ULTRASTRUCTURAL CHANGES IN NERVE TISSUE CELLS AFTER EXPOSURE TO BLOOD SERUM FROM PATIENTS WITH INFANTILE CEREBRAL PALSY IN VARIOUS MODEL SYSTEMS

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Several investigations have shown that the blood serum (BS) of patients with infantile cerebral palsy (ICP) contains a pathological transfer factor of peptide nature [1-4] By means of this factor the donor's motor disturbances can be reproduced in a recipient in an experimental model. Research workers are currently displaying great interest in this problem, but there are as yet no direct experimental data to shed light on the source and mechanism of formation of these transfer factors. The method of interaction of transfer factor with the recipient's brain neurons still remains an unsolved problem and there have been no investigations of the ultrastructural changes taking place in the CNS after injection of BS from patients with motor pathology.

It was accordingly decided to carry out the investigation described below, in order to study the effect of BS from healthy individuals and patients with ICP on the ultrastructure of the brain. Organotypical cultures of spinal ganglia and the developing brain of chick embryos were used as different kinds of models.

EXPERIMENTAL METHOD

In experiments on chick embryos the effect of BS on ultrastructure of the archistriatum was studied There were four series of investigations: on intact 18-day embryos, on 18-day embryos into which BS from patients with ICP was injected on the 10th day of incubation, 18-day embryos into which BS from healthy individuals was injected on the 10th day of incubation, and 18-day embryos into which BS from patients with ICP was injected on the 18th day of incubation.

BS in a dose of 0.1 ml was injected through a puncture of the shell above the air chamber of the egg. Pieces of brain were fixed in 2% OsO_4 solution, made up in cacodylate buffer, then washed in 30° alcohol, dehydrated in alcohols, and embedded in Epon. Sections 700 Å thick were studied in the IEM-100B microscope.

To obtain organotypical cultures, 10-week human embryos obtained at artificial termination of pregnancy were used as the source of nerve tissue. The ganglia were removed and trsnsplanted on to coverslips coated with collagen, and cultivated at 37°C in a CO_2 incubator, containing 5% CO_2, 25% O_2, and 70% N_2. The composition of the nutrient medium was: DMEM medium (Gibco) 60% Hanks' salt solution 20%, fetal calf serum 15%, glucose 6 mg/ml, glutamine 6 mg/ml, insulin 0.4 μg/ml, and gentamicin 50 μg/ml. BS (20% of the volume of nutrient medium) was added to the nutrient medium BS from patients was used in the experiment, BS from healthy individuals in the control. To sterilize the BS it was passed through a 0.22 μ filter BS was added on the 4th day of cultivation and the culture was grown until the 21st day, with medium changed twice a week. Twelve cultures each were used in experiment and control. Specimens for electron-microscopic analysis were fixed in 2.5% glutaraldehyde solution (Merck) in 0.05 M cacodylate buffer, with the addition of sucrose and postfixation with 1% OsO_4. After dehydration the cultures were embedded in Araldite. Sections were stained with lead citrate and uranyl acetate and studied in the electron microscope.

