DIURNAL VARIATIONS IN MITOTIC ACTIVITY IN MICE

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The diurnal variations in the number of mitoses in the tissues are related to various factors (the diurnal photoperiodicity, the motor activity of animals the pattern of functional activity of the cells, changes in metabolic reactions, etc.) [2]. The relationship between the division and functional activity of the cell has long attracted the attention of researchers. When an organ is subjected to moderate functional strain, its mitotic activity diminishes [8, 10, 13]. During more prolonged work the mitotic activity increases [3]. It is not yet clear what predominates in the natural diurnal mitotic rhythm: a fall or rise in mitotic activity.

In the present article we shall attempt to discover the relationship between the functional activity of an organ and the multiplication of its cells throughout the 24 hours in mice.

EXPERIMENTAL METHOD

Experiments were carried out on albino mice aged 2½ months. The animals were subdivided into 8 groups, each containing 5 or 6 mice. The mitotic activity was determined at the following times of day: 8, 11, 14, 17, 20, 23, 2, and 5 hours. One group of mice was sacrificed every 3 hours. The degree of mitotic activity was judged by the number of dividing cells in a constant area, the relative proportion of the various phases of mitotis (in %), and the phase coefficient. The intensity of cell proliferation at different times of day was determined in the duodenal epithelium, the serous and mucous divisions of the salivary gland, the exocrine part of the pancreas, the epithelium of the principal divisions of the kidneys, and the epidermis of the skin. Concurrently, E. V. Abramson determined the mitotic activity in the corneal epithelium*. The number of dividing cells in the epithelium of the intestine, the cornea, and the skin was counted in an area of 1.65 m^2, and that in the kidney, the salivary gland and the pancreas in an area of 3.3 mm^2.

Before the animals were sacrificed, certain functional tests were performed on the organs to be studied. The intensity of the digestive function of the mice was estimated by the amount of food eaten by the animals and by the number of times they visited the feeding bowl. The food remaining in the feeding bowl was weighed every 3 hours. The visits to the feeding bowl were recorded (together with the general activity of the mice) in a special cage equipped with an electric contact and a counter. The volume of water drunk by the mice was also recorded every 3 hours by a special floating counter with transmission through a system of levers. The diuresis was measured daily by weighing filter paper placed under the perforated floor of the cage. This method of recording the diuresis was not accurate, and the results which it gave merely showed the trend of the diuresis.

These functional tests were carried out for 2 weeks before the animals were sacrificed, and on the actual day of the experiment. All the animals were kept in identical conditions. The mice received the food and water strictly at the same time (9 A.M.).

EXPERIMENTAL RESULTS

The experiments showed that the mitotic activity in the various organs of albino mice undergoes regular changes throughout the 24 hours.

The intensity of cell proliferation in the digestive organs tested (the salivary glands, the intestinal epithelium, and the pancreas) showed similar changes. The curve of the diurnal changes in mitotic activity of these organs had

* These figures are included in this article through the courtesy of E. V. Abramson.

a well-defined bimodal character (Fig. 1). The first peak of mitotic activity took place at 8 A.M. The mitotic activity then gradually declined to a minimum at 11-17 hours. The second maximum of the number of mitoses was seen at 20-23 hours. A second minimum was observed during the night (2-5 hours). The differences between the levels of mitotic activity (maximum and minimum) were obvious. The probability (P) of random variation did not exceed 0.001-0.002. The bimodal character of the diurnal changes in the number of mitoses in the intestinal epithelium was described some time ago by Klein and Geisel [12]. The conflicting results obtained by other workers [4, 6, 7] may presumably be explained by differences in the methods of feeding the animals in the various laboratories, or by the longer intervals (6 hours) between observations.

