total preparation. Magnification: ocular 5x, objective 100x.

4000 cells. By means of drawings we determined the area of the nuclei of the cells of the pulmonary epithelium, which enabled us to judge their hypertrophy.

EXPERIMENTAL RESULTS

Regeneration of the lung after simultaneous removal of the left and half of the right lung.

In the animals of this series 2 months after operation the lung was shaped like a small sack, the distal end of which was rounded and dilated. In its color and blood content, there was little difference between the operated and unoperated lungs. At the place of union of the edges of the wound in the lung there was a small white scar, but in no case were outgrowths of lung tissue observed from the raw surface.

Besides these findings a considerable increase in the weight of the regenerating lung took place (Fig. 1). The increase in weight of half of the regenerating lung by comparison with half of the normal lung was 101% (18.9-9.4 mg). We observed changes in weight of the same order on comparing the relative weight of the operated and normal lungs. This increase in weight was statistically significant.

The mean absolute and relative length of the resected lung was almost unchanged by comparison with the length of half the normal lung.

The increase in the size of the residual portion of the lung took place largely on account of changes in its width, which increased by 18%. The regenerating lung differed in its histological structure from the undamaged lung of the triton: its walls were thicker than the walls of the normal lung, and its muscle cells were hypertrophied. Whereas in the normal lung the cells of the pulmonary epithelium were encountered in groups containing 2, 3 or at the most 4 nuclei, in the regenerating lung larger collections were found, containing 8-10 nuclei. Also, the number of these collections in the regenerating lung per field of vision was less than in the normal. The nuclei of the pulmonary epithelial cells were of different shapes and sizes, whereas the nuclei of the pulmonary epithelial cells in the normal lung were characteristically of average size and oval in shape.

The increase in the number of nuclei in the pulmonary epithelial cells and their polymorphism all indicate the presence of proliferative processes. It was found that the mitotic coefficient (MC) in the epithelium of the regenerating lung in the animals of the 2nd series was 250% higher than that in the epithelium of the lungs of the control tritons. This was suggestive of intensive hyperplasia of these cells.

The ratio between the phases of mitosis was the same as in the lungs of normal tritons. No special zones of mitotic activity could be identified, and only along the course of the pulmonary artery were dividing cells

TABLE 1

Changes in the Regenerating Hypertrophied Lung 2 Months after Operation

Series of experiments	Type of operation	Number of animals	Period of observation (in months)	Right lung						
				Length (in cm)	Width (in cm)	Mitotic coefficient (in %)	Area of nuclei (in relative units)	Increase in mitotic activity (in %)	Degree of hypertrophy of nucleus (in %)	Ratio of intensity of hyperplasia to hypertrophy
1	Removal of half of the hypertrophied lung	17	2	1.7	1.08	20.2	76	260	10	26.0
2	Simultaneous removal of the left lung and half of the right control	27	2	1.5	1.08	19.6	78	250	13	19.2
3	Total removal of the left lung	20	4	3.1	1.09	4.4	86	22% below normal	24	—
	Control	15	—	3.1	0.92	5.6	69	—	—	—

TABLE 2

Degree of Compensatory Hypertrophy in the Lung in Relation to the Time after Removal of the Opposite Lung

Type of operation	Period of observation (in months)	Right lung		
		Weight (in mg)	Increase in mitotic activity (in %)	Degree of hypertrophy of the nucleus (in %)
Removal of the left lung	2	31.6	422	43
	4	22.8	22% below normal	24

observed slightly more often than in the remaining lung tissue. Proliferation of the cells of the pulmonary epithelium was not confined to division of cells by mitosis: their proliferation by amitosis could also be observed (Fig. 2, 3).

Comparison of the area of the nuclei of the pulmonary epithelial cells in the lungs of the experimental and control animals showed that hypertrophy of the nuclei of the cells of the regenerating lung took place (Table 1).

In summing up, it must be pointed out that in the regenerating lung of the triton after removal of the opposite lung, the processes of proliferation of the pulmonary epithelial cells and of hypertrophy of their nuclei were intensive and prolonged.

