VITAL FIXATION OF DYE BY TISSUES AND DIURNAL PHYSIOLOGICAL RHYTHM

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A diurnal rhythm of vital fixation of neutral red by the liver, thyroid, and brain tissues of mice and liver tissue of frogs is found. The amplitude of diurnal fluctuations in fixation of dye by the mouse tissues averaged 30%, rising to a maximum at 9 P.M. and falling to a minimum at 3 A.M. Fixation of dye by frog liver tissue reaches a maximum at night and a minimum at 3 P.M.

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One of the clearest signs of a paranecrotic state is an increase in vital staining of the tissues [9]. An increase in deposition of vital dye in the cells is observed not only after injury, but also in excited tissue [8, 12]. The use of this cytophysiological method of investigation by D. N. Nasonov, and his pupils and followers, has resulted in the accumulation of a vast experimental material demonstrating the general biological significance of the paranecrotic state. However, this method has not been used to investigate the character of vital fixation of dye by the tissues of healthy animals at different times of day, i.e., the pattern of fixations of vital dye by tissue cells in the diurnal physiological rhythm has not been studied.

The object of the present investigation was to examine the dynamics of fixation of a vital dye by the tissues in the biological 24-h rhythm, to compare the results with those of our previous histochemical investigations [5, 6], and to deduce conclusions regarding the mechanism of fixation of vital dye.

EXPERIMENTAL METHOD

The liver, thyroid, and brain of 400 albino mice and the liver of 100 frogs were investigated. At different times of year (winter, fall, summer, spring) 11 series of experiments were undertaken with the liver, 7 series with the thyroid, and 5 series with the brain of the albino mice, and three series of experiments with frogs' liver were carried out in the spring. Each series of experiments was carried out on animals of the same sex and weight. The cytophysiological method of vital staining of the tissues (Nasonov's method as modified by A. A. Braun [1]) was used in the investigation. At different times of day and night the animals were decapitated, the organs to be tested were quickly removed and rinsed in Ringer's solution, after which they were stained with 0.025% neutral red made up in soda-free Ringer's solution (at 37° for mice, at room temperature for frogs). After staining for 15 min the tissue was gently dried with filter paper and immersed in 70° alcohol acidified with 2% sulfuric acid. After 24 h photometry of the alcoholic extracts of dye from the tissues taken from the animals decapitated at different times of day and night was carried out on a wedge photometer. The concentration of dye extracted from the tissues was expressed in conventional photometer (extinction) units and calculated per 100 mg weight of tissue (both dry and moist weight). The mean diurnal extinction was then calculated for each tissue in each series of experiments and the increase or decrease in the degree of fixation of vital dye by the tissue calculated as a ratio of this extinction for each investigated time of the 24-h period. The dynamics of fixation of dye by the liver of the albino mice was investigated every 3 h, that of the thyroid 3 times daily (at 3A.M. and at 3 and 9 P.M.) and that in the frogs' liver twice daily (at 3 A.M. and 3 P.M.). These times were chosen for investigation of the pattern of fixation of vital dye by the tissues because it was at these times that the most significant differences in concentration of ribonucleic acid were found by the use of histochemical methods [5, 6]. At each time not less than 50 animals were investigated; in addition, when studying adsorption of vital dye by the liver of the mice and frogs, not less than 3 or 4 pieces of about equal weight and size were cut from the

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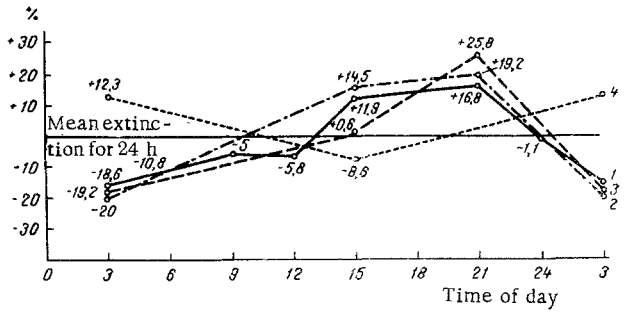

Fig. 1. Diurnal rhythm of fixation of vital dye by tissues (% of mean extinction for 24 h). 1) Mouse liver; 2) mouse thyroid; 3) mouse brain; 4) frog liver.

liver tissue of each animal with a razor blade. Consequently, at each time of investigation at least 150 measurements were made for the liver. The experimental results were analyzed by statistical methods.

