THE EFFECT OF DIVISION OF THE NERVES OF THE HINDLIMB ON THE RATE OF HEALING OF SKIN WOUNDS AND ON THE MITOTIC ACTIVITY OF THE REGENERATING EPIDERMIS

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The influence of the peripheral nervous system on the rate of healing of skin wounds, and especially on the changes in mitotic activity of the regenerating tissues has received very inadequate study. Research into these subjects is, however, of essentail importance to an understanding of the laws governing regenerative processes and cell division.

The data in the literature on these questions are few in number and contradictory. Some authors [1, 11] report acceleration of healing of skin wounds when the nerve supply of the tissues is disturbed, whereas others [5, 9, 10, 14, 15] under the same conditions observed retardation of the processes of wound healing. Even less work has been done on the study of the changes in the mitotic activity of the cells in process of regeneration when the nerve supply of the tissues is disturbed. It is only in a paper by L. I. Sazanov [10] that quantitative findings are given on the changes in the mitotic activity of the tissues during the healing of skin wounds in different animals after division of the peripheral nerves.

The aim of the present investigation was to study the changes in the rate of healing of skin wounds in the mitotic activity of the regenerating epidermis when the nerve supply to the limb was disturbed.

EXPERIMENTAL METHOD

Experiments were carried out on 67 sexually mature male white rats (weighing on the average 185 g). Denervation of the left hindlimb was performed in the experimental animals by excision of a portion of the nerve about 0.5 cm long. The femoral nerve was excised at the level of Poupart's ligament, the obturator nerve in the pelvis and the sciatic nerve directly at the point of its emergence from the pelvis. All the operations were conducted under ether anesthesia.

The changes in the dimensions of skin wounds during healing were studied by outlining their contours on a celloidin film, cutting out pieces of the film along the lines corresponding to the edges of the wound, and then weighing them on torsion scales. The results of daily measurement of the wounds were converted into percentages of the value obtained for the area by measurement of the wound within 24 hours of its infliction.

In order to study the changes in mitotic activity at different periods of regeneration, pieces of skin were excised from an area adjacent to the wound surface. The material was fixed in Bouin's fluid and embedded in celloidin-paraffin wax. Sections were cut to a thickness of 7μ and stained with hematoxylin by Carazzi's method. The mitoses were counted in the epidermis only in areas of skin lying next to the wound. The mitotic coefficient was calculated per 1000 from not less than 3000 cells in each case. To count the mitoses we used a 90 × objective and a 7 × ocular.

When comparing the mitotic coefficients and the other numerical values obtained, the probability P of chance variation of their values was obtained by the Fisher-Student method.

EXPERIMENTAL RESULTS

In the first series of experiments we made a comparative study of the rate of healing of skin wounds in the denervated and undenervated limbs of rats. For this purpose, in the experimental animals (14 rats) we divided the nerves in accordance with the method described above, and in the controls (14 rats) we divided only the soft tissues of the left limb. On the 10th day after the operation, an area of skin measuring 4 × 8 mm was excised from both forearms and both shins of all the animals.

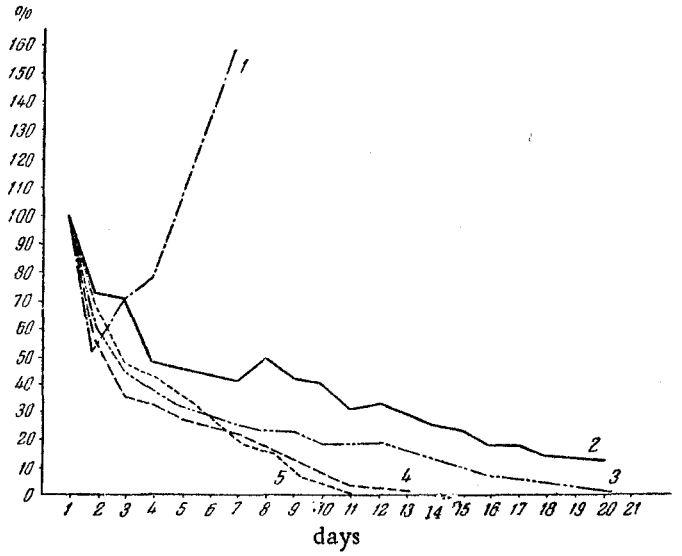

Fig. 1. Rate of healing of skin wounds in experimental rats. (Along the ordinate axis, dimensions of the skin wounds as a percentage of their area in the first day after operation). 1) Changes in the dimensions of the wounds of the left hindlimb during their conversion into ulcers; 2) changes in the dimensions of the wounds of the left hindlimb during healing; 3) changes in the dimensions of the wounds of the right hindlimb during healing; 4) changes in the dimensions of the wounds on the right forelimb during healing; 5) changes in the dimensions of the wounds on the left forelimb during healing.

It was found that the wounds on both the fore- and hindlimbs of the control animals healed at approximately the same rate. In 12 of the 14 control rats, for instance, complete healing of the wounds was observed on the 8th day after operation, and in two only, on the 10th day.

We obtained different results in the experimental animals. The changes in the dimensions of the wounds at different times are shown in Fig. 1.

