ON THE REGENERATION OF THE NEURONS OF THE INTRAMURAL PLEXI OF MAMMALS

N. M. Shestopalova

From the Laboratory of Growth and Development (Head – Prof. M. A. Vorontsova) of the Institute of Experimental Biology (Director – Prof. I. N. Maisky) of the Academy of Medical Sciences of the USSR, Moscow

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The problem of the development and regeneration of the neurons of adult mammals is extremely complicated and very difficult. It was considered established that neurons, once developed, function throughout the life of the organism, without being replaced by new ones when old or injured.

Most authors explain the inability to replenish neurons by a complicated morphological and functional differentiation and by the absence of a cambial source in nervous tissue [14, 3].

Some authors concede the possibility of the existence of cambial cells in nervous tissue [8, 11]. Due to development of these cells during the postnatal period, dying neurons of the adult organism are replaced. The amount of cambial cells depends on the age, the area of the nervous system, and on the systemic condition of the animal.

The widely known ability of epithelial and connective tissue to regenerate was explained by their more ancient origin and by their uncomplicated morphological and functional differentiation. But the tissues which appeared at later stages of phylogenetic development were considered incapable of regeneration. Included among these were muscular (smooth and somatic) and nervous tissues.

However data are available indicating the inapplicability of this concept. It is known that the smooth muscles of the blood vessels [4], uterus [19], and intestines [15, 4] are able to regenerate. Their regeneration occurs by amitotic and karyokinetic division of the smooth muscle cells and myoblasts. Equally able to regenerate [12] is skeletal muscle. The removal of a muscle under certain conditions is accompanied by its regeneration.

The ability of the spinal cord to regenerate, i.e., the nervous tissue, during regeneration of the tail in lower vertebrates, has been described [17, 2, 6]. Differentiation of neuroblasts and development of ganglions take place in the embryonic brain tissue of birds when cultured outside of the organism [5]. The ability of the peripheral nerve process and endplates ro regenerate, as well as the neogenesis and growth of new processes of neurons, is widely known in the higher vertebrates [1, 18, etc].

In the present work an attempt was made to discover what happens to the neurons of the intramural plexi of the intestinal tract when the organism is injured [1, 18, etc.].

EXPERIMENTAL METHOD

The work was carried out on 120 white rats. The operation was carried out by the method worked out earlier [15]. The operated area was carefully marked by surgical silk in the form of knots on the four sides of the wound. The material was fixed immediately after the operation, 3, 24, 48, 60 hours later, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 20, 23, 27, 28, 30, 42, 54, 63, 68 and 70 days after infliction of the wound. As fixatives were used 12% neutral formalin and Zenker's fixing fluid with added formalin. The impregnation was

carried out according to Buke and Bielschowsky-Gross with subsequent gold staining. The series of paraffin sections was stained with toluidine blue in order to show the tigroid substance, hematoxylin with additional staining with picrofuchsin, eosin blue, and according to Feulgen.

Two series of investigations were carried out. In the first series, a penetrating wound with an incision $4 \times 3\ mm^2$ was inflicted on the animal, in the second an area of the intestinal wall $4 \times 7\ mm^2$ in area was crushed to the state of a thin transparent film (by compressing it many times with Pean's forceps).

EXPERIMENTAL RESULTS

Study of the operated area and the zone adjacent to it showed that liquidation of the wound defect occurred in 10-15 days. At the same time, completere-establishment of the removed portion of the intestinal wall took place. The external surface of the operated area became smooth at the end of the second week and was covered with serosa. Villi were seen on the inner surface of the mucous membrane when examined with a stereoscopic magnifying glass in reflected light. They were located throughout the entire operated area and differed only in the center from the surrounding ones by their lesser height. The defect in the muscular coat was filled in by regenerating smooth muscle tissue. Regenerating intramural plexi were noted in the newly-formed smooth muscle tissue in the injured area.

The regeneration of all portions of the intestinal wall took place by means of regeneration of the tissues from the edges of the wound.

When a penetrating wound was inflicted, the edges of the wound remained separate during the first days. The space between the edges was filled with effused blood. In addition to fibrin and blood elements, there are destroyed tissues in the wound. Later, the fibrin undergoes organization, the remains of the destroyed tissues become necrotic and undergo resorbtion in the process of the developing inflammation. The inflammatory process spreads to tissues adjacent to the wound also. Degenerative phenomena are observed in the tissue cells of the border zone. The connective tissue surrounding the nodes of the intramural plexi becomes separate fibers. In part of the ganglion cells of the nodes is observed wrinkling of the cell body, vacuolization of the protoplasm, argentophilia increases, staining with toluidine blue by Nissl's method increases. The nuclear membrane becomes more sharply and coarsely evident. The fine structure of the nucleus is destroyed. Dark lumpy nuclei appear when stained according to Feulgen. Degenerative phenomena in the ganglions of the zone surrounding the wounded area are observed during the first week, individual cases are found at later dates as well. Simultaneously with the degenerative processes in the uninjured tissues and in those not involved in the inflammatory process, reactive changes arise: the cellular elements increase in size, the ability to stain with basic dyes appears or this ability increases, the ability to proliferate appears, the fine structure of the cellular elements changes. Regenerative processes begin at the tissues which changed reactively. Connective tissue and blood-vessels grow. An intensive process of amitotic division of the smooth muscle cells is observed in the muscular coat during the first two days. On the second day, a large amount of mitotic division of the smooth muscular cells and myoblasts is noted. The myoblasts which are formed move from the edges to the center of the wound, thus liquidating the defect in the muscular coat.