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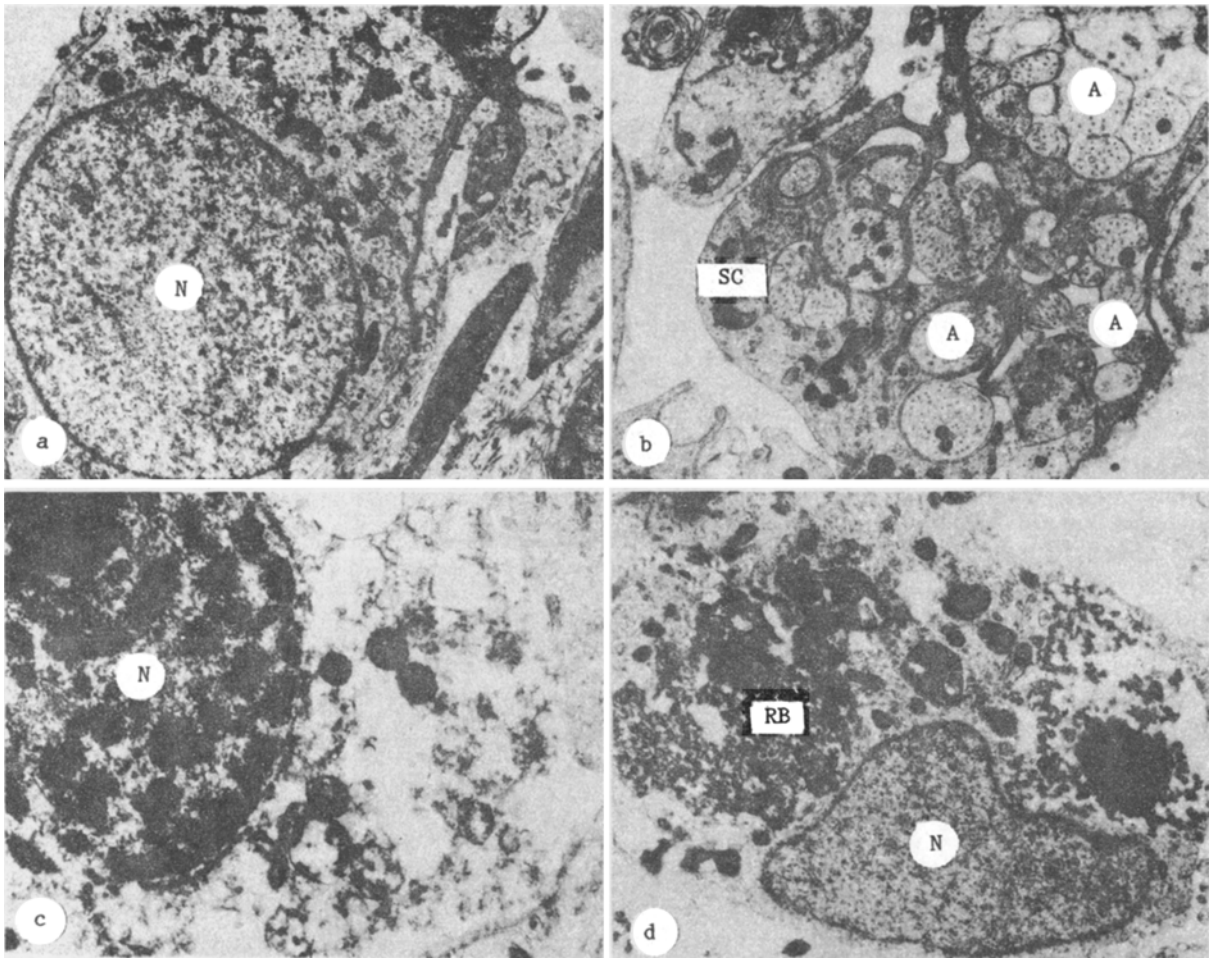

Fig 1. Use of a culture of human embryonic spinal ganglia to analyze action of BS factor on nerve cells. Ultrastructural state of nerve cells on addition of BS from healthy individuals (a, b) and patients with ICP (c, d). N) Nucleus, Magnification 7400; SC) Schwann cells, A) axons. Magnification 13,100, c) destruction of nerve cell accompanied by aggregation of chromatin, destruction of membranes (indicated by +). Magnification 11,900, d) accumulation of numerous residual bodies (RB) in glial cell. Magnification 8100.

EXPERIMENTAL RESULTS

A comparative study of cultures growing in the presence of BS from patients with ICP and from healthy individuals revealed significant differences in the time course of differentiation of nerve cells.

For instance, during culture of the ganglia with the addition of the control BS progressive differentiation of neuroblasts into neurons was observed. Nerve cells in different stages of development could be seen (Fig. 1a) in the 21-day culture. During maturation of the cells the nucleocytoplasmic ratio decreased, the total number of organelles increased, the Nissl's substance developed and axons were formed. The axons were surrounded by bodies of Schwann cells (Fig 1b).

