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# NEOCORTICAL ULTRASTRUCTURE DURING REHABILITATION AFTER LONG-TERM PROTEIN-CALORIC DEFICIENCY

D. I. Medvedev, I. I. Babichenko, I. Z. Eremina, and A. I. Kravtsova

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The principal method of treatment of patients suffering from protein-caloric deficiency in contemporary medical practice is still food rehabilitation; as a rule, however, this does not lead to complete recovery of the morphology and functions of the individual concerned [11]. Attempts to utilize hormones and, in particular, thyroid hormone, pituitary growth hormone, and adrenocortical hormones, for rehabilitation have been described [2, 13]. However, their use has not achieved widespread popularity. Previous investigations have shown that a combination of dietary rehabilitation with the addition of carnitine to the diet goes a long way toward restoring the tissue-structural changes arising in the brain after protein-caloric deficiency [8].

In the investigation described below the possibilities of dietary rehabilitation involving the use of a balanced synthetic diet and a diet with the addition of carnitine, in order to reverse the changes caused by protein-caloric deficiency, were studied.

## EXPERIMENTAL METHOD

Experiments were carried out on 34 CBA mice which received an experimental diet containing 5% of casein and a control diet with 10% of casein from the 10th through the 40th day of life [5]. Six experimental and six control animals were killed immediately after the experiment, eight experimental mice received a balanced synthetic diet for 1 month after underfeeding (dietary rehabilitation), and seven mice, in addition to a balanced diet, also were given carnitine in a dose of 833 mg/kg of food, corresponding to a dose of 0.5 mg carnitine/g body weight [6]. Seven animals receiving a balanced diet from the 10th through the 70th days of life served as the control. The carnitine was provided by the Laboratory of Pharmacology (Head, Dr. Med. Sci. V. M. Avakumov), "Vitaminy" Research-Production Combine.

Material for electron-microscopic investigation was processed by the method described previously [8].

## EXPERIMENTAL RESULTS

Several changes of destructive and compensatory character were described in previous publications describing a study of the effect of malnutrition on brain ultrastructure, a series of changes of destructive and compensatory nature was described [8, 9, 12]. For instance, a decrease in the number of ribosomes and tubules of the rough endoplasmic reticulum was found in the cytoplasm of neurons of layer V of the neocortex in mice kept on a low protein diet from the 10th through the 40th day of life. Widening of the cisterns of the lamellar complex and an increase in the number of vesicles surrounding them took place. Many mitochondria had a very pale matrix and their cristae were partly destroyed (Fig. 1). Meanwhile in the perikaryon of the neurons of underfed animals many large secondary lysosomes, from 1 to 1.5 μ in diameter, and also lipofuscin granules and vacuoles appeared. The most dramatic changes in neuropil ultrastructure were found in the structure of synapses on spinous processes of the dendrites. The width of the synaptic spaces and of the postsynaptic condensations of the membranes was reduced. The spinous apparatus was substantially modified,

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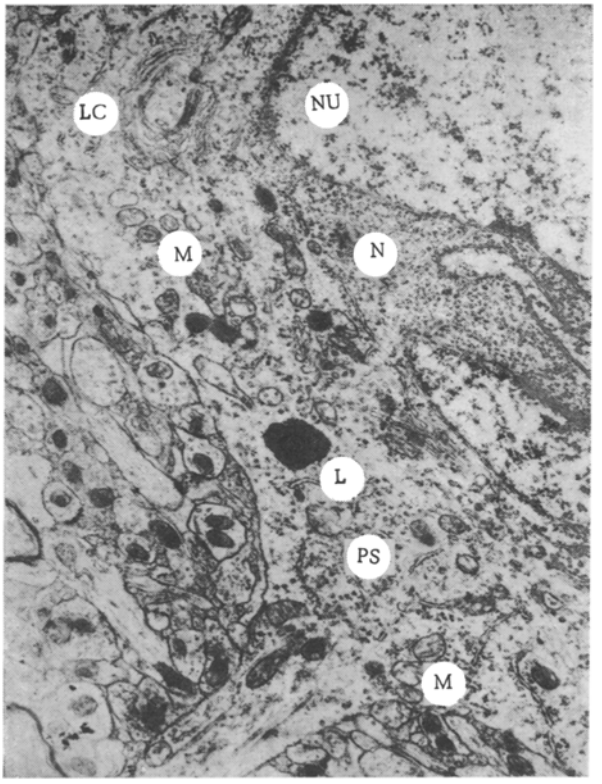


Fig. 1. Neuron in layer V of neocortex of 40-day-old mouse, underfed from 10th through 40th days of life (15,200×). L) Secondary lysosomes; M) Mitochondria; N) neuron; LC) lamellar complex; PS) polysomes; NU) nucleus.

its cisterns were widened and irregular in shape, and the lamellae of electron-dense material were hardly distinguishable [8].

