RELATIONSHIP BETWEEN DIURNAL RHYTHM IN THE NUMBER OF BINUCLEAR CELLS IN THE RAT LIVER AND ITS GLYCOGEN-FORMING FUNCTION

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UDC 612.35.014.3:612.6]-02:612.352.1

By varying the feeding and lighting arrangements for rats during the 24-h period a direct relationship was shown between the formation of binuclear cells and the glycogen content in the liver of these animals.

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A previous investigation [2] showed that the number of binuclear cells in the liver varies in the course of the 24-h period just as regularly as the mitotic activity of the cells. One of the factors determining the diurnal rhythm of mitosis is function of the organ. Most authors have observed an inverse relationship between mitotic division and function of the cell [1, 4, 5, 8, 9].

No data could be found on the relationships between function of an organ and the number of binuclear cells during the natural diurnal cycle of the organism. Observations have been made indicating a relationship between fluctuations in the number of binuclear cells in the liver and its physiological state. Petrov [7], for instance, in animals with experimental degeneration of the liver found a marked decrease in the number of binuclear cells. Similar results were obtained by Zaletaeva [3], who studied the ratio between the numbers of binuclear cells and mitoses in fasting animals during the 24-h period. According to her observations the number of binuclear cells in the liver fell by half during starvation, while the mitotic activity remained unchanged. Comparison of these data suggests the existence of a definite relationship between the number of binuclear cells in the liver and the level of its function.

To test this hypothesis the diurnal rhythm of the number of binuclear cells and glycogen content in the liver was studied in normal animals and during modification of the feeding and lighting arrangements.

EXPERIMENTAL METHOD

Three series of experiments were carried out on albino rats aged 2-2.5 months. In the experiments of series I and II the animals were kept under normal lighting conditions with natural alternation of day and night. For one month the animals of series I received food at 7 a.m. and the animals of series II at 7 p.m. In the experiment of series III the animals were kept in complete darkness during the day time (7 a.m.-7 p.m.) and in artificial illumination at night (7 p.m.-7 a.m.), and as in series I they received food at 7 a.m. In all experiments the same diet was given without restriction of quantity. The rats were sacrificied in groups of 5-6 animals every 4 h (at 3, 7, and 11 a.m. and 3, 7, and 11 p.m.). For 3-4 days after sacrifice, at each time of investigation the quantity of food eaten by the animals was determined.

Binuclear cells were counted in sections (5 μ) under the binocular microscope (ocular 10, objective 90). The coefficients were calculated in promille. Glycogen in the liver cells was detected by Shabadash's method. Sections preliminarily treated with salivary amylase were used as controls. The glycogen content was judged from the results of photometric examination of the sections in a specially adapted MF-2 microphotometer. Statistical analysis of the results was carried out by the Fisher – Student method.

EXPERIMENTAL RESULTS

Animals kept under normal laboratory conditions (series I) were more active during the morning (3-11 a.m.). This was reflected in the quantity of food eaten (Fig. 1A). Parallel to this digestive activity,

Department of Histology, Khabarovsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 68, No. 9, pp. 98-100, September, 1969. Original article submitted February 14, 1969.

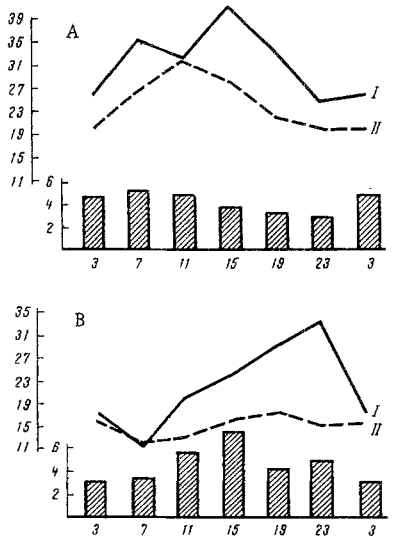

Fig. 1. Diurnal changes in number of binuclear cells (I) and glycogen content (II) in liver of rats fed in the morning (A) and in animals with reversal of the normal photoperiodicity (B). Abscissa, time of day; ordinate, number of binuclear cells (in %) and glycogen content. Columns show quantity of food eaten (in g).