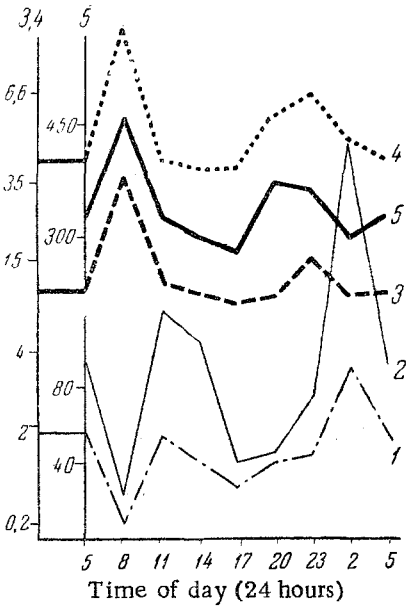

Fig. 1. Curves showing diurnal variations in the number of mitoses and the functional activity of the digestive organs. 1) Amount of food ingested; 2) number of visits to feeding bowl; 3) mitotic activity of the salivary gland; 4) mitotic activity of the pancreas; 5) mitotic activity of the intestinal epithelium.

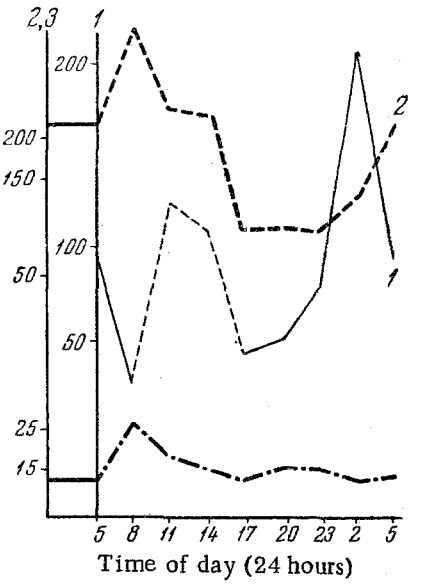

Fig. 2. Curves of the diurnal variations in the number of mitoses and the functional activity of the kidney. 1) Amount of water ingested; 2) mitotic activity of the kidney.

Comparison of the diurnal changes in the number of mitoses in the digestive organs with their functional activity discloses that these indices are in an inverse relationship with one another. The curves of functional activity of the digestive organs, like the curves of the diurnal variation in the number of mitoses, are bimodal in character. The maxima of ingestion of food and frequency of visits to the feeding bowl by the animals coincided with the minima of the indices of mitotic activity (11 and 2 hours). Conversely, the minimal indices of functional activity at 8 and 17-23 hours coincided with a high intensity of multiplication of the cells. These comparisons show that the natural diurnal rhythm of the number of mitoses in the digestive organs is characterized by a predominately inverse relationship between the division and work of the cell.

Closely similar results were obtained when the diurnal variations in mitotic activity were studied in the renal cortex. The mitotic activity of the kidney in mice (Fig. 2) reached a maximum at 5-8 A.M. and fell to a minimum at 11-17 hours ($P = 0.001$). During the evening a second, slight increase in mitotic activity was observed (20 hours). In contrast to the corresponding increase in the digestive organs, this second increase between 20 and 23 hours was much smaller ($P = 0.08$). The curve of diurnal ingestion of water by the mice was bimodal in character, and two maxima were also found on recording the diuresis. Judging by these approximate figures, the maxima of renal functional activity evidently occurred at 23-2 hours and 8-11 hours. The nocturnal maximum of renal function coin-

cided with the minimal indices of mitotic activity. The morning minimum of functional activity of this organ coincided with the highest level of mitotic activity. The morning increase in the functional activity of the kidney (between 8 and 11 hours) did not coincide precisely with the minimum of mitotic activity. The diurnal decrease in the water intake and the excretion of urine coincided approximately with the very slight increase in mitotic activity at this time. Thus in the kidney too, as noted by other writers [8], an inverse relationship was observed between function and cell division. In contrast, however, to the digestive organs this relationship, which was so well defined during the night hours, was less apparent during the day.

