HISTOCHEMICAL STUDIES OF GLYCOGEN IN THE NERVE CELLS OF THE RABBIT AFTER SECTION OF THEIR AXONES

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By

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Introduction

It has been generally known that the so-called chromatolysis, wholly or in part, occur in the nerve cells under various pathological conditions. Previously it was reported by Toryu (2) that the disintegration of the Nissl's bodies in the nerve cells of Guinea pigs after section of their axones is accompanied by glycogenolysis, and when the recovery of the Nissl's bodies takes place the glycogen granules of the same form and arrangement as Nissl's bodies reappear, showing that glycogen in the nerve cells is contained in Nissl's bodies. Shimizu and Kumamoto (5), however, reported that glycogen is demonstrated homogeneously and diffusively, and is principally located between the Nissl's bodies.

In the present investigation, the occurrence of glycogen in the nerve cells after section of their axones was morphologically and cytochemically examined, with special reference to the relation of the appearing glycogen in the recovery from the chromatolysis, as the part of investigations concerning the relation between the glycogen and the Nissl's bodies.

Materials and Methods

The spinal ganglia and the spinal cords of the cervical region obtained from eight healthy adult rabbits were used. In these animals the nervous branches on the right side arising from the brachial plexus going to the right fore limb were cut off for about 1.5 cm long. They are branches of *N. suprascapularis*, *Nn. subscapulares*, *Nn. pectorales*, *N. musculocutaneus*, *N. medianus*, *N. ulnaris*, *N. radialis* and *N. axillaris*.

At fixed intervals from 4 to 49 days after the operation the rabbits were killed by cutting the carotid artery and performance on the neuloectomy of

the nervous branches was found to be excellent. The spinal ganglia and the spinal cord of the right cervical region were collected. The ganglia of the left side which had not been injured by the operation were used for the control.

All the materials were fixed with alcohol-formalin saturated with sodium acetate (Toryu), embedded in celloidin, and cut at 7 μ. The following stains were used: 0.5 per cent toluidin blue for the Nissl's bodies, periodic acid-Schiff method (PAS) modified by Lillie for glycogen.

Results and Discussion

1. Result obtained for the glycogen under normal condition.

The results obtained from the sections stained by PAS method are as follows:

In the nerve cells of the spinal cord in the cervical region the PAS-positive substance, though it was weakly stained, was demonstrated in two types. The first type was fine granules uniformly dispersed in the cytoplasm. The second type was also fine granules, but they were condensed in the Nissl's bodies. Since all the PAS-positive substances disappeared after the treatment with 50 per cent saliva solution at pH 7, they were found to be glycogen. The morphological relation between these glycogen granules and the Nissl's bodies were studied as follows: The sections previously stained for the glycogen by PAS method were stained for Nissl's bodies with 0.5 per cent thionin or with 0.5 per cent toluidin blue. The condensed glycogen granules were not found on account of the dark shade of the Nissl's bodies stained with thionin or toluidin blue. The small glycogen granules, however, were demonstrated among the Nissl's bodies. The fact that the condensed glycogen granules coincide with the Nissl's bodies in their form, size and position, clearly showed that Nissl's bodies contained the glycogen. In the nerve cells of the spinal ganglia, a similar result was also obtained.

In the previous paper, Toryu (1—3) reported that glycogen is contained in the nerve cells of the horse, Guinea pig and chick embryo at least as one of the elements of the Nissl's bodies. He used Best's carmine stain for demonstration of glycogen. Shimizu and Kumamoto (5) histologically studied the glycogen in the nerve cells using the lead-tetra-acetate-Schiff method and concluded that the glycogen in the nerve cells is mainly demonstrated homogeneously and diffusively, and probably is located among the Nissl's bodies. Toryu and Tamate (4) studied the histochemical distributions of the glycogen and ribonucleic acid in various animals, and stated that the glycogen is mainly present conjugated with the ribonucleic acid in the cells in Protochordata and Vertebrata.

In the present investigation, it was noticed that the glycogen is contained in the nerve cells as the pure element, not combined with the Nissl's bodies. Thus, the fine glycogen granules were condensed in the Nissl's bodies, and also uniformly distributed among them. The difference between the results previously obtained (1–4) and those in the present study is probably due to the different technics employed. A further study on this problem is desired.

2. Results obtained for the appearance of glycogen in the nerve cells after section of their axones.

In this experiment, the spinal ganglion cells were used. The transfer of the nuclei and the disappearance of the Nissl's bodies in the nerve cells of the operated rabbit were studied with the following results: In from 4 to 8 days after the operation, when the chromatolysis began, the unclei were situated near the axone or to the opposite of it. In from 14 to 21 days after the operation the chromatolysis reached the maximum, when the Nissl's bodies almost disappeared throughout the entire body of the cells. The size of the normal nerve cells was about from 10–60 μ. As shown in Fig. 1, however, the cells increased in size with the progress of the chromatolysis, ranging from 20–70 μ. In from about 28 to 35 days after the operation the nerve cells showed the recovery of the Nissl's bodies, and in about 49 days their form and arrangement were found to be normal.

