DEGENERATION AND REGENERATION OF SYNAPSES AFTER DAMAGE BY PENETRATING RADIATION

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A study of degeneration and regeneration at synapses on neurones due to irradiation extends our knowledge of the radiation sensitivity of the nervous system and presents a completely unexplored aspect of the pericellular apparatus. Our previous observations on argentophil synapses after irradiation made it possible to follow their subsequent structural changes.

METHOD

The experiments were carried out on two groups of animals. The first consisted of 15 cats in which a study of degeneration of the synapses was made, and the second of 46 cats for a study of their regeneration. The operation was performed three days after irradiation with 30, 80, 100, or 500 r. The irradiation was obtained from a RUM -11 apparatus operating at a voltage of 140 kv and a current of 15 ma, with the animal at 45 cm from the target electrode. The operation consisted of dividing the sympathetic truck in the neck 1 cm caudad to the superior cervical ganglion, and in animals used for a study of degeneration we removed a 1 - 2 cm length of nerve, to prevent regeneration. In the second case, the cut was made with a shapr razor or scalpel, the ends of the nerve were brought to within 1 - 2 mm of each other, and the wound sewn up in layers. The operation was always performed on the right side, and the left side served as control. The right and left ganglion were fixed at the same time and treated by the method of Golgi-Deinek, or by that of Bielschowsky-Gros. Because the process of regeneration and the closely associated degenerative process depend on the age, nutrition and living conditions of the animals, we standardized these conditions as far as possible. As a control over the animal's general condition, before operation and at various times afterwards we made a blood analysis. In all cases we determined precisely the level at which the sympathetic trunk had been divided, and the position of the cut ends. For this purpose we impregnated the region of the scar, and from sections determined the positions of the central and peripheral ends. We studied the regeneration of the synapses in 12 unirradiated cats kept together with the experimental group.

EXPERIMENTAL RESULTS

From our own observations [1, 2], and from published reports [3, 6], we formed the opinion that degeneration of the synapses after division of preganglionic fibers takes place as follows: after 15 - 17 hours, an argentophilia of the synaptic terminations occurs, after 24 hours they swell and so increase their size, after which vacuolization and fragmentation occurs, and finally, after 4 - 5 days, there is a granular disintegration.

Previously we had studied the condition of the synaptic apparatus of the superior cervical ganglion of the cat after a single exposure to 30, 100, or 500 r. We observed an argentophilia of most of the synapses, which occurred 7 - 9 days after irradiation according to the dose. After 1 - 1½ months, these changes disappeared, and the synaptic apparatus resumed its normal appearance.

After division of the sympathetic trunk and irradiation, the degeneration of the synapses differed from the corresponding process in the controls. Thus, in animals irradiated with a dose of 30 r, all the degenerative stages occurred considerably earlier than in the control group. The argentophilia was observed as early as 8 hours after the operation (4 days after irradiation), and 24 hours after the operation most of the synapses were grossly swollen and misshapen. The degenerative changes progressed rapidly, and by the third day granular disintegration of the synaptic terminations was complete. There was a striking increase in the number of satellites around the ganglion

cells. On the third day after the operation there was a mild leucocytosis, with 19,000 leucocytes per mm^3 instead of the normal 16,000.

In animals irradiated with 100 r, we observed no changes in the times of degeneration as compared with the control group. We found only that in the former the synapses were impregnated more intensely.

Further increase of the radiation dose led to a marked retardation of the degeneration of the synapses. In cats irradiated with 500 r we were frequently able to find synapses, even as late as the 9th day after the division of the sympathetic trunk (Fig. 1). It is true that the synaptic endings were clearly abnormal and many of them were at a stage of breakdown, but, as was pointed out above, in the nonirradiated animals, the pericellular apparatus had completely broken down by the 4 - 5th day after division of the sympathetic trunk. The number of Schwann cells remained unchanged. In the blood there was a leucopenia, with a white cell count of 14,000 per mm^3.

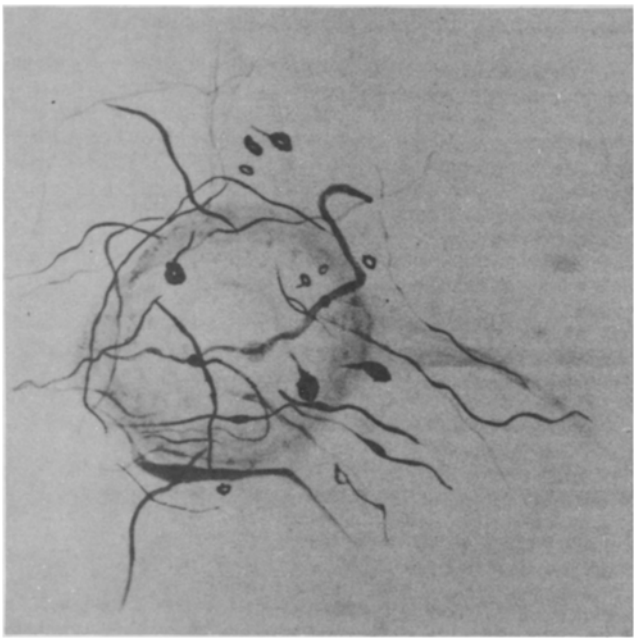

Fig. 1.. Degeneration of synapses on a neurone of the superior cervical sympathetic ganglion of a cat 9 days after section of the sympathetic trunk in the neck. Irradiation 500 r. Golgi-Deinek impregnation. MBI-3, objective 100, ocular 7X.

