SPLEEN REGENERATION IN MICE FOLLOWING REMOVAL OF THE MAJOR PART OF THE ORGAN

L. D. Liozner and G. V. Kharlova

Laboratory of Growth and Developments (Head–Doctor of Biological Science L. D. Liozner) of the Institute of Experimental Biology (Director–Prof. I. N. Maiskii) AMN SSSR, Moscow
(Presented by Active Member of the AMN SSSR N. N. Zhukov-Verezhnikov)
Translated from Byulleten Éksperimental'noi Biologii i Meditsiny Vol. 49, No. 4, pp 96-100, April, 1960
Original article submitted March 12, 1959

Despite the fact that regeneration of the spleen of mammals has been studied since the middle of the XIX century, there is still no unified opinion about its capacity for restoration following partial resection [1, 2, 5, 6, 7]. In our laboratories É. I. Mun'kina and G. I. Sol'ts [4] and B. M. Levitina [3] observed the regeneration of the spleen in certain mice and rats after the removal of half the organ However, a detailed description of the structure of the regenerating organ was not given in these works.

In an attempt to eliminate the indicated shortcoming we set up experiments on the regeneration of the spleen in mice, removing the major part of the organ (80-90%), since the study of a small fragment of the spleen makes it easier to acquire impression of the structure of the regenerating organ.

METHOD

The removal of 80-90% of the organ was performed on 160 white pubertal male mice, 15-18 g in weight. Both ends were amputated from the externalized spleen, leaving in the middle, at the site of vascular entry, a piece 1-2 mm in width. The portion of the spleen removed was weighed. In order to gain an impression of the weight of the fragment remaining in the animal's body, a piece of the removed portion of the spleen was cut out, approximately equal to that fragment in its dimensions. We will subsequently arbitrarily call the weight of this piece the original weight of the retained fragment. This piece was fixed and subjected to histological study. Each mouse had its marking, so that it was possible to know how much the residual fragment weighed (on the basis of the weight of a piece comparable to it) as well as the weight of the spleen regenerating from it.

The animals were sacrificed by decapitation on the 2nd, 4th, 6th, 14th, 21st, 28th, 38th, and 73rd days after the operation. Normal mice were sacrificed on the 14th, 28th, 38th, and 73rd days at the same time as the experimental animals, these serving as controls. Upon the death of the animals several of the spleens were fixed in Zenker's solution, imbedded in paraffin, and the sections stained with hemalaun plus eosin. In these preparations the number of mitoses and Malpighian bodies were counted. The average measurement of the Malpighian body was 350-400 μ, and thus they were counted in every 50th section (width of the section was 7 μ). The mitoses were counted in a center section across a Malpighian body, using 100 visual fields (ocular 10×, objective 90 ×; diapragm in the ocular with a quadratic aperture measuring 7 × 7 mm).

For a clearer outlining of the white pulp from the red, several regenerating spleens were fixed in 12% formalin and treated by the method of Lepene (histochemical reaction to peroxidase). Longitudinal and cross sections of the spleens were prepared; 5-10 sections, taken from each piece, were drawn on paper, using the Edinger apparatus, noting by different shading the parts corresponding to the white and red pulp. Then the pieces corresponding to the white and red pulp were cut out and seperately weighed and the ratio between the areas that they occupied computed.

Data on the measurements and weighings of the spleens and remaining fragments are presented in Tables 1 and 2. They give evidence of the fact that during the period of observation regeneration processes took place in the residual fragments.

Two days after the operation the weight of the residual fragment increased by an average of two times. Up to that time, as can be seen from Table 3, the ratio of the area occupied by the white pulp to the area occupied by the red was less than in the normal; this is apparently evidence of the increased blood storage of the fragment. In all fragments there were observed processes of destruction, manifested to a varying degree.

Further increase in the regenerating fragment was observed after 4-6 days. At this time the processes of break-down had somewhat diminished. Proliferation was noted in the reticular tissue, and the processes of myelopoiesis, lymphopoiesis and erythropoiesis were proceeding intensively. The dimensions of the small Malpighian bodies increased to a certain degree. The ratio of the area of the white and red pulps was equal to 0.74 (one fragment examined), i.e., was within normal limits. Beginning with two weeks, in the majority of animals there was observed such significant increases in the dimensions and weights of the organs that it was already possible to decide with certainty in which cases regeneration occurred.

