RATIO BETWEEN THE NUMBERS OF POLYPLOID AND BINUCLEAR LIVER CELLS DURING THE 24-HOUR PERIOD

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It was still believed only comparatively recently that physiological regeneration does not take place in the parenchyma of the liver [8, 12]. Most authorities now hold the opposite view, although the mechanism of replacement of the liver cells has not been explained. Some workers consider that the cells divide only by mitosis [6, 9], others – only by amitosis [7, 10], and finally, considerable attention has been paid recently to endomitosis [3].

In researches of considerable interest the changes in the number of mitoses [4] and in the number of binuclear cells [11] in the liver were studied during the 24-hour period. These investigations showed that the periodicity of mitoses and emitoses during the 24-hour period (more accurately, so far as the amitoses are concerned, the number of binuclear cells) is equally well defined: the maximum of the number of mitoses and binuclear cells fell during the night and morning, and the minimum during the afternoon.

In our investigations of regeneration of the liver in rats after starvation for 7 days we found that besides changes in the number of binuclear cells, sharp changes also occurred in the number of cells with large (octoploid) nuclei, and also in the number of cells which died or were on the way to death. These findings suggested that the binuclear, polyploid, and dying cells are in some way interconnected, and this relationship is more easily apparent during examination of the changes in the liver of normal animals during the 24 hour period.

To study this problem experiments were carried out, during which animals were sacrificed at different times of day or night and the mitotic activity and numbers of binuclear, octoploid, and dying cells in the liver.

EXPERIMENTAL METHOD

The investigation was carried out on the livers of male albino rats weighing about 150 g. Altogether 40 animals were used. To ensure standard conditions, for 24 h before the experiment the animals were placed in cages in laboratory conditions and kept on an ordinary diet. The animals were sacrificed by decapitation at intervals of 3 h: at 11 A.M., 2, 5, 8, and 11 P.M., and 2, 5, and 8 A. M. Pieces of the left lateral lobe of the liver were fixed in Carnoy's fluid. Paraffin-wax sections were cut to a thickness of 5 μ and stained by Feulgen's method.

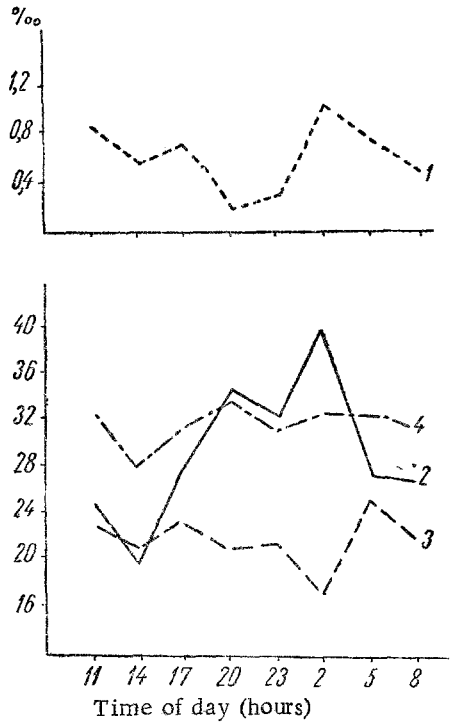
To count the cells an ocular diaphragm measuring 8 × 8 mm was used. The total number of nuclei and the numbers of mitoses, binuclear cells, large polyploid (presumably octoploid) and disintegrating nuclei were counted. The latter included pycnotic nuclei, nuclei with a large vacuole in the center or a fragmented membrane, and also light nuclei, optically empty in appearance, with a few chromatin granules situated at the inner side of the membrane. In each case the counts were made in 100 fields of vision, and the results were expressed in terms of 1000 nuclei. The numerical results were analyzed statistically by the Fisher-Student method. The number of binuclear cells was not calculated from Pfuhl's formula, for according to some writers [7] this calculation does not yield the true number of these cells.

EXPERIMENTAL RESULTS

The table shows that the mitotic activity of the liver cells of the rats varied during the 24 hour period. The largest number of mitoses was observed at 2-5 A.M. Other authors observed the maximal number of mitoses at 4-7 A.M. [2], 8 A.M. [1], and 10 A.M. [5], probably in connection with differences in the conditions of keeping and feeding. The minimal number of mitoses was observed between 8 and 11 P.M.

