DIURNAL RHYTHM OF ACID DEOXYRIBONUCLEASE ACTIVITY IN RATS' SPLEEN AND THYMUS

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Activity of acid deoxyribonuclease (DNAase II) was investigated in tissues of the rat thymus and spleen (at intervals of 3 h during the 24-h period). The animals were kept in artificial lighting from 5 A.M. to 5 P. M. Investigations of the lymphoid tissue revealed a well-marked diurnal rhythm of DNAase II activity. The greatest increase in activity of this enzyme was observed during the evening and night (8 P. M.-2 A. M.), with a smaller peak at 11 A. M. Activity diminished in the morning (5-8 A. M.) and afternoon (5 P.M.). Analysis of the literature on acid DNAase function in animal tissues and of personal observations suggested a relationship of cause and effect between the diurnal rhythm of DNAase II activity and processes of DNA catabolism in rapidly renewed tissues.

A problem of current importance in chronobiology is the nature of rhythmic processes in the cell. According to one view [11], the biochemical basis of mechanisms of diurnal rhythms is formed by the breakdown and synthesis of biologically important macromolecules (proteins and nucleic acids).

This causative link between the diurnal rhythm of multiplication and renewal of cells and nucleic metabolism is reflected in the literature on the diurnal periodicity of synthesis of nucleic acids and their concentration in animal tissues [3, 4, 6, 12].

Remembering that the catabolism and anabolism of nucleic acids takes place through a system of enzymic reactions, it can be postulated that the diurnal metabolism of these acids is controlled by changes in the activity of the participating enzymes.

In the investigation described below the diurnal activity of one of the enzymes present in animal tissues with a high intensity of DNA metabolism, acid deoxyribonuclease (DNAase), was studied.

As a hydrolase, acid DNAase is mainly responsible for the hydrolytic breakdown of DNA [8]. The enzyme not only participates in the disposal of nuclear material in physiologically dying cells but also, as Pokrovskii and Tutel'yan have shown, it has a reconstructive function [5]. Fragments formed during hydrolysis can be used to form new DNA required for the vital activity of the cell. The role of DNA depolymerases not only in the catabolic but also in the anabolic metabolism of DNA must also be mentioned [10].

EXPERIMENTAL METHOD

Experiments were carried out on 80 male Wistar rats weighing 150-200 g. For 1 month the animals were kept under strict conditions of illumination (artificial lighting, intensity 150-200 lx, duration 12 h daily – from 5 A. M. to 5 P. M.). Every 3 hours five animals from each of the two experimental groups were decapitated. The spleen and thymus were removed, and their tissues analyzed for acid DNAase activity by a spectrophotometric method based on the increase in content of acid-soluble products with enzymic hydrolysis of DNA optically active at 260 mμ [15]. DNA with a molecular weight of 6×10^6, isolated by the detergent method [13], was used as the substrate.

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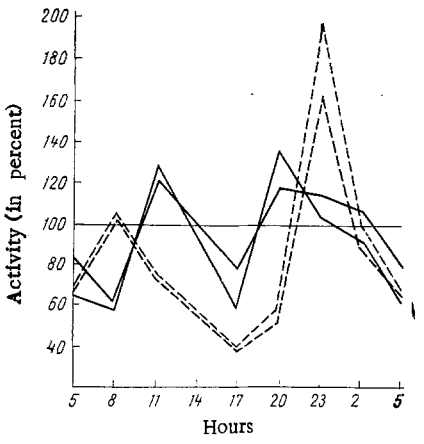

Fig. 1

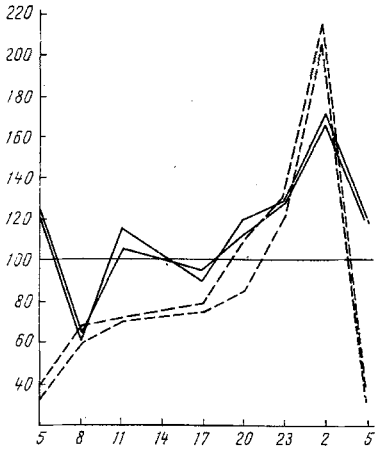

Fig. 2

Fig. 1. Diurnal rhythm of acid DNAase activity in rat thymus. Continuous line represents enzyme activity in tissue homogenates, broken line – nonsedimentable activity. Empty and filled circles* correspond to experimental points obtained for different groups of animals. Abscissa, time of day (in h); ordinate, enzyme activity (in percent of mean for 24-h period).