At a different time of the year conflicting results were obtained. Thus when the experimental animals

TABLE 1. The Average Weight of the Fragment and of the Spleen Regenerating from It at Different Intervals After the Operation

Duration of the expt. (in days)	Total no. of animals in the series	Number of animals in which regeneration occurred	Original wt. of the spleen (in mg)	Wt. of the residual fragment of spleen at time of operation (mg)	Weight of the regenerated spleen (mg)
2	6	6	76,0	9,5	18,2
4	5	5	84,2	11,2	23,4
6	6	6	82,5	10,3	41,1
14	13	10	104,0	11,4	42,0
	10	8	83,0	8,7	28,3
	21	9	114,0	9,4	27,7
21	7	5	53,7	6,2	30,0
28	14	11	53,0	7,3	43,1
	12	10	95,7	10,5	49,7
38	25	21	64,4	5,0	42,8
73	10	10	86,0	10,0	54,1

TABLE 2. The Average Dimensions of the Fragment Remaining After Operation and of the Regenerated Spleen

Length of expt. (days)	Size of residual spleen fragment (in mm)			Size of regenerated spleen (in mm)		
	length	width	thickness	length	width	thickness
2	4,0	2,0	1,5	4,4	2,9	2,0
4	4,4	2,1	1,3	4,6	3,4	2,0
6	4,1	1,9	1,1	6,3	3,8	2,8
14	4,6	2,1	1,3	5,1	3,3	2,3
21	3,5	1,7	—	4,4	3,3	—
28	4,6	2,1	—	5,7	4,6	—
38	4,7	1,1	1,7	6,1	4,0	2,7
73	4,8	2,1	1,9	6,0	4,5	3,8

were sacrificed in the spring, after 14 days, regeneration of the spleen was observed in two different series in 80 and 77.7% of all cases, while after the same number of days in the fall, the residual fragment of spleen increased in only 43.4% of the mice; in the remaining animals during the elapsed two week period the fragment decreased or underwent complete destruction. The weight of the regenerated fragment two weeks after the operation increased by an average of 3 to 3 $^1/_2$ times. In one animal the regenerated fragment of spleen exceeded the weight of the entire organ on the day of operation: 31 mg in place of 30 mg.

TABLE 3. The Ratio of the Area Occupied by the White and Red Pulp in Sections Across the Spleen

Residual fragment of spleen	Duration of regeneration	
	2 days	14 days
0,99	0,81	0,52
0,91	0,81	2,09
0,71	0,89	0,74
0,98	0,68	0,79
0,74	0,65	0,50
0,86	0,70	0,81
0,85	—	0,90
0,86	—	0,83
0,98	—	1,21
0,97	—	0,92
0,91	—	1,29
0,96	—	1,51
1,11	—	—
0,96	—	—
0,72	—	—
0,75	—	—
0,89	0,76	1,01

In cross sections it was possible to observe a significant increase in the residual fragment; the sections did not have a triangular form, but rather a round shape, the angles being rounded out. The longitudinal sections were

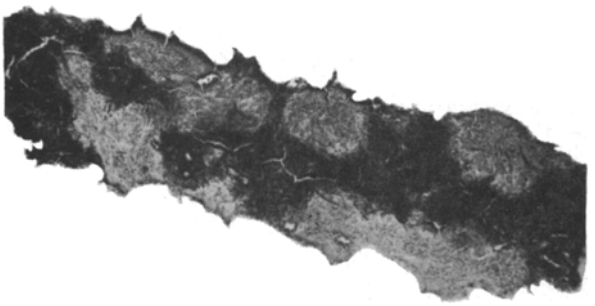

Fig. 1. Longitudinal section of the fragment of spleen remaining after operation. Magnification: ocular 5×, objective 4×. Stained according to the method of Lepene.

Fig. 2. Longitudinal section of a regenerated spleen 14 days after the operation. Magnification: ocular 5×, objective 4× Stained according to the method of Lepene.

especially indicative, in which the mass of the regenerated fragment was markedly increased (Fig. 1 and 2), as was the reticular stroma and the number of Malpighian bodies.

A count of the number of Malpighian bodies showed that two weeks after the operation the number increased by 2 $^{1}/_{2}$ times in comparison with the control. In the original fragment the number of Malpighian bodies on the average (14 animals) was equal to 8.9, while in the regenerated fragment it reached 22.4 (8 animals).

The number of mitoses in the Malpighian bodies per 100 visual fields also increased significantly in comparison with the control: from 8 (6 animals) to 31 (4 animals).

It was possible to note 3 types of interrelationships between the red and white pulps in the regenerated spleens. In several animals the ratio of the area of the white pulp to the area of the red did not differ from that characteristic for the controls; in others there was observed a considerable increase in the area occupied by the white pulp; in 2 animals the ratio of the area of white and red pulp was less than in the control.

It was possible to note further increase in the weight of the regenerated spleens 3-4 weeks after the operation. In several series the weight of the spleen increased by 4-5 times. However, the proliferative processes at this time had quieted down somewhat. The number of mitoses 3 weeks after the operation decreased to 12.5 (average for 4 mice).

Upon sacrificing the experimental animals 38 days after the operation regeneration of the organ was observed in 84% of the cases. In 4 mice the weight of the regenerated organ considerably exceeded the weight of the spleen at the time of operation. However, the average weight of the regenerated spleens (76, 104, 68, and 86 mg in place of 53, 54, 55, and 70 mg respectively) at this time had still not attained the weight of the spleens in the control animals, which was equal to an average of 160 mg.