Changes in Number of Mitoses and of Binuclear and Octoploid Cells in the Liver of Rats during the 24 hour Period

Time of day	Number per 1000 cells			
	mitoses	binuclear cells	octoploid nuclei	disintegrating nuclei
11	0,84	25,4	22,9	32,9
14	0,61	19,2	20,7	27,9
17	0,70	27,5	23,1	31,0
20	0,22	34,7	21,3	33,9
23	0,30	32,25	21,8	31,4
2	1,0	38,8	17,7	32,1
5	0,78	27,7	25,7	33,2
8	0,56	27,5	22,0	31,0



Changes in the number of mitoses (1) and of binuclear (2), octoploid (3), and dying liver cells (4) during the 24 hour period.

Significant changes took place in the number of binuclear cells during the 24 hour period. Their number was lowest (19.2) at 2 P.M., after which it gradually rose to a maximum, like the number of mitoses, at 2 A.M. (38.8, or twice as high as at 2 P.M. The differences are statistically significant (P = 0.001). At 5 P.M. the number of binuclear cells again began to fall.

The behavior of the octoploid cells was different. Their number changed only slightly (differences not statistically significant) during the afternoon and evening, but at 2 A.M. (P = 0.005), i.e., when the number of binuclear cells was at its peak, it fell sharply. The curves of the change in the number of octoploid and binuclear cells bore a mirror-image relationship to one another (see figure). At the time of the next examination, at 5 A.M., the number of octoploid cells again increased, reaching a maximum (P = 0.004). The number of dying cells remained at approximately the same level throughout the 24 hour period.

The most interesting feature was the ratio between the numbers of octoploid and binuclear cells at night. The time between 11 P.M. and 5 A.M. was the period of most intensive cytological transformations in the liver associated with physiological regeneration. It was not accidental that the maxima of the numbers of mitoses and of binuclear cells occurred at the same time. The decrease in the number of octoploid cells cannot be attributed to death, for the number of disintegrating nuclei did not increase. In general, death of octoploid cells was not observed.

The binuclear cells evidently developed from polyploid cells, but not by mitosis for cell division by mitosis was extremely rare, the usual method being by endomitosis and subsequent division. However, the number of binuclear cells did not stay at the same level. Three hours after the sharp rise in their number at 2 A.M. it fell by one third, with no visible increase in the number of disintegrating nuclei. This can only be ascribed to the conversion of some of the binuclear cells into mononuclear (although cytotomy was not observed). At the same time a fresh increase took place in the number of octoploid cells.

It may be concluded from these experimental findings that physiological regeneration of the liver evidently takes place in two ways: by mitotic division and by division of polyploid nuclei with subsequent separation of the resulting binuclear cells into two mononuclear cells. It should be noted that far more cells appeared in this way than by mitosis (in 1000 cells there were 38 binuclears and only one mitosis). In normal conditions division of liver cells probably takes place without exclusion of the cells from function. This is achieved by division of the octoploid nucleus, followed

by the formation, initially of binuclear, and later of mononuclear cells. The relative part played by mitotic activity in the general balance of physiological regeneration is extremely small. In reparative regeneration, on the other hand, the mitotic activity of the liver rises sharply and leads to rapid growth of the liver parenchyma.

The processes of physiological regeneration (both mitosis and amitosis) take place most intensively at night.

SUMMARY

Experiments were staged on male albino rats weighing 150 gms. Animals were sacrificed at 11, 2, 5, 8, 11, 1 and 8 o'clock. Pieces of the left lobe of the liver were obtained for the examination. The number of binuclear and of the large polyploid (octoploid) cells were counted: the number of mitoses and destroying nuclei and their correlation at different time of the day was also estimated.

The greatest number of mitoses (1 per 1,000 cells) and of binuclear cells (38.8 per 1,000 cells) was noted at 2-5 A.M. At the same time the polyploid cell count decreased sharply. At 5 o'clock in the morning the number of binuclear cells reduced by one third, whereas the polyploid cell count reached the maximum. The number of destroying cells exhibited almost no change during the 24 hour period. Evidently, the restorative processes in the liver occur mainly at the expense of endomitosis, the specific weight of which is much greater than that of mitosis.

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