# ELECTRON-CYTOCHEMICAL STUDY OF ACTIVITY OF ENZYMES OF ENERGY METABOLISM IN DARK AND PALE NEURONS OF THE RAT CEREBRAL CORTEX

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Hyperchromic or "dark" cells appear in the brain under various experimental conditions and in certain pathological processes [5]. Most workers nowadays consider that the appearance of "dark" neurons is a universal and nonspecific response of nerve cells to the action of a physiological and pathological factor which characterizes a certain functional state of the neurons. It is also known that hyperchromic cells may exist in the brain of intact animals [4, 8]. However, it has not yet been decided whether the functional activity of these cells is depressed or enhanced. Only in sporadic publications have the parameters of protein and nucleic acid metabolism in hyperchromic neurons of intact animals been determined [4, 8, 10]. Meanwhile, knowledge of the various aspects of the function of these neurons in intact animals can help to elucidate the functional role of these cells, when they appear in various pathological states.

The aim of this investigation was to study the ultrastructural localization of succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) activity in dark and pale cortical neurons of the intact rat brain.

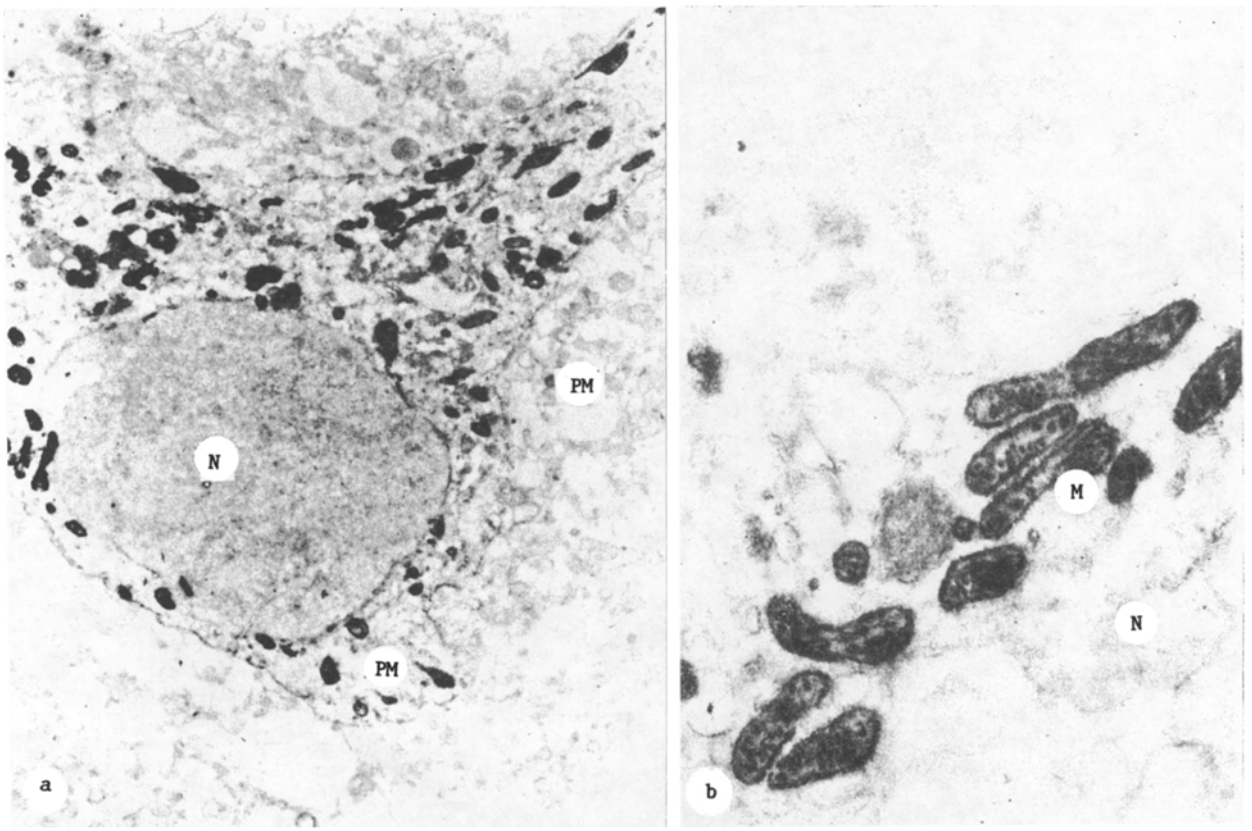


Fig. 1. Distribution of SDH activity in "pale" neurons in layer III of rat cerebral cortex. N) Nucleus, M) mitochondria, PM) plasma membrane; magnification: a) 3000×, b) 10,000×.