Simultaneously with the movement of the regenerating smooth muscle tissue, the regenerative processes develop in all the structural components of the intestinal wall. At the ends of the cut fibers of the nerve plexi 30 hours after the operation growth bulbs are evident. Later growth bulbs appear in the tissues filling the wound. An excess amount of chromatin is noted in the nuclei of the nerve cells of the ganglions in the bordering zone. Frequently nuclei with 2-3 nucleoli occur; oval nuclei and those contracted in the middle occur. The structure of the cell body changes; a light area of neuroplasma appears near the nucleus, the appearance of protoplasmic nodules is observed in the peripheral areas of the cell; a large number of binucleated neurons and cells connected by bridges are present.

On the 7th day of regeneration after injury, in the nodes of the intramural plexi were observed mitotic division of the ganglion cells, various stages of karyokinesis were observed(Fig. 1-3). Later mitosis was observed on the 10th and 11th day also. Neurons appeared between the regenerating muscular layers and in the tissues filling the wound. They were located at first singly, then their number increased and rods of 3-4 cells appeared. Amitotic division was observed in the cells of the rods.

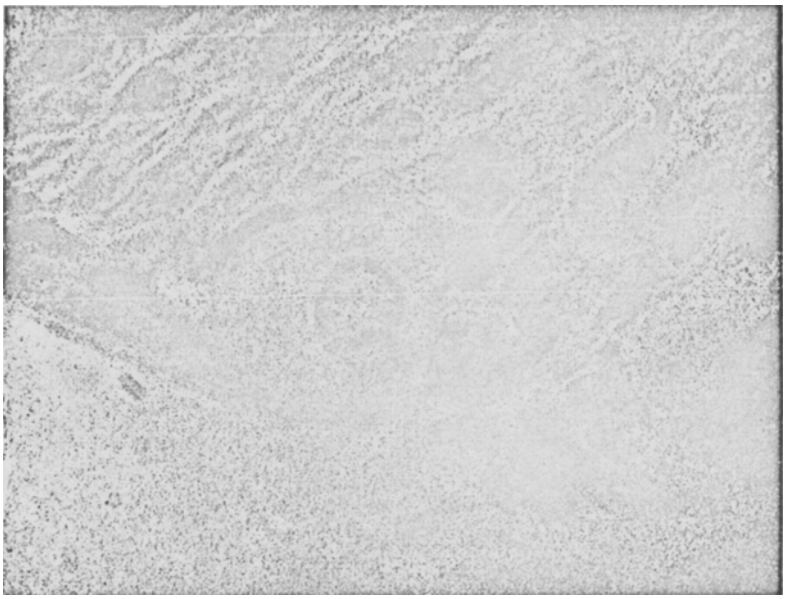

Fig. 1. Ganglion cell of a perivascular plexus. The peripheral location of chromatin in the nucleus can be seen: beginning of mitotic division. Zenker's formol, eosin blue. Microphotograph. Enlargement: ocular 5x, objective 100x.

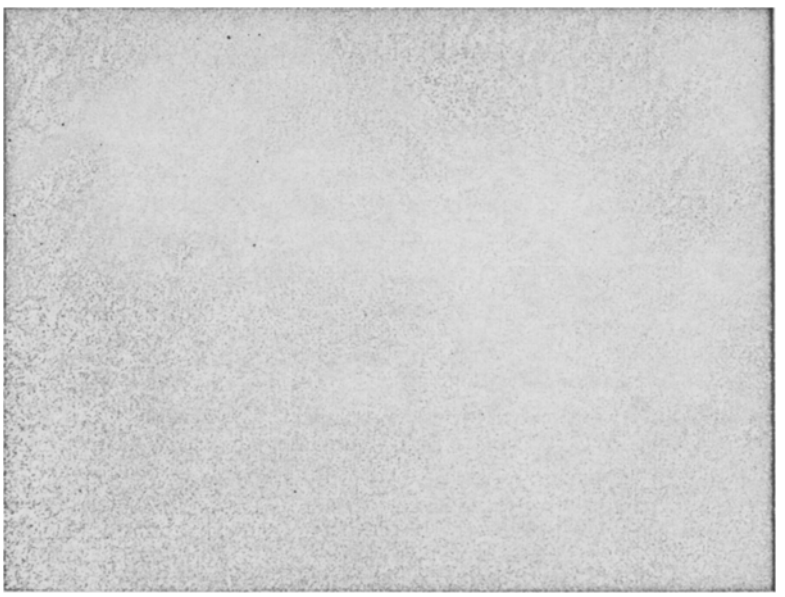

Fig. 2. Ganglion cell from the node of Auerbach's plexus in a state of mitotic division. Prophase, beginning of chromosome orientation in the equatorial plane. Zenker's formol, eosin blue. Microphotoraph. Magnification: ocular 5x, objective 100x.

