THE LEVEL AND DAILY RHYTHM OF MITOTIC ACTIVITY IN HYPOPHYSECTOMIZED RATS

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Although it is an established fact that the growth of animals ceases after hypophysectomy, the problem of the influence of the hypophyseal hormones on the mitotic division of cells has not yet been adequately explained. Several workers [4-7] have reported that proliferative processes can be induced in hypophysectomized rats, especially those associated with physiological regeneration.

In this connection it was necessary to determine the extent to which the processes of physiological regeneration, as reflected by their mitotic activity, are disturbed after hypophysectomy. This requires the study not only of the general level of mitotic activity of the organs, but also its daily fluctuations, for failure to take account of the latter will mean an incomplete picture of the mitotic regime.

In addition to organs characterized by a high mitotic activity (cornea, esophagus, tongue), we also used organs from adult rats in which most writers have described either absence of mitosis or the presence of only occasional mitoses (the liver, kidney, and external orbital gland). In extending the scope of our investigation in this way, we were profiting by recent findings [2,3] that significant mitotic activity is found in these organs at certain times of the day.

EXPERIMENTAL METHOD

Since one research from our series, devoted to the study of the mitotic activity of the epithelium of the crypts of Lieberkuhn in the small intestine, has already been published [4], we shall give here only a brief description of our experimental method.

Mitotic Activity (Pro Mille) in Various Organs of Hypophysectomized and Intact Rats

Organ	Rats	Time of day					Mean mitotic coefficient
		4 AM	7 AM	10 AM	6 PM	10 PM	
Cornea	Control	3.9	6.4	9.3	6.8	4.9	6.2
	Experimental	6.4	9.6	7.4	4.9	3.8	6.4
Tongue	Control	9.1	13.1	20.5	3.1	8.6	10.9
	Experimental	14.7	18.2	12.7	7.3	2.2	11.0
Esophagus	Control	6.0	10.2	10.5	3.7	3.7	6.8
	Experimental	2.5	6.0	7.2	1.0	2.7	3.9
Liver	Control	0.1	0.6	0.9	0.1	0.2	0.4
	Experimental	0.01	0.05	0.03	0.06	0.0	0.03
Orbital gland	Control	0.4	0.3	1.1	0.08	0.2	0.4
	Experimental	0.06	0.02	0.08	0.01	0.0	0.03
Kidney	Control	0.13	0.22	0.09	0.05	0.03	0.16
	Experimental	0.01	0.0	0.12	0.02	0.0	0.03

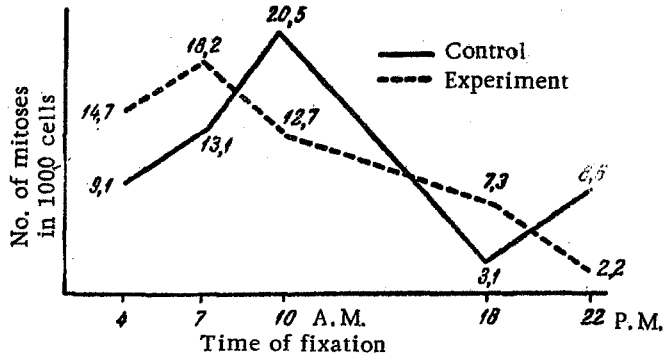

Fig. 1. Curve showing the daily fluctuations in the mitotic activity of the tongue in hypophysectomized and intact rats.

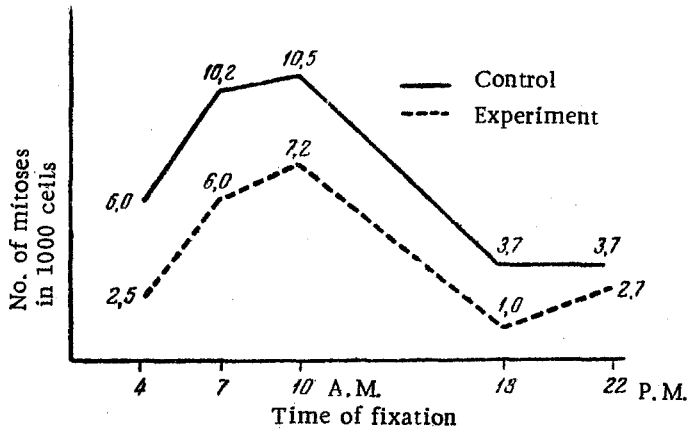

Fig. 2. Curve showing the daily fluctuations in mitotic activity of the esophagus of hypophysectomized and intact rats.

