ON THE CAUSES OF REGENERATION ABILITY LOSS IN THE AXOLOTL EXTREMITY AFTER X-RAYING

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That the axolotl or triton extremity loses its ability to regenerate after exposure to large doses of roentgen rays is known. After the irradiated organ has been amputated, the wounded surface of the skin closes, and the morphogenetic process normally present, which leads to the rebuilding of the lost structures of the organ, is gone. Biological interpretation of the processes occurring in the irradiated organ has been recently done by G. S. Strelin and his colleagues [5, 6], and also by other authors.

However, in none of these hypotheses as to how the regeneration ability is lost by irradiated tissues is there any clear explanation of the reasons for this phenomenon [1, 8, 9, 11].

Such a situation is explained by the difficulties of studying the irradiated organ.

With the doses of radiation customarily used to suppress the regeneration ability of the axolotl or triton extremity (5,000-10,000 r), the morphological picture of the reaction to irradiation is rather indefinite. In some cases, the reaction was observed from the standpoint of the cutaneous sheath: due to the partial destruction of pigment, the skin became a whitish shade. Sometimes the reaction was observed from the standpoint of the digits: resorption of their tips occurred. Essential morphological changes could not be found by the usual methods of study (fixation by Bouin's fluid, staining according to Van Giesen, Mallory, or with hematoxylin-eosin) even in the internal tissues of the extremity.

Thus, histological study of the irradiated limb gives no reason to connect the loss of the regeneration ability with any of the visible morphological changes.

One must note that the sensory and motor reactions were completely retained after irradiation. One can only judge whether the irradiated extremity has lost its regeneration ability after the latent period (3-4 weeks) and subsequent amputation.

The absence of important morphological changes in the tissues of the irradiated extremity, the preservation of the sensory and motor reactions, the presence of a latent period – all these complicate the explanation of the reasons for the loss of the regenerative ability.

In our study, we decided to analyze the role of the nervous system in the process of tissue loss of regenerative ability after irradiation.

There are reports in the literature that, normally, the nervous system plays an important role in the differentiation of the regenerated axolotl limb and in the sequence of formation of the organs's structures [4, 7, 10, 12, 13]. There is also data to indicate that in a denervated axolotl extremity, which has been irradiated by roentgen rays, restoration of the motor and sensory functions occurs more slowly than in nonirradiated extremities [2].

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Retarded restoration of function in the denervated axolotl extremity was observed not only when the actual extremity was irradiated, but when the portion of the spinal cord from which the nerves innervating the extremity originate was irradiated. The same irradiation of another portion of the spinal cord (for example, the lumbar section, when an anterior extremity has been denervated) did not retard functional restoration of the extremity [3].

To explain to what extent the loss of the regenerative ability in the irradiated portion is connected with changes in the nervous system, one must examine the condition of all parts of the nerve course. The changes caused by irradiation can affect the structural features of the peripheral nerve endings or can even impinge on the central nervous system.

EXPERIMENTAL METHODS AND RESULTS

To examine the condition of the nerve endings in the irradiated organ, we used the stump of an axolotl extremity which did not regenerate after amputation. The irradiated extremity (radiation dose, 5,000 r) was amputated a month after the action of the radiation dose. After 4 months, the amputated extremity showed no signs of regeneration. Only at the most distal end of the stump was a small cutaneous growth of from 2 to 3 mm in size formed. The stump was fixed in 12% neutral formalin. The method of E. I. Rasskazova (a modification of the Bielschowsky method) was used to impregnate the nerve fibers. We were not able to detect visible morphological changes in the nerve fibers innervating the striated muscles, the connective tissue, and the skin of the stump. However, in the distal portion of the stump, growth of individual nerve fibers was observed, so strong that the nerve fibrils enveloped the nonregeneration ends of the muscle fibers in an unbroken net. The individual nerve fibers permeated the entire cutaneous protuberance which had formed on the wounded surface (Fig. 1, a, b).

