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SYNTHESIS OF RIBOSOMAL RNA IN PALE AND DARK CEREBRAL CORTICAL NEURONS OF RATS AFTER THERMAL TRAUMA

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Many publications have been devoted to the problem of dark and pale cells (see the surveys [2, 6]), and give comparative data on enzyme activity, content of organelles, proteins, lipids, mucopolysaccharides, glycogen, and certain other substances in them [9]. These data, it must be pointed out, give only an indirect idea of the functional difference between pale and dark cells. Direct measurement of functional activity (the velocity of protein synthesis) by dark and pale neurons was undertaken by Meitner et al. [11], who showed autoradiographically that pale cells in normal rats are functionally more active (incorporate labeled leucine more rapidly).

Having chosen dark and pale neurons as the test objects, the authors attempted to analyze how one of the most important processes — RNA synthesis, ultimately responsible for the specific function of the neuron and regeneration of structures necessary for the performance of this function, takes place in these cells in the course of a disease. Previously a significant increase in the rate of synthesis of nucleolar RNA was found in neurons of burned animals [3].

This paper gives the results of an electron-autoradiographic study of the development of this response in dark and pale neurons separately.

EXPERIMENTAL METHOD

Under ether anesthesia a burn of the IIIb-IV degree (20% of the body surface) was inflicted on noninbred albino rats weighing 180 g. RNA synthesis was investigated in intact animals (control) and 1, 12, 72, and 144 h after burns (five animals at each time). Altogether 25 animals were used.

For the electron-autoradiographic investigation of RNA synthesis a special needle was introduced into the animals' brain (cerebral cortex, cutaneous-motor area, area PA^m), and

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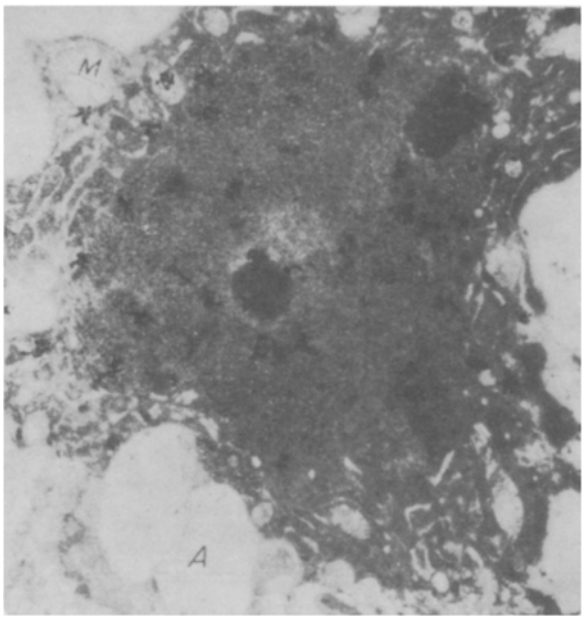

Fig. 1

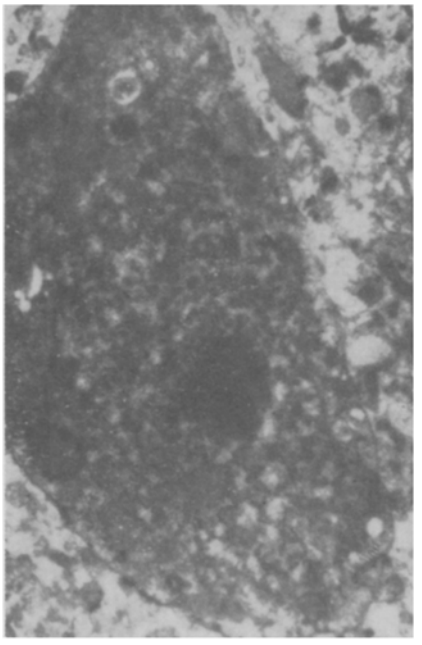

Fig. 2

Fig. 1. Dark neuron from layer V of cerebral cortex of rat 1 h after burn covering 20% of body surface. Edema of astrocyte processes (A), deformation of mitochondrial cristae (M). Label concentrated chiefly in nucleus. Nucleoli do not differ appreciably from nucleoplasm in concentration of label, 15,000×.

