Stimulation of the Growth and Differentiation of Axons and Dendrites of the Spinal Neurons in Tissue Culture

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Regeneration of axons and dendrites after damage to the spinal cord results in partial restoration of the nervous connections and functional activity. Some authorities have used substances of different nature which actively affecting the processes of differentiation of the nervous system in order to stimulate the restoration processes. Experiments in transplanting embryonic spinal cord to the damaged region of the spinal cord of mature animals have also been performed. The recovery of functional activity was not observed in the majority of cases, although active growth of axons was noted [5,6,13]. Now, in addition to nerve growth factor, the classical agent stimulating neuronal differentiation in the spinal and sympathetic ganglia, a group of substances affecting the growth of axons and their collaterales has been studied. Serotonin [15], dalargin [7], fusaric acid [10], insulin [14], cytosine arabinoside [12], substance P [9], and GABA [11] all possess different degrees of stimulation.

The present investigation was undertaken to study the effect of blood serum from human umbilical cord (BSHUC), of dalargin, and of supernatant of somatic muscle tissue on the growth of axons and dendrites of spinal cord motoneurons.

MATERIALS AND METHODS

BSHUC contains an activated agent in the form of α-globulins and serves as a routine component of the nutrient medium. We used an increased concentration of the serum (20%), which exhibited a high stimulative effect in preliminary experiments on tissue cultures. Dalargin (an analog of leu-enkephalin) stimulates the migration of nerve and glial cells [7]. Supernatant of somatic muscle tissue accelerates the differentiation of spinal neurons [2]. For the study, transverse fragments of the spinal cord of a 14-day chick embryo were cultivated in Maksimov chambers at 37°C. Four series of inoculation were performed. The nutrient medium of standard composition for control cultures (the first series) comprises Eagle's medium (60%), medium 199 (30%), BSHUC (10%), and glucose (0.1%). An increased concentration of BSHUC (20%), dalargin (10^{-8} mol/liter), and supernatant of somatic muscle (30% of

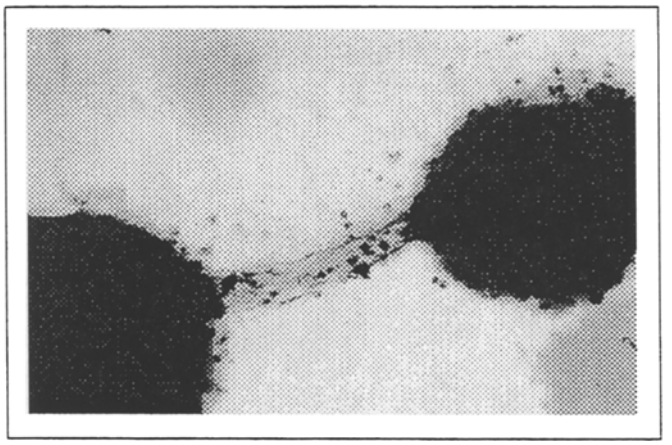

Fig. 1. Formation of a neurite bridge between explants of the spinal cord after the addition of 20% BSHUC to the nutrient medium. Phase contrast, ×100.

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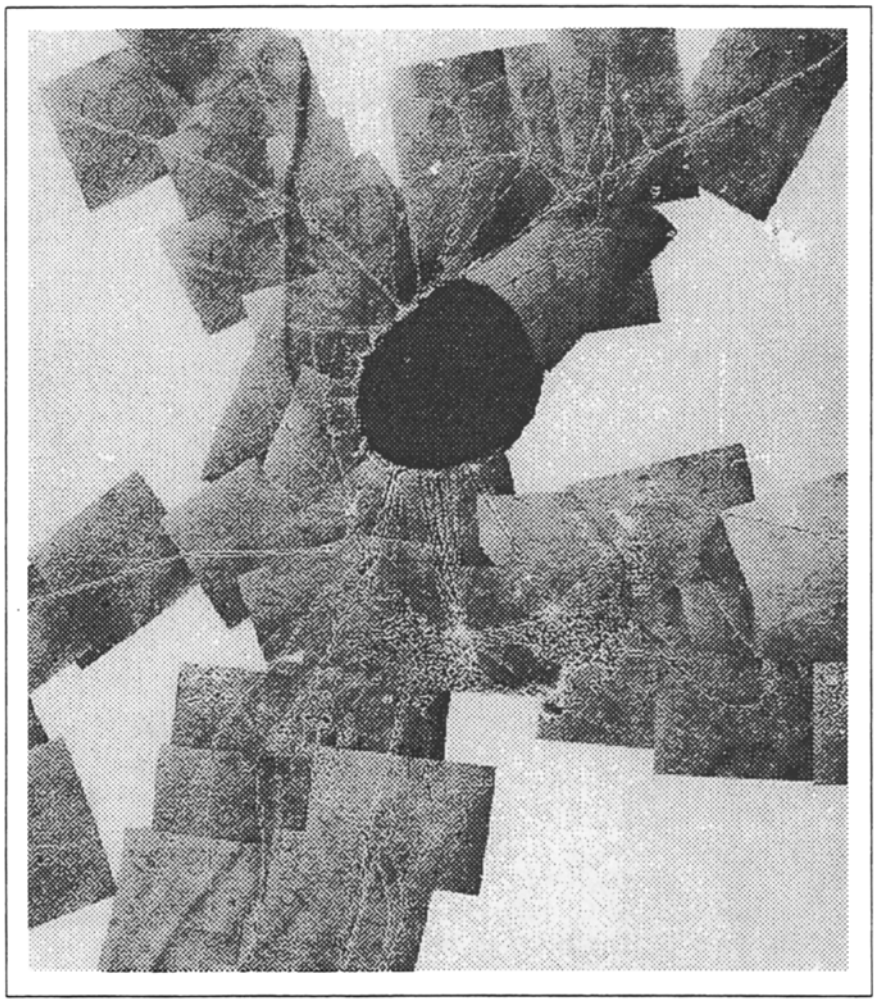

