EXPERIMENTAL BIOLOGY

THE MITOTIC BEHAVIOR OF THE LIVER DURING REPARATIVE REGENERATION

L.D. Liozner, Z.A. Ryabinina and V.F. Sidorova

From the Laboratory of Growth and Development (Head – Prof. L.D. Liozner) of the Institute of Experimental Biology (Director – Prof. I.N. Maiskii) of the AMN SSSR, Moscow

(Received August 12, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

Most workers have come to the conclusion that regeneration of the mammalian liver after injury takes place on the basis of the mitotic proliferation of the cells of the residual part of the organ. At the same time, the findings of certain workers [4, 6, 7] on the time of appearance of the mitoses and their number are in disagreement, although in the majority of experiments, a standard operation was used (extirpation of $\frac{2}{3}$ of the liver by the Higgins and Anderson method). These differences may be partly explained by the fact that, in the analysis of the experimental findings, no allowance was made for the time of fixation of the tissues or the time of injury. Nevertheless, these important factors must not be ignored, as has been shown by the findings of a number of workers [1, 2, 3, 5, 9] who studied the physiological regeneration of various tissues, and by the special experiments of Jaffe [8]. Jaffe discovered a well-marked diurnal periodicity of the mitotic activity of the liver cells during regeneration of the liver in rats. According to his results, the maximum number of mitoses is found during the mourning hours. Jaffe also attempted to establish a relationship between the mitotic activity and the time of operation, but came to the conclusion that, generally speaking, no such relationship exists.

We set out to ascertain how widespread is the phenomenon of diurnal periodicity of mitotic activity during regeneration of the liver, and, for this purpose, we carried out experiments, not on rats as used by Jaffe, but on mice. We also considered it necessary to investigate, in mice, the influence of the time of operation on the subsequent mitotic activity of the liver cells.

EXPERIMENTAL METHOD

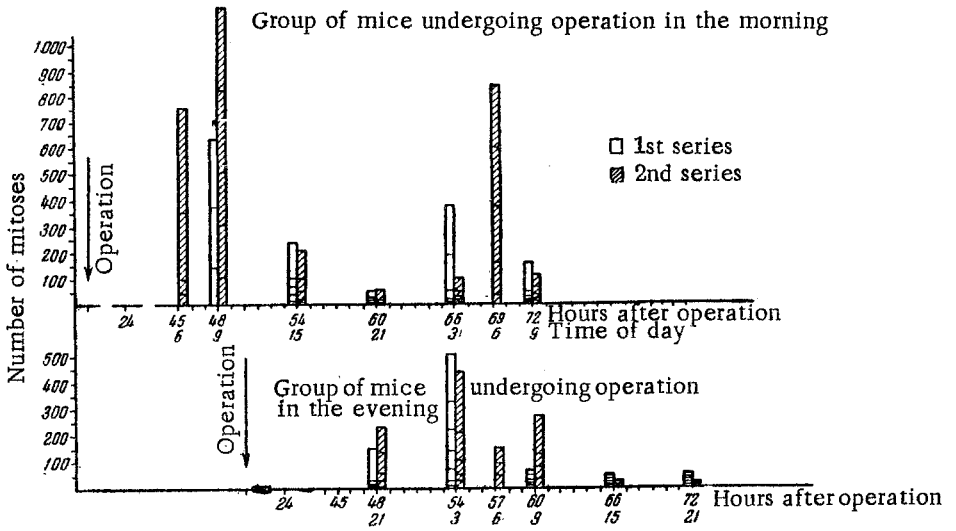
Experiments were performed as follows. The left lateral and central lobes of the liver, amounting to 60-70% of the total weight of the organ, were completely removed from white mice. One group of mice was subjected to this type of operation between 9 A.M. and 12 noon (morning group) and a second group between 6 P.M. and 9 P.M. (evening group).

The regenerating liver was fixed for histological examination 48, 54, 60, 66 and 72 hours after operation (5-7 animals at each time). The times of fixation of the morning group of mice were 9 A.M. 3 P.M., 9 P.M. 3 A.M. and 9 A.M., and of the evening group the corresponding times of fixation were 9 P.M., 3 A.M., 9 A.M., 3 P.M. and 9 P.M. (see table). The animals were killed (on the 2nd-3rd day after operation) at times selected on the basis of research by Yakoyama et al. [10], which showed that the maximum number of mitoses is observed in the regenerating liver of the mouse at this time.