Fig. 2. Pycnomorphic neuron in layer V of cerebral cortex of rat 72 h after burn covering 20% of body surface. Floccular distribution of chromatin in nucleus. Increased osmiophilia of cytoplasm, destruction of cell ultrastructures. Depression of RNA synthesis, 15,000×.

the RNA precursor uridine-5-^{3}H (specific activity 26 Ci/mmole) was injected through it. The labeled uridine (50 μCi) was dissolved in 0.05 ml of Ringer's solution. Pieces of cortex 3 h after injection of uridine-^{3}H were fixed in 2.5% glutaraldehyde solution made up in phosphate buffer, pH 7.4. Next, for 24 h the pieces were washed with buffer, with frequent change of solution, and postfixed in 1% OsO_4 solution. After dehydration in alcohols the pieces were embedded in Epon. Light-microscopic autoradiographs were prepared to begin with on semithin section and, on the basis of the results of their analysis the region for cutting ultrathin sections (layer V) was chosen. Serial electron-autoradiographs were prepared by means of type M emulsion by the method of Sarkisov et al. [4, 5]. After exposure for 1 month the preparations were developed and examined in the JÉM-100B microscope. All labeled pale and dark neurons with no significant technical defects were photographed one after the other. The area of cross section (product of maximal and minimal diameters of the nucleolus and nucleus of the neurons) was determined on the negatives, and the labeling density in the nucleolus and nucleus (the ratio of the number of grains of reduced silver found above these zones to their area of cross section) was calculated in conventional units. The quantitative experimental results were subjected to statistical analysis on the M-220 computer [1].

EXPERIMENTAL RESULTS

Grains of reduced silver reflecting incorporation of uridine-^{3}H in both pale and dark cells of the control rats were concentrated mainly above the nuclei. This feature of distribution of the label also was preserved after burns. In most pale neurons of burned animals trophic disturbances were observed: swelling of the mitochondria, an increase in the number of lysosomes, slight edema of the astrocyte processes surrounding the neuron. However, individual cells were found with destructive changes — partial vacuolation of the karyoplasm, fragmentary karyorrhexis, deformation of mitochondrial cristae. Electron-microscopic analysis showed that when the karyoplasm was destroyed the nucleolus remained completely intact. Trophic disturbances were observed in most dark neurons, expressed as dilatation of the tubules of the rough endoplasmic reticulum, an increase in the number of lysosomes, intensification of satellitosis, deformation of mitochondrial cristae, and neuronophagy. Many dark neurons were sur-