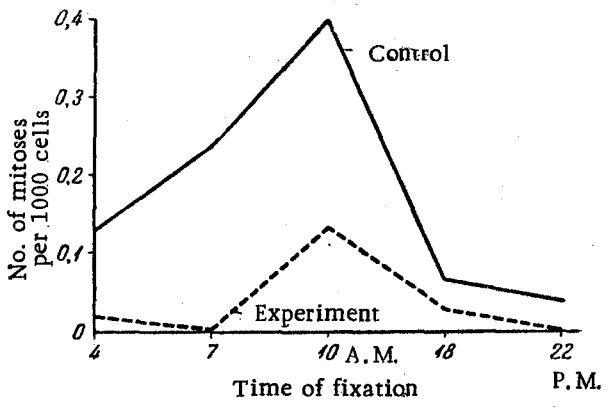

Fig. 3. Curve showing the daily fluctuations in mitotic activity of the convoluted tubules of the kidney in hypophysectomized and intact rats.

The hypophysis was removed from male rats weighing 170-240 g by Smith's method, as modified by Gasanov [1]. The rats were sacrificed by decapitation 19 days after the operation, at 4, 7, and 10 AM and at 6 and 10 PM. Between 4 and 6 experimental, and 8 control animals were sacrificed at each time. The organs of only those animals in which inspection post mortem revealed no traces of the hypophysis after the operation, and in which atrophy of the testes had taken place, were subjected to the following treatment. The rats' organs were fixed in Zenker's or Bouin's fluid, embedded in paraffin wax, and cut into sections which were stained with hematoxylin and eosin. Total preparations of the cornea were obtained and stained by the same method. The number of mitoses was counted in a definite number of cells: in 10,000-15,000 in the cornea, in 4000 in the lingual epithelium, in 4000 in the esophageal epithelium, in 6000 liver cells, in 15,000 in the epithelium of the external orbital gland, and in 15,000-18,000 cells of the epithelium of the convoluted tubules of the kidney. In the tongue, mitoses were counted in the epithelium of the upper surface in transverse sections, and in the esophageal epithelium also in transverse sections.

EXPERIMENTAL RESULTS

Mitoses were found in all the organs of the hypophysectomized rats (see table), and the mitotic activity, moreover, showed definite changes during the 24 hours. Comparison with the controls was made easier by the fact that in all organs of the intact rats the changes in mitotic activity were expressed by a unimodal curve with a maximum at 10 AM and a minimum occurring in the period between 6 PM and 4 AM.

It may be seen from the table that removal of the hypophysis was reflected differently in the mitotic activity of different organs. According to the character of the change in the level of mitotic activity and of its daily rhythm, three groups of organs can be distinguished or, more accurately, three types of epithelium. The first group consisted of the cornea and tongue. In these organs the level of the mitotic activity after hypophysectomy remained unchanged, and the experimental and control values of the mitotic coefficient were very close. The daily fluctuations in mitotic activity were also of a similar character (Fig. 1). The only difference between the experimental and control curves was a shift of the peak of mitotic activity to an earlier time (7 AM instead of 10 AM). This shift was statistically significant.

The second group included the esophagus, in the epithelium of which the level of mitotic activity fell after hypophysectomy. The mean mitotic coefficient in the control series was 6.8, and in the experimental 3.9. The difference between these values is statistically significant. Hence, the mitotic activity in the esophageal epithelium fell by approximately 45%. At the same time, the curve of the daily fluctuations of mitotic activity was of the same

character as in the control rats (Fig.2). The peaks and the minima of the curves of mitotic activity occurred at the same times of day or night.