Before fixation, the liver was weighed. The residual lobes of the liver were enlarged $1\frac{1}{2}$ to $2\frac{1}{2}$ times. On the average, the weight of the regenerating liver amounted to 58-78% of the weight of the liver in control animals. The material was fixed in Zenker's fluid and the sections were stained with hematoxylin eosin. The number of mitoses per 4500 liver cells was counted in a small piece of tissue excised from the caudate lobe of the liver.

EXPERIMENTAL RESULTS

On microscopic examination of the regenerating liver, it was found that on the 2nd-3rd day after operation, besides intensive mitotic division of the liver cells, in a number of cases, the cells were enlarged. The hypertrophy of the cells was shown particularly clearly at the periphery of the hepatic lobules, where a disturbance of the proper arrangement of the cells in trabeculae was often observed. The liver lobules were larger than normal; in the experimental animals, the distance between the central veins in cross sections of the lobules was on the average 42.5 divisions of the ocular micrometer, and in the control animals, 35.8. There was no regular feature of the distribution of the dividing cells to be seen in the lobule. Besides mitoses, amitoses were also found in the liver cells, although these were rarer. We also found, rarely, mitotic divisions in the epithelium of the bile ducts and in the Kupffer cells.



Changes in the number of mitoses, in the course of the second and third days after operation, in the liver of mice undergoing operation in the morning and evening hours. Each column represents the total number of mitoses found in the liver of all mice killed at that particular time.

The results of the mitosis counts in the liver cells at different times after operation are shown in the table and in the figure (see data of the 1st series). Attention is drawn, in the first place, to the fact that at hardly any time of regeneration was it possible to obtain absolutely consistent results in respect to the number of mitoses. Whereas the liver of some mice showed high mitotic activity, an almost complete absence of mitoses was observed in that of other mice. There were, therefore, no grounds for speaking of a regular diurnal periodicity of mitosis in the regenerating liver of mice. Nevertheless, the results obtained did show certain regular features about the mitotic activity. The distribution of the number of mitoses among the different times of fixing was, undoubtedly, uneven. Periods could therefore be distinguished in which enhanced mitotic activity was observed. We accepted, to some extent conventionally, that increased mitotic activity could be defined as the presence of 100 mitoses each in the liver of not less than 2 mice. When the table is scrutinized from this point of view, it is easy to be convinced that in the mice undergoing operation in the morning two peaks of mitotic activity were observed – at 9 A.M. and 3 A.M. on the 2nd and 3rd days after operation. In the mice undergoing operation in the evening, it is possible to find only one peak of mitotic activity of the liver cells, at 3 A.M. on the 3rd day after operation. At the remaining times of day, a very small number of dividing cells was to be observed. Only in individual mice were larger numbers found. On the average, the number of mitoses in the "morning" group of mice was greater than in the "evening" group. The average number of mitoses per mouse undergoing operation in the morning was 57.3; the average, per mouse, in the evening group – 26.9. If in calculating the average number of mitoses, only the figures obtained at 4 intervals of time, i.e., in the course of 24 hours, were used, then the average number of mitoses per "morning" mouse was 54.1, and per "evening" mouse 31.6.

TABLE

Number of Mitoses in the Liver of Individual Mice at Different Times after Operation

Time of operation	Morning							Evening					
Time of fixing	6	9	15	21	3	6	9	21	3	6	9	15	21
Duration of regeneration (in hours)	45	48	54	60	66	69	72	48	54	57	60	66	72
	First series												
Number of mitoses in individual mice		259	130	29	186		109	123	181		18	12	12
		231	34	10	136		17	17	110		17	13	9
		138	29	5	35		12	5	76		10	9	7
		3	26	3	15		10	5	72		8	3	7
		0	10	1	2		3	0	44		7	3	5
									21			0	5
									4			0	
Average		126	45	10	74		30	30	72		12	6	7
Average number of mitoses per mouse	57,3							26,9					
	Second series												
Number of mitoses in individual mice	394	320	108	40	52	238	48	90	222	54	139	6	15
	259	313	50	5	19	221	24	83	110	44	48	4	3
	54	203	34	5	12	217	14	28	50	19	46	3	0
	41	197	6	3	12	124	10	21	27	16	30	3	0
	2	107	6	0	2	31	6	6	26	10	0	0	0
Average	150	228	41	11	19	166	20	45	87	28	54	3	4
Average number of mitoses per mouse	90,9							37					

In order to verify our findings, we repeated our experiments after one year under the same conditions (see table, second series). We added one further time of fixing the material, namely 6 A.M., in order to find out whether the peak of mitotic activity of the liver cells persisted throughout the whole interval from 3 to 9 A.M. As may be seen from the table, the results obtained were similar to those of the previous series. There were, however, a few variations. In the "morning" mice, we found no peak of mitotic activity at 3 A.M. on the 3rd day. An increase in the number of divisions was found later, namely at 6 A.M., In the "evening" mice, only one peak of mitotic activity was now found – at 3 A.M. on the 3rd day after operation. The average number of dividing cells per mouse undergoing operation in the morning was now 90.9, and per mouse undergoing operation in the evening – 23.7. If the additional fixing time (6 A.M.) was excluded, the corresponding average value would then be 63.8 and 38.7, which were close to the results of the first series.