Fig. 2. Diurnal rhythm of acid DNAase activity in rat spleen. Legend as in Fig. 1.

Activity of the enzyme was determined in tissue homogenates and supernatant (nonsedimentable activity) obtained after ultracentrifugation of a tissue suspension at 105,000 g. Activity was calculated per gram wet weight of tissue.

EXPERIMENTAL RESULTS

The results of investigation of the dynamics of diurnal enzyme activity in the animals of the two experimental groups are shown graphically in Figs. 1 and 2. (Each point on the curves was obtained by analysis of tissues taken from five animals.) The results show marked fluctuations in the diurnal activity of acid DNAase in the organs studied.

The curve of enzyme activity in homogenates of the thymus (Fig. 1) is bimodal. Its maxima fell at 11 A. M. and 8 P. M. and its mimina at 8 A. M. and 5 P. M. The diurnal rhythm of acid DNAase activity in homogenates of the spleen was basically of the same character as in the thymus. The only exception was the position of the second maximum, which was displaced to the night (2 A. M.). The maximal deviation of enzyme activity from the mean value for the 24-h period for the thymus was about 30% at the maximum and 40% at the minimum, while for the spleen it was 40% at the two extremes. Consequently, differences in the acid DNAase activity at the points of maximum and minimum for the two organs were similar and amounted to 70-80%. However, compared with the spleen, the diurnal rhythm of acid DNAase activity in thymus homogenates was more clearly defined, evidently because of the more homogeneous cellular composition of the tissue of that organ.

Acid DNAase is localized mainly in lysosomes [9], where it occurs in an inactive, latent state. Since activation of the enzyme is largely connected with its liberation from membrane structures, the diurnal rhythm of this process was investigated. It follows from the curves (broken lines) in Figs. 1 and 2 that nonsedimentable activity in the tissues studied, just as in the homogenates, does not remain constant during the 24-h period. The highest increase in activity (by more than 100%) was observed at night (11 P. M. – 2 A. M.), whereas in the morning and afternoon the activity of the enzyme was below the mean value for the 24 hours. The results indicate a possible role of membrane mechanisms of enzyme activation in the regulation of the diurnal activity of the acid hydrolases.

The observed diurnal rhythm of acid DNAase activity correlates with the intensity of DNA metabolism in the tissues. Diurnal periodicity of the enzyme was observed only in rapidly renewed tissues (spleen,

*As in Russian original. Circles not present in figure in original. – Publisher.

thymus). Attempts to detect a diurnal rhythm of acid DNAase activity in the liver were unsuccessful. The activity of the enzyme in this organ was practically the same at all times of day and night.

The basic problem for solution is to identify with which aspect of DNA metabolism – anabolic or catabolic – the diurnal rhythm of acid DNAase activity is connected. According to the literature [1, 7], the curve of the diurnal rhythm of mitosis in the lymphoid tissues of animals is unimodal in character, by contrast with the bimodal diurnal curve of enzyme activity; the time of the maximum and minimum of mitosis does not coincide with the extremes of the diurnal rhythm of DNAase activity obtained in the present experiments. It can accordingly be postulated that the diurnal periodicity of DNAase activity is not connected with one of the essential components of the biochemical mechanism of mitotic rhythm, namely anabolism of DNP [2]. This hypothesis is supported by information in the literature showing that acid DNAase activity is unchanged during the intensification of synthesis caused by increased proliferation of cells in the regenerating liver [14]. Meanwhile, special experiments to determine the percentage of dead cells in the spleen and thymus during the 24-h period revealed patterns analogous to the diurnal periodicity of acid DNAase activity (coincidence with the times of the extreme points of the bimodal diurnal curves). Evidence that the diurnal periodicity of the enzyme is connected with DNA catabolism is also given by the periodicity of localization of the enzyme during the 24-h period; this is reflected in changes in the nonsedimentable activity, which may be connected with autolytic breakdown of the cells during their physiological death.

Consequently, the most probable hypothesis is that the diurnal periodicity of acid DNAase activity detectable in lymphoid tissues is connected mainly with the periodicity of DNA catabolism.

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