DAILY RHYTHM OF MITOSIS AND AMITOSIS IN THE ESOPHAGEAL EPITHELIUM OF THE GUINEA PIG

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Several investigations of the daily rhythm of mitosis have been undertaken, but only fragmentary reports of the daily rhythm of amitosis are available.

In 1923, Smith [14] showed that the number of amitoses in the hyphae of the fungus Saprolegnia reaches a maximum between 10 PM and 2 AM. Staemmler [15] considers that an increase in the frequency of binuclear cells and amitotic constrictions around the nuclei reflects the periodicity of rhythm of waves of amitosis. It has been stated that the number of binuclear cells in the liver of albino rats arising as a result of amitosis rises by night and falls by day [13]. A similar conclusion was reached by Wada [16], who found that the number of binuclear cells in the liver varies with the time of day, reaching a minimum at 10 AM, and a maximum at 2 AM. Fujiwara [12] found a bimodal curve of amitotic activity in the transitional epithelium of the urinary bladder of the albino rat.

Having undertaken the study of the daily rhythm of amitosis, we considered that a suitable test object would be one having numerous binuclear cells constantly present, arising as a result of mitosis. We know that many binuclear cells are constantly present in the esophageal epithelium of certain rodents – the beaver [6] and the guinea pig [10].

EXPERIMENTAL METHOD

The present investigation was conducted on the esophageal epithelium of male guinea pigs weighing about 400 g. Forty animals were used. To ensure standardized conditions, the animals were kept for 24 h before the experiment in cages in laboratory conditions and on a normal diet. Animals (5 at a time) were sacrificed at 3-h intervals, as follows: 8 and 11 AM, 2, 5, 8, and 11 PM, and 2 and 5 AM. The animals were killed with ether vapor supplemented by the injection of 2 ml of ether into the lungs from a syringe. A part of the upper third of the esophagus was taken for investigation. The material was fixed with Susa's fluid and embedded in paraffin wax. Sections were cut strictly transversely (the accuracy of the cuts was checked from the sections of the muscle fibers of the muscular coat of the mucous membrane), to a thickness of 5μ, and stained by Carazzi's hematoxylin with counterstaining with eosin, by Heidenhain's iron-hematoxylin, and by Mallory's method.

The amitoses were counted as follows. The field of vision of the microscope (immersion objective 100×, eyepiece 10×) was demarcated by a square diaphragm measuring 8 × 8 mm, incorporated in the eyepiece, and the total number of nuclei, nuclear amitoses, and mitoses was counted. In each case, 3000 nuclei were counted. The amitotic coefficient and mitotic activity were expressed in terms of 1000 nuclei. The numerical results were treated statistically by the Fisher–Student method.

When the amitoses were counted, only cells with nuclei in contact and lying in the same plane (Fig. 1) were considered. No account was taken of cells with two widely separated nuclei or of cells whose nuclei contained only constrictions or grooves. We were thus concerned with only one stage of amitosis – the stage of completion of nuclear division.

EXPERIMENTAL RESULTS

The curves obtained after analysis of the numerical results are shown in Fig. 2.

The curve of mitotic activity shows that very little change took place in the esophageal epithelium of the guinea pig during the 24 h. The mitotic coefficient remained practically on the same level (8-13 pro mille). These

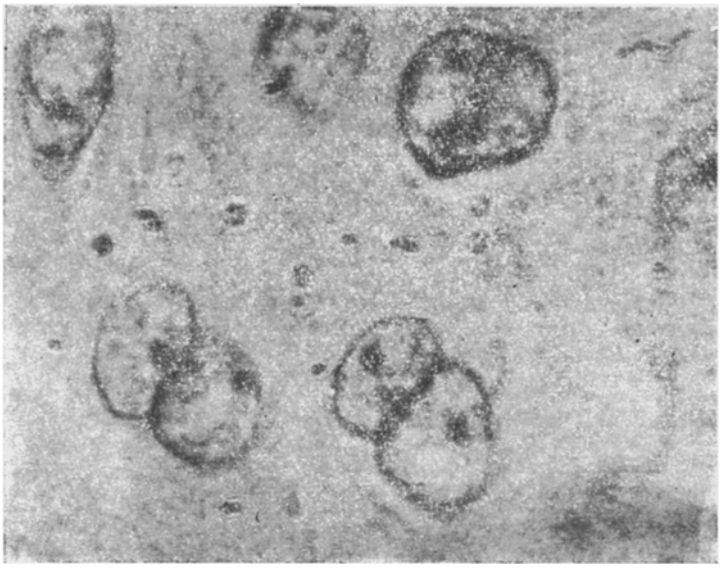

Fig. 1. General appearance of nuclei undergoing amitotic division. Photomicrograph. Fixation with Susa's fluid; stained with hematoxylin-eosin. Objective, immersion, 100×; eyepiece 10×.