Electron-microscopic study of the neurons of layer V of the neocortex after dietary rehabilitation showed that their ultrastructure was largely restored to normal. However, many large, secondary lysosomes and lipofuscin granules still remained in the cytoplasm of these cell. In a study of the neuropil, widening of the cisterns of the spinous apparatus, located in spinous processes of dendrites, was observed (Fig. 2e), whereas the width of the synaptic spaces and of the postsynaptic condensations of the membranes in synaptic junctions remained much less than in the control animals (Fig. 2b, c). Many of the axon terminals composing the neuropil had no special features, but some of them, unlike in the control, contained electron-dense inclusions.

In the neurons of layer V of the neocortex in mice undergoing dietary rehabilitation with the addition of carnitine, a number of ultrastructural changes were discovered for the first time. For instance, an increase in the density of distribution of chromatin and the extent of the nuclear membrane was observed in the nuclei of these neurons, on account of its considerable tortuosity. All the principal cell organelles were well developed in the cytoplasm of the neurons. There were many more free ribosomes than in the control animals. Tubules of the rough endoplasmic reticulum were very numerous and contained many ribosomes on their outer surfaces. The cytoplasm of the neurons contained a well developed lamellar complex, many mitochondria, and also microtubules and neurofilaments, which were quite difficult to find because of the large number of densely packed ribosomes. Besides the organelles described above, a considerable number of lysosomes and lipfuscin granules was still present in the perikaryon of the neurons (Fig. 3).

Unlike in the control animals and mice undergoing simple dietary rehabilitation, in mice whose dietary rehabilitation was combined with the addition of carnitine to the diet, the number of cisterns of the spinous apparatus was significantly increased (6-7 cisterns compared