Besides changes in the times of degeneration of the synapses, there was also a change in their staining properties. The degenerating terminations stained completely black with silver. This was evidently the result of the combined action of the two types of damage—irradiation and section.

Turning now to study regeneration of the damaged nerve fibers, we had in mind that their recovery depended to a large extent on the condition of the scar tissue.

Therefore, in all cases, studies were made of the scars, which were impregnated by the method of Bielschowsky-Gros, and counter-stained with Mallory. On such preparations we could clearly see collagenous fibers, cords of Schwann cells, and blood vessels. It was found that with the greater doses of radiation, the scar was less dense, and also smaller. This effect was particularly clearly shown when a comparison was made between the scars of the control animals and those of rats exposed to a radiation dose of 500 r. It follows that the scar may exert either more or less resistance to growing axons.

In the first set of experiments in which the radiation given was 30 r, the first regenerating preganglionic fibers were found in the scar on the 5th day after the operation. Fine unmedullated fibers passed through the scar within the cords of Schwann cells, giving off a large number of collaterals on the way. Some of the branches ran parallel with the main fiber, often rejoining it, while others left the cord, and grew out into the surrounding connective tissue. All these fibers pursued a tortuous course and terminated in rings of flask-shaped endings. Ultimately, the collaterals degenerated, and along the outgrowing preganglionic fibers, many dozen of isolated terminal structures could be seen.

In the second set of experiments with irradiation of 100 r, the growing neurites in the scar were seen on the 7th day after the operation. In the 3rd set, with irradiation of 500 r, this effect was not observed until the 11th day. There was then a marked reduction in the number of collaterals from the outgrowing fibers.

In the caudal part of the ganglion, regenerating fibers were found in the first set of experiments two weeks after the operation. In the second and third sets of experiments, the time was extended to three and to four weeks. The growing axons could clearly be distinguished from the outgrowths of the ganglion cells by their marked argentophilia and by slight swellings at a small distance from each other. Equally characteristic was the occurrence of 4 - 5 fibers in a bundle. Having entered a ganglion, the regenerating axons began to divide, and the fibers formed which had growth cones on their ends gradually approached neurones of the ganglion and made contact with them.

The growth cones of the preganglionic fibers varied in shape, but usually took the form of reticular swellings or plaques. Their diameter varied from 6 to 14μ. In the first set of experiments, such contacts were observed 17 days after the operation, in the second set after 22 days, and in the third after 29 days.

Thus, after small doses of radiation (30 r), recovery of the synapses was accelerated from a time of 20 days in the unirradiated group to 17 days in the animals receiving irradiation. The moderate dose of 100 r slightly slowed regeneration, and large doses of 500 r depressed it considerably. Possibly, the accelerated regeneration in the first

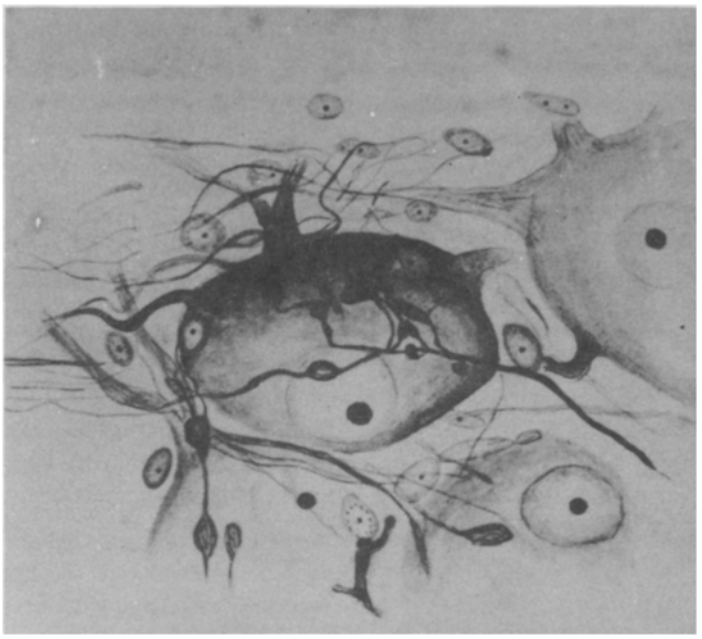

Fig. 2. Regenerating synapses on a neurone from a ganglion, 36 days after the operation. Irradiation 100 r. Impregnation Golgi-Deinek. MBI-3, objective 100 ×, ocular 7 ×.

group of experiments was due to a suppression of mitotic activity of the connective tissue elements of the scar, or to the stimulating influence of small doses of radiation of the nervous system. This problem is not yet solved. However, it is known medical pratice, that small doses of irradiation may be used to accelerate regeneration of peripheral nerves after damage.

