REGENERATION OF THE LIVER IN ANURA

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The comparative aspect of regeneration of the liver has hardly been studied. While the mammalian liver is one of the favorite objects of investigation, liver damage reactions in other classes of vertebrates has been studied only by a few authors, and not sufficiently in full [4,6]. Because of this it is difficult to have a correct concept of the evolution of the regenerative ability of the liver among a number of vertebrates, and consequently to study the peculiarities of liver regeneration in mammals. In particular, the study of regeneration of the liver has suffered greatly because the amphibian liver, an object so easily available for experimentation, has not been used.

There are some data, although insufficiently detailed, on the regeneration of the liver in Anura. Heberlein [5] found that reconstruction of liver in Axolotl takes place as a result of increase in size of the remainder of the organ, i.e., its hypertrophy. R. P. Zhenevskaya [3] is of the same opinion. An analogous condition occurs in tritons [4]. The weight of the liver is restored in approximately 2-3 months.

The data on liver regeneration in frogs are contradictory. N. I. Grogor'ev [2] affirms that in the grass frog the liver is fully restored in 1-3 months after half of its posterior left lobe is removed. On the other hand, R. P. Zhenevskaya [3], having removed half of the posterior left and right lobes of the liver, found a slight hypertrophy of the intact lobes and lack of any restoration of the defect, although her experiments lasted for $3\frac{1}{2}$ months.

That author considers that regeneration of the liver in Amphibia is much weaker than that in mammals, and is not as complete.

In order to clarify the problem as to how the liver is regenerated in frogs, we have conducted special experiments, employing different species of animals and inflicting damage upon different lobes of the liver.

METHOD

In these experiments we have used adult, sexually mature Rana temporaria, Rana esculenta, Rana ridibunda of different ages, Bombina bombina, as well as one year-old Rana temporaria. In most experiments both left lobes and half of the right one were removed completely, in some half of the left posterior lobe or half of the right one were removed. Experiments were of two types.

In the first type of experiment two groups of animals were used, the control group (without liver damage) and the experimental group (with a part of the liver removed). When the animals were killed at different intervals after the partial hepatectomy, the weights of livers in the experimental and the control animals were compared.

In the second type of experiment there were no control animals. Preliminary weighings have shown that the weight of both left lobes and half of the right lobe constitutes 2/3 of the total weight of the liver. With this knowledge we removed the above lobes, and basing on their weight, calculated the original weight of the liver. Later we determined the weight of the regenerated liver, and compared it with the original weight, thus calculating the percentage of weight restoration. This method has several advantages. It allows the investigation of weight restoration of the liver in individual frogs, and as a result, there is no need to use large numbers of animals in these experiments. It must be remembered, however, that the weight of the liver in frogs depends on the season. Therefore, experiments based on observations on individual frogs were conducted during the same time of year, summer, and the frogs were fed regularly.

RESULTS

Our observations have shown, first of all, that, regardless of which lobe of the liver was damaged and whatever amount of liver tissue was removed, there was never any restoration of the amputated part of the liver. The inflicted

damage was retained for the entire period of study, (maximum period was 6 months). As the extent of damage had no tendency to decrease, while the regenerative process was by that time almost over, it may be definitely concluded that restoration of the liver, after it had been damaged, does not take place in frogs. Thus, our conclusions are in complete accord with those of R. P. Zhenevskaya [3].

TABLE 1. Restoration of Liver Weight During its Regeneration in Bombina

duration of regeneration (days)	original weight of liver	amount of tissue removed	weight of regenerated liver	weight of regenerated liver (% of original wgt)
		in milligrams		
22	150	100	180	120
22	174	116	212	122
22	348	233	298	86
26	147	98	150	102
26	240	161	143	59
26	195	131	73	37
26	240	160	107	45
30	138	92	140	101
31	253	169	235	93
31	240	160	238	99
37	333	222	293	88

The conclusions of N. I. Grigor'ev [2] that a complete restoration of the removed portion of the liver is possible in frogs, are, in our opinion, based on erroneous observations. The original shape of the liver is not restored not only in adult frogs, and in yearlings, but also in tadpoles, as had been shown by us earlier [4]. Our observations have shown that regeneration of the liver in frogs takes place not as a result of outgrowth from the damaged surface, but through an increase of the remaining part of the organ. This process is known as regenerative hypertrophy [1].

As a result of the regenerative hypertrophy the weight of the remaining part of the liver increases considerably, and may reach the original value. As an example of this we are giving results of one of a series of experiments on Bombina in which 2/3 of the liver was removed (Table 1).

As seen in Table 1, the results of the experiments are not identical in some cases. The original weight of the liver was restored in 6 out of 11 animals, in 2 the weight of the liver increased considerably and became very near the original weight, in 2 the increase of weight was very small and in 1 there was no increase.

