DAILY RHYTHM OF MITOTIC DIVISION OF THE ESOPHAGEAL EPITHELIAL CELLS OF ALBINO RATS

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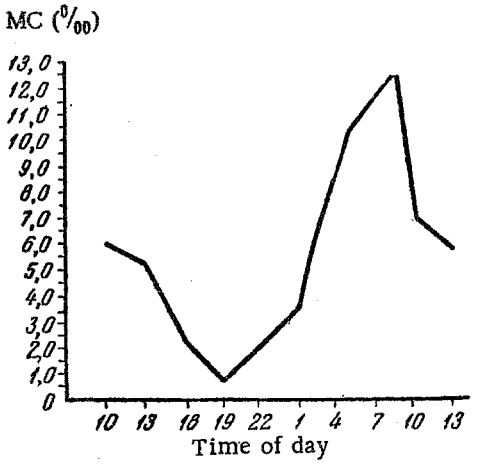
Determination of the mean daily mitotic activity of tissues provides important information about the intensity of their physiological regeneration. However, it has proved impossible to use the available evidence in the literature on the daily rhythm of mitosis for estimating the rate of physiological regeneration of several tissues because of the actual technique used to determine the mitotic activity. This was to count the number of mitoses in a conventional unit area of a histological section or unit volume of tissue.

This method can be used to determine the variations in the number of mitoses throughout the day and to assess the effect of different factors, but it cannot be used to compare the mitotic activity of different tissues and to elucidate the role of mitoses in physiological regeneration. The solution of these problems obviously requires the determination of the proportion of the number of mitoses to the total number of cells, so that, knowing the duration of mitoses, the duration of interkinesis can be deduced and, consequently, the mean time required for renewal of the cell population can be calculated.



Mitotic activity in the esophageal epithelium of rats at different times of day.

Little work has been done on the study of the daily fluctuation in the number of mitoses in the esophageal epithelium of mammals. Hardly any numerical data on the physiological regeneration of this tissue is available. Bullough [4] found the maximal number of mitoses in the esophagus of adult mice at 2 PM and 6 AM, and the minimal at 10 AM and 10 PM. Sinha [5] studied the relationship between the daily mitotic rhythm in the rat's esophagus and the pattern of illumination. He showed that with the normal alternation of natural and artificial illumination, the maximal number of mitoses was observed at 10 AM to 2 PM and the minimal at 6 to 10 PM. Prolonged and complete inversion of the illumination pattern led to inversion of the daily mitotic rhythm. I. A. Alov [1], who fixed the esophagus from mice and rats at 8 AM and 8 PM, showed that the mitotic activity was significantly higher in the morning than in the evening. Prolonged inversion of the illumination pattern also led to inversion of the daily fluctuations in the number of mitoses.

In contrast to these researches, in which the number of mitoses was determined in a convention unit area of a histological section, in an investigation by L. D. Liozner and co-workers [2], the mitotic activity of the rat's esophagus was determined as the ratio between the number of mitoses and the total number of epithelial cells in the esophagus. These workers plotted the mitotic activity during the 24 hours and found it to be a unimodal curve with a maximum number of mitoses at 7-10 AM.

EXPERIMENTAL METHOD

We studied the daily rhythm of mitotic activity in the epithelium of the lower third of the esophagus in male albino rats weighing 160-170 g. The animals were kept in an insulated room with natural conditions of lighting for a few days before being sacrificed. Natural food was given once a day (at 10:30 AM) in sufficient amount to ensure that some remained in the cages until the next morning. The animals were sacrificed in groups of 10 at 3-hour intervals.

Daily Fluctuations in Mitotic Activity in the Esophageal Epithelium of Albino Rats

Time of fixation	Total Nos. of mitoses	MC (pro ‰)	P
1st Day			
10 AM	868	6.15	0.33
1 PM	1020	5.39	0.0001
4 PM	477	2.33	0.001
7 PM	162	0.79	0.05
10 PM	402	1.96	0.21
1 AM	691	3.32	0.001
4 AM	2142	10.42	0.07
7 AM	2593	12.69	0.0001
2nd Day			
10 AM	1536	7.14	0.40
1 PM	1391	6.54	

The esophagus, stomach, and duodenum were all fixed for histological examination at the same time. Immediately after decapitation, the abdominal and thoracic cavities of the rats were opened. The esophagus was freed from surrounding tissues and a ligature applied around its upper half. A second ligature was passed beneath the esophagus near the stomach. Meanwhile, a ligature was applied to the duodenum, 1-2 cm away from the stomach. Carnoy's fluid was injected above this ligature into the lumen of the bowel under sufficient pressure to distend the walls of the esophagus, stomach, and duodenum. The second ligature was then drawn tight around the esophagus, and the complete preparation was extracted and kept for 1-2 h in a vessel containing Carnoy's fluid for subsequent fixation of the tissues.