Fig. 2. Explant of the spinal cord after 7 days of culturing in nutrient medium, containing 20% BSHUC. Phase contrast, ×50.

the total volume of nutrient medium) was added to the test cultures (series II, III, and IV) for stimulation. Cultures were examined 14, and 48 h and 3, 5, 7, and 11 days after inoculation of medium under phase contrast (Ienaval) and with the use of Nomarskii optics (MRI-5). For the morphometric analysis the reconstruction of the growing zone of explants was performed. The length of dendrites and axons and the number of their collaterals were determined.

RESULTS

During the culturing of explants in nutrient medium containing 10% BSHUC, the active migration of glial cells was observed, mainly of plasmatic astrocytes, and the growth of axons, regardless of the number of cultured fragments of spinal cord, although this usually does affect the nature of axon growth. A more ordered growth of axons as a thick neurite bridge connecting two explants (Fig. 1) was noted in the latter case.

Single explants cultured in medium with an increased concentration of BSHUC (20%) exhibited an extremely intensive diffuse growth of axons and dendrites in the growing zone (Fig. 2). BSHUC had a stimulative effect not only on the development of axons, but also on the growth of dendrites. The arborization of dendrites was intensive, main branches sprouting offshoots. Up to ten bifurcations of each branch was observed, as a result of which the dendritic system of one neuron encompassed a significant area of the growing zone.

In experiments with administration of dalargin, its capacity to stimulate migration of cells from an

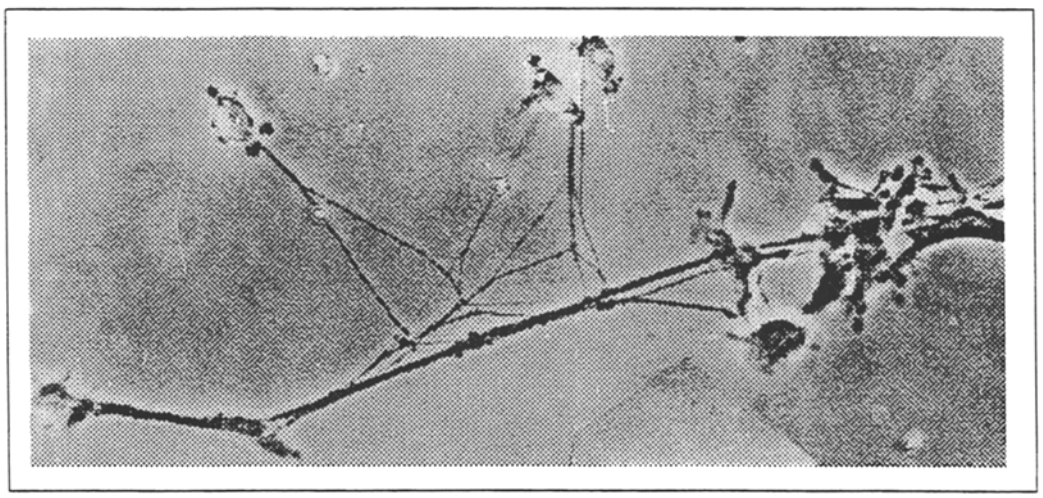

Fig. 3. Astrocytes making contact with the growing ends of axons in the growing zone of a spinal explant after the addition of dalargin to the nutrient medium. Phase contrast, ×150.

explant was noted. Cytoplasmic astrocytes migrated into the growing zone particularly actively, some of them preserving contacts with collaterals of growing axons (Fig. 3). The capacity of dalargin to stimulate the migration of glial cells may be explained by the presence of corresponding receptors on the cell membranes, which is consistent with data of biochemical studies [1].

Adding supernatant of the somatic tissue to the nutrient medium confirmed the data obtained previously [2]. Supernatant stimulate the migration of neurons, the growth of axons, and the formation of branches. Neurons preserved the capacity for differentiation and their axons participated in the formation of the neuronal network. These processes were more intensive for combined culturing of two fragments of spinal cord, when the establishment of mutual contacts was noted (Fig. 4).

The comparison of quantitative parameters of axon growth confirmed the high stimulative effect of the nutrient medium containing 20% serum. The length of some axons in