Data obtained in other experiments have shown that lack of regeneration of the liver is encountered in a small percentage of cases. As a rule, already one to two weeks after the operation, the weight of the regenerated liver constitutes 50-80% of the original weight. Twenty-five to thirty days are usually required for a complete restoration of the liver weight, but the latter does not take place in all the specimens.

A similar conclusion may be derived from the data in Table 2, in which is shown the gradual increase of mean percentage of restoration with time. Species differences are revealed during the course of regeneration. Thus, in Bombina the liver regenerates better than in the lake, pond and grass frogs. The following data bear witness to this.

TABLE 2. Restoration of the Original Liver Weight in Bombina at Different Periods of Regeneration

duration of regeneration (days)	number of animals	percentage of restoration of the original wgt of the liver
7	10	52
14	21	65
21	10	69
2	12	76

In experiments on lake frogs, from which 2/3 of the liver were removed, 50-60 days after the operation the following percentage of restoration of the original weight of the liver in individual frogs was recorded: 32,32,33,33,39,40,83,86, 101,108. The average percentage of regeneration was 59, while in Bombina 28 days after the operation it was equal to 76. It will be seen from the data presented, that in half of lake frogs regeneration had not even started by the time they were killed. In similar experiments on pond frogs, 14 days after the operation we obtained an average degree of liver regeneration in 15 specimens, equal to 49%, and with grass frogs it was equal to 53%(17 animals).

Regeneration is more rapid when relatively small portions of the liver are removed, than after more extensive amputations. As an example, in Table 3 are given the results of two series of experiments on Bombina. In the first series 22-42 mg of liver were removed from a total weight of 115-250 mg, i.e. about 1/5 of the liver. In the second series 2/3 of the liver were removed.

As seen from the table, the restoration of the original weight of the liver, when 1/5 of the organ was removed, occurred in 6 days. Differences in the weight of experimental and control frogs in this series lie within the limits of random variations. On the other hand, when 2/3 of the liver were removed, the weight of the regenerated liver was much lower than the weight of the liver in controls, even 40 days after the operation.

Comparing the speed of liver restoration in frogs and in mammals, it may be stated that in the species of frogs used by us, the regeneration of the liver after extensive amputations (removal of 2/3 of the organ) is worse than in rabbits, rats and mice. In these species of mammals the weight of the liver reaches the original level in approximately 2 weeks - 1 month after the operation. It is possible, however, that there may be species of frogs in which liver regeneration may be more intensive. According to Strett [6] in the leopard frog (Rana pipiens) the restoration of the original weight of the liver, after a removal of 1/3 of it, occurs in 30 days. It would be premature to make a generalization about weaker liver regeneration in frogs than in mammals. It can be only affirmed that in a number of mammals, the regeneration of the liver, after 2/3 of it had been removed, is faster than in some species of frogs.

TABLE 3. Restoration of Liver in Bombina after Removal of Varying Amounts of Liver Tissue

Experiments series	group of animals	duration of experiment (days)	amount of tissue removed	number of animals	average relative weight of the liver (percentage)
First	control	6	0	10	2.90
		6	1/5	11	2.82
second	experimental	40	0	12	2.93
		40	2/3	17	2.09

TABLE 4. Number of Cells in 100 Microscope Fields in Experimental and Control Bombina

Control	Experimental
2198	2253
2277	2379
2326	2209
1830	2762
1987	2278
	1704
Average 2124	2264

The increase in weight of the liver, after its damage in frogs, as well as in mammals, depends on the increase in the number of cells as a result of their intensive reproduction, and not on the direct consequences of the operation, such as edema, inflammation, etc. The following facts bear witness to this. We have counted the number of cells in sections of regenerated livers of Bombina 26 days after removal of 2/3 of the liver. In these frogs the original weight of the liver had been restored. The cells were counted in 100 microscope fields (ocular 7×, objective 90×, diaphragm in ocular 7 × 7 mm). The results are given in Table 4.

The data given in Table 4 show that the average numbers of cells in the livers of experimental and control frogs are almost identical, and consequently, the liver cells in frogs of both groups are approximately of the same size. It can be concluded from this that the increase of the liver weight in the experimental frogs, as compared to the weight immediately after the operation, is dependent on the increase of the number of cells. The general histological picture of liver structure was the same in the experimental and the control animals. There were no pathological changes in the structure of the regenerated livers.

Thus, the liver of frogs is capable of regeneration. The latter occurs, as in mammals, as a result of regenerative hypertrophy, without an outgrowth of tissues from the damaged surfaces. A future investigation will include the study of the course of restoration at different periods of regeneration and the accompanying cellular changes.

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