# REGENERATION OF THE LIVER IN LARVAE OF *Triturus cristatus*

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UDC 612.35:612.6.03-019:597.9

Regeneration of the liver after resection in triton larvae takes place by regeneration hypertrophy through mitotic division of liver cells with no increase in their size. The shape of the liver is not restored, but its weight comes close to that of the control.

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The study of the regenerative power of the larval liver of the triton is important in connection with discovery of the ontogenetic and phylogenetic principles governing regeneration processes. The method of regeneration of the liver in caudate amphibians has for a long time remained a matter for discussion [1].

The possibility of adequate regeneration of the liver, with restoration of its shape and the dynamics of regeneration of the triton liver depending on the volume of tissue removed were studied in the present investigation.

## EXPERIMENTAL METHOD

Larvae of the crested triton (Triturus cristatus) between stages 50 and 60 of development (Gluecksohn) were used in the experiments. In the experiments of series I the mamillary process of the liver, about 10% of the total liver tissue, was resected. The operations were carried out without anesthesia. The animals were sacrificed after 5, 14, 25, and 60 days. In the experiments of series II the left lateral part of the organ (30% of its total mass) was resected. The animals were sacrificed 5, 10, 15, and 21 days after operation. The liver was resected by bringing it through a transverse incision (3-mm) on the ventral aspect a little posteriorly to the forelimbs. After resection the liver was restored to its original position and no sutures were inserted. After the operation the larvae were kept for 1-2 h in a refrigerator, improving their chances of survival. The liver was weighed, its outline drawn, and fixed in Carnoy's fluid. Paraffin

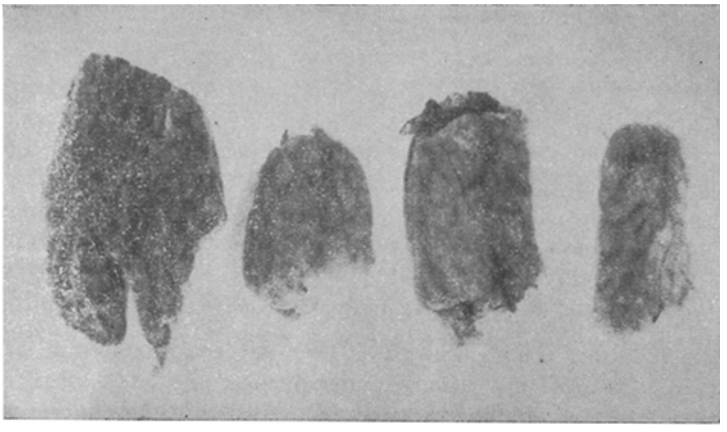

Fig. 1. Liver of triton larvae. First from the left: liver of intact triton, next three: liver of experimental animals one month after resection of distal portion of the organ.

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TABLE 1. Dynamics of Changes in Liver Weight of Triton Larvae After Resection of 10% of Liver Tissue

| Time after operation (in days) | Experiment | | | Control | | | Relative weight of liver in experiment as a percentage of relative weight of control liver |
|---|---|---|---|---|---|---|---|
| | No. of animals | weight of residual part of liver (in mg) | relative weight of liver (in % of body wt.) | No. of animals | weight of liver (in mg) | relative weight of liver (in % of body wt.) | |
| 7 | 5 | 21.6 | 2.33 | 14 | 27.2 | 3.28 | 71 |
| 14 | 5 | 18.2 | 2.48 | 9 | 22.2 | 3.32 | 74.6 |
| 25 | 4 | 37.7 | 3.86 | 4 | 33.0 | 4.09 | 95 |

TABLE 2. Dynamics of Changes in Liver Weight after Resection of 30% of Liver Tissue

| Time after operation (in days) | Experiment | | | Control | | | Relative weight of liver in experiment as a percentage of relative weight of control liver |
|---|---|---|---|---|---|---|---|
| | No. of animals | weight of residual part of liver (in mg) | relative weight of liver (in % of body wt.) | No. of animals | weight of liver (in mg) | relative weight of liver (in % of body wt.) | |
| 5 | 5 | 9.4 | 2.42 | 11 | 13.3 | 2.84 | 85.2 |
| 10 | 4 | 16.7 | 2.72 | 12 | 17.3 | 2.86 | 95.4 |
| 15 | 3 | 22.3 | 3.29 | 12 | 27.1 | 3.63 | 90.6 |
| 21 | 7 | 35.5 | 3.80 | 8 | 39.5 | 4.42 | 86.0 |

sections 7 $\mu$ in thickness were stained with hematoxylin-eosin. The mitotic index was determined by counting the number of mitoses in 3000 cells. At 7 a.m. on the day of sacrifice the experimental and control tritons received an intraperitoneal injection of 0.05 ml 0.004% colchicine solution through the muscle at the base of the tail. The animals were sacrificed at 11 a.m. According to data in the literature, mitotic activity of the liver cells in the regenerating liver is much higher in the morning than in the evening [2]. To study the dimensions of the liver cells, their outlines were traced from histological sections under the microscope (360 ×) by means of the RA-5 drawing apparatus on squared paper. In each case 100 cells were drawn. The outlined cells on the paper were cut out and weighed on VT torsion scales. Their area was then calculated.

