MORPHOLOGY AND PATHOMORPHOLOGY

THE EFFECT OF ACTH ON THE RESTORATION OF MYONEURAL JUNCTIONS UNDER CONDITIONS OF REPARATIVE REGENERATION

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Investigation of the compensatory-adaptive and restorative reactions in myoneural synapses upon a change of the internal environment of an organism is of interest in that this problem is inadequately studied. The plastic properties of tissues and the possibility of their adaptation to changes in metabolism of the environment is most fully revealed under conditions of the formation and differentiation of tissue elements. The expediency of investigating the effect of ACTH on the development and formation of motor-nerve endings under conditions of reparative regeneration is also determined by the needs of practical medicine in connection with the use of this hormone in surgery and neuropathology [1-4, 6, 10, 11].

In the present work an attempt has been made for the first time to given a quantitative evaluation of the relationship of the muscular and connective-tissue components of regenerated tissue in order to analyze the data on the distribution of newly formed myoneural synapses in the traumatic area.

METHOD

The experiments were carried out on 60 male rabbits weighing 600-800 g. The object under investigation was the anterior tibial muscle of both hind legs. Standard damage of the muscle was caused in all animals under rigorously aseptic conditions: a hole 5 mm in diameter was cut out by a special round punch in the center of the muscle. The cutaneous edges of the wound were sutured. One group of rabbits (experimental) was injected intramuscularly with ACTH from the first day of the operation until the end of the experiment. In order to avoid the adverse effect of the hormone on the nitrogen equilibrium in the organism [5,8], small doses from 3 to 6 units were used. The animals of the other group (control) were injected with a physiological salt solution. The rabbits were killed at various period (4-30 days) after the operation by injecting air into the vein of the ear. The muscles of the right leg were fixed with 12% neutral formalin and the motor-nerve endings were elicited by the Bielschowsky-Gros-Lavrent'ev silver-staining technique. Infected material was not included in the experiments. The method of counting the myoneural junctions in the region of the regenerated tissue was as follows: each section was studied through an ocular with a square iris diaphragm; using the movable stage we examined the entire zone of restoration (in one preparation we investigated from 80 to 100 fields). The arrangement of the motor end-plates detected in a square of the field was plotted on a special grid on paper in conformity with the histotopography of the investigated area of the regenerated tissue. The data obtained in the microscopic study of the individual sections were subsequently transferred to one general grid and a map of the regenerated tissue was compiled with an indication of the number of newly formed myoneural junctions and their arrangement in the zone of restoration. The muscles of the left leg were fixed with Zenker formol; paraffin-embedded sections were stained with hematoxylin-eosine, Heidenhain's iron hematoxylin, and by Mallory's method. Outline sketches of the connective-tissue areas of the regenerated tissue on the 30th day postoperation were made by means of a drawing apparatus with a total enlargement of $42\frac{1}{2}$ times from preparations stained by Mallory's method. On the drawings we also marked the boundary of the "old" and "young" muscle fibers, i.e., the outline proper of the entire regenerated tissue. The areas of the connective tissue indicating its maximal development in the region of healing were measured for each rabbit by means of a planimeter. The adrenals were fixed with Becker's fluid and stained with Sudan III by Daddi's method.

