DIURNAL RHYTHM OF MITOTIC ACTIVITY OF THE EXOCRINE EPITHELIUM OF THE RAT'S PANCREAS

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The value of counting the variation in the intensity of cell division, as a method of obtaining a more precise idea of the mitotic behavior of an organ, has been demonstrated in many researches [1]. In particular, it has given a totally different interpretation of the ability of organs such as the liver [3, 4], kidneys [2], and lacrimal glands [3] to undergo mitotic division. Whereas it was formerly held that in normal adult animals the parenchymal cells of these organs very rarely undergo mitotic division, it has now been recognized that they have a definite diurnal rhythm of mitotic activity, and also, moreover, that in some cases the mitotic coefficient at times of maximal activity may reach a considerable figure [3].

In the present paper we give the results of a study of the mitotic activity of the exocrine epithelium of the rat's pancreas.

We found very few references to investigations of the number of mitoses in this tissue in adult animals. Bullough [6] points out that the number of mitoses in the pancreas of female mice is 0.01/mm^2 area of section in diestrus, and increases the proestrus and estrus to reach a maximum in metestrus (0.21), i.e., the mitotic activity is very low. There are frequent reports in the literature that in fully grown animals the pancreatic cells under normal conditions cease to undergo mitotic division [5, 8]. However, Messier and Leblond [7] have shown that after administration of tritium-labeled thymidine to rats, the label can be found, in particular, in the acinar cells of the pancreas.

EXPERIMENTAL METHOD

Experiments were carried out in July on albino rats weighing 160-180 g, kept in natural conditions of illumination. Before and during the experiments the animals received food pellets and water ad lib. Every 3 hours for a period of 2 days 10 rats were sacrificed by decapitation.* The material was fixed in Zenker's fluid, embedded in paraffin wax, and sections cut to a thickness of 8μ were stained with hemalum and eosin. The mitoses were counted in the acinar cells, for which purpose 500 fields of vision were examined in each case, with a magnification of the microscope of 90 × 7 × 1.5 and a diaphragm in the eye-pieces having an aperture 7.5 × 7.5 mm (an average of 32,000 nuclei in each animal). In each case the number of nuclei in 10 fields of vision was counted and the mean value for individual fixation times calculated. Since most cells in the rat's pancreas are binuclear, the mitotic coefficient was taken to be the number of mitoses in 1000 nuclei, and not 1000 cells. The degree of variation of the mitotic coefficient at the different periods was characterized by calculation of the coefficient of variation. The results were treated statistically by the Fisher-Student method.

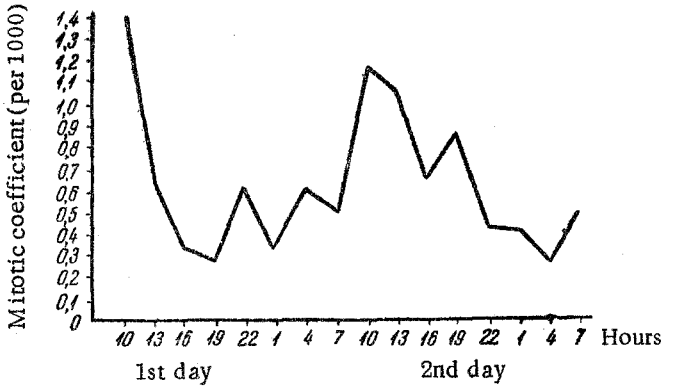
EXPERIMENTAL RESULTS

The results of determination of the mitotic activity in the acinar cells of the pancreas for a period of 48 hours are shown in Fig. 1.

At 10 A.M. on the first day of the experiment the mitotic coefficient was relatively high, namely 1.4 per 1000, after which a fairly sharp fall was observed to 0.28 per 1000 at 7 P.M. ($P = 0.01$) and a further rise, although not so

*In this investigation we used the pancreases of rats on which experiments were conducted concurrently by other members of the laboratory staff, who determined the diurnal rhythm of mitotic activity for a period of 2 hours in the cornea and kidney [2], the lacrimal gland [3], the liver [3], and the adrenal and lymph glands.

considerable (0.63), yet statistically significant (P = 0.005) at 10 P.M. At 1 A.M. the mitotic activity fell (P = 0.015), but later rose again to reach a high level again at 10 A.M. on the following day. The curve of mitotic activity on the first day of the experiment was thus bimodal in character.