As may be seen from Fig. 1, healing of the wounds of the forelimbs took place at the same time and was completed on the 10th-12th day, i.e., slightly later than in the same limbs in the animals of the control group. Healing of the wounds of the undenervated shin, especially in the middle periods of the process of regeneration, took place much more slowly than on the forelimbs, and in the majority of animals it was only completed on the 16th-20th day.

The wounds of the denervated limb healed still more slowly. On the 20th day after operation, for instance, in the majority of rats the wounds of this limb had not completely healed, and in some animals, after a brief reduction in the size of the wounds, they began to enlarge rapidly and to be converted into ulcers. This

Mitotic Coefficient in the Epidermis of Rats during Healing of Skin Wounds of the Shin in Control and Experimental Animals

Time of regeneration (days)	Animal No.	Mitotic coefficient in control animals		Animal No.	Mitotic coefficient in experiment animals	
		left skin	right skin		denervated skin	undervated skin
1st	26	19.8	25.9	19	21.6	18.6
	29	18.2	19.8	20	12.8	15,6
	27	21.7	18.0	18	12.3	15.0
				17	7.0	14.2
				16	9.2	13.5
Average	—	19.9	21.2	—	12.6	15.3
3rd	36	16.3	20.9	22	13.1	11.0
	31	19.6	11.5	24	2.5	9.8
	33	12.3	10.6	21	10.3	8.8
				23	12.9	6.8
				25	6.9	3.9
Average	—	16.1	14.5	—	9.2	8.0
5th	14	19.7	18.1	15	15.8	16.0
	40	6.2	8.9	11	14.0	15.2
	37	10.0	7.9	2	13.7	11.6
				1	9.8	6.8
				10	8.5	5.6
Average	—	12.2	11.6	—	12.4	11.1
7th	48	4.8	8.7	41	10.7	12.0
	47	10.3	6.9	42	12.5	7.9
	51	5.8	5.3	45	12.7	7.7
				44	10.4	6.7
				43	14.8	5.4
Average	—	7.0	7.0	—	12.2	7.9
9th	59	9.0	10 2	54	6.6	9.6
	60	11.2	7.9	56	4.5	8.6
	62	9.7	6 9	53	6.3	5.9
				57	13.7	3.7
Average	—	10.0	8.4	—	7.8	6.8

difference in the changes in the dimensions of the wounds is illustrated in Fig. 1 in the form of two curves, one of which shows the changes in the area of the gradually healing wounds (10 rats), and the other, changes in the dimensions of wounds unhealed after a considerable length of time (4 rats). Fig. 2 illustrates the external appearance of one of these ulcers arising at the site of infliction of a wound.

Fig. 2. Nonhealing ulcer arising at the site of infliction of a skin wound on the denervated limb of a rat.

The aim of the second series of experiments was to make a comparative study of the mitotic activity of the epidermis during regeneration of skin wounds of denervated and undenervated limbs. In accordance with the times of regeneration, the animals of this series were subdivided into 5 groups, each of which consisted of 3 control and 5 experimental rats. In the experimental animals, as in the first series, the left hind-limb was denervated, and in the control animals the soft tissues alone were incised. On the 10th day after operation, skin wounds were inflicted on all the animals, on the posterolateral aspect of the upper third of both shins. The mitotic activity was studied on the 1st, 3rd, 5th, 7th and 9th days after operation.

The results showin the changes in the mitotic coefficient in the epidermis of the experimental and control animals are given in the table. As can be seen from the table, the average mitotic coefficients in the epidermis of both limbs of the control rats revealed no essential difference at any period of regeneration. The highest mitotic coefficient was found in the first 24 hours of regeneration; thereafter it diminished slightly. In the experimental animals the average mitotic coefficients in the epidermis of the denervated and undenervated limbs likewise showed no difference at any period of regeneration (the very small variations which we observed were not significant). We have previously [8] shown that division of nerves leads to a fall in number of dividing cells in the epidermis of the denervated limb by comparison with the undenervated limb of the same animal. This phenomenon was not observed in conditions of regeneration.

At the same time it follows from the table that the average mitotic coefficient in the experimental rats on the 1st, 3rd and 9th days of regeneration was lower than in the corresponding limbs of the control animals.

The slower healing of the wounds in the experimental animals was thus accompanied by a lower mitotic activity of the epidermis at the various periods of the regenerative process.

It is interesting that the mitotic coefficient was highest in the first 24 hours of regeneration. Other workers [4, 7, 12, 13] have obtained identical results in mammals. At the same time, in experiments carried out mainly on amphibia [2, 3, 6], a considerable fall in the number of cell divisions was observed in the first days o of the process of regeneration. It is difficult to say at this stage whether this is due to species differences or to some other factor affecting the healing of wounds.

The results described in the present paper demonstrate the fundamental role of the nervous system in the processes of wound healing and in the regulation of the mitotic activity of the tissues of the body.

SUMMARY

Ischial, femoral and obturator nerves were divided unilaterally in rats. On denervated extremities the healing of skin wounds was much slower than in the one with the nerves intact. In the latter case the skin wounds heal slightly slower than in control animals. On the 1st, 3rd and 9th days of regeneration the mitotic coefficient of the epidermis adjacent to the wound surface is almost the same in denervated extremities as in those with the nerves intact, but is lower than in the limbs of control animals.

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