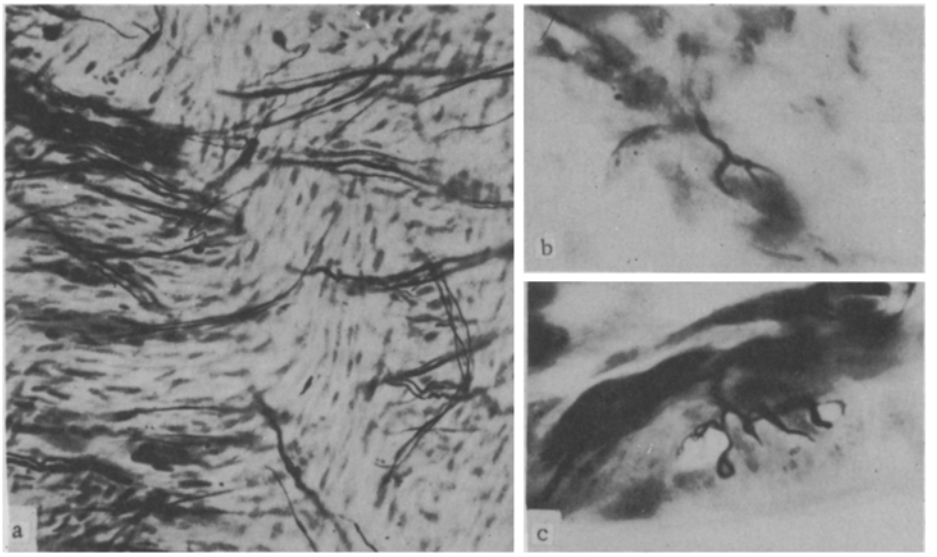

Fig. 1. Development of nerve fibers and motor end-plates in the traumatic area of the muscle. a) Experiment, 15th day postoperation. Development of nerve fibers in area of wound. Objective 20, ocular 7; b) control, 30th day postoperation. A developing motor end-plate. Objective 90, ocular 7; c) experiment, 30th day postoperation. Well differentiated newly formed motor end-plate. Objective 90, ocular 7. Staining of the preparation by the Bielschowsky-Gros-Lavrent'ev method.

RESULTS

The observation of the condition of the control and experimental rabbits showed that the injection of small doses of ACTH during the entire postoperation period did not impede an increase in the weight of the animals and did not lead to an increase in the number of infected wounds. It was found upon studying the histological structure of the adrenal cortex that under the effect of ACTH sudanophilia of the zona fasciculata and the zona reticularis noticeably diminished (a regular reaction to the injection of the hormone); at the same time the sudanophilia of the zona glomerulosa increased in connection with the enhanced production of lipoids.

In the histological investigation of the muscle on the 4-7th day postoperation we could note that both in the control and in the experiment all nerve elements damaged by the wound underwent various pathological changes. The most gross destructive processes were observed in the small intramuscular nerve trunk distad from the region of the wound: their nerve fibers degenerated, and the decomposition products of the axons were resorbed. An appreciable portion of the nerve elements situated proximad from the wound remained viable; reactive changes in them were expressed primarily by higher argyrophilia of the axons and by the appearance of varicose enlargements along their course.

On the 7th day after the operation we could clearly note, in all animals, the growth of young axons from the proximal ends of the transsected nerve trunks; the newly formed nerve twigs grew along the Schwann syncytium. At the terminal branches of the axons it was possible to detect primitive endings in the form of rings and buttons situated among the cells of the granulation tissue or close to the young muscle element—myosymplasts. On the 15th day the nerve fibers of the control and experimental rabbits grew into the wound zone for an appreciable distance (Fig. 1, a). In the peripheral areas of the zone we could see at this period numerous muscle tubules and young muscle fibers; the central part of the wound was filled with connective tissue.

On the 20th day after the operation the newly formed nerve fibers began to gather into bundles: they reached the distal end of the wound and grew into the glial scar which formed by proliferation of the cords of Schwann cells from the peripheral segments of the nerve trunks. In the experimental animals an orientation of the Schwann and muscle nuclei was noted in the peripheral areas of the regenerated tissue where there were more differentiated young muscle fibers (in the region of the dichotomous branches of the axons on the ends of the terminals).

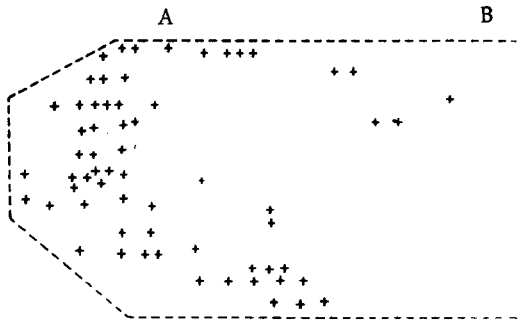

Fig. 2. Diagram of the arrangement of newly formed motor end-plates in the zone of regenerated tissue. Experiment, 30th day postoperation. Rabbit No. 11. A) Proximal area of wound; B) distal area of wound.