It follows from these findings that periodic increases in the mitotic activity of the liver cells could be detected in the liver of mice undergoing operation. Between 3 A.M. and 9 A.M. the number of mitoses was higher than at the other times of day. This feature was apparent, however, in far from every animal. It cannot, therefore, be asserted that by fixing the liver at a particular period of time, it will always be possible to find a large number of mitoses. Nevertheless, if this interval of time is examined, there is no doubt that it is characterized

by an increase in mitotic activity. We may point out that, in his paper, Jaffe found a constant periodic increase in mitotic activity thanks to the fact that he injected the animals, 4 hours before fixing, with colchicine which led to a gradual accumulation of mitoses.

We found an increase of mitotic activity only on the 2nd and 3rd days after operation and at the beginning of the 4th day; at 9 A.M., the number of dividing cells was small. Examination of sections of the regenerating liver on the 5th and 6th day also revealed a small number of mitoses.

The second conclusion from our work was that the mitotic activity of the liver cells depended on the time of operation. The "evening" mice had only one peak of mitotic activity of the liver cells, taking place at 3 A.M. (54 hours after operation). Neither at 6 nor at 9 A.M. did they show an increase in the number of dividing cells. Meanwhile, the "morning" mice showed a peak of mitotic activity at 6, 9, and 3 A.M. (45, 48, and 66 hours after operation). As a result, more mitoses appeared, on the average, in the "morning" than the "evening" mice, i.e., their mitotic activity was higher. The impression was created that there were times at which the mitotic activity of the cells of the regenerating liver was greatest (3-9 A.M.), although it must be remembered that the appearance of mitotic activity within these limits depended on the time of operation.

Our findings showed that the behavior of the mitotic division of cells during regeneration of the liver in mammals is quite complex. This has obviously been largely responsible for the differences in the results obtained by individual authors.

Although, in a few mice, we obtained no enhanced mitotic activity of the liver cells, it would be premature to conclude that other methods of production of new cells, namely amitosis, had any considerable part to play. Since it was not possible to observe a regular diurnal periodicity in the liver of the mice, it is not excluded that mitotic activity may take place in individual mice at another time of day. Our results raise the question of the necessity of making a more detailed analysis of both the methods of increase in the number of cells during regeneration of the liver and the conditions regulating mitotic activity. One of these conditions, previously unrecognized, which must also be borne in mind is the time of operation.

SUMMARY

Mitoses in the regenerating liver were observed on the 2nd–3rd day after the removal of $^2/_3$ of this organ, mainly from 3 A.M. to 9 A.M. There is not much mitotic activity during the rest of the 24 hours, large number of mitoses being revealed only in individual animals. There were many more mitoses revealed in the liver of the mice operated at 9 to 12 in the morning than in those operated during the evening hours (at 6 to 9 P.M.).

LITERATURE CITED

[1] M. T. Golobova, Byull. Éksptl. Biol. i Med., 46, No. 9, 118-122 (1958).*

[2] I.A. Utkin et al., Byull. Éksptl. i Med. 41, No.4, 62-66 (1956).*

[3] C.M. Blumenfeld, Arch. Path., 1942, v.33, p.770-776.

[4] A.M. Brues, B.B. Marble, J. Exper. Med, 1937, v. 65, p.15-28.

[5] W.S. Bullough, Phil. Transactions of the Royal Soc. London, 1946, v.231, p.453-516.

[6] R.D. Harkness, Brit. Med. Bull., 1957, v.13, p.87-93.

[7] G.M. Higgins, R.M. Anderson, Arch. Path., 1931, v.12, p.186-202.

[8] J. Jaffe, J. Anat. Rec., 1954, v. 120, p.935-954.

[9] H.R. Mühleman, S. Hartl, Bull. Schweiz. Akad. med. Wiss, 1955, Bd. 11, S.379-385.

[10] H.O. Yakoyama, M.E. Wilson, K.K. Tsuboi and R.E. Stowell, Cancer Res., 1953, v.13, p.80-85.

* Original Russian pagination. See C.B. Translation.