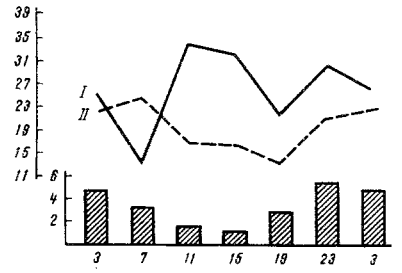

Fig. 2. Diurnal fluctuations in number of binuclear cells (I) and glycogen content (II) in liver of rats fed in the evening. Legend as in Fig. 1.

changes occurred in the glycogen content in the liver cells. At night (7 p.m.-3 a.m.) the cells were poor in glycogen. Later, as the quantity of food eaten increased, the glycogen content in the liver rose to reach a maximum at 11 a.m. ($P = 0.03$). The cytoplasm of the cells at this period was filled to the limit with large granules of glycogen. Starting at 3 p.m. the reserves of glycogen gradually diminished. The number of binuclear cells in the liver reached a maximum between 7 a.m. and 3 p.m. At night (11 p.m.-3 a.m.) their number reached a minimum ($P = 0.008$).

When the lighting conditions were reversed (series III), the character of the diurnal changes in the indices studied was modified. The digestive activity of the rats now reached a maximum at a different time, from 7 a.m. to 3 p.m. (Fig. 1B). The dynamics of the liver glycogen content was correspondingly changed. Its content was increased from 3 to 7 p.m. (maximum at 7 p.m.) and reached a minimum between 7 and 11 a.m. ($P = 0.014$). These periods coincided with those at which the number of binuclear cells reached a minimum (7 a.m.). Starting from 11 a.m. their number increased, to reach a maximum at 11 p.m. ($P = 0.001$).

In the experiments of series II the rats were kept under nearly natural conditions characteristic of nocturnal animals. With a change in the feeding arrangements, the general activity of the animals also was modified. The rats ate food most actively at night, with a maximum at 11 p.m. (Fig. 2). Activity reached a minimum in this direction from 11 a.m. to 3 p.m. During this period the glycogen content was low (minimum at 7 p.m.) in the liver cells. The glycogen content was high at night and in the morning, reaching a maximum at 7 a.m. ($P = 0.028$). The number of binuclear cells reached a minimum at 7 a.m. and a maximum between 11 a.m. and 3 p.m., when the liver was poor in glycogen, and again at 11 p.m., when the intensity of glycogen formation by the liver had increased.

Analysis of these results reveals a clear effect of the conditions under which the animals were kept on the diurnal fluctuations in the glycogen reserves and number of binuclear cells in the liver. Comparison of the changes in the glycogen content with the intensity of formation of binuclear cells in the liver during the 24-h period reveals a direct relationship between these two parameters. This was particularly obvious in the experiments of series I and III, when maximal numbers of binuclear cells coincided with the maximal glycogen content; the same pattern was observed also with regard to the minima. In the experiments of series II this general pattern was slightly disturbed. However, this applied only to the changes taking place in the liver during the first half of the day (7 a.m. to 3 p.m.). Starting from 3 p.m., fluctuations in the glycogen reserves and the number of binuclear cells were almost completely synchronized.

The diurnal rhythm of the liver glycogen content, as these observations show, depends primarily on the feeding arrangements; diurnal photoperiodicity also has some effect on the feeding activity of the animals. Possibly the arrival of a large quantity of raw material required for glycogen synthesis in the liver acts as the trigger mechanism for the formation of binuclear cells possessing a higher level of physiological activity than mononuclear cells. The views of Lutsenko [6], who regards the presence of a large number of binuclear cells in the liver as a special adaptation of the organ, developed during evolution and enabling it to carry heavy functional loads with a comparatively slight degree of cell proliferation, can be fully accepted. The increase in number of binuclear cells and the increase in number of mitoses are different methods of physiological regeneration, probably determined by different conditions. Whereas an

inverse relationship is found between mitotic activity and cell function, in most cases the relationship between physiological activity and the formation of binuclear cells is a direct one.

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