Intravital phase-contrast microscopy revealed inhibition of growth of neurites, differentiating in nutrient medium after the addition of BS from patients with ICP. Ultrastructural analysis revealed considerable slowing of differentiation of some neurons and degenerative changes in others. Culture of the ganglia in the presence of BS from the patients was accompanied by inhibition of the transition from neuroblast to the young neuron stage. Only single cells continued to differentiate, and no mature neurons were formed. The few axons present were not gathered into bundles. Parallel with inhibition of differentiation, death of the nerve cells also was observed (Fig. 1c). Meanwhile well-marked differentiation of glial cells was noted. The Schwann cells surrounded the axons present with their bodies. Increased phagocytic activity of the glial cells was evident, aimed at ingesting breakdown products of nerve cells (Fig. 1d).

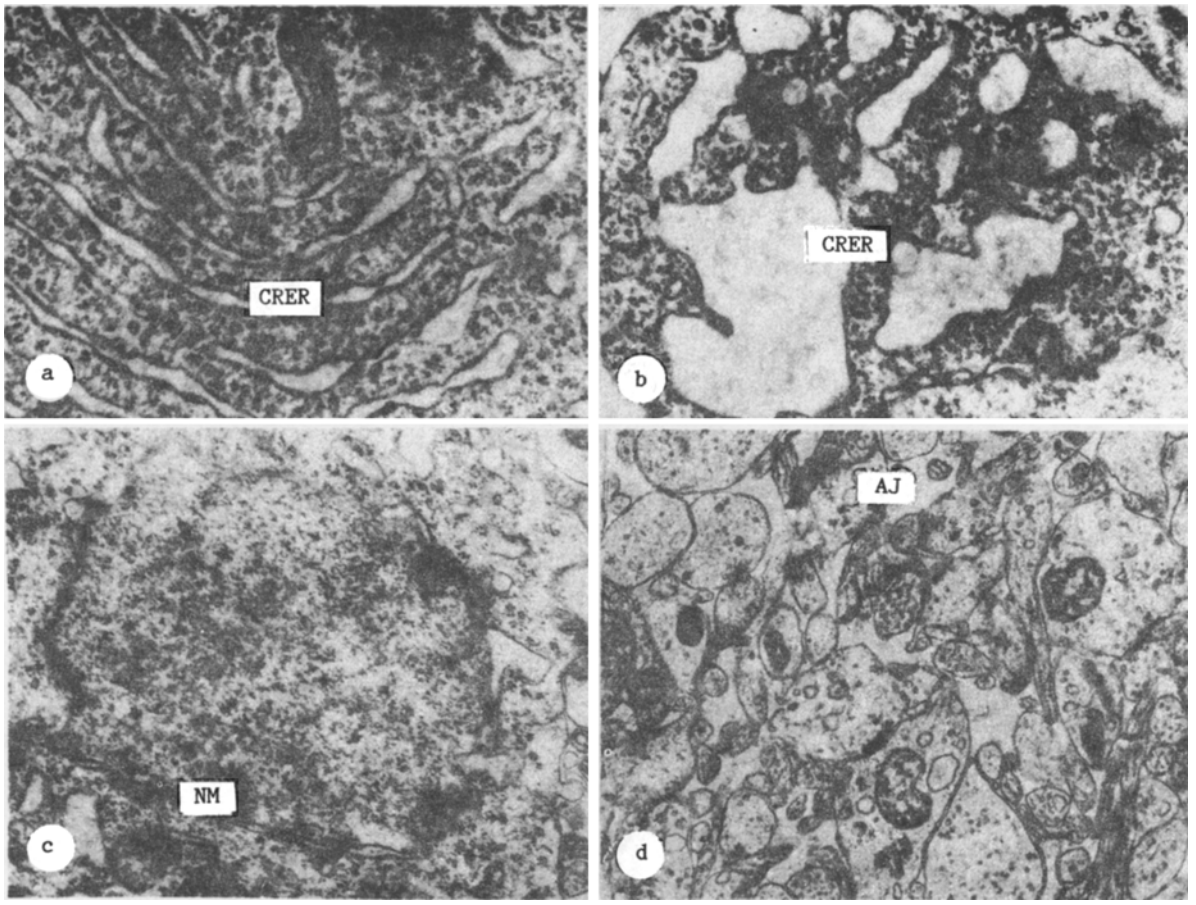

Fig. 2. Ultrastructure of cells and neuropil of 18-day-chick embryo (archistriatum). a) Part of body of a neuron of intact embryo, b) part of body of neuron in archistria tum of 18-day embryo after exposure to BS from patients with ICP, c) part of nucleus of neuron of 18-day embryo after exposure to BS from patients with ICP, d) neuropil of archistriatum of 18-day embryo after exposure to BS from patients with ICP. CRER) Cisterns of rough endoplasmic reticulum, R) ribosomes, N) nucleus, NM) nuclear membrane, AJ) avesicular junctions Magnification 10,000.

The results of the experiments on chick embryos enabled the initial stages of development of the pathological process in the brain structures under the influence of BS from patients with ICP to be studied.

The writers showed previously that neurons differing in their degree of morphological maturity are present in the archistriatum of intact 18-day-chick embryos. They differ from one another in size and in the structure of their endoplasmic reticulum. Three groups of cells can be distinguished: large, small, and medium-sized neurons [5].

Part of the body of a large neuron from the archistriatum of an intact 18-day embryo is shown in Fig. 2a. In the cytoplasm cisterns of the rough endoplasmic reticulum are arranged in distinct rows, lying parallel to the cell membrane. This arrangement of the cisterns is characteristic only of the large, best differentiated neurons. In small and medium-sized cells cisterns of the rough endoplasmic reticulum do not form regular rows, and they are usually short, with a wider lumen.

