

THE MITOTIC ACTIVITY OF THE EPIDERMIS OF THE LIMB IN RATS AFTER DENERVATION

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Many investigations have shown that the nervous system plays a considerable part in the regulation of the proliferation of cells. Changes in mitotic activity have been demonstrated in experiments in which the innervation of tissues was destroyed [1, 3, 5, 6, 8]. The conflicting nature of the findings do not, however, permit any conclusions to be drawn on the character of the influence of the nervous system on cell division. We accordingly set out to study the changes in mitotic activity occurring during certain forms of interference with the peripheral nervous system.

In the present paper are described the results of observations made on the changes in mitotic activity in the epidermis after denervation of the limb.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature male white rats (average weight 180 g). The nerves of the hind limb of the experimental animals were divided on the left side: — the femoral nerve at the level of Poupart's ligament, the obturator nerve in the pelvis and the sciatic nerve immediately at its point of emergence from the pelvis.

In each case a portion of the nerve about 0.5 cm long was resected. All the operations were carried out under ether anesthesia. Sixty-one rats were used in the experiments.

On the 10th day after operation pieces of skin measuring 4 x 8 mm were excised from the upper third of the outer side in both limbs. At this time the operation wounds had completely healed. The material was embedded in celloidin paraffin. Sections were cut to a thickness of 7 μ and stained with hematoxylin by Caracci's method.

The mitotic coefficient was calculated (the ratio between the number of dividing cells and the total number of cells, expressed in per thousand). In each case not less than 3000 cells were counted. In certain series we also determined the number of amitoses and measured the thickness of the epidermis. The counting of the cells and mitoses was done under a 90x objective and a 7x eyepiece. When comparing the mitotic coefficients and the other indices also, the significance of the random variation of their values — P — was determined by the Fisher-Student method. The differences were regarded as significant when $P = 0.001$.

EXPERIMENTAL RESULTS

In the 1st series (23 rats) we studied the changes in mitotic activity of the epidermal cells of the skin after section of the nerves of the limb as indicated above. The results obtained (see Table) show that the mean mitotic coefficient for the epidermis of the intact (not denervated) limbs (7.3 per 1000) was higher than that for the

TABLE

Mitotic Coefficient in the Epidermal Cells of the Denervated and Intact Limbs in Rats

Experimental animal number	Intact limb		Denervated limb		Changes in the mitotic coefficient of the denervated limb compared with the intact, in %
	Number of mitoses	mitotic coefficient (per 1000)	Number of mitoses	mitotic coefficient (per 1000)	
14	8	2.1	6	1.8	85.7
38	12	3.7	7	2.2	59.5
88	12	3.8	17	5.1	134.2
57	14	4.0	8	2.5	62.5
23	14	4.2	17	5.3	126.2
54	14	4.4	11	3.5	79.8
86	14	4.6	8	2.5	54.3
24	15	5.0	9	2.8	56.0
47	18	5.4	11	3.5	64.8
59	17	5.5	24	7.4	134.5
81	18	5.6	20	6.7	119.6
36	20	6.1	18	5.5	90.2
7	22	6.6	17	5.5	83.3
76	23	7.7	11	3.6	46.8
50	25	7.8	16	5.0	64.1
62	29	8.5	12	3.6	42.4
82	29	9.0	18	4.5	50.0
52	28	9.2	10	3.2	34.8
17	32	10.5	23	7.3	69.5
30	38	12.0	22	6.9	57.5
11	37	12.1	20	6.6	54.5
63	47	14.1	33	10.5	74.5
2	53	15.1	14	4.5	29.8
Average	23.4	7.3	15.3	4.8	65.7

epidermis of the denervated limbs (4.8 per 1000). This difference was statistically significant ($P = 0.009$).

A count of the number of mitoses by phases showed that the differences in the mean percentage values for each phase of mitoses in the epidermis of the comparable limbs were extremely insignificant.

The number of early prophase in the epidermis of the intact and denervated limbs, for instance, was respectively 25.4 and 29.5%, prophase - 31.9 and 28.7%, metaphase - 29.9 and 29.6%, anaphase 6.3 and 6.0% and telophase - 6.5 and 6.3%. At this period of time after operation, denervation of the limb thus has no essential effect on the rate of any of the phases of mitosis. This suggests that the reduction in the number of mitoses in the epidermis of the denervated limb is due to a reduction in the number of cells starting to undergo division.