## EXPERIMENTAL RESULTS

The macroscopic investigations showed the formation of adhesions after 7-14 days in the region of trauma, proliferation of blood vessels, and accumulation of small granules of black pigment. In the control series the liver was bright brown in color. After 25 days its color remained unchanged. After 2 months the liver of the experimental and control tritons was pale gray in color and it contained equal amounts of pigment. In no case did the liver recover its original shape (Fig. 1). Data for changes in the weight of the residual liver at various periods after resection of 10% of its mass are given in Table 1.

As Table 1 shows, the weight of the regenerating liver in the experimental animals was close to the weight of the control liver 25 days after operation. Consequently, one month after resection of 10% of the liver tissue it was almost completely restored through hypertrophy of the remaining part of the organ.

In the experiments of series II the original shape of the organ was likewise never restored. The changes were similar to those observed in the experiments of series I, i.e., the weight of the residual organ was increased, but within a shorter time (Table 2).

As Table 2 shows, the weight of the liver in the experimental series was almost up to the control level after 10 days. However, no further recovery took place later. The probable reason for this was metamorphosis (triton larvae began to change into adult forms), and this presumably was also reflected in the rates of regeneration.

TABLE 3. Mitotic Index (in %) in Liver Cells After Resection of 10 and 30% of the Mass of the Organ

| | Resection of 10% of liver | | | Resection of 30% of liver | | | |
|---|---|---|---|---|---|---|---|
| | Time after operation (in dogs) | | | | | | |
| | 7 | 14 | 25 | 5 | 10 | 15 | 21 |
| Control | 0,43 | 4,99 | 0,45 | 1,7 | 0,41 | 0,13 | 1,26 |
| Expt. | 1,06 | 2,58 | 0,41 | 3,4 | 1,16 | 1,61 | 1,91 |
| *P* | 0,0001 | 0,11 | 0,7 | 0,21 | 0,04 | 0,09 | 0,08 |

TABLE 4. Dimensions of Liver Cells (in $mm^2$) in Experimental and Control Series at Various Times of Investigation After Resection of 30% of Liver Mass

| Time after operation (in days) | Control | Expt. | *P* |
|---|---|---|---|
| 5 | 87,0 | 81,3 | 0,929 |
| 21 | 182,4 | 172,4 | 0,7 |

To determine the level of proliferative activity of the liver cells in the intact liver, the mitotic index (MI) in the liver was compared in control tritons of two groups: receiving and not receiving colchicine (fixation at 11 a.m.). These experiments showed that mitoses are in fact present in the intact liver of triton larvae (MI = $0.16‰$) and they are revealed more clearly in animals receiving colchichine (MI = $1.26‰$; P = 0.008).

Investigation of MI in the regenerating liver after removal of 10% of the organ yielded the results given in Table 3.

In the experimental series the value of MI after 7 days was 240% higher than in the control. These results are in agreement with those obtained on adult tritons. After 25 days the mitotic activity of the liver cells was the same in the experimental and control animals (P = 0.7). After resection of 30% of the liver mass (Table 3) an increase in MI was observed at all periods of the investigation, and its value had not fallen by the 21st day after resection of the liver.

As a rule mitoses were observed in the liver cells at the periphery of the organ nearer to the cortical layer, in which intensive division of hematopoietic cells was observed at all times of the investigation. As well as hepatocytes, the reticulo-endothelial Kupffer cells divided no less actively. Sometimes binuclear and multinuclear liver cells were observed, the latter probably the result of incomplete amitosis. The comparative sizes of the liver cells in the experimental and control series after resection of 30% of the liver mass remained practically unchanged in the early and late periods after operation, although the true dimensions of the cells increased with growth of the animal (Table 4).

Regeneration of the liver in larvae of Triturus cristatus thus evidently takes place mainly through mitotic division of liver cells.

After resection of the liver in larvae of this animal, regeneration of the organ thus takes place by regeneration hypertrophy, without restoration of the original shape of the organ, and accompanied by a compensatory growth of its residual part. The speed of recovery is directly dependent on the volume of tissue removed. Regeneration of the liver is largely due to stimulation of mitotic division of the liver cells.

## LITERATURE CITED


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2. L. D. Liozner et al., Regenerative Processes in Vertebrates [in Russian], Moscow (1959), p. 240.