EXPERIMENTAL BIOLOGY

THE PHYSIOLOGICAL REGENERATION OF THE MAMMALIAN LIVER

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According to the results of many investigations, mitoses are either not found in the liver of adult mammals [8] or they are found in very small numbers: 1 mitosis per 10,000-20,000 cells [7, 3]. In this connection, the problem of the method of renewal of the cellular composition of the liver remains unsolved. Some research workers consider [8, 13, 14] that the liver is a "stable" organ in which no replacement of cells takes place, while others [3, 8, 12] incline to the view that liver cells die in the process of the vital activity and are then replaced with new cells, formed as the result of amitotic division. Insufficiently clear data has, however, been submitted in support of this point of view.

Our attention was attracted to work in which the diurnal rhythm of mitotic activity was studied in various organs characterized by the presence of obvious proliferation. It was shown, in particular, [1, 2] that in rats and mice the mitotic activity in the cornea, the epidermis, and the mucous membrane of the small intestine was minimal at midday and increased sharply during the night and morning hours. We found a similar pattern in work which we carried out jointly with Z. A. Ryabinina [4], on the investigation of changes in the mitotic activity of the regenerating liver of the mouse. Studies of the effect of hypnotics on the mitotic activity of the liver cells of rats showed [6] that a large number of mitoses could be found during the hours of night in many of the control animals which were untreated in any way. There are reports of the diurnal periodicity of the mitoses in the liver during growth [15].

When examining the liver of animals acting as controls to mice used in the work cited above [4], our attention was also directed to the fact that mitoses could easily be found in some of them during the hours of morning and night.

These findings led us to postulate that the replacement of the liver cells in mammals is brought about by mitotic division, although this cell division takes place mainly during the night and morning, and by virtue of the fact that the majority of research workers have fixed the liver of their experimental animals during the daytime, they have been unable to find any mitoses.

In order to test this hypothesis, we carried out a special series of experiments in which animals were sacrificed at various times of the day or night, and then the mitotic activity of the liver cells was investigated.

EXPERIMENTAL METHODS

Eighty sexually mature male white mice, weighing on the average 22 g, and taken recently from the nursery, were divided into 8 groups, each of 10 mice. The animals were kept on ordinary rations and subjected to no form of interference. The mice were killed by decapitation at intervals of 3 hours throughout the 24 hours. The liver

TABLE

Mitotic Activity of the Liver Cells in Adult Mice at Different Times of Day and Night (November, 1958)

	Time of fixation of liver (hours)							
	12	15	18	21	24	3	6	9
Number of mitoses per 6000 cells in individual animals	0	0	0	3	0	1	2	4
	0	0	0	0	0	10	1	4
	0	2	0	1	0	0	9	0
	0	0	0	0	0	3	5	5
	1	1	2	0	0	1	0	1
	4	1	0	1	1	6	10	1
	1	1	1	0	0	4	18	1
	0	2	0	0	0	2	5	2
	1	0	1	2	1	0	15	0
	0	0	1	1	1	—	9	0
Average	0.7	0.7	0.5	0.8	0.3	2.7	7.4	1.8

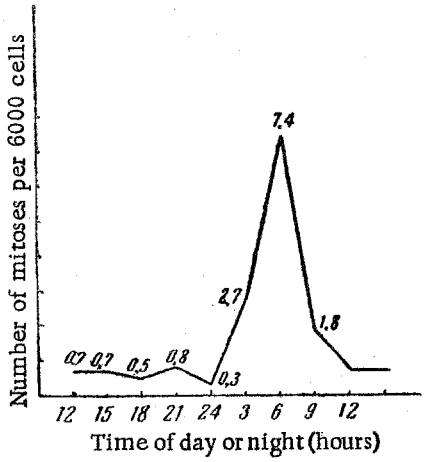

Fig. Changes in the mitotic activity of liver cells at different times of day and night.

was fixed in Zenker's fluid with acetic acid. A piece of the left lateral lobe of the liver, measuring 16-20 mm^2, was excised from the liver which weighed on the average 1300 mg. The pieces of liver were embedded in paraffin and sections were cut to a thickness of 6 μ and stained with hemalum and eosin. Mitoses were counted in 300 fields of vision (ocular 7, objective 90, the diaphragm in the ocular fitted with an aperture measuring 7 mm square). Under these conditions a field of vision contained about 20 cells, so that about 6000 cells were examined altogether. Twenty sections were always examined, each 8th-10th being selected, in order to prevent the same mitosis being counted twice. Only mitoses in liver cells were counted.