On the 30th day processes of myelinization of the axons were clearly evident in all operated rabbits, whereas in the control animals the formation of motor endings was just beginning (Fig. 1, b). At this period in the experiment it was possible to see well-differentiated motor end-plates (Fig. 1,c) in which arborization of the terminals is quite evident, and the nucleosarcoplasmic base of the plate is formed. Not more than 2-3 primitive motor endings were counted in the region of the regenerated tissue of the control animals,* whereas in the regenerated tissue of the experimental rabbits there were 8, 16, 62, and 13 motor endings. The newly formed motor endings were situated primarily in the proximal and lateral areas of the regeneration zone (Fig. 2). About 40% of all motor endings contained not less than 5 terminals and on the average 7 nuclei each in the base.

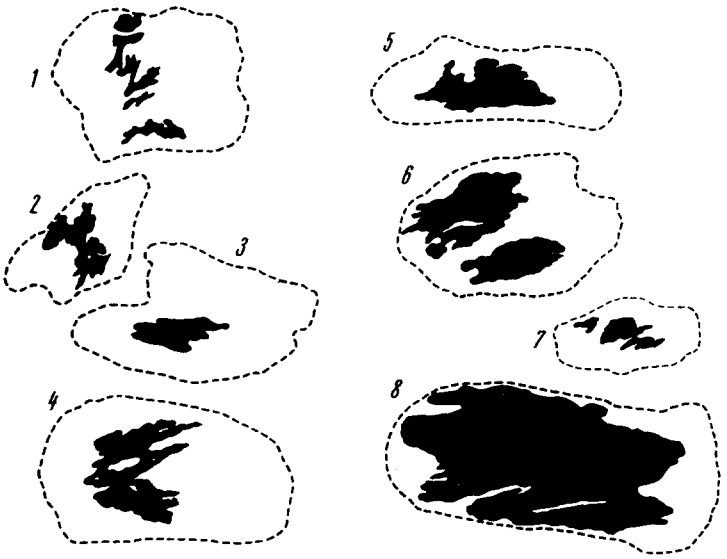

Fig. 3. Outline sketches of the area of the regenerated tissue. Connective-tissue zone;---boundary of the "old" and "young" muscle fibers. 30th day postoperation. 1, 2, 3, 4,—Experiment (rabbits Nos. 9, 10, 11, 12); 5, 6, 7, 8—control (rabbits Nos. 13, 14, 15, 16).

An analysis of the data on the relationship of muscle and connective-tissue components in the regenerated tissue of the control and experimental rabbits showed the following: in the animals which received ACTH we did not observe a significant proliferation of connective tissue within the limits of the regenerated tissue, which substantially changes the relationship in behalf of the muscle components (Fig. 3, see Table).

Thus, by the start of the formation of motor end-plates a difference was elicited in the degree of differentiation of the myoneural synapses in the experiment and in the control. In the rabbits which received ACTH numerous motor end-plates had developed by the 30th day postoperation in the regenerated tissue which were more differen-

*In one of the control rabbits no newly formed motor endings were discovered. In the proximal portions of the anterior tibial muscle of this rabbit the normal tissue structure of the muscle fibers and nerve elements was retained, which precludes the supposition of the possibility of denervation of the muscle at the time of operation.

Results of Measuring the Areas of the Connective Tissue Reflecting its Maximal Development in the Region of the Regenerated Tissue (30th day postoperation)

No. of rabbit (control)	Area † (in cm^2)	No. of rabbit (experimental)	Area† (in cm^2)
13	20.4	9	11.2
14	40.5	10	9.27
15	5.8	11	8.6
16*	147.7	12	23.4
M ± m‡	22.2 ± 6.7	M ± m	13.0 ± 2.3‡

*Rabbit No. 16 was not included in the calculation of the arithmetic mean.

†Area of the outline sketch is 1806- $(42.5)^2$ times greater than the corresponding area of the preparation.

‡m-probable error.

tiated than the newly formed motor end-plates of the control animals. Taking into account that the rate of differentiation of motor end-plates is determined to a certain extent by the morphologic maturity of young myons, we can consider the rapid formation of motor end-plates as due to healing in the experimental rabbits in relation to the predominant development of the muscle areas of the regenerated tissue. We cannot preclude the possibility of the manifestation of the specific action of ACTH [7, 9, 10].

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