EFFECT OF x-RAYS ON THE REGENERATIVE POWER OF THE IRRADIATED PERONEAL NERVE OF THE RAT

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The effect of irradiation in highly differentiated tissues with low proliferative activity consists mainly of the depression of the process of posttraumatic regeneration [8, 10, 11]. Examples of tissues reacting in this way to irradiation are muscle and bone [5, 9, 12]. For this reason the study of the posttraumatic regeneration of the irradiated peripheral nerves of animals is of great interest.

Marked inhibition of the posttraumatic regeneration of nerves has previously been demonstrated in axolotls [3] after irradiation of the nerves or the thoracic segments of the spinal cord of these animals in doses of 10,000-20,000 R. Meanwhile, following irradiation of the larvae of spotted salamanders and tritons, no such effect was found [13]. Following irradiation of rabbits in a dose of 300-900 R depression of regeneration of nerve trunks was observed [1]. Similar conclusions have been reached in experiments on dogs [3] irradiated in doses of 300-350 R. The histological study of the site of injury of peripheral nerves has revealed marked inhibition of the formation of a traumatic neuroma for the extent of 4-5 months, when the sciatic nerve was injured by operation 3, 20, and 60 days after irradiation. V. M. Lavrinenko [6] irradiated albino rats in a dose of 600 R and observed a lowering of the intensity of regeneration of nerves. M. N. Meisel' [7] studied the regeneration of nerves in the irradiated skin of mice and found no significant depression of regeneration. He showed that nerve tissue may respond by morphological changes to changes in the external environment. E. I. Zaitsev [4] irradiated rabbits in a dose of 1000 R and observed no significant disturbances in the rate of regeneration of injured nerves.

Hence, there is no generally accepted opinion in the literature concerning the action of radiation on the posttraumatic regeneration of the peripheral nerves. It must be noted that none of the authors cited above analyzed the process of regeneration quantitatively. In the present investigation experiments were carried out on rats to study the effect of x-rays on the regeneration of a nerve after mechanical trauma, using quantitative method for assessing the changes arising after irradiation in different situations.

EXPERIMENTAL METHOD

Experiments were carried out on 185 male Wistar rats weighing 160-180 g. The animals were irradiated with an RUM-3 apparatus operating at a voltage of 180 kV, a current of 17 mA, skin-focus distance 30 cm, dose rate 7.8 R/min, and filters 0.5 mm Cu and 1 mm Al. In the experiments of series 1 the hind limb of the rats was irradiated in a dose of 3000 R. In series 2 the region of the spinal cord was irradiated from the dorsal aspect in the same dose. The zone of irradiation comprised the segments L_4–S_1 innervating the hind limbs. The remaining part of the animal's body was screened with lead. Control rats were not irradiated. In the experiments in which the limb was irradiated, the unirradiated opposite limb of the animal acted as the control.

The peroneal nerve was resected for a constant length of 0.4 cm. The nerve was divided in the region of origin of its muscular branch and at the point of entry of the sciatic nerve into the popliteal fossa, ensuring minimal separation of the nerve ends after the operation. The tibial nerve remained intact. Trauma was applied 24 h and 20, 60, 127 (in the experiments in which the limb was irradiated), and 170 days after irradiation. The traumatized nerves

Quantitative Assessment of Regenerative Process Caused by Resection of Peroneal Nerve of a Rat after Irradiation of Hind Limb or Spinal Cord in a Dose of 3000 R (18th day after mechanical trauma)

Time of trauma after irradiation	Number of animals	Site of irradiation	Size of connective-tissue scar (in relative units)	Size of neuroma without connective-tissue capsule (in relative units)	Density of neuroma (in relative units)
Control	43	Hind limb	106.3 ± 4.3	71.4 ± 4.4	31.3 ± 1.3
24 h	39		33.1 ± 2.1	22.5 ± 2.5	18.3 ± 1.3
20 days	11		55.3 ± 7.6	37.1 ± 4.1	32.2 ± 2.2
60 "	14		50.7 ± 4.7	26.4 ± 5.4	26.4 ± 2.4
127 "	12		37.2 ± 7.2	31.1 ± 6.1	28.0 ± 4.0
170 "	10		61.0 ± 9.8	60.6 ± 9.6	30.7 ± 1.7
24 h	19	Spinal cord	75.8 ± 5.8	57.7 ± 4.7	30.6 ± 1.6
20 days	11		83.0 ± 5.0	68.0 ± 6.0	34.1 ± 1.1
60 "	16		100.6 ± 7.6	61.7 ± 4.7	27.6 ± 0.6
170 "	10		69.1 ± 3.1	66.7 ± 7.7	30.5 ± 3.5

were fixed along with the underlying muscle in 20% neutral formalin. On a freezing microtome sections were cut parallel to the plane of the underlying muscle to a thickness of 30 μ (20-25 sections from each nerve) and impregnated by the Gros–Bielschowsky–Lavrent'ev or Kampos method as modified by Shubin, with counterstaining with hematoxylin-eosin. Some sections were stained by Spielmeyer's method. The connective-tissue scar was fixed in Zenker-formol and stained with azure-eosin or with Heidenhain's azocarmine.