The third group included the liver, the external orbital gland, and the convoluted tubules of the kidney. Hypophysectomy caused a sharp fall in the mitotic activity of these organs. The mitotic activity of the liver and orbital gland fell to approximately one-thirteenth, and that of the kidney to one-fifth, of its original level. So far as the daily fluctuations of mitotic activity are concerned, these were difficult to estimate, for the level of activity was very low. With such low values of mitotic activity, the changes in the number of mitoses at the different times of day could be assessed only from a large series of cases, by counting the number of mitoses in not less than 40,000 to 50,000 cells. In the liver, in which fewer cells were counted, the changes that were found were not consistent in character. In the orbital gland and the kidneys, where many more cells could be counted, the curve of the daily fluctuations of mitotic activity was evidently similar in character to that of the corresponding organs of the control rats (Fig. 3), although no final decision could be made on this problem.

Our results showing the level of mitotic activity and its daily fluctuations in the various organs of the intact animals were in general agreement with those given in the literature [2,3]. A small correction is required only in respect of the epithelium of the external orbital gland, for the use of more frequent times of fixation would have shown that the curve of daily fluctuation of mitotic activity is bimodal in character, with an additional small maximum during the evening. This fact, however, does not interfere with our main conclusions.

So far as the mitotic activity of the organs of the hypophysectomized rats is concerned, no experiments have been conducted by other workers in the same conditions. The work of S. S. Raitsina [4] shows that yet another type of epithelium may be distinguished (that of the crypts of Lieberkuhn in the intestine), in which neither the level of mitotic activity nor its daily fluctuations underwent any modification after hypophysectomy.

Our results show beyond doubt that the processes of physiological regeneration in the phase of repair (proliferation of cells) take place without hindrance in certain organs after hypophysectomy. They also show that in other organs (liver, orbital gland, kidneys) these processes are disturbed. The fact that these organs undergo atrophy after hypophysectomy has been known for a long time, but no accurate information was available of the changes in their mitotic activity. We may ask whether the proliferation of the cells of the liver, orbital gland, and kidneys found in the intact animals characterizes the growth of these organs, continuing in the adult animals, or the renewal of the cells, their physiological regeneration. Experiments involving hypophysectomy ought to throw light on this problem, for after hypophysectomy the growth of these organs ceases, and yet some degree of mitotic activity, admittedly slight, is found in them. However, the conclusion that the mitotic activity in the cells of the liver, the orbital gland, and the kidneys characterizes the growth of these organs and not their physiological regeneration would be premature. It must not be forgotten that in hypophysectomized rats these organs undergo atrophy, and their condition, in consequence, is greatly disturbed. Further investigations are, therefore, required in order to demonstrate more clearly the relationship between the processes of growth and renewal of cells in these organs.

Taken as a whole, our results show that in hypophysectomized animals cell division by means of mitosis continues. This means that some reappraisal of the mechanism of the effect of the hypophyseal hormones on animal growth is required. The inhibition of growth after removal of the hypophysis cannot be explained, it seems, purely by the fact that in the absence of its hormones cell proliferation cannot take place. Special investigations are therefore necessary in order to discover what is responsible for the inhibition of growth after hypophysectomy.

SUMMARY

Subject to assessment was the mitotic activity in the epithelium of the cornea, tongue, esophagus, liver, outer orbital gland, renal convoluted tubules of adult hypophysectomized rats (170-210 gm). It was studied at 4, 7, and 10 AM and at 6 and 10 PM; mitoses were calculated as per 4-18 thousand cells. Mitotic activity proved to be the same in the corneal and lingual epithelium of experimental and control animals. Following hypophysectomy the curve of the daily mitotic activity changes exhibited but insignificant changes, i.e., its peak shifted to earlier hours. Mitotic activity level decreased by 45% in the esophageal epithelium; however, its daily rhythm here remained unchanged. Mitotic activity of the epithelium in the rest of the organs decreased 5-12 times, the curve of the daily changes evidently remaining unchanged. Thus, hypophysectomy did not eliminate mitosis, even had no decreasing effect on this activity in a number of organs.

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