Sacrifice of the animals 73 days after operation showed that the fragment of spleen increased by an average of 5 times; in several cases it reached the weight of the spleen in the control mice. The ratio of the area of white and red pulps was equal to 1.1.

The regenerated fragments of spleen and the spleen of the control mouse are presented in Fig. 3. The weight

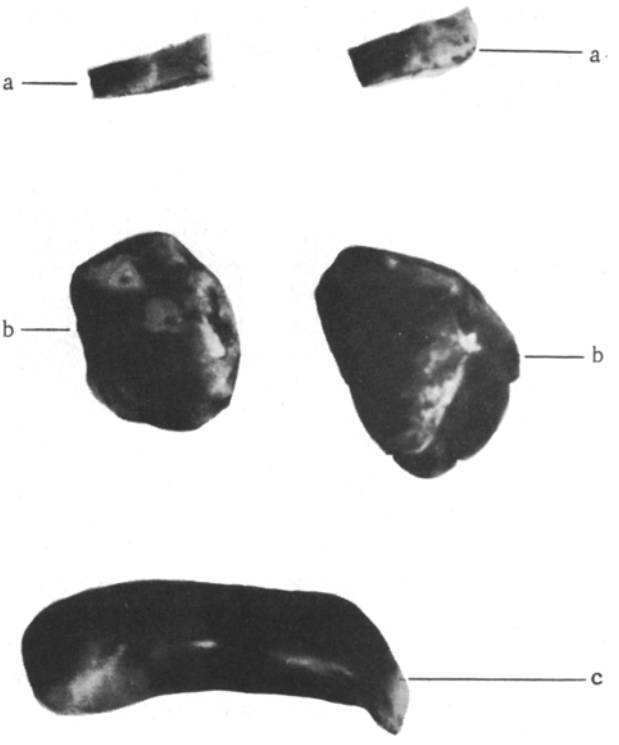

Fig. 3. Fragments of spleen (a), remaining after operation (weight 6 and 9 mg); regenerated spleens (b) 73 days after operation (weight 82 and 97 mg); spleen (c) of the control animal (weight 95 mg).

of the regenerate was 97 mg, the original fragment being 6 mg; the length of the regenerate was 6 mm, of the original fragment, 1 mm. The original form of the organ was not reconstructed; the fragment increased evenly in all three measurements. The ratio of the white and red pulps in this fragment was equal to 0.95.

The data obtained bears evidence of the capacity of the spleen of mice for regeneration after removal of 80 to 90% of the organ. This may be adjudged on the basis of the marked increase in the weight of the spleen. Histological investigation showed that the increase in the weight of the fragment of spleen is not caused by blood engorgement, but by an increase in the mass of the organ's parenchyma. The ratio of the mass of the white and red pulp characteristic for the normal was preserved in the regenerating spleen, and the number of Malpighian bodies increased; the latter, however, did not reach the normal, which indicates a certain hypertrophy of the Malpighian bodies in the course of regeneration.

Our observations permit, to a certain degree, the explanation of the conflicts present in the literature on the question of the capacity of the spleen for regeneration. As is shown by the data presented, regeneration of the spleen does not occur in all cases, and, at different times of the year, it is manifested to different degrees. It is also possible that several authors denied the capacity of the spleen for regneration on the basis of the fact that they did not account for the peculiar means of its reconstruction, wherein along with well manifested regeneration phenomena the original form of the organ is not reconstructed (regeneration hypertrophy).

SUMMARY

White mice were subject to spleen amputation from both ends, with a fragment thereof left intact, equal to about 1/8 to 1/10 of the initial weight of the organ. A considerable increase in the weight of the spleen was noted in the mice sacrificed 14 to 73 days after the operation. In individual instances the weight of the regenerating spleen equalled that of the spleen of control mice. The ratio between the area occupied by the white and the red pulp approached normal. The number of Malpighian bodies was increasing but would not reach their initial level. The spleen failed to recover its original shape, i.e., its restoration pursued the course typical for regenerative hypertrophy.

LITERATURE CITED

[1] M. B. Ambrosovskii, Operative Approaches to the Spleen in Bullet Wounds [in Russian] Diss. Kand. (Leningrad, 1948).

[2] B. Brandsburg, Zhurn. Sovrem. Khir. 1, 5-6, 611 (1926).

[3] B. M. Levitina, Data from the Reports of the Conference of Young Scientists of the Inst. of Exper. Biol. AMN SSSR (Moscow, 1958) p. 10.

[4] E. I. Mun'kina and G. I. Sol'ts, In the book; M.A. Vorontsova, The Regeneration of Lost Organs in Animals and Humans [in Russian] (Sov. Nauka, 1953) p. 105.

[5] A. G. Sosnovskii, Injuries of the Spleen in Clinical Practice and Under Experimental Conditions [in Russian] Diss. Dokt. (Odessa, 1940).

[6] R. M. Calder, J. Path. a Bact. 49, 351 (1939).

[7] G. R. Cameron, Pathology of the Cell (Springfield, 1952).