In the first group of animals after 24 days, and in the other groups after 35 - 40 days, more complex types of contact were observed (Fig. 2.). It can be seen that numerous mushroom-like outgrowths have budded off from the primary synaptic plaques, and they have established many new contacts with the neurones. The separate presynaptic threads have grown out strongly and have shrunk, and the internal reticular structure of most of them can no longer be made out.

After 2½ - 3 months, the pericellular apparatuses revealed no longer differ essentially from the synapses of the control group. We could also observe that the synapses were rather more numerous than usual in the intercellular spaces, and some of them were not quite the normal shape (Fig. 3).

When comparing regneration of the synapses in the nonirradiated and irradiated group, we found that in the former the regenerating fibers underwent but little morphological change after they had made contact with neurones, whereas in the irradiated animals considerable alteration followed this first contact. The coarse enlarged synaptic endings gradually formed slender synaptic rings, or reticular flask-shaped endings.

As the preganglionic fibers grew out into the ganglion and synaptic connections were restored, impaired function recovered. Tests for recovery were: 1) the position of the nictitating membrane, and 2) pupil size. The nictitating membrane, which was paralyzed after division of the sympathetic trunk, gradually contracted, and came to occupy its normal position, and the pupil, which was initially contracted, expanded to normal size. Recovery of function ran parallel with the recovery of the synaptic connections, and was complete when the synapses had finally regenerated.

SUMMARY

Cats were irradiated with a single dose of 30, 100, or 500 r of x-rays; the sympathetic trunk was divided in the neck, and a study made of degeneration and regeneration of synapses in the superior cervical sympathetic ganglion. After small doses, both degeneration and regeneration were accelerated, but were delayed after large doses. The synapses which were the first to recover underwent considerable structural changes subsequently, and became small and delicate. After large doses of x-rays, the scar at the site of the nerve section became considerably smaller and less dense.

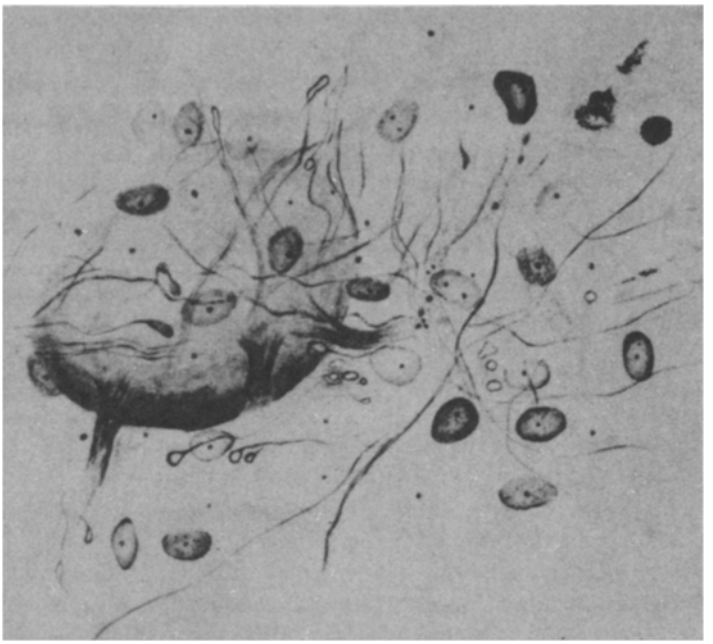

Fig. 3. Restoration of a synapse 80 days after division of the preganglionic fibers. Irradiation 500 r. Impregnation Bielschowsky-Gros. MBI-3, objective 100 x, ocular 10 x.

LITERATURE CITED

1. V. P. Babmindra, The Connection between the Superior Cervical Sympathetic Ganglion and the Central Nervous System. Candidates dissertation. Leningrad (1957).
2. V. P. Babmindra, Izv. AN SSSR. Seriya biol., No. 4, (1960), p. 505.
3. B. J. Lawrentje, Z. mikr-anat., Forsch., Bd. 35, S. 78 (1934).
4. F. Gastro, Trab. Lab. Invest. biol. Unw. Madr., Vol. 26 (1939), p. 357.
5. W. Gibson, J. Neurophysiol., Vol. 3 (1940), p.237.