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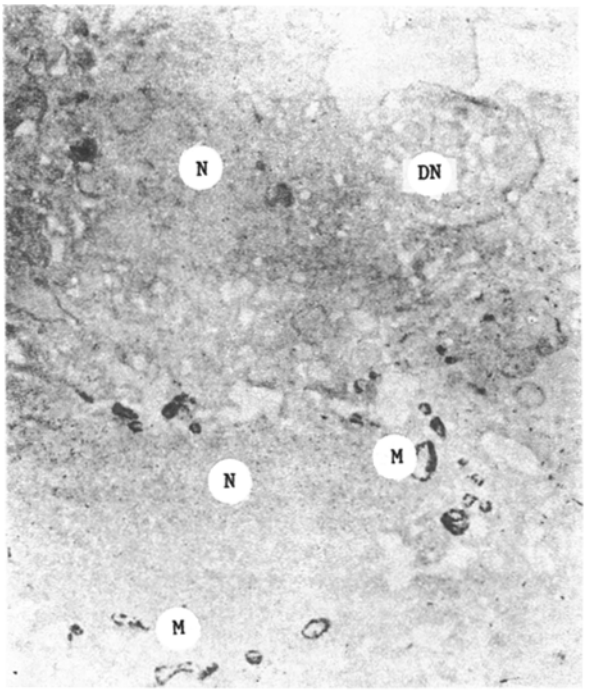


Fig. 2. Distribution of SDH activity in "pale" neurons (no SDH activity found in "dark" neuron. DN) "Dark" neuron, N) nucleus, M) mitochondria. 2000×.

## EXPERIMENTAL METHOD

Pale and dark neurons of layer III of the frontal cortex of five intact noninbred male rats weighing 180-200 g were studied. The animals were killed by decapitation under ether anesthesia. SDH activity was revealed by the method in [14] and LDH by the method in [13] in our modification [9]. After embedding of the material in Araldite, the specimen was polymerized for 2 days at 56°C. Sections out on a Reichert ultratome were examined in the Hitachi-9 electron microscope with accelerating voltage of 60 kV.

## EXPERIMENTAL RESULTS

SDH activity was determined both in the bodies and in the processes of "pale" neurons. The cells were heterogeneous as regards the degree of their enzyme activity. Intracellularly, the reaction product was distributed in the mitochondria and in areas of the plasmalemma (Fig. 1a). Mitochondria varied in the level of their enzyme activity. Besides mitochondria completely filled with a precipitate of copper ferrocyanide, there were others with only a small quantity of electrondense reaction product. SDH activity was located on the inner and outer mitochondrial membranes and on the cristae (Fig. 1b). No SDH activity could be detected in "dark" neurons (Fig. 2).

LDH activity in "pale" neurons was found both in the bodies and in the processes of the cells. The intensity of the reaction differed in different neurons. Intracellularly, the electron-dense reaction production was found in mitochondria, regions of the plasma membrane, and the hyaloplasm (Fig. 3a). The mitochondria varied in their degree of enzyme activity. The reaction product was distributed mainly on the outer and inner mitochondrial membranes. The intensity of the reaction in most neurons was lower than that of SDH. Activity of the enzyme in the hyaloplasm was found in only a few cells. LDH activity was not found in either the bodies or the processes of "dark" neurons (Fig. 3b).

Thus the "pale" neurons of the frontal cortex of rats possess marked SDH activity, which is localized in the mitochondria and parts of the plasmalemma, and rather lower LDH activity, localized in the mitochondria, parts of the plasma membrane, and the hyaloplasm. Neither SDH nor LDH activity is present in "dark" neurons.

The localization of SDH and LDH activity thus revealed in "pale" neurons agrees basically with that described previously [11, 13, 14]. We also found activity of these dehydrog-