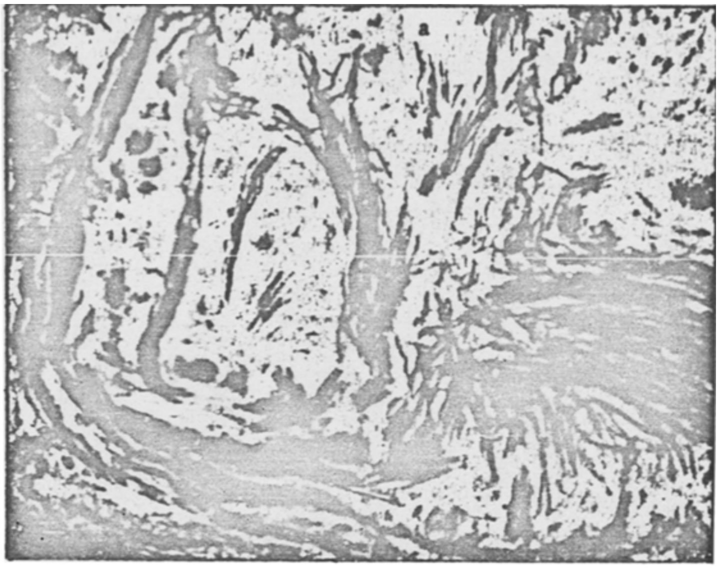
The results of the experiments led to the notion that roentgen rays in the dose we used do not suppress the growth ability of nerve fibers. However, one could propose that the vigorous growth of the nerve fibers in the distal portion of the stump was pathological, representing a response to the action of radiant energy. The absence of visible morphological changes in the actual fibers still is not sufficient reason to conclude that the response is absent from the nervous system.

In our experiments, both the receptors and the effectors could be damaged as a result of irradiation, i.e., the whole terminal nervous apparatus of the extremity. In both cases, the normal nervous connections with the the center were changed. Allowing for such a possibility, we decided to denervate the irradiated extremity by sectioning the nerves of the lumbo-sacral plexus, in order to cause atrophy of the irradiated terminal nerve apparatus. Due to the regeneration of the nonirradiated nerve, the terminal nerve endings also must be restored. Thus, we could, so to speak, restore the peripheral section of the extremity's nervous system, replacing the irradiated peripheral nerve fibers and the terminal apparatus with nonirradiated regenerated ones.

If the loss of the regeneration ability in the irradiated portion is only a consequence of the injury to the peripheral nerve fibers and to the terminal apparatus, then the regeneration of new nerve fibers should restore this ability. In other words, when the irradiated portion of the limb is amputated after the regeneration of the nerve fibers, regeneration may be hoped for. Cutting through the nerve trunks produced somewhat higher branching places in the 16th, 17th and 18th spinal cord nerves (Fig. 2).

Denervation was carried out on 12 axolotls. The right irradiated extremity was denervated; the left irradiated extremity was used as the control (5,000 r dose). Irradiation was done two months before denervation. The denervated extremity lost the ability of independent movement and sensibility. It hung, like a whip-lash, and did not help the animal to swim. When the denervated extremity was pricked with the sharp end of pincers, the axolotl did not withdraw it, whereas the control extremity, when pricked, responded quickly to the irritation.

Restoration of motor and sensory fibers in the denervated organ proceeded rather slowly. The appearance of a sensory and motor reaction in the denervated irradiated extremities was observed only by the 50-70th day. In 4 axolotls, disintegration of the irradiated extremity tissues occurred due to denervation, and these animals were consequently excluded from the experiment. In the remaining 8 axolotls, when motion and sensibility were restored to the denervated extremity, simultaneous amputation of the experimental and control extremities was done. During a period of four months after the amputation (observation period), regeneration of the amputated extremities did not occur.



