

# SEASONAL RHYTHM OF MITOSES IN CELLS OF THE RETINA AND LENTICULAR EPITHELIUM OF *Rana temporaria* L.

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Investigation of mitotic activity of retinal and lenticular epithelial cells in *Rana temporaria* revealed a well-marked seasonal rhythm. Mitoses were found throughout the year in the corneal epithelium and epidermis of the eyelids.

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Our investigations of the cause of increased radiosensitivity of the peripheral zone of the retina in adult grass frogs [5] revealed the presence of mitoses in retinal cells. In the peripheral zone mitoses were numerous (as observed previously [6]), whereas in the central part of the retina they were solitary [4]. The presence of mitoses in the retina of adult amphibians has been reported by only a few of the workers who have studied this organ, and this suggested the existence of circadian and seasonal periodicity in its cell division.

The present investigation was carried out to study seasonal periodicity of mitosis in the retina and lens of adult frogs (*Rana temporaria* L.).

## EXPERIMENTAL METHOD

Material was collected every month from June, 1964 to May, 1965, with additional collections in April and May, 1966. Colchicine was injected into the dorsal lymph sacs of the adult frogs in a dose of 0.07 ml of 0.1% solution per gram body weight. The material to be investigated was kept as homogeneous as possible: male and female frogs weighing 25-30 g. Since no sex differences were found in the number and distribution of mitoses, the results obtained with males and females are considered together.

The animals were kept at room temperature, and sacrificed 25 h after injection of colchicine. Fixation took place at the following times after injection of colchicine: in 1964 - June 13, July 3, August 5, September 11, October 7, November 10, December 7; 1965 - January 13, February 11, March 11, April 16, May 28; and 1966 - April 16 and April 26. At each period 3 or 4 animals were sacrificed, so that from 6 to 8 eyes were investigated. In addition, on April 27 and June 3, 1965, 50 frogs were sacrificed at each time without injection of colchicine. The enucleated eyes were fixed in a formalin-alcohol-acetic acid mixture in the ratio of 3:1:0.3. Paraffin sections, 6  $\mu$  in thickness, were stained with Heidenhain's iron-haematoxylin. The number of mitoses in the retina was calculated per 50 sections of each eye. Total preparations of the lenticular epithelium and cornea were made as we described previously [3].

## EXPERIMENTAL RESULTS

The distribution of mitoses among the zones and layers of the retina was the same as described previously [4]. A well-marked seasonal periodicity was observed in the mitotic activity of the retinal cells: in winter, from November through March, mitoses were absent from the retina; they were first noted in spring in the middle of April, near to the time of spawning, and their number reached a maximum in June, followed by a decrease until October (Fig. 1).

An equally clear seasonal periodicity, also described previously [1, 2], was observed in the mitotic activity of the lenticular epithelial cells. In winter, mitoses were absent from the lens. In spring they appeared much later in the lens than in the retina. On April 16, 1965 and April 16 and 26, 1966, mitoses were

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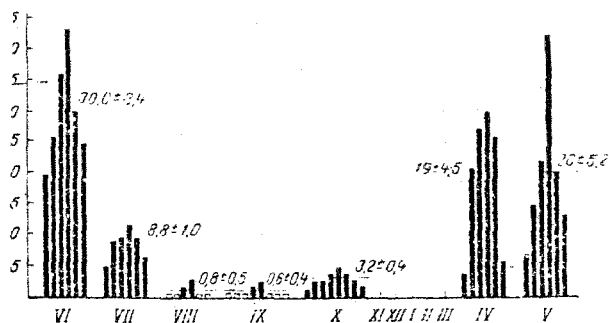


Fig. 1. Seasonal changes in mitotic activity of retinal cells of *R. temporaria*. Shaded columns show number of mitoses per 50 sections of retina. Unshaded columns represent frogs with absence of mitoses in their retina. Numbers at base of columns indicate months of year.

ference between results of the present and previous investigations may be that we used animals which had only just been caught, whereas in the previous studies, because of the experimental conditions, the animals had been kept in the laboratory for some time. It is conceivable that changes in external conditions (primarily temperature) may hasten the onset of cell division in the lenticular epithelium.

Mitoses were observed in the corneal epithelial cells throughout the year, although they were significantly fewer in the winter months than in the summer. Mitoses also were found all the year round in the epidermis of the eyelids. We did not make accurate counts of the number of mitoses in the lenticular and corneal epithelium or in the epidermis of the eyelids.

The pattern of mitotic activity thus varies in different parts of the complex system of the eye. Coordination of growth processes of these two important parts of the eye is perhaps manifested in coordination between the mitotic cycles of the lens and retina. We consider that the higher lability of the times of appearance of mitoses in the cornea than in the lens and, in particular, the delay in the appearance of spring mitoses in the lens compared with the retina, merit further study. Delay in mitosis in the lens is not a rigid phenomenon but can easily be removed by a change in external conditions, but it has so far proved impossible to bring the time of onset of mitosis in the lenticular epithelium forward so that it takes place before spawning [1, 2].

#### LITERATURE CITED

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found in the retina of all animals investigated at these times, whereas none were found in the lenticular epithelium. On May 28, 1965, mitoses were found in the retina of the 4 animals tested, while the lens contained no mitoses in two of these animals, a few in one, and numerous mitoses in one frog. On April 27, 1965, about 100 lenses were investigated from frogs not receiving colchicine and sacrificed at different times of day, and no mitoses were found in them. On June 3, 1965, a similar investigation was carried out on 100 lenses and mitoses were numerous in all of them. Hence, mitoses did not appear in the lenticular epithelium in April, at the time of spawning, but much later, toward the end of May.

Somewhat different results were obtained in the investigations cited above [1, 2], for mitoses began to appear in the lens in the middle of April, i.e., near to the time of spawning. The reason for the dif-