In 18-day embryos receiving an injection of BS from patients with ICP on the 10th day, submicroscopic changes were found in the cytoplasm of the large neurons (Fig. 2b). The regular arrangement of the cisterns was disturbed, their shape altered, and their lumen greatly widened. The number of free ribosomes and polysomes was considerably increased and the cytoplasm acquired a dense, dark matrix. Changes also took place in the structure of the smooth endoplasmic reticulum: its cisterns were dilated and partly fragmented into individual vacuoles, numerous small vesicles appeared, and the lamellar complex was enlarged.

Besides the response of the rough and smooth endoplasmic reticulum, submicroscopic changes were observed in the karyolemma (Fig. 2c). The tortuosity of the membranes was increased, numerous pores appeared, the quantity of chromatin was increased, and varicose expansions appeared between the membranes and formed evaginations toward the cytoplasm.

The ultrastructural study of the neuropil revealed differences in the composition of the synapses in the experimental series compared with the control. Characteristically the archistriatum of 18-day intact embryos contained axodendritic synapses differing in their degree of maturity Most synapses at this time had distinct active zones and synaptic vesicles. In the experimental material there were few polarized synapses, and those that were present were poorly differentiated. The commonest form of synapse consisted of avesicular junctions (Fig. 2d). These were formed between small branches, did not contain synaptic vesicles, and had small, pinpoint thickenings on the contacting membranes Incidentally, these thickenings could often be seen on membranes of processes not in contact with other processes, but facing the intercellular spaces; the latter, moreover, were much wider than normally. We know from the literature that substances, including biologically active substances, pass along the intercellular spaces, either entering or leaving the cells; the rate of penetration of the substances is several orders of magnitude higher through the thickened areas than in neighboring, unthickened parts of the membrane [6-9].

The avesicular junctions which we found in large numbers were similar to the initial stages through which the chemical synapse passes in its development. In the early stages its formation begins with the appearance of small, symmetrical thickenings on the contacting membranes, and later one or two synaptic vesicles appear in the preterminal.