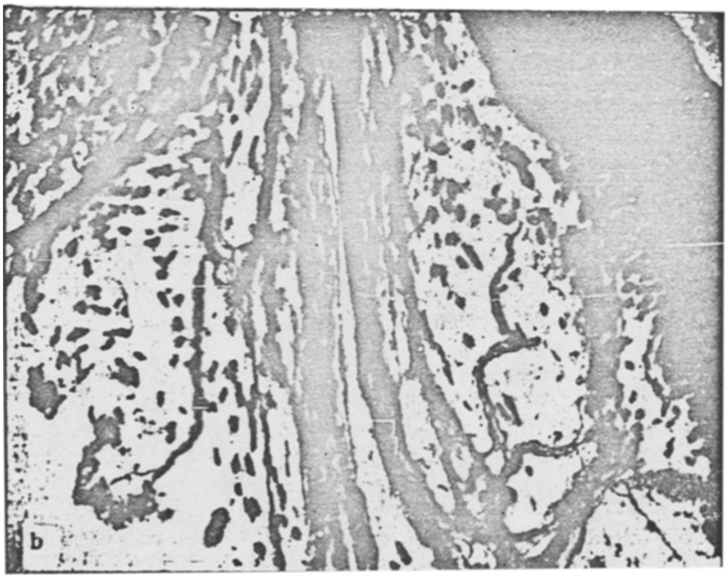


Fig. 1. Growth of nerve fibers in the stump of the irradiated extremity of an axolotl.
a) in the distal portion of the stump muscles; b) in the skin protuberance.

One could propose that not only the peripheral nervous system suffers from irradiation, but other higher orders of nervous connections, that provide for regeneration, as well (the spinal cord, the nerve ganglia). To prove this proposal, we set up the following experiment. The right rear extremity in 10 axolotls was denervated before irradiation. This, to a considerable extent, excluded the possibility of direct transmission of strong stimulation (in the case of irradiation) to the central nervous system. The left rear extremity was not denervated, and was used as the control.

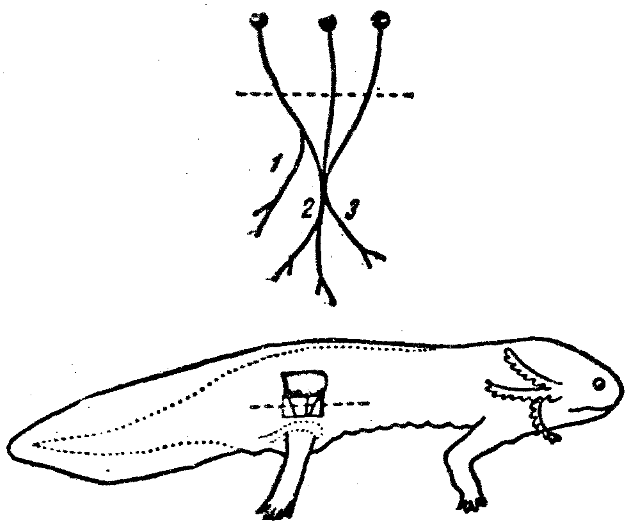


Fig. 2. Plan of nerve sections in the lumbo-sacral plexus of the axolotl. 1) n. cruralis anterior; 2) n. ischiadicus; 3) n. fibularis. The sectioning line is shown by the dots.

A week after denervation, both rear extremities of the axolotls were irradiated (5,000 r). Six days after irradiation, signs of tissue destruction in the denervated extremity began to appear in two of the axolotls.

In the remaining axolotls, restoration of the sensory and motor reactions of the right extremity occurred, just as in the preceding experiment, 50-70 days after denervation.

After this, both rear extremities of the axolotls were amputated at the distal third of the femur. In 12-14 days, epithelization of the wounded surfaces was noted on both extremities of all the axolotls. Organotypical regeneration was not observed in any of the axolotls during a period of three months after the amputation.

One could propose that, because of the way this experiment was conducted, we could not fully exclude the transmission of the stimulation, which occurred as a result of irradiation, to the central nervous system. Consequently, the regeneration ability was not restored, in spite of the sectioning of the nerves of the lumbosacral plexus before irradiation.

It is also possible that the loss of the regeneration ability is not connected with the disruption of nervous connections, but with some other changes of a different sort.

SUMMARY

It was demonstrated that the amputation of an irradiated organ does not bring about its regeneration in the case of the denervation of the axolotl extremity x-rayed with the dose of 5000 r.

Under the conditions of provisional denervation of the intact extremity followed by irradiation, and amputation after innervation restitution, no regeneration was found. The loss of regeneration ability in the axolotl extremity is, apparently, unconnected with the change of peripheral nerve fibers in the stump.

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