The development of myelin sheaths and the biosynthesis of myelin and its components in the white matter of the spinal cord of the rats in the course of time are known to correlate with the development of the myelinization glia. It is formed in the spinal cord during the first 10-12 days of the animal's life [1]. Presumably, during asphyxia, injury to the myelin sheath on the nerve fibers is due not only to damage to the nerve cells whose axons form these fibers, but also to damage to the myelinization glia as a result of injury to the glioblasts.

Asphyxia of the embryos in the last quarter of the intrauterine period of development thus leads to chronic pathological changes in the nerve cells and fibers of the spinal cord.

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ELECTRON-MICROSCOPIC AUTORADIOGRAPHIC INVESTIGATION OF INTRACELLULAR RNA SYNTHESIS IN THE MOUSE CEREBRAL CORTEX

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Electron-autoradiographic investigation of DNA synthesis in mouse cerebral cortical neurons showed the highest concentration of label in the nucleolus. Many grains of silver were concentrated above the nucleoplasm. The content of radioactive substances in the cytoplasm of the neurons 2.5 h after injection of uridine-5-^{3}H into the animals was lower than in other types of cells after the same length of contact with the labeled precursor. A considerable difference was observed in the number of grains of silver above serial sections of a single nucleolus and in the character of distribution of the label in neurons situated side by side.

KEY WORDS: *neurons; RNA metabolism; electron-microscopic autoradiography.*

The study of the dynamics of intracellular processes in neurons is of great importance to the further explanation of the principles governing the function of the nervous system. At the present time new prospects are being opened in this field with the opportunities offered by a combination of electron microscopy and autoradiography, whereby metabolic processes taking place in particular cell structures can be observed. One such possible line of research is the study of RNA metabolism, because the synthesis of RNA and its distribution between different parts of the cell are the chief method of regulation of intracellular processes. However, such electron-autoradiographic studies of the nervous system as have been published deal mainly with DNA synthesis during histogenesis of the brain [9], protein metabolism [6-8], and the localization of enzymes [11] and mediators [4, 5, 12].

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This paper gives some data relating to RNA metabolism in neurons.

EXPERIMENTAL METHOD

For the electron-autoradiographic investigation of RNA synthesis the RNA precursor uridine-5-^{3}H (specific activity 16.3 Ci/mmole) was injected by means of a special needle into the brain substance (the fifth layer of the cortex of the cutaneous-motor zone, area PA^m) of noninbred albino mice weighing 25-28 g under ether anesthesia. The dose of the labeled precursor (4 μCi/g) was dissolved in 0.05 ml Ringer's solution. Pieces of cortex 2.5 h after injection of the uridine-5-^{3}H were fixed in a 2.5% solution of glutaraldehyde made up in phosphate buffer, pH 7.4. For the next 24 h the fragments were washed with buffer with frequent changes of the solution and postfixed in 1% OsO_4 solution. After dehydration in alcohols the fragments were embedded in Epon. Serial electron-autoradiographic sections were prepared with M emulsion by the method described earlier [2]. After exposure for 1.5-2 months the preparations were developed and examined in the JEM-100B microscope.

EXPERIMENTAL RESULTS

Analysis of the electron-microscopic autoradiographs showed that the site of most active RNA synthesis in the neurons is the nucleolus (Fig. 1). The property of the nucleolus of synthesizing RNA more intensively than other structures of the cell was first discovered by autoradiographic investigation of a culture of connective-tissue cells in the light microscope [10] and was subsequently confirmed in many other types of cells in experiments *in vitro* and *in vivo*. In this respect neurons of the cerebral cortex are no exception. Meanwhile it must be emphasized that different zones of the nucleolus differed sharply in their density of labeling. Grains of silver were distributed very densely above the nucleolonema but were virtually absent above the heterochromatin connected with the nucleolus (Figs. 1 and 2). This fact indicates the metabolic inertia of the latter. The absence of label above the heterochromatin reflects not only the character of function of the nucleolus but also the accuracy of localization of the source of radiation during analysis of the elctron-microscopic autoradiographs. Since the heterochromatin is in direct contact with the nucleolonema, the site of the highest concentration of newly synthesized DNA, it will not contain label only if, first, RNA synthesis is absent or takes place very slowly in it and, second, there is no appreciable migration of the grains relative to the source of radiation.