EXPERIMENTAL RESULTS

The results obtained are shown in the table and also illustrated graphically (see figure).

It can be seen from the figures in the table that the mitotic activity of the liver cells of the mice varied at different times of day and night. It was low in the period from 12:00 to 24:00 hours. In many animals at this time mitoses were completely absent and in the rest, as a rule 1-2 mitoses were seen. On the average there was 0.3-0.8 mitosis per animal (or more accurately per 6000 cells), which agrees fully with the findings in the literature. A different picture can be observed in livers fixed at later times: 3:00, 6:00 and 9:00 hours. At these times a large number of mitoses could be seen. Its average values were 2.7, 7.4, and 1.8 respectively. Mitoses were especially numerous in the group of mice at 6 a.m., when the mitotic activity was maximal. Of 74 nuclei of liver cells dividing at this time, 20 were at the prophase, 35 at the metaphase, 3 at the anaphase, and 16 the telophase. The phase ratio of the mitoses was similar to that observed during reparative regeneration of the liver, i.e., the greater number of nuclei were in the early stages of division [7]. Meanwhile, in some animals at this same period mitoses were rare or absent altogether.

It was shown by statistical treatment of the results obtained that the difference between the number of mitoses at 3:00 hours and at the preceding times of the experiment was significant ($P = 0.0000$). The difference

between the number of mitoses at 24:00 and 6:00 hours was also significant. The difference between the number of mitoses at 3:00 and 6:00 hours was close to significant (P = 0.03).

The findings suggest that our hypothesis was confirmed. The mitotic activity in the liver of mice was reasonably well marked in the period from 3:00 to 9:00 hours. Accurate limits of the period of increased mitotic activity were not defined, for the intervals of time used in the investigation were large, namely 3 hours. Mitotic activity, although on a far lower scale, was also present at the other times of day and night. Rough calculations show that, if the duration of mitosis in the liver cells is taken to be 1 hour, the cell complement of the liver may be completely renewed in 100 days. The rate of physiological regeneration in the liver is slower than in some other organs, but the replacement of its cells is an undoubted fact.

It must be pointed out that we could find no special "cambial" areas in the liver, in which particularly intensive division of liver cells might take place. Mitoses were found everywhere throughout the lobules, and no tendency was shown for them to accumulate at the periphery. Replacement of the Kupffer cells, the cells of the stroma, and the cells of the bile ducts took place as well as of the liver cells, as was shown by the frequent discovery of mitotic figures of division of their nuclei. We therefore consider that there are no grounds for regarding the liver as a "stable" organ, in view of its physiological regeneration.

It is interesting that the curve which we obtained showing the changes in mitotic activity of the liver cells was extremely similar to the curves of mitotic activity of the other tissues investigated by M. T. Gololobova [1, 2].

Naturally, the discovery of mitotic activity in the liver cells does not disprove the possible occurrence of amitotic division in the liver, but the detection of amitosis is difficult on account of the lack of precise criteria of their presence. The diurnal differences in mitotic activity may be to some extent associated with findings of diurnal changes in the biochemical and histochemical indices of the normal liver [5, 9, 10].

The discovery of the method by which the physiological regeneration of the normal mammalian liver is effected makes it imperative that the diurnal changes in the mitotic activity of other organs be studied, in which no mitoses are found during the daytime.

SUMMARY

Sexually mature white mice, weighing 22 gms, were sacrificed every 3 hours within the 24-hour period.

Mitoses in the liver were counted in 300 fields of vision (in about 6000 cells).

The mitotic activity is very low during the period from 12:00-24:00 hours. It is increased at 3:00 hours, reaches its maximum at 6 (7.4 mitoses) and is reduced at 9:00 hours. These data demonstrate that replacement of cells in the liver occurs by mitotic division during the night and morning hours.

The rise of mitotic activity during the period from 3:00 to 9:00 hours is statistically significant.

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