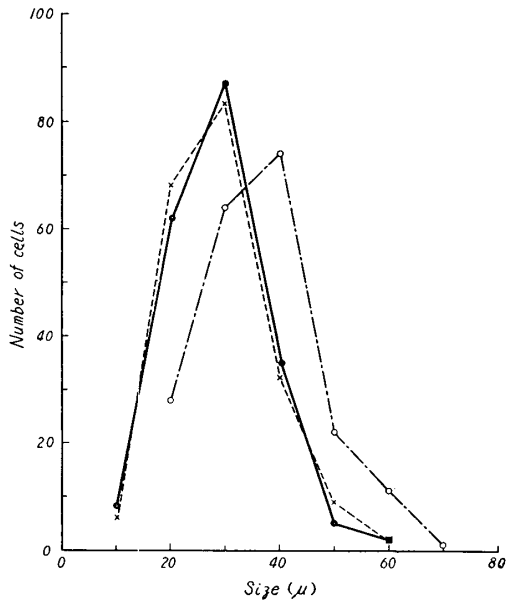

Fig. 1. The curve showing the increase in size of the nerve cells.
●——Control.
×——7 days after the operation.
○——14 days after the operation.

To obtain the data concerning the relation of the glycogen to the Nissl's bodies, the morphological changes of glycogen in the nerve cells after section of the axone, with reference to the chromatolysis, was investigated. The microscopic examination was made on the sections stained by PAS method after the decolorization of the Nissl's bodies, which were previously confirmed by thionin stain. In from 7 to 21 days after the operation the glycogen was not affected, but the Nissl's bodies began to disappear. In from 14 to 21 days after the operation the Nissl's bodies completely disappeared, while the glycogen was still present uniformly in the cytoplasm. In the earlier stage of the recovery, in about

28 days after the operation, the Nissl's bodies reappeared around the nuclei as large granules (Fig. 2). At the same time the glycogen also collected around the same nuclear zone, but with a little larger diameter than the Nissl's pattern (Figs. 2, 3). In about 35 to 49 days after the operation, the Nissl's bodies completely reappeared in the cytoplasm, when the glycogen also recovered its normal pattern.

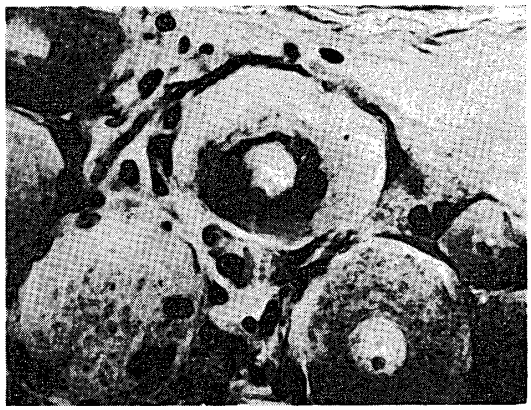

Fig. 2. The spinal ganglion cells 28 days after the operation. Thionin-PAS stain. ×600. The Nissl's bodies reappeared around the nuclei as large granules. The glycogen also appeared in the same zone with a little larger diameter than the Nissl's bodies.

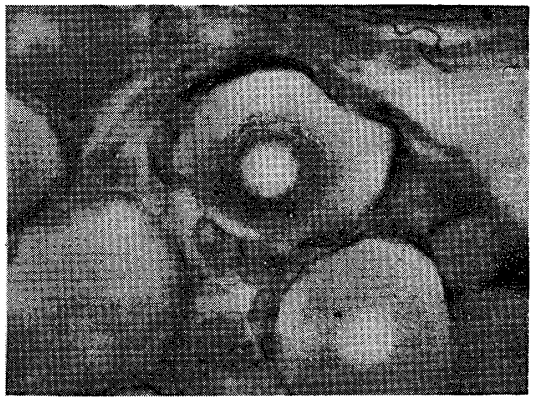

Fig. 3. Glycogen in the same cells as those in Fig. 2. PAS reaction. ×600. Glycogen collected around the nuclear zone.

Toryu (3) studied the appearance of the glycogen in the nerve cells of the chick embryo and stated that the appearance of the Nissl's bodies agreed with that of the glycogen. In the present investigation, the reappearance of the Nissl's bodies in the earlier stage of the recovery from chromatolysis was accompanied with the collection of the glycogen around the nuclear zone. From the fact just stated, it was concluded that the glycogen contained in the nerve cells plays an important role for the recovery of the Nissl's bodies.

Summary

1. Two types of glycogen were found; the first type was of fine granules uniformly dispersed in the cytoplasm, and the second type was also of the same form as the above, but was condensed in the Nissl's bodies.

2. Chromatolysis occurred in the spinal ganglion cells after section of their axones, reaching its maximum in about from 14 to 21 days after the operation. The complete recovery takes place within about 49 days after the operation.

3. During the process of the chromatolysis the glycogen was not affected at all, and at the early stage of the recovery from the chromatolysis, the glycogen was collected around the same nuclear zone as that of the recovering Nissl's bodies.

Reference

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