Changes in the mitotic coefficient of the exocrine epithelium of the pancreas of a rat during a period of 48 hours.

The curve illustrating the change in mitotic activity during the second day of the experiment repeats the main features of the curve obtained on the first day. The fall in mitotic activity after 10 A.M. took place more gradually, and the evening rise was observed not at 10 P.M., but at 7 P.M., which slightly masks the bimodal character of the curve. Other workers have also reported a similar change in the time of the increase in mitotic activity. In particular, in work by V. N. Dobrokhotov and co-workers [3], conducted on the same animals, a well marked bimodal character of the curve of diurnal changes in the mitotic activity of the lacrimal gland cells was observed, and just as in our experiments, the evening rise in mitotic activity on the first day of the experiment took place at 10 P.M., and on the second day at 7 P.M. This interesting phenomenon – the shift of the peaks of mitotic activity in time – requires further study.

The individual fluctuations in mitotic activity in the pancreas were considerable throughout the whole period, but they were especially large just before a sharp fall or rise in the mean mitotic coefficient. For instance, at 10 A.M. on the first day of the experiment and at 7 A.M. on the first and second days, the coefficient of variation of the mitotic coefficient was greater than 80%.

During the counting of the mitoses observations were made, still only preliminary, which we consider to be of definite interest. It has already been mentioned that most cells in the rat's pancreas are binuclear. In the prophase stage both nuclei of the cell were always at the same level of development. In cases when the number of mitoses was high, cells with 2 metaphase plates were also frequently seen. We never saw two anaphases or telophases in the same cell. Hence it may be assumed that as a result of the combination of the material of both nuclei at the stage of metaphase, by the division of the binuclear cells two mononuclear cells are formed, having a double set of chromosomes. These cells are subsequently, it seems, changed into binuclear cells. Three ways for this transformation may be suggested: 1) mitosis not terminating in division of the cytoplasm (however, in our preparations at the stage of late telophase or reconstruction of the nucleus clear division of the cell body was always seen); 2) fusion of two cells without fusion of the nuclei; in this case the chromosome number of the cells would increase continually during subsequent divisions; and 3) amitotic division of the tetraploid nuclei formed as a result of mitosis not accompanied by division of the cytoplasm. We consider that the last method is the most probable, although no definite evidence can yet be adduced in its favor. We can only cite Ries and Gersch [8], who are of the opinion that the cells of the pancreas in fully grown animals divide by amitosis. This problem requires further study.

Analysis of the results shows that the view that mitoses do not occur in the exocrine epithelium of the pancreas in normal adult animals is erroneous, or at least it is so in rats. In our experiments on these animals a diurnal rhythm of mitotic activity was found in the exocrine epithelium of the pancreas. The curve representing this rhythm was bimodal in character.

A morning rise in mitotic activity was observed at 10 A.M. This was constant, and relatively high, and evidently corresponded to the morning rise in mitotic activity taking place in other organs, of our experimental rats (the cornea, liver, kidney, and lacrimal gland [2, 3]). The evening rise was smaller in absolute terms, less stable, and coincided in time with the evening peak of mitotic activity found in the lacrimal gland [3].

By carrying out the experiment for 48 hours we were able to show that even in animals kept in identical conditions the curve of the diurnal mitotic rhythm may vary within wide limits. The mean mitotic coefficient on the first day of the experiment was slightly lower than on the second day (0.6 and 0.67 per 1000).

If the mean mitotic coefficient for the 24 hours is known, an approximate estimate can be made of the rate of renewal of the cells in an organ. If it is accepted that mitosis does not last longer than 1 hour, then with an average mitotic coefficient of 0.63 per 1000, every hour 63 cells in 100,000 divide. Consequently, division of all 100,000 cells (doubling of their number) takes place in the course of 100,000 : 63 = 1587 hours, or roughly in 66 days.

On the basis of the fact that in their experiments labeled nuclei were found in the acinar cells of the pancreas, after administration of thymidine-H^3, throughout the experimental period (95 days), Messier and Leblond [7] concluded that these cells are not renewed and that mitosis of these cells brings about only growth of the organ. We consider that there is little ground for this conclusion. From our own counting, and taking into consideration the diurnal rhythm of mitotic activity, we may conclude that the life span of the individual cells of the pancreas is sufficiently long, but that the cells are, nevertheless, systematically renewed. Otherwise an extreme enlargement of the pancreas in the course of time would have to be admitted, which in fact is not observed.

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