Glycogen Deposits in Motorneurones of Young Chickens following Peripheral Nerve Section

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Summary. Spinal cord motorneurons of young chickens were examined 2—93 days after section of the brachial plexus nerves.

The maximal reaction to axotomy was found 7—14 days after the operation. The majority of cells showed nuclear eccentricity, displacement of Nissl substance towards the periphery and accumulation of mitochondria and other organelles in the cytocentrum. The most conspicuous alteration, however, in a large number of neurones, was the occurrence of numerous dense granules corresponding in size and staining characteristics to glycogen. These granules most often formed large deposits in marginal regions of cells. In the light microscope the presence of polysaccharides, apparently glycogen, was demonstrated with the PAS reaction.

From 14 to 93 days after the operation most perikarya regained their normal ultrastructural appearance. Increased amounts of glycogen granules could be seen in some of the cells till the end of the fourth postoperative week.

The development of glycogen deposits in axotomized perikarya indicates that considerable alterations in the breakdown and/or synthesis of glycogen apparently occur in young animals after neurotomy.

Key-Words: Electron Microscopy -- Motorneurones -- Neurotomy -- Chromatolysis -- Glycogen Deposits.

Introduction

Changes in the fine structure of nerve cells after section of the peripheral nerve are characterized by a peripheral shift of nuclei, enlargement of nucleoli, marginal displacement of the Nissl substance and accumulation of mitochondria and other organelles in the cytocentrum (for review see Cole, 1968; Zelená, 1971). However, it is known from light microscope studies that the intensity and character of the reactive changes after peripheral nerve injury vary considerably with many factors including the type of neurone (Cammermeyer, 1963) and the age of the animal (Brodal, 1939; Romanes, 1946; La Velle and La Velle, 1958).

The ultrastructural changes in different types of neurones following peripheral nerve section have been extensively studied (Hartmann, 1954; Andres, 1961; Hudson, Lazarow, and Hartmann, 1961; Evans and Gray, 1961; Smith, 1961; Cer- vós-Navarro, 1962; Pannese, 1963a, b; Mackay, Spiro, and Wiener, 1964; Takano, 1964; Barron, Oldershaw, and Bernsohn, 1966; Barron, Doolin, and Oldershaw, 1967; Holtzmann, Novikoff, and Villaverde, 1967; Lentz, 1967, 1969; Kirkpatrick, 1968; Sechrist and La Velle, 1969; Zelená, 1971), but spinal motorneurones have received relatively little attention (Porter, Bowers and students, 1963; Bodian, 1964; Barron, Chiang, and Daniels, 1969). Since profound changes are known to develop in motorneurones following peripheral nerve section early in the
postnatal period (Romanes, 1946; La Velle and La Velle, 1958), it seemed of interest to investigate the retrograde changes in spinal motorneurons of young animals.

Material and Methods

The operations were performed in young chickens 2—8 days after hatching. All peripheral nerves of the brachial plexus were transected on the right side and small pieces of nerves were excised. At intervals of 2, 4, 5, 6, 7, 10, 12, 14, 20, 29, 42 and 93 days after section of the brachial plexus, the animals were anaesthetized with Nembutal (50 mg/kg) injected intraperitoneally then perfused through the aorta with several millilitres of buffered saline solution, followed immediately by the fixative, 1 % paraformaldehyde and 1 % glutaraldehyde solution in phosphate buffer (pH 7.3). 2 to 4 h after perfusion the spinal cord segments between the 13th to 15th spinal nerves were removed and placed for another 2 h in fixative in a refrigerator at 4 °C. Blocks of tissue were then rinsed in buffered dextrose solution and thick transverse sections were cut with a razor blade. The control side was marked by a small incision. The material was postfixed in buffered 2 % osmium tetroxide for 2—3 h, dehydrated and embedded in Epon. As controls, the spinal cord was removed from unoperated chickens 1, 4, 6, 12, 14, 20, and 22 days after hatching and treated in the same way.

Sections 0.5 μ thick were cut on a Porter-Blum ultramicrotome and stained with 0.1 % toluidine blue solution in 1 % sodium borate and examined by the light microscope and sections 0.5—1 μ thick from the same blocks were examined after exposure to the periodic-acid-Schiff (PAS) reaction according to Thiéry (1967). Perikarya with chromatolytic changes and large granular aggregates at their periphery were selected for thin sectioning. Thin sections were mounted on grids and stained with 1 % uranylacetate in ethanol and 0.1 % lead citrate in 0.1 N sodium hydroxide. In several instances serial sections were made, some sections being stained alternately with uranylacetate and with uranylacetate and lead citrate. The sections were examined in a Tesla B 413 electron microscope at 80 kV, using an objective aperture of 50 μ.

Results

1. Ultrastructural Appearance of Normal and Axotomized Perikarya

a) In normal motorneurons of young chickens (Fig. 1) the nuclei with dense nucleoli are generally located in the centre of the cell. Numerous aggregates of tubules of rough endoplasmic reticulum surrounded by ribosomes, i.e. Nissl bodies, are distributed throughout the cytoplasm. In some of the cells Nissl bodies are relatively scarce in peripheral regions. Mitochondria and dense bodies are randomly scattered in the cytoplasm. Individual delicate neurofilaments and microtubules occur in the cytoplasmic spaces between the Nissl bodies. Very occasional dense granules 150—400 Å in size are dispersed throughout the cytoplasm of some cells.

b) In axotomized perikarya during the first post-operative week the Nissl bodies are slightly reduced in size but remain scattered throughout the cytoplasm. Mitochondria and dense bodies generally increased in number are also dispersed in the cytoplasm. However, in some cells there is a significant decrease of Nissl substance accompanied by accumulation of mitochondria, dense bodies and other organelles. Numerous dense granules 150—400 Å in size appear in some neurones 4 days after nerve section and occur in a large number of neurones at the end of the first post-operative week.

During the second week after the operation chromatolysis occurs in the majority of perikarya. The nuclei are displaced from their central position and in some instances almost touch the cytoplasmic membrane. Only small islets or