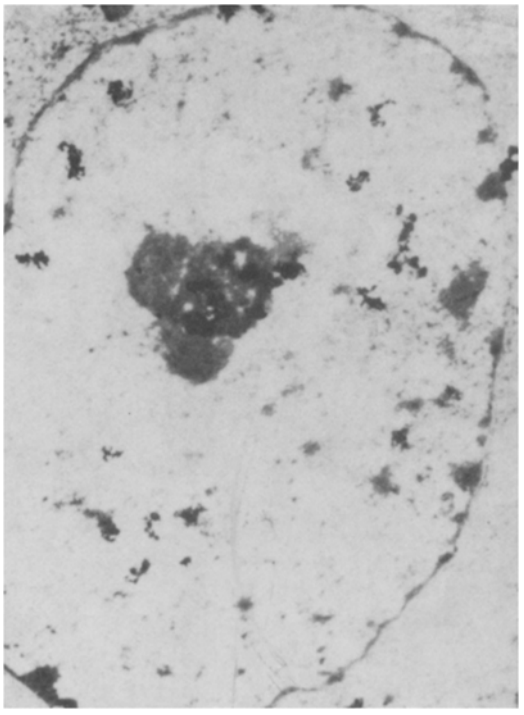

Fig. 3

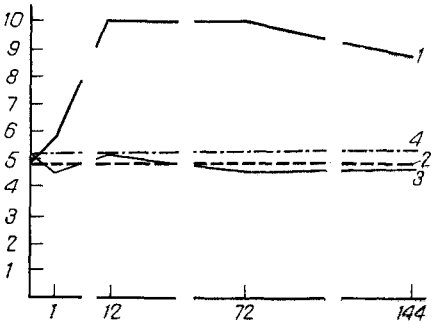

Fig. 4

Fig. 3. Pale neuron in layer V of cerebral cortex of rat 1 h after burn covering 20% of body surface. Concentration of label in nucleolus much higher than in extranucleolar zone of nucleus, 20,000 ×.

Fig. 4. Rate of synthesis of ribosomal RNA in pale and dark cortical neurons of rats after thermal trauma: 1, 2) pale neurons in experiment and control, respectively; 3, 4) dark neurons in experiment and control, respectively. Abscissa, time after burning until injection of isotope (in h); ordinate, ratio of labeling density in nucleolus to labeling density in extranucleolar zone of nucleus.

rounded by edematous astrocyte processes (Fig. 1). Neurons of this sort were found more frequently in the early periods after burns (1 and 12 h).

Pycnomorphic cells were found with destructive changes in the nucleus and organelles of the cytoplasm. RNA synthesis in these neurons was considerably depressed or ceased altogether (Fig. 2). The relative density of labeling in the nucleoli and extranucleolar zone of the nucleus of dark and pale neurons is illustrated in Table 1 and Fig. 3. Normally the ratio between the density of labeling in nucleolus to nucleus is a little higher in dark neurons than in pale neurons.

It has recently been shown that ribosomal RNA is synthesized in the nucleolus. Consequently, the correlation studied between labeling densities reflects the level of synthesis of ribosomal RNA. After burns, this level in dark neurons was substantially unchanged, with nothing more than a small decrease which was not statistically significant. Pale neurons responded quite differently under pathological conditions, for after thermal trauma RNA synthesis in the nucleolus was sharply accelerated (Fig. 4). The rate of synthesis of nucleolar RNA 12 h after trauma was doubled and it remained at almost the same level until the end of the observations (144 h). The absolute values of labeling density in the nucleolus and nucleus under the experimental conditions used were not basically important, for they depended not only on the rate of incorporation of precursor, but also in removal of the test cell from the site of injection. Nevertheless, it must be pointed out that in both types of cells the absolute values of labeling density both in the nucleolus and in the nucleus increased after burns. Consequently, it is quite probable that thermal trauma stimulates intraneuronal RNA synthesis. However, whereas the labeling density in the nucleolus and in the nucleus increased approximately equally in dark neurons, in pale neurons the increase in labeling density in the nucleolus preceded that in the nucleus.

TABLE 1. Mean Ratio of Density of Labeling in Nucleolus to Density in Extranucleolar Zone of Nucleus in Pale and Dark Neurons in Layer V of Cerebral Cortex (M ± m)

Type of cells	Time after burn when isotope injected, h	Number of cells tested	Nucleolus/nucleus
Pale	Control	155	4,802±0,567
Dark	Control	70	5,224±0,756
Pale	1	109	5,780±0,596
Dark	1	153	4,532±0,587
Pale	12	155	9,903±0,453*
Dark	12	130	5,102±0,239
Pale	72	66	9,893±1,460*
Dark	72	54	4,522±0,431
Pale	144	69	8,655±0,941*
Dark	144	1722	4,661±0,210

*P < 0.05 compared with corresponding control.

These observations thus showed that after thermal trauma the intensity of synthesis of ribosomal RNA differs in pale and dark neurons. In dark neurons it either remains unchanged or it increases to match the increase in the general level of RNA synthesis. In pale neurons, however, the rate of synthesis of ribosomal RNA determines the rate of synthesis of nuclear RNA, so that it can be concluded that the functional activity of these cells is greater.

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