EXPERIMENTAL RESULTS

A study of the dynamics of fixation of vital dye by the liver of the mice throughout the 24-h period revealed (Fig. 1) that at night (3 A.M.) the degree of fixation of dye is minimal and significantly below the mean adsorption of dye (difference 18.6 ± 1.76%).

Vital fixation of the dye then began to increase, but from 6 A.M. until 12 noon it remained below the mean daily level. After 3 P.M. fixation of dye by the liver tissue increased considerably (+11.9 ±8.2%), the difference between the mean percentages of the adsorption values at 3 P.M. and 3 A.M. being statistically significant (M_{diff} = 30.5 ±0.4%). Maximal fixation of neutral red by the liver tissue was found at 9 P.M. (+16.8 ±6.4%). The difference between the percentage increase in dye fixation at 9 P.M. and the percentage decrease in adsorption at 3 A.M. was 35.4 ± 6.68%, and was statistically significant. After 12 midnight, as a rule fixation of dye by the mouse liver tissue began to decrease, although in some series it remained slightly above the mean daily extinction, while in others it was lower.

In the course of the 24 h, the liver tissue of healthy mice thus showed variation in its ability to fix neutral red, minimal adsorption being found at night and during the first half of the day, while maximal ability to adsorb the dye was observed in the second half of the day starting at 3 P.M. and reaching a maximum at 9 P.M. The amplitude of fluctuation in the degree of vital fixation of dye by the liver of the albino mice was over 35%.

The dynamics of fixation of neutral red by the thyroid of albino mice in the course of the 24 h proved to be very similar to that for the liver. At 3 A.M., for instance, the level of fixation of vital dye was 20 ± 9.3% below the mean daily level. At 3 P.M. the adsorption of neutral red showed an increase of 14.5%, and at 9 P.M. it was 18.2 ± 8.4% above the mean daily level. The difference between the mean percentages at 3 A.M. and 9 P.M. was 39.2 ± 2.6 and was statistically significant.

A similar picture was found during the study of the dynamics of neutral red fixation during the 24-h period of mouse brain tissue: at 3 A.M. a statistically significant decrease of 19.2 ±3.45% was found in the fixation of the vital dye, followed by a tendency for adsorption of the dye to increase (+0.6% at 3 P.M.). Vital fixation of the dye by the mouse brain at its maximum exceeded the mean daily level by 25.8% at 9 P.M., and the difference between the mean percentages of dye fixation at 9 P.M. and 3 A.M. was 45 ± 16.3%.

Consequently, a definite diurnal and synchronized rhythmic fluctuation is found in the ability of the liver, thyroid, and brain tissues of mice to fix vital dye, the amplitude between maximum and minimum reaching 30%.

The character of fixation of vital dye by the frog liver during the 24-h period was found to be different: maximal adsorption of neutral red was observed at 3 A.M. (+ 12.3 ± 5.5%), and at 3 P.M. fixation of the dye was 8.6 ± 0.33% below the mean daily level. The difference between mean percentages at 3 A.M. and 3 P.M. was 20.9 ± 5.9%. The amplitude of diurnal fluctuations in dye fixation was considerably less for frog liver tissue than for mouse liver tissue.

With what is the diurnal rhythm of vital staining of mouse and frog tissue associated? Since the injury factor was excluded in these experiments with intact animals, it must be assumed, in accordance with the theory of paranecrosis, that the increase in dye fixation by the tissues at certain times of day is connected with the diurnal rhythm in the state of excitation of the organism, with its phases of waxing and waning of activity. According to our experimental results, an increase in fixation of vital dye by the tissues is observed at times of day or night at which the animals exhibit greatest physiological activity (in mice during the second half of the day, particularly in the evening, and in frogs at night).

The question of the mechanism of fixation of vital dye by the tissue remains open. Some investigators, notably the authors of the denaturation theory of injury [2-4, 10, 13, 15] consider that vital dye is fixed by proteins. There are indications that vital fixation of dye is due to lipids [18]. Finally, some evidense has been obtained to show that a decisive factor in the mechanism of vital fixation of dye is the nucleic acids [7, 11, 14, 16, 17]. Comparison of the results of the present investigation with those of cytochemical studies of the dynamics of changes in RNA content in the tissues of mice [5, 6] and frogs during the 24-h period suggest that RNA plays a highly important role in the mechanism of fixation of vital dye by the tissues, because dye fixation reaches a maximum at the times of day and night when the RNA concentration in the cells of these tissues reaches a maximum and, conversely, when the RNA concentration reaches a minimum, so also does the fixation of vital dye in the cells of these tissues. Our results show that nucleic acids are to some extent responsible for vital staining of tissues.

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