For the quantitative evaluation of the developing neuroma and connective-tissue scar two methods were used: the first consisted of measurement of the size of the neuroma and connective-tissue scar from the outlines traced by means of a drawing apparatus, while the second was based on determination of the density of the neuroma. This was estimated from the number of intersections of the developing nerve fibers with a given straight line of definite length, always pointing in a direction perpendicular to the course of the fibers. In each series of sections 10-20 measurements and drawings were made. The degree of development of the neuroma was expressed by the greatest area on the sections and the mean density of distribution of the fibers in conventional units. All the measurements in the control and experimental series were made on the 18th day after resection. The morphology of the regeneration processes was investigated at the same times. The size and density of the neuroma in the control and experimental series were subject to considerable variation, as a result of which many repetitions of the experiments were necessary.

EXPERIMENTAL RESULTS

Analysis of the histological material from the experiments in which the limb was irradiated showed marked depression of the regenerating power of the nerve if it was divided 24 h and 20, 60, and 127 days after irradiation. The young nerve fibers spread for a very short distance into the connective-tissue scar, whereas in the controls at the same periods they reached the peripheral segment, forming loops with many branches. The degenerative processes in the peripheral and central segments were indistinguishable in development from those in the control series. The process of removal of disintegration products was greatly inhibited. This was clear from the considerable infiltration of the cytoplasm of the phagocytic Schwann cells with myelin droplets. Inhibition of the regenerative processes of the injured nerve after irradiation of the limb directly was demonstrated particularly clearly by quantitative measurements of the developing neuroma and the connective-tissue scar. For example, in the experiments in which the operation was performed 24 h after irradiation, the average size of the neuroma on the 18th day of regeneration was only 31% of the control value, the size of the connective-tissue scar was 33%, and the density of the neuroma was 59% of the control value (see table).

Hence, radiation injury to a nerve, as manifested by the depression of its regenerative power, continues unchanged for a long time. Not until 170 days after irradiation does the regenerative power of the nerve show a tendency to recover.

In all the series of experiments in which the spinal cord of the experimental rats was irradiated, as in the control (unirradiated) animals, microscopic examination of the developing neuroma on the 18th day of regeneration showed that young nerve fibers had spred throughout the connective-tissue scar, and that some of them, entering the Buengner's bands of the peripheral segment, terminated in bulbs of growth of different sizes. In the peripheral segment Wallerian degeneration of the fibers could be clearly seen. In contrast to the experiments in which the limb was locally irradiated, after irradiation of the spinal cord, as also in the control animals, on the 18th day after the operation the processes of removal of the products of disintegration were almost complete, and ovoids were visible in only a few fields. The conclusion that the regenerative processes developed in the same manner in the injured peroneal nerve as in the control experiments was also drawn from a quantitative analysis of the developing neuroma. The differences between the mean values of the density and size of the neuroma in the experimental and control series were within the limits of natural variation (see table).

During the study of the morphological picture of the irradiated spinal cord and spinal ganglia (stained with thionine and toluidine blue by Nissl's method) no gross destructive changes were observed in the nerve cells. When the peroneal nerve was injured mechanically 24 h and 20, 60, and 170 days after irradiation, isolated cells with moderate chromatolysis in their cytoplasm were found in the anterior horns of the spinal cord. In this case the nuclei of the cells were slightly displaced towards the periphery. In the cells of the spinal ganglia also signs of primary irritation were observed by Nissl's method. Evidently these changes were not due to irradiation, but to the infliction of mechanical injury to the peripheral nerve, and they were the primary signs of the retrograde reaction usually observed following operative trauma to a peripheral nerve.

The absence of marked morphological changes in the nerve cells of the irradiated spinal cord and spinal ganglia, and the fact that in these experiments no inhibition of regenerative processes was observed in the divided peripheral nerve, cast doubt on the suggestion that after local irradiation of a nerve in a dose of 3000 R the depression of regenerative capacity is due to injury to the axons. Depression of neuroma formation can be more probably attributed to depression of the regenerative power of the auxiliary tissue elements of the nerve trunk, the connective tissue and the Schwann syncytium. Injury to the Schwann cells is directly revealed by the depression of their phagocytic activity following irradiation of the nerve, as mentioned above.

Injury to the connective-tissue components in the developing scar at the site of injury may be judged from the results of supplementary experiments in which double resection of the peroneal nerve was carried out in the limbs of animals 24 h after irradiation in a dose of 3000 R. The first resection was performed at the usual place. The second resection of the same nerve was carried out 1 cm proximally to the first, in the region of the third trochanter. This operation made it possible to exclude growth of young nerve fibers in the region of the distal resection.

Morphological and quantitative analysis of the regenerating nerve revealed a marked depression of the development of this process. The scar was reduced in size by an average of 58% on the 18th day under the influence of irradiation. Hence, the proliferation of connective tissue, of which the regenerating nerve consisted almost entirely, was depressed by the action of radiation.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