On the 8th day proliferating nerve fibers and neurons, similar in structure and location to the cells of Meissner's plexus, were observed in the tissues of the wounded area. By the 10th day, rods of 5-6 and more cells were observed in the tissues filling the wound. In transverse sections, their location along the developing muscular layers was seen. Several groups of nerve cells were evident near the regenerating blood vessels of the mucous membrane in stained longitudinal sections through the wounded area. Some of them lay alone, others were interconnected into nerve fibers. On the 20th day, accumulations of nerve cells were found between the newly-formed muscular layers. The nerve cells were joined into ganglions, which were connected by nerve fibers.

Thus, cell division was found in all the regenerating tissues. The time when the dividing cells appeared in the various tissues and their number varied. We observed karyokinetic division of the smooth muscle cells and myoblasts in large numbers beginning on the second day; division of the neurons was noted beginning with the 7th day after injury.

Attention should be paid to the fact that the regeneration of the ganglionic apparatus proceeds simultaneously and parallelly with the regeneration of all the components of the intestinal wall. The external muscular and serous coats regenerate. The regenerated smooth muscle tissue does not have the correct separation into the longitudinal and transverse layers at early stages of development. First a single layer of muscular tissues is formed in which the smooth muscle cells are not oriented. During the regeneration of the smooth muscular tissue, myoblasts appear which have the ability to divide mitotically.

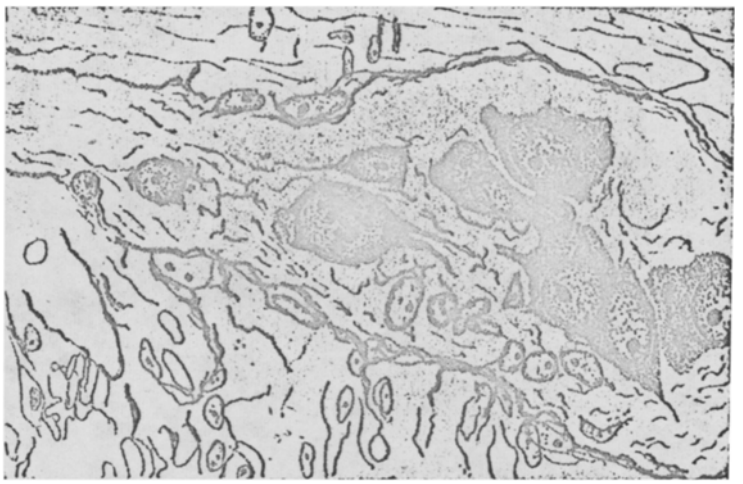

Fig. 3. Ganglion cells of Auerbach's plexus in a state of mitotic division. Metaphase. Beginning of chromosomal movement to the poles. Zenker's formol, toluidine blue. Magnification: ocular 5x, objective 100x (drawn with Abbe's drawing apparatus).

In the process of regeneration of the intestinal wall, regeneration of the mucosa takes place, including regeneration of the normal villi. The regeneration processes of the structural components of the intestinal wall are interrelated and ensured by their simultaneous development as units of a single whole.

Summarizing the results which were obtained, it must be observed that the regeneration processes which take place when a penetrating wound is inflicted and on crushing proceed in a similar way in general. The difference consists of the fact that regeneration of the intestinal wall in some cases takes place a few days earlier. The reason for this, apparently, is that in these cases undestroyed collagen fibers remain sometimes at the place where the defect was made. Apparently, they serve as a channel for the regenerating cells and so speed the entire regenerative process.

Analyzing the results which were obtained and correlating them with the data available in the literature, it should be noted that information has appeared in print during the last few years which indicates the possibility of the multiplication of neurons in adult organisms of higher vertebrates.

V. V. Troitsky, M. V. Rudenskaya [13], L. B. Levinson and M. M. Leikina [9], Z. S. Katsnelson [7], A. F. Nikiforov [10] and others found amitotic division of the neurons of the extramural ganglions and of the brain.

Thus, regeneration of the nodes of Auerbach's and Meissner's plexi takes place after infliction of trauma on the wall of the small intestine of white rats. In the ganglions in the zone surrounding the wounded area reactive changes occur which lead to an increase in the number of neurons. In the edge and regenerated zones, karyokinetic and amitotic division of the neurons is noted. In addition, in the regenerated muscular tissue of the external muscular coat and near the regenerated blood vessels, nodes of the intramural plexi are found.

Consequently, the intramural ganglionic apparatus of the intestinal wall of higher vertebrates has the ability to regenerate.

SUMMARY

After the infliction of deep and contused wounds on the wall of the small intestine of white rats regeneration of the ganglia of Auerbach's and Meissner's plexi took place along with the restoration of the principal structures of the intestinal wall. Karyokinetic and amitotic division of neurons were noted in the zone surrounding the wound as well as in the regenerated zones.

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