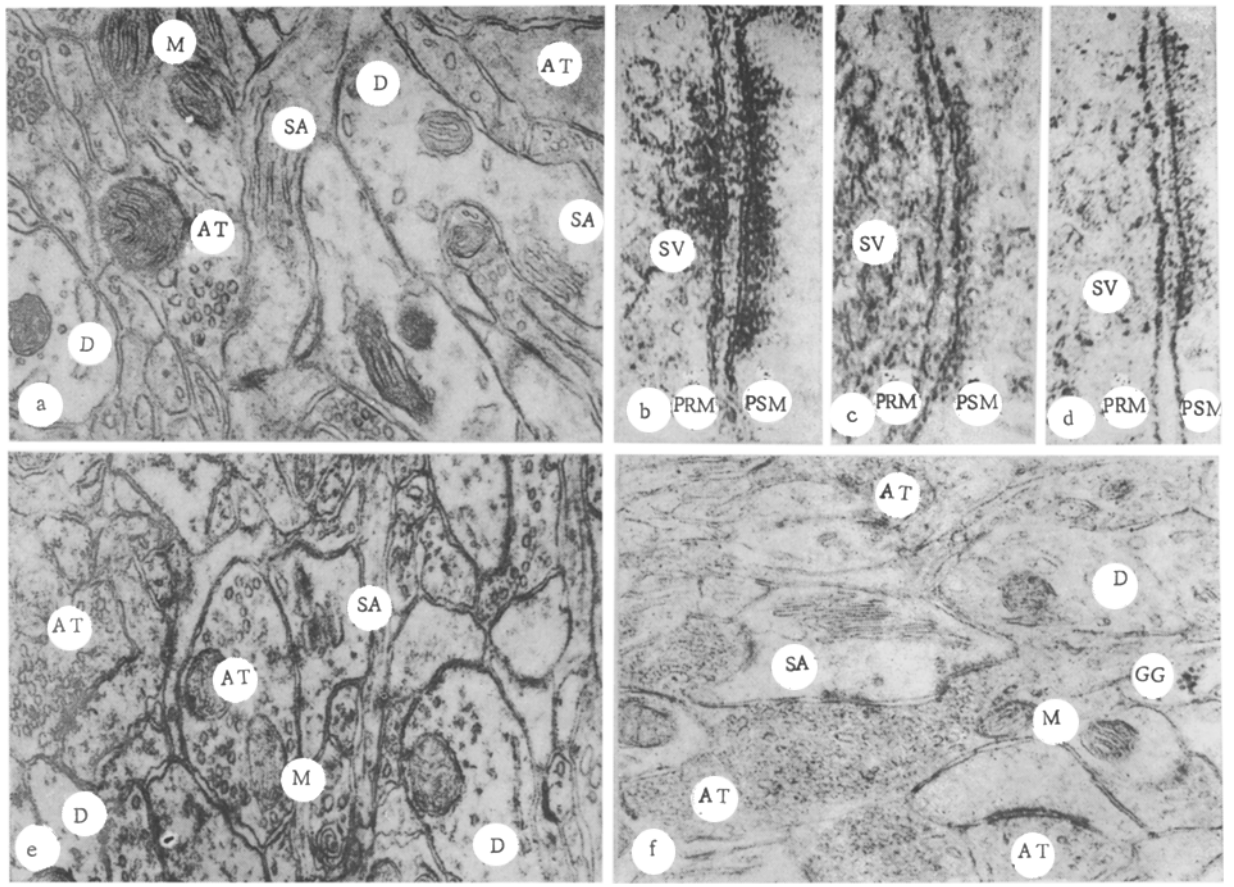


Fig. 2. Ultrastructural changes in neuropil of neocortex of 70-day-old mouse in control and after rehabilitation. a) Area of neuropil in layer V of neocortex of 70-day-old control mouse (34,000×); b, c, d) synaptic junctions on spinous processes of dendrites in control, after dietary rehabilitation, and after dietary rehabilitation with carnitine respectively (16,000×); e) area of neuropil of layer V of neocortex of 70-day-old experimental mouse after dietary rehabilitation (34,000×); f) area of neuropil of layer V of neocortex of 70-day-old experimental mouse after dietary rehabilitation with carnitine (34,000x). AT) Axon terminal; GG) glycogen granules; D) dendrite; M) mitochondrion; PRM) presynaptic membrane; PSM) postsynaptic membrane; SV) synaptic vesicles; SA) spinous apparatus.

with 3-4 in the control and during dietary rehabilitation alone; Fig. 2f) in the structure of the neuropil in the spinous processes of the dendrites. In the region of the synaptic junctions, synaptic vesicles were in close apposition to the presynaptic membranes, but the width of the synaptic spaces and of the postsynaptic condensations, just as after simple dietary rehabilitation, was reduced compared with the control (Fig. 2d).

This action of carnitine was evidently associated with the fact that in protein-caloric deficiency gluconeogenesis and the mobilization of nonesterified fatty acids are sharply intensified [1]. This is confirmed by recent biochemical investigations, which showed that during food deprivation in young monkeys the free fatty acid concentration in the brain is increased [3]. At the same time, oxidative phosphorylation processes in the mitochondria are inhibited [1], and this is reflected morphologically in ultrastructural changes in the cell organelles, described in this paper.

Under these conditions of cell metabolism there is a sharp increase in the demand of the cells for carnitine acetyltransferase, which stimulates the passage of fatty acids from the cytoplasm inside the mitochondria, and thus leads to marked activation of intracellular energy-producing processes [7]. This explains the positive effect of carnitine on dietary rehabilitation after malnutrition, found at the ultrastructural level.

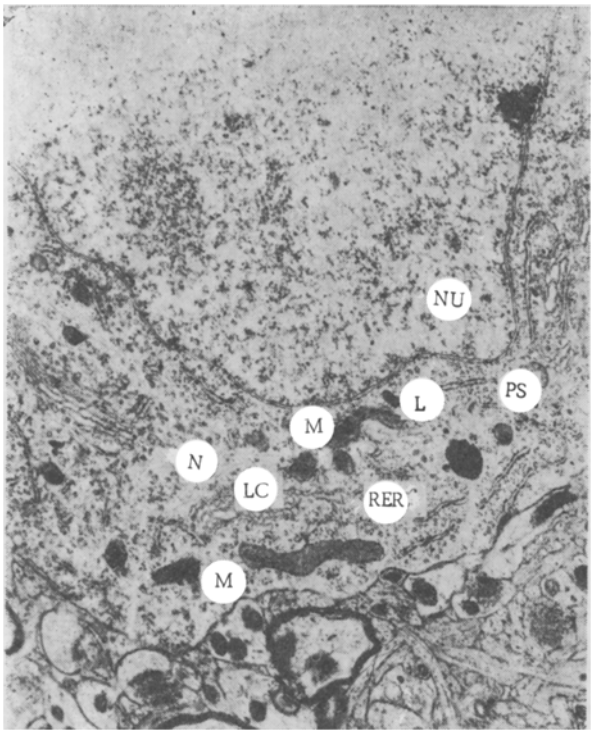


Fig. 3. Neuron in layer V of neocortex of mouse underfed for 70 days, after undergoing dietary rehabilitation with the addition of carnitine (14,600×). RER) Rough endoplasmic reticulum. Remainder of legend as to Fig. 1.

The present investigation also showed that the use of carnitine under these conditions does not restore the normal width of the synaptic spaces or of the postsynaptic condensations of the membranes in highly specialized synaptic junctions, located on the spinous processes of dendrites. In all probability, in order to reverse these structural changes, additional and adequate physiological stimulation of the growing organism is necessary (see in [3, 4] for indirect evidence in support of this view).

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