A considerable difference was noted in the number of grains of silver above serial sections through the nucleolonema of the same nucleolus (Figs. 1 and 2). This is evidence that the rate of synthesis and, consequently, the content of the newly synthesized product differed in different parts of the nucleolonema at the moment of fixation. In that case, to obtain a complete picture of the state of biosynthesis in the structure, not even the most accurate quantitative analysis of one section was sufficient, but a three-dimensional study of the structure throughout its volume, based on examination of all serial sections, was necessary.

The density of the label above the nucleoplasm of the neurons was very high compared with cells of other types (fibroblast, histiocyte, hepatocyte). As regards the cytoplasm, only single grains of silver were found above it (Fig. 3). With the circulation time of the labeled precursor chosen for the experiments described above (2.5 h) the concentration of

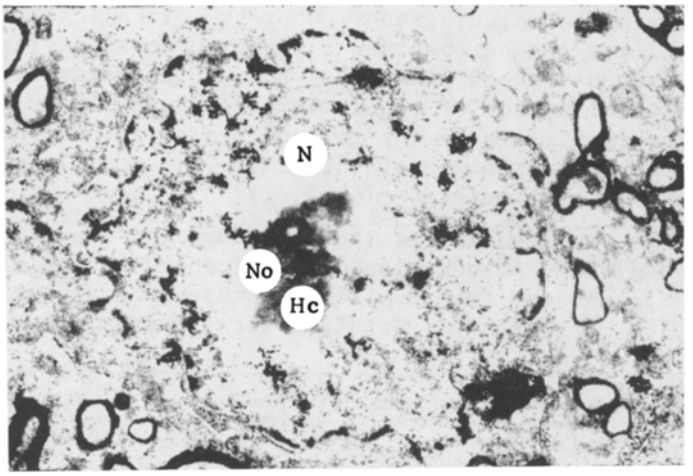

Fig. 1. Electron-microscopic autoradiography reflecting distribution of newly synthesized RNA in neuron of cerebral cortex. Numerous grains of silver above nucleoplasm (N) and, in particular, above nucleolus (No, nucleolonema) indicate active RNA synthesis in those zones. Heterochromatin (Hc) connected with nucleolus contains no label; 18,000×.

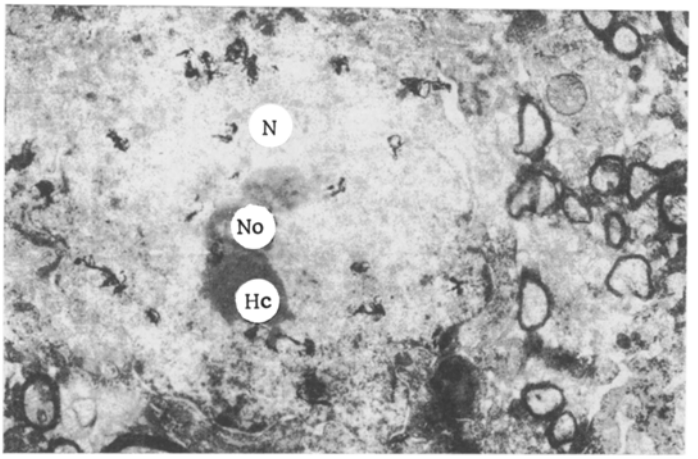

Fig. 2. Another section through neuron illustrated in Fig. 1. Although arrangement of grains above nucleoplasm is similar there is a sharp difference in number of grains above nucleolus (a single grain at boundary between nucleolonema and heterochromatin). Legend as in Fig. 1; 18,000×.