Total film preparations were obtained from the esophageal epithelium. The resected portion of the esophagus was washed in alcohol and distilled water, and kept for 24 h in a refrigerator in 1% acetic acid. The organ was then cut open longitudinally and, using ophthalmic forceps, the epithelial layer was removed together with a thin layer of the underlying connective tissue, spread onto a strip of strong paper and transferred to 70° alcohol, in which it could be kept indefinitely. To obtain permanent preparations, the epithelial film was washed in water, stained with Carazzi's hematoxylin, taken through alcohols of increasing strength, followed by xylol, and mounted in balsam under a cover glass.

This method of obtaining total preparations, which has been used by one of us (A.G.K.) to study the mitotic activity of several epithelial tissues, has advantages over the method of studying mitotic activity in histological sections. It is less laborious, it permits the examination of mitoses undamaged by section cutting, facilitates the topographical orientation of the mitoses and determination of the plane of division of the cells, etc.

The mitoses in the esophageal epithelial cells were counted under the binocular microscope (objective 101 ×, eyepiece 7×), the eyepiece of which incorporated a square diaphragm measuring 8 × 8 mm. All phases of mitoses from early prophase to late telphase were counted. The mitotic coefficient (MC) was calculated per 1000 cells. Mitoses were counted in 100-125 fields of vision. Cell counts in 200 fields of vision of the microscope showed that the mean number of cells in one field of vision was 192. Consequently, the mitotic coefficient in each case was calculated in relation to 19,000-20,000 cells. The numerical results were treated statistically by the Fisher-Student method.

EXPERIMENTAL RESULTS

The results obtained are given in the table.

The table shows that the considerable mitotic activity observable at 10 AM fell steadily throughout the afternoon and reaches its minimal value at 7 PM. At 10 PM, and to a slightly greater extent at 1 AM, it rose again. Consequently, the evening hours (4 to 10 PM) may be regarded as the period of minimal mitotic activity. From 4 to 7 AM the mitotic activity rose sharply to reach its maximum at 7 AM. Consequently, the early morning hours (4 to 7 AM) were the period of maximal mitotic activity. At 10 AM, and even more so at 1 PM, on the next day the mitotic activity again decreased. The differences between the mean values of the mitotic coefficients at 10 AM and 1 PM on the first and second days of the investigation were not statistically significant. Hence, the daily changes in the mitotic activity of the esophageal epithelium in the rat may be represented by a unimodal curve (see figure).

The mean mitotic coefficient during the first day of the investigation (from 10 AM to 7 AM next day) was 5.34 pro mille. The relative proportions of the various phases of mitosis, expressed as percentages of the total, varied only very slightly from one time of investigation to another, and their average values were as follows: early prophase, 12.7%; prophase, 9.6%; metaphase, 39.3%; anaphase, 7.7%; telophase, 30.7%. The ratio between early and late phases of mitosis also varied insignificantly.

It should be pointed out that the character of the daily fluctuations in the number of mitoses in the esophageal epithelium and the relative proportions of the different phases of mitosis were in very close agreement with the corresponding indices of the corneal epithelium of albino rats [3], despite the fact that the mean mitotic activity in the corneal epithelium was higher than in the part of the esophageal epithelium which we studied.

We could find no data in the literature concerning the duration of mitosis in the esophageal epithelium. It is evident, however, that it does not exceed 45 min to 1 h, as has been established for many mammalian epithelial tissues. Since the mean daily mitotic activity was 5.34 pro mille, if the duration of mitosis is 45 min, the duration of interkinesis and, consequently, the mean rate of renewal of the cells of this tissue, will be 5.8 days, and if the duration of mitosis is 1 h, the figure will be 7.7 days. The simplicity of obtaining total film preparations of the esophageal epithelium, the clarity of the morphological pictures of the mitotic figures in this tissue, and the large number of mitoses with relatively small individual variations in their number at each time of investigation, make the esophagus a convenient object for the study of the various problems of regulation of mitotic activity in the organism, especially during the study of the action of factors stimulating or inhibiting mitotic activity.

SUMMARY

Twenty-four hour periodicity of mitotic activity was shown on the total mounts in the epithelium of the lower third of the esophagus of adult male albino rats. The maximal number of mitoses was detected at the early morning hours (4-7 AM) and the minimal in the evening (4-10 PM). No essential changes were revealed in the percentage ratio of various mitotic phases. Complete renovation of the cellular elements of the esophagus (epithelium) occurs approximately every 6-8 days.

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