ON THE PROBLEM OF REGENERATION OF THE LUNGS IN CAUDATE AMPHIBIA

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In spite of the fact that there have been extensive investigations of the regenerative capacities of amphibia, there has been very little study on the regeneration of internal organs in these animals. In particular, the data on lung regeneration are in conflict. E. Muftich recorded lung regeneration in frogs, toads and salamanders, but not in tritons. Lung regeneration in tadpoles was obtained by K. Westphal [2]. G. Geberlein, working on axolotls came to the conclusion that they do not regenerate lungs.

The solution of the question as to whether amphibia are capable of regenerating lungs has been hampered by faulty conclusions made by a number of authors. Thus, A. Weismann [1] maintained that the internal organs are incapable of being regenerated. To confirm this opinion he performed a number of experiments; in part, he attempted to study lung regeneration in tritons.

A. Weismann performed experiments on only 3 animals; his conclusion was that triton lungs do not regenerate. However, a careful perusal of his data shows that A. Weismann had already formed prejudicial opinions before he analyzed his results. Thus, in one of his 3 cases presented in his study, there really was some apparent lung regeneration. In the triton he removed a right lung area of 2 cm dimension. Autopsy 10 months later showed the left lung to be 4.4 cm in length while the right lung measured 3.9 cm; i.e., the difference in lung lengths was smoothing out. In spite of the entirely unconvincing experimental presentation, the conclusion of A. Weismann in regard to the impossibility of the regeneration of internal organs, lungs especially, has found a widespread acceptance.

In the last few years, a whole series of Soviet authors have demonstrated the capacities for regeneration of internal organs in various vertebrate animals. In view of this, we decided to re-examine the problem of lung regeneration in caudate amphibia.

The basic portion of our work was done on the great crested newt, Triturus cristatus.

The first experimental series consisted in amputation of the right lung in adult tritons. The operation was performed in the following manner. At the base of the right anterior extremity, where there is the line dividing the transition of the dark color into the yellow, one blade of the scissors was used to penetrate the skin coverings and then make a short incision some 2-3 mm in length. The peripheral portion of the lung was then drawn up gently to the level of the anterior extremity. The amputation was performed at the place where the lung pouched out from the laryngo–tracheal juncture.

Two months following the operation, 2 tritons were sacrificed. It could be seen with the naked eye that some lung regeneration had taken place. After 9 months, 12 tritons were sacrificed. In 10 of them the observed regeneration was such that the regenerated lungs were almost the size of the normal ones; in only 2 instances, where the amputation had apparently been too high was there no lung regeneration to be observed.

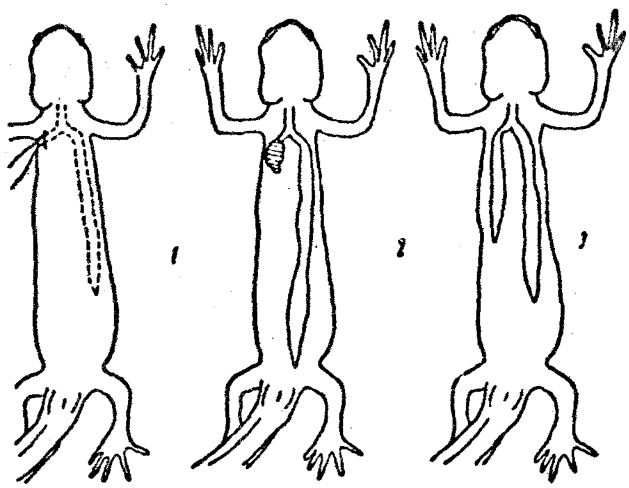

Fig. 1. Diagrammatic representation of the surgery performed and extent of lung regeneration in the triton.
1) Diagram showing place where lung was amputated; 2) three months' regeneration of the lung; 3) twelve months' regeneration of the lung.

The 3-month lung regeneration took the form of a small outpouching (Fig. 1), the color of which changed in correspondence with the cardiac rhythm. The regenerated portion was sharply demarcated from the old portion both in color and size (i.e., it was much wider). The left lung was markedly hypertrophied.

The lungs were fixed in an inflated state in Bouin's fluid and then imbedded in paraffin. Some of the sections were stained with hematoxylin – eosin, some with Weigert resorcin – fuchsin, and some according to Mallory.