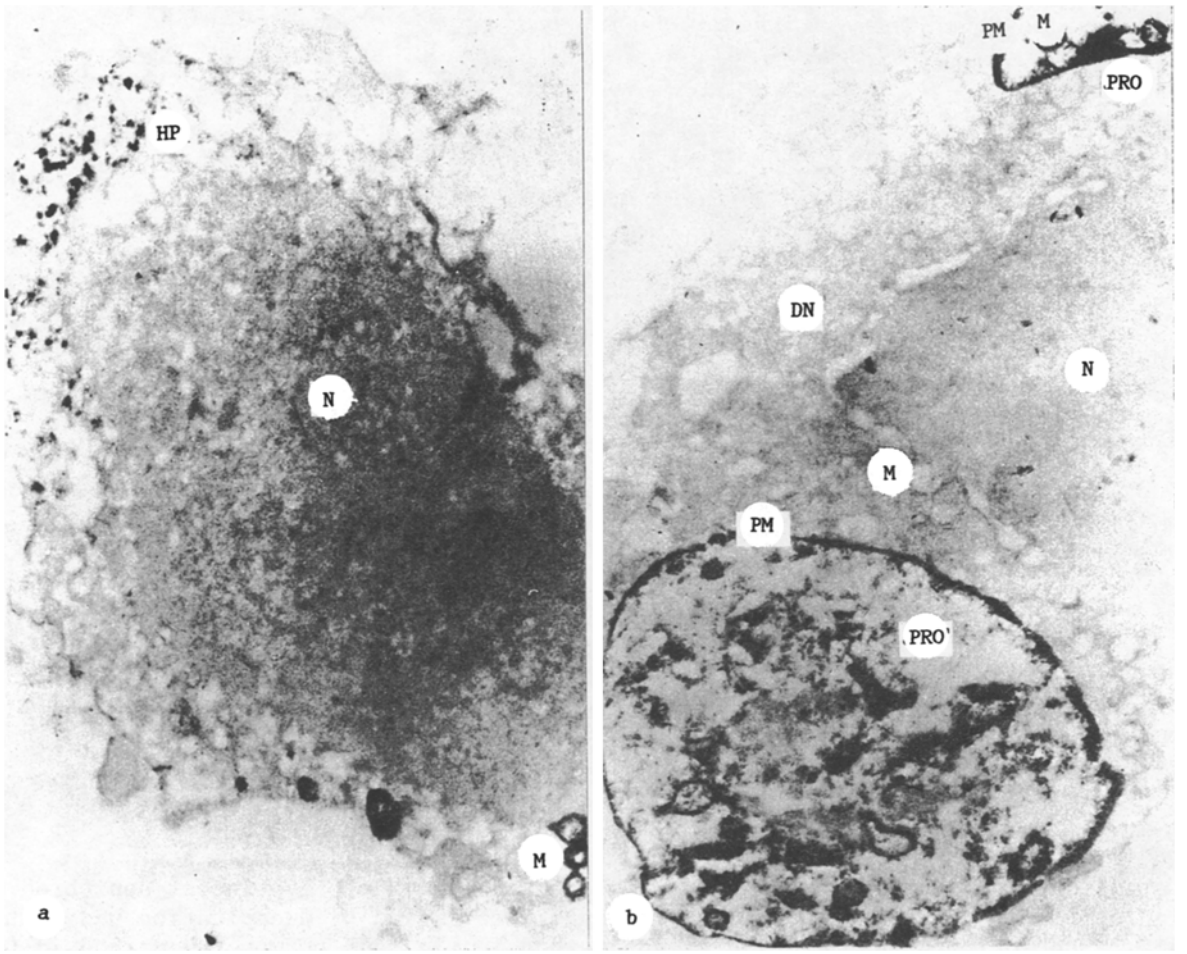


Fig. 3. Distribution of LDH activity in perikaryon (a) and processes (b) of "pale" neurons in layer III of rat cerebral cortex. PRO) Processes, DN) "dark" neuron, N) nucleus, M) mitochondria, PM) plasma membrane, HP) hyaloplasm. 5000×.

enases in the plasma membrane of the neurons. Participation of the plasmalemma in enzyme activity was observed by the writers previously in human embryonic brain cells [7, 9]. The very small proportion of activity discovered in the plasma membrane may be linked with the work of other dehydrogenases oxidizing endogenous substrates, and also with activity of cytochrome C-ferricyanide reductase [15]. LDH activity was weaker than SDH activity in "pale" neurons. This evidently confirms the view that an aerobic pathway of glucose oxidation predominates in neurons [12], and the very low LDH activity in the hyaloplasm corresponds to data indicating that the extramitochondrial form of LDH has greater affinity for the glial cells of the brain, and that the mitochondrial form of the enzyme functions predominantly in neurons [2].

It is a very interesting fact, in our view, that activity of the two dehydrogenases was not found in a single "dark" neuron. Thus besides their considerably depressed level of RNA synthesis [8], these cells are also characterized by a modified energy metabolism compared with that in the actively functioning "pale" neurons. Returning to the problem of role of hyperchormic neurons it can be tentatively sugested that they are in an inactive state. This may probably be reflected also in specific activity of the brain. We know, for example, that the number of these cells is increased in experimental animals in a state of deep sleep, and it has been claimed that hyperchromia is the equivalent of inhibition taking place in cells [3]. It has also been established that the number of "dark" neurons is increased under various experimental conditions, including in hypoxia [1, 6]. We found that the number of "dark" neurons is considerably increased in animals subjected to total ischemia, and that these cells, as in the brain of intact animals, possess neither SDH nor LDH activity, i.e., under various experimental conditions the number of inactive neurons in the brain increases. Meanwhile the preserved ultrastructure of these cells [8] is in harmony with the view that this is a functional and not a pathological state of the neuron.

These cells perhaps constitute an inactive neuron pool at the expense of which brain functions may be restored in the course of time, for we know that the number of these cells decreased after the end of the experiments, i.e., the state of hyperchromia is transient [10], and the passage of neurons into it is one of the ways of protecting cells against further injury.

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