The total number of amitotic figures in the epidermis of the denervated and intact limbs (3 and 14 amitoses respectively) was so small that it ruled out the possibility of making any conclusions about the influence of section of the nerves of the limb on the amitotic activity of the epidermis.

No essential differences were found in the thickness of the epidermis in the denervated and intact limbs.

In order to reveal any possible effect of trauma on the mitotic activity of the epidermis, we carried out a second series of experiments. The animals in this series were divided into two groups. In the first group of 10 rats the same operation of division of the nerves of the left limb was performed as in the animals in the 1st series of experiments; in the animals of the 2nd group (11 rats) the soft tissues of the left limb were incised and the

nerves left undamaged. The material in this case also was fixed on the 10th day after operation.

It was found that the animals in which only the soft tissues were incised showed a mean mitotic coefficient in the epidermis of both limbs which was almost the same: in the epidermis of the left limb, for instance, it was 12.1 per 1000 and in that of the right — 11.6 per 1000. In the first group of animals in this series of experiments, the mitotic coefficient in the epidermis of the denervated limbs was 7.7 per 1000, and in the epidermis of the intact limbs — 10.2 per 1000. This difference, like that in the 1st series of experiments, was statistically significant ($P = 0.003$). The higher value of the mitotic coefficient in the epidermis of those animals in which only the soft tissues were incised (12.1 per 1000) than that in the epidermis of the intact limbs of the experimental animals (10.2 per 1000) is not significant ($P = 0.105$).

Thus the results of the two series of experiments demonstrate a fall in the mitotic activity of the epidermis of the denervated limb. At the same time it is still not clear whether this fall is on account of a reduction in the number of dividing cells in the denervated limb, or of an increase in the mitotic activity of the epidermis of the opposite (intact) limb. It is true that a comparison of the mitotic coefficients of the epidermis of the undamaged limbs in the animals of the 2nd series of experiments (11.6 and 10.2 per 1000) shows no such increase. However the considerable individual variations in the mitotic activity of the animals prevents these findings from being regarded as sufficiently convincing. For this reason we attempted, in a third series of experiments, to elucidate this problem by comparing the mitotic activity in the epidermis of the fore-and hindlimbs of the same animal.

In the experimental animals of this series (12 rats) the left hindlimbs was denervated, and on the 10th day the mitotic activity was studied in the epidermis of all 4 limbs. Control animals (5 rats) were not subjected to any form of interference. In the control animals the number of mitoses in the epidermis of the fore-and hindlimbs was found to be almost the same.

The mean mitotic coefficient in the epidermis of the forelimb for instance, was 2.6 per 1000, and in the epidermis of the right hindlimb 2.1 per 1000 and the left hindlimb — 2.4 per 1000.*

The number of mitoses in the epidermis of both forelimbs and in the epidermis of the intact hindlimb of the operated animals also showed no significant differences. The mean mitotic coefficient in the epidermis of the right forelimb was 4.9, the left forelimb 5.4 per 1000 and the right hindlimb 5.3 per 1000. Meanwhile, in the epidermis of the denervated limb, the number of mitoses was considerably lower (2.9 per 1000). This fall in the number of mitoses is significant by comparison with their number in the right hindlimb ($P = 0.003$), the right forelimb ($P = 0.01$) and the left forelimb ($P = 0.004$).

SUMMARY

Unilateral section of the nerves of the hindlimb carried out under the above mentioned experimental conditions, does not, at a given period of observation, lead to any form of change in the mitotic activity of the epidermis in the contralateral limb; this does not, however, rule out the possibility of such changes as have been shown by several workers in experiments performed under different conditions [2, 4, 6, 7]. The conclusion may be drawn from the results of all 3 series of experiments that interruption of the innervation reduces the mitotic activity in the epidermis of the denervated limb.

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* The relatively low values of the mitotic coefficient in this series of experiments may probably be due to the fact that the experiment was performed in the winter.

* * See English translation.

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