Study of the histologic preparations proved that typical lung regeneration had taken place. The microscopic studies of the regenerated tissues show the difficulties of differentiating them from those of the normal (Fig. 2). In these sections, just as in normal lung, are seen the typical regions divided by the partitions. The lumen is lined with a single layer of cubical epithelium. In the walls and partitions of the regenerated organ are seen strands of smooth muscle tissue.

However, along with the indications of normal structure, typical of organ regeneration, there are some observable differences. Thus, the wall of the lung remnant is much thicker than the wall of the regenerating lung. In the regenerating lung, the transverse and longitudinal partitions are much thicker than in the normal lung and contain a much larger quantity of fibrous connective tissue as can be especially well seen on the section stained by Mallory. The partitions have vessels with larger lumens as compared with the normal lung; apparently, this vessel widening lends the red coloration to the regenerating tissue especially as the walls of the regenerating lung are thinner. The lumens within the partitions of the regenerating lung are larger than in the normal lung but their number is less. In the regenerating lung large partitions are formed much more proximally than is the case with normal lung.

The regenerating lung is more like the distal portions of a normal lung in its architecture; the proximal portion of the regenerating lung does not have short, thick partitions but forms partitions winding immediately and forming large loops, i.e., the functional component of the lung forms first. In addition, the distal portion of the regenerating lung shows a large accumulation of connective tissue elements.

Thus, our first series of experiments demonstrated that tritons have the ability to regenerate lungs. The regenerated lung was proven to be fully functional when, 6 months after the lung had been permitted to regenerate, the second normal lung was extirpated. The animals survived for a long time with two regenerated lungs.

Fig. 2. Section through regenerated lung in a triton 9 months after amputation (microphoto).

The second series of experiments was performed on the larvae of the crested triton. It is known that in the triton larvae breathing is accomplished by means of gills, the skin surface and the mucous membrane of the mouth. The lungs of the larvae have a relatively simple structure. The young larva has a lung pouch lined smoothly with loose connective tissue having occasional cells with large nuclei. Both the lung and peritoneal epithelium of the lung sac are represented by a single layer of flattened cells which are hard to tell apart from the underlying connective tissue.

The right lung was amputated in the larvae of the tritons at the level of the anterior extremity. Two larvae were sacrificed 2 months after the operation; no lung regeneration could be demonstrated. Twenty larvae which had already metamorphosed into adult tritons were sacrificed 9 months after the operation and again no lung regeneration could be demonstrated there being seen in some, at the stump only an insignificant sausage-shaped swelling.

Thus, we were unsuccessful in obtaining regeneration of lungs in triton larvae.

The third series of experiments consisted in taking a group of Amblystoma mexicanum and amputating their right lungs at the level of the anterior extremity. The operation was performed as with the adult tritons.

The animals were sacrificed at varying postoperative intervals: one, 3 months after the surgery, at which time it was found that the regenerating lung was $1/3$ the length of the normal one; two, 4 months after surgery, at which time the regenerating lung was $1/2$ the length of the normal one; two, 6 months after surgery, at which time the regenerating lung was in excess of $1/2$ the normal lung. Histological studies in all instances confirmed typical lung regeneration.

Thus, the results of this experimental series showed that amblystoma lungs, just as is the case with adult tritons, are capable of organ specific regeneration.

The fourth series of experiments was performed on larvae of amblystoma-axolotl, the right lung being amputated at the level of the anterior extremity. The technique of the operation was the same as with the tritons.

The operated animals were sacrificed at the following times: 1 axolotl, 2 months after surgery, at which time no lung regeneration was observed; 4 axolotls, 8 months after surgery, at which time also no regeneration could be observed.

The fifth series of experiments consisted in taking 3 axolotls and amputating their right lungs at the level of the base of the anterior extremity. In 2 other axolotls both lungs were amputated simultaneously at the level of the bases of their anterior extremities. The animals were sacrificed after 2 years and they all showed marked lung regeneration. In the cases where both lungs had been removed simultaneously the regenerated lungs looked like large red sacs reminiscent of frog lungs. In width the regenerated lungs exceeded 4 to 5 times the normal axolotl lungs.