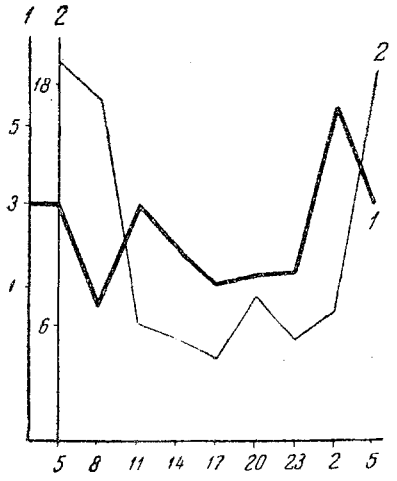

Fig. 3. Curves showing the diurnal variations in the number of mitoses in the epithelium of the cornea and skin and the general activity of the animals. 1) Number of visits to feeding bowl; 2) mitotic activity in the corneal epithelium; 3) mitotic activity of the epidermis.

The diurnal variations of the mitotic activity in the epithelium of the cornea and skin followed a parallel course. As other workers have found [5, 6, 7, 9], the curve of the diurnal rhythm of mitotic activity in these organs was unimodal in character (Fig. 3). The maximum of mitotic activity took place at 8 hours and the minimum at 17-23 hours. The high values of mitotic activity in the morning hours coincided with the first maximum of mitotic activity in the digestive organs. In contrast, however, to the latter no midday minimum (11-17 hours) and evening rise (20-23 hours) in mitotic activity could be found in the epithelium of the cornea and skin. These differences suggest that synchronism of the diurnal rhythm of mitotic activity occurs only in organs belonging to the same or to closely related functional systems.

Comparison between the mitotic activity of the epithelium of the cornea and skin and the general activity of the mice (the curve of the number of visits to the feeding bowl, which also serves as an actogram) shows that an inverse relationship between functional activity and cell division was present in these organs also. The maximal number of cell divisions in the surface epithelia coincided with minimal activity of the mice (8 hours). A low level of mitotic activity, on the other hand, was observed in the period of general activity of the animals. This relationship was disturbed only by the morning increase in the activity of the mice, which was not reflected in the diurnal rhythm of mitoses in the epithelium. This increased activity of the animals were "digestive" in nature, associated with the feeding time, and created artificially in the laboratory. Preliminary experiments showed that the time of appearance of this peak could be altered by changing the feeding time.

By taking into account the character of the morning activity of the animals, we can understand the differences in the diurnal rhythms of mitotic activity in the digestive organs and in the surface epithelium. Feeding the animals in the morning led to the appearance of a second minimum and maximum of mitotic activity in the digestive organs. These changes were related only to digestive activity, and were only slightly reflected in the kidney, and had no effect whatever on the proliferation of the cells of the surface epithelium. It has been shown previously that a complete change in the feeding habits of the animals (feeding the mice only during the daytime) modifies the diurnal variations in the number of mitoses in the digestive organs without affecting those in the epithelium of the cornea and skin [2].

If we compare the curves of the diurnal rhythms of mitotic activity of the digestive organs and the surface epithelia, we may observe that differences arise between them only as a result of the additional maximum and minimum caused by digestive activity. This activity, artifically produced in the morning hours in the digestive organs, complicates the natural diurnal rhythm of mitotic activity typical of nocturnal animals.

The results of these experiments thus show that the mitotic activity varies in the course of the 24 hours. In the digestive organs (and less obviously in the kidneys) the curves of the diurnal rhythm of mitotic activity are bimodal in character. The diurnal curves of mitotic activity in the digestive organs are related to the feeding habits of the animals in the laboratory and to the digestive activity. In the course of the 24 hours an inverse relationship is seen between cell division and the functional activity of the organ.

SUMMARY

A study was made of the regularities in the 20-hour changes in the mitotic activity of intestinal epithelium of the salivary glands, pancreas, kidney, epidermis and corneal epithelium of mice. Changes of the functional activity of these organs were recorded simultaneously. Regular 24-hour changes of mitotic activity were noted in various test organs. The 24-hour mitotic rhythm curves had two peaks in the case of digestive organs; in case of the kidney the curves were also double-peaked, the peaks, however, being less marked. The second peak of the cellular mitotic activity curve is connected with the regimen of the animal feeding. The curves of the 24-hour rhythm of the number of mitoses had a single peak in the case of corenal epithelium and epidermis. In the 24-hour changes of the cellular mitotic activity there existed a reverse relationship between the number of dividing cells and the functional activity of the organ.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