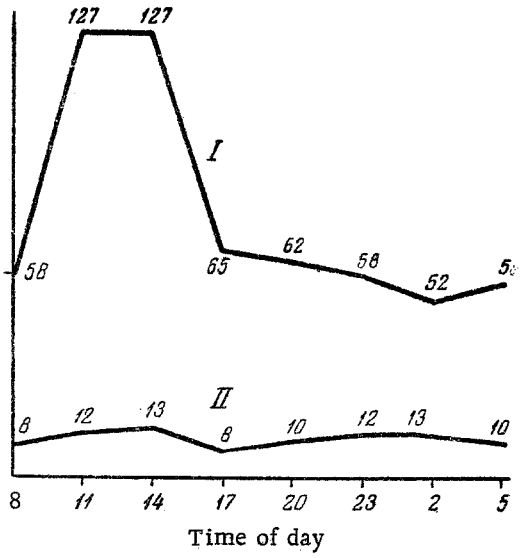

Fig. 2. Curves of amitotic (I) and mitotic (II) activity.

results were in full agreement with those reported by other workers studying the pattern of mitotic division in guinea pigs: absence of daily rhythm [4,5], slower course of mitosis [3], absence of inhibiting action of certain agents on mitosis [7], etc. The absence of a daily rhythm of mitosis in the esophageal epithelium of the guinea pig affords further confirmation of the biological peculiarities of the processes of cell division inherent in this animal.

The curve of amitotic activity was quite different in its character. The first feature to strike the eye was that amitoses were more numerous that mitoses. A clear difference was observed between the numbers of amitoses in the course of the 24 h; a statistically significant and sharp increase was observed in the amitotic activity of the esophageal epithelium during the daytime. The minimal number of amitoses (52 pro mille) was observed at 2 AM, the maximal (127 pro mille) between 11 AM and 2 PM. From 2 to 8 AM there was a slow but statistically significant increase in amitotic activity. During the three hours from 8 to 11 AM the number of amitoses rose sharply (from 58 to 127 pro mille). It remained at this level for the next three hours (until 2 PM), and then fell sharply to 65 pro mille at 5 PM, after which it fell gradually to a minimum at 2 AM.

The curve of the amitotic activity of the esophageal epithelium of the guinea pig thus gave evidence of a well-marked daily rhythm. The daily rhythm of cell division is a reflection of the course of the proliferative processes which, in normal conditions, serve to some extent as an index of the intensity of physiological regeneration. Because of the conditions in which it functions, the esophagus is an organ in which physiological regeneration must take place intensively. This cannot be achieved adequately, however, by mitosis alone, as is also the case with physiological regeneration of the cornea [3, 4, 5, 9]. I. A. Utkin [9] points out that the complete replacement of the cells of the corneal epithelium in the guinea pig on account of mitoses must occupy 10.5 days, and in the mouse and rat only 2 to 3 days. It is quite clear that the course of physiological regeneration as a result of mitotic division in the esophagus of the guinea pig is so slow that it cannot meet the demands of renewal, for the wear and tear of the esophagus must be quite considerable.

Different suggestions have been put forward to explain the mechanism of the daily mitotic rhythm – for example, the view that reactive stimulation [8] is responsible, or that this rhythm is a manifestation of the daily periodicity of the various physiological processes in the body [1, 2, 11]. So far as the daily rhythm of amitosis is concerned, the evidence is still inadequate to form any reliable interpretation. At the present time all we can say is that such a daily rhythm is present.

SUMMARY

As established, the daily mitotic activity of the epithelium of the guinea pig esophagus is almost unchanged and remains on a relatively low level (8-13 pro mille). Amitotic coefficient (only the stage of contiguous nuclei was employed) is characterized by a marked 24-h rhythm with a maximum of 127 pro mille during the period from 11 AM to 2 PM and the minimum of 52 pro mille at 2 AM.

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