Histologic studies showed the presence of organ-typical regeneration. There might be noted a few morphological peculiarities in the regenerated lungs as compared with the normal. The lung wall is thick. In the regenerated lung are encountered many more cylindrical epithelial cells. The epithelium carries a more transitional appearance. The crowded state of a large number of elongated cylindrical epithelial cells is testimony to the lung being in a markedly collapsed state. In the epithelium are seen oval light nuclei with, among them, some dark, rod-like nuclei; among the epithelial cells are many of the goblet type which produce the mucous entering as drops into the lung lumen.

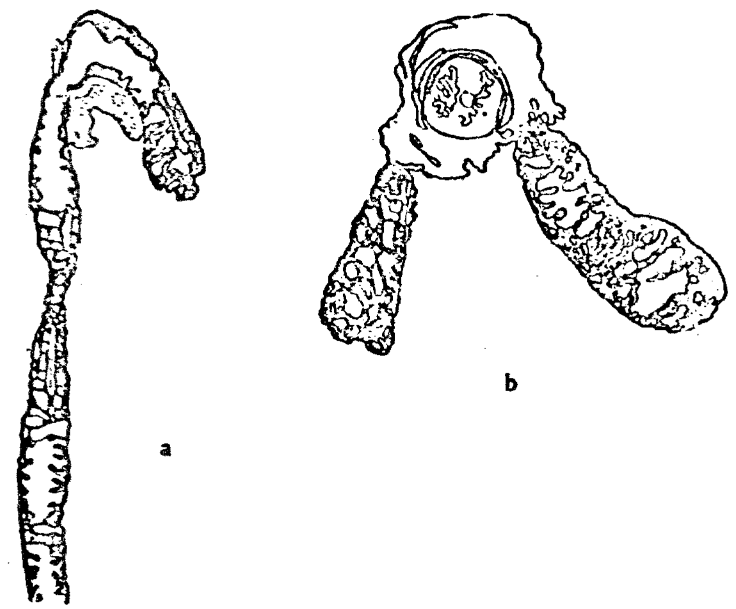

Fig. 3. Graphic reconstruction of the lungs in a triton 3 months after amputation (a) and in an axolotl 2 years after amputation (b).

In the epithelium are many, mainly lymphoidal migratory elements, also encountered are some polymorph-nucleated leucocytes. In places, are seen spaces filled with hematogenous elements, this being especially true in the distal end of the regenerating lung. Individual capillaries are packed with lymphoidal elements.

The blood vessel net is more markedly developed in the regenerating lung and it indents the lung lumen. Such a marked vascularity brings to mind the thought that we are dealing with a compensatory adaptation of the blood capillary network. Figure 3 shows graphically the reconstructed lung in a triton and an axolotl.

In addition to the peculiarities observed in regenerating lung of axolotl as compared with the normal lung, there may be noted some differences in lung regeneration in an axolotl as compared with amblystoma.

In amblystoma regenerating lungs do not attain the width while being longer (up to $^2/_3$ that of the normal lung), at a time when the axolotl regenerating lung is shorter.

Summarizing our results we can say that in adult tailed amphibia regeneration of organ-typical, normally functioning lungs takes place. Triton larvae did not show lung regeneration at all, while in axolotls lungs regenerate more slowly than in amblystoma.

The experimental data obtained indicate the great importance of the age of the animals and also the significance of functional differences producing regeneration or else failing to do so. To a certain extent, our data help to explain the contradictions in the views of various authors as to the possibility of obtaining lung regeneration among amphibia.

SUMMARY

Contrary to previously expressed opinions, a series of experiments, performed on adult crested newts, Triturus cristatus, demonstrated that entire lungs could be amputated and then made to regenerate into fully functional new organs.

The importance of the age factors and the functional state of the lungs at the time of amputation was demonstrated by the failure to obtain regeneration in triton larvae.

Amblystoma mexicanum and axolotl were similarly tested in other experimental series. The axolotl was shown to regenerate lungs but more slowly than amblystoma.

LITERATURE CITED

[1] A. Weismann. Anat. Anzeiger 22, 20-21, 425-431 (1903).

[2] K. Westphal. Ztschr. d. ges. Anat. u. Entw.–Gesch. 77, 144-163 (1925).