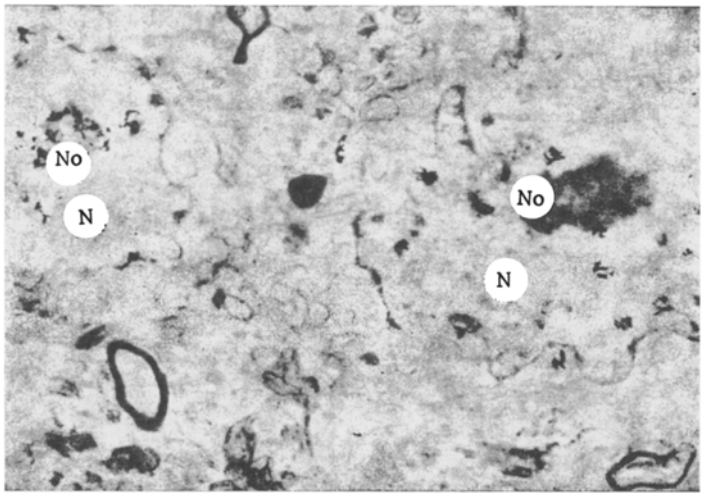

Fig. 3. Difference in character of distribution of grains of silver (reflecting RNA synthesis) in nuclei of two neurons. Grains in one neuron are concentrated almost entirely above nucleolus, in another above nucleoplasm. One grain of silver lies above cytoplasm of each neuron. Legend as in Fig. 1; 17,000×.

grains of silver above the cytoplasm of cells such as fibroblasts, histiocytes, and hepatocytes would be considerably greater [2, 3]. In other words, compared with other types of cells, a smaller fraction of the RNA synthesized in the nucleus moves into the cytoplasm of the neuron, and the overwhelming mass of newly synthesized RNA evidently breaks up inside the nucleus. According to Georgiev's view [1], the fraction of RNA which breaks up in the nucleus is synthesized in regions of the genome not containing information directly concerned with protein synthesis, but which are the site of application of influences controlling this synthesis. In the light of this view, the high concentration of grains above the nucleoplasm and the small number of them above the cytoplasm can be explained on the grounds that the neuron, as a cell controlling the functions of other cells, in order to perform this role must obtain a much greater flow of information than other cells. Accordingly, the zone of the genome which receives such information and on which RNA not migrating into the cytoplasm but breaking up in the nucleus is synthesized is much wider in the neuron. Of course the possibility cannot be ruled out that the small number of grains above the cytoplasm of the neuron must be attributed not to the small amount of RNA penetrating into it from the nucleus, but merely to the slower rate of such penetration than in other cells.

Frequently neurons lying side by side differed significantly in the character of distribution of silver grains in the cell. For instance, in one of the two neurons illustrated in Fig. 3, many grains are concentrated above the nucleoplasm but none above the nucleolus (nucleolonema); in the other neuron, conversely, a high concentration of grains is observed above the nucleolus and only a few above the nucleoplasm. It was recently suggested [3] that the difference in the character of distribution of label in neighboring cells is due to the asynchronous nature of their RNA synthesis. This asynchronous activity reflects the different timing (alternation) of the functional activity of individual structures that ensures optimal conditions for their physiological regeneration.

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DYNAMICS OF THE DNA CONCENTRATION IN HEART MUSCLE CELL NUCLEI OF RATS WITH EXPERIMENTAL MYOCARDIAL INFARCTION

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The DNA concentration was determined microspectrophotometrically in heart muscle cell nuclei of rats at different stages of experimental myocardial infarction. In the intact rat heart some nuclei (1.5-1.8%) of myocytes had a tetraploid DNA complement. Myocardial infarction activates the polyploidization of the nuclei of the muscle cells, especially those lying around the area of injury. The highest intensity of polyploidization of the muscle nuclei was found during the first week of myocardial infarction. Later during the experiment the degree of ploidy of the myocytes increased.

KEY WORDS: *regeneration; myocardial infarction; DNA content; polyploidization.*

In the modern view, regeneration of mammalian heart muscle takes place mainly on account of restoration and hyperplasia of the ultrastructures of the muscle cells [3, 6, 7]. It can accordingly be considered that an increase in the functional load on the myocytes would lead to a corresponding increase in all components of the cell and, in particular, to an increase in the quantity of genetic material. One form of increase of the DNA content in the nuclei of muscle cells is polyploidization. For instance, muscle cell nuclei of the human myocardium are diploid at birth but in the adult they are mainly tetraploid, and they reach a high degree of ploidy in the hypertrophied heart [9, 10, 14]. In experimental hypertrophy of the myocardium the number of polyploid nuclei in the rat heart has been shown to increase, although by a lesser degree than in man [11]. Several workers [4, 12, 13] have observed DNA synthesis in some muscle cell nuclei in the zone of the heart surrounding the infarct.

It was decided to study the increase in the DNA content in heart muscle cell nuclei of rats with myocardial infarction and also to ascertain the time of appearance of polyploid

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