The result of this was an increase in the weight and volume of the residual organ. It might be thought that two processes were taking place simultaneously in the residual organ: compensatory hypertrophy, since restoration took place in the absence of the other paired organ, and regeneration, since part of the lung had been removed.

It was, therefore, natural to consider that restoration of the traumatized organ in an experiment under these conditions was the result of these two processes taking place in harmony and in mutual association.

Also, we were inclined to think that the deciding factor in this case was the injury to the lung, the presence of which was responsible for the special features of the restorative process.

Regeneration of the lung took place along the lines of regenerative hypertrophy, like that which was shown in a series of investigations proving that restoration of the internal organs (lungs, kidneys, pancreas and salivary glands) of mammals takes place mainly by regenerative hypertrophy [1, 2, 3, 4, 5].

Regeneration of the hypertrophied lung. In the experiments of series I, after removal of half of the hypertrophied lung the same results in general were obtained as after simultaneous removal of the whole lung and the half.

The regenerating hypertrophied lung weighed on the average 18.7 mg. If it is considered that at the moment of resection this lung weighed on the average 31.6 mg, as shown by the appropriate data (Fig. 1), then the half of the regenerating hypertrophied lung increased in weight on the average by 2.9 mg (18%) in comparison with the part left behind after operation (18.7-15.8). Furthermore, the regenerating hypertrophied lung overtook in weight the lung of the control animals, which weighed 18.8 mg. In relation to the half of the control lung, the increase in weight of the regenerating lung averaged 98% (18.7-9.4 mg). Such a gain in weight, however, might be regarded as the result either of regeneration or of continuing compensatory hypertrophy. The latter hypothesis breaks down, since comparison of the results obtained in animals killed 2 and 4 months after removal of one lung suggested that the process of compensatory hypertrophy not only did not progress but actually gradually receded: the weight of the hypertrophied lung was reduced, and both the mitotic activity of the pulmonary epithelium and the degree of hypertrophy of the nuclei decreased. What is more, the mitotic activity of the pulmonary epithelium of the lung in which compensatory hypertrophy had lasted for 4 months was less than that of the pulmonary epithelium of the lungs of the control tritons (Table 2).

Thus during the same period of time (2 months) the regenerating hypertrophied lung increased in weight by 2.9 mg (18%) in relation to its original condition, whereas the lung in which compensatory hypertrophy continued not only did not gain in weight but actually weighed 8.8 mg (28%) less than the left lung, the compensatory hypertrophy in which had been going on for longer than 2 months. This indicated that the processes observed in the resected and in the undamaged hypertrophied lungs were dissimilar in their nature. Hence the increase in weight of the injured hypertrophied lung was the result of regeneration and not of compensatory hypertrophy.

It follows from the results described that the hypertrophied lung of the triton is capable of regeneration which takes the form of regenerative hypertrophy, and during this process the ratio between the indices of hypertrophy and hyperplasia of the cells of the pulmonary epithelium remains almost the same as during regeneration of the lung which has not previously undergone any form of operation but with the opposite lung removed.

Attention must be drawn to the possible appearance of regenerative powers in the hypertrophied lung in spite of the extinction of the process of compensatory hypertrophy in the lung.

The fundamental hypothesis may therefore be put forward that the stimulus which mobilizes the reserve powers of the body and which is capable of displaying the regenerative powers of the hypertrophied lung is the factor of trauma and the removal from the body of a definite amount of functioning lung tissue.

SUMMARY

Compensatory hypertrophy was induced in the right lung of tritons by removing the left lung. Two months after the operation half of the hypertrophied lung was resected. Comparison of the data obtained in this series of experiments and in the series where regeneration of the lung occurred after simultaneous removal of the whole lung on one side and one half on the other demonstrated that the hypertrophied lung of tritons is able to regenerate. Regeneration runs a course of regeneration hypertrophy. The relationship of the indices of hypertrophy and hyperplasia of the cells of the pulmonary epithelium is almost the same in both series of experiments.

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