Many avesicular junctions and widened intercellular spaces also were observed in the experimental material from 18-day embryos receiving BS from patients with ICP on the 18th day of incubation (40 min before inspection), Meanwhile the membranous structures of the neuronal cytoplasm showed no marked ultrastructural changes.

The study of the archistriatum of 18-day embryos into which BS from healthy individuals was injected on the 10th day of incubation revealed no changes in the karyolemma and no significant changes in synapse-architectonics of the neuropil.

Thus, activity of the protein-synthesizing system is sharply increased in brain neurons of chick embryos developing for 9 days in the presence of BS from patients with ICP; the process begins, moreover, with an increase in permeability of the dendritic membranes bounding the intercellular spaces. The processes taking place can be represented schematically as follows: transfer factor of patients' BS — capillaries — intercellular spaces — dendritic membranes — body of neuron — axon — avesticular junctions between processes. The excessive intensification of metabolism in the neuronal cytoplasm, induced by the position of the transfer factor contained in BS from healthy individuals may lead to death of the cells (as has been shown in tissue culture), but the formation of numerous avesicular junctions leads to changes in synapse architectonics of the neuropil; later superfluous chemical synapses, not characteristic of the neuropil of the normally developing embryo, may be formed from these avesicular junctions.

The results obtained on different model systems thus indicate that BS from individuals with motor pathology does in fact contain a factor with neurospecific action. However, the character of its effect is largely determined by its concentration and by the characteristics of its target cells, i.e., by their localization in the recipient, their structural and functional state, their degree of maturity, and also the time of exposure. Realization of the action of the factor can be correspondingly expressed as a whole spectrum of changes: from a change in functional activity of the organelles to their complete destruction.

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RESTORATION OF MOTOR FUNCTION AFTER PARTIAL DECORTICATION AND DURING EXPOSURE TO TWICE THE GRAVITATIONAL LOAD

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The search for specific treatment of disturbed functions after brain injury remains an urgent problem even today. Methods of transplantation of brain tissues and cerebrospinal fluid therapy, which involve the obtaining of material from donors [2, 3], are evidently the most promising approaches. This paper describes an attempt to activate the injured victim's own powers in order to optimize the course of recovery.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 180-200 g. The zone of representation of the limbs in the motor cortex of the left hemisphere was removed from all the rats by suction [6] under hexobarbital anesthesia, in a dose of 70 mg/kg, intraperitoneally; the mean values for the limits of the extirpated zone were from 4 mm rostrally to 4 mm caudally to the bregma, and from 0.5 to 4 mm laterally to the sagittal suture. There were two series of experiments: I) with exposure to twice the gravitational load daily for 10 days from the 2nd day after the operation, and for 20 min each day, II) with exposure for 40 min daily for 30 days starting on the 1st day after the operation. There were two series of control experiments, which corresponded to the experimental series but without exposure to the increased gravitational load. The double gravitational load was created by spinning the animals (they preferred to move facing the direction of spinning) in the horizontal plane in the clockwise direction when viewed from above. The animals either went willingly into the cabin (measuring $6 \times 8 \times 15$ cm) of the "roundabout," or they did not resist being placed inside it. The direction of the force of gravity was dorsoventral, and during spinning the rats sat in a normal, restful position. The supporting function was tested by measuring the distance between the first and fifth digits of the hind limbs while the rat held itself in the vertical erect position for 10 successive measurements [4]. The mean value for the day and for the series and the confidence interval at the $p = 0.95$ level were calculated. After the end of the experiments the animals' brain was removed for morphological examination.

EXPERIMENTAL RESULTS

The experiments showed that post-traumatic pathology, identified by testing as a decrease in the distance between the digits of the limb whose cortical projection area was damaged was reduced in rats of the experimental series compared with the controls. In the case of daily exposure for 20 min this decrease was small and was visible only during the first days after the operation (Fig. 1). Exposure for 40 min led to a greater difference between the data of the experimental and control series (Fig. 1). In series II the differences remained to a greater or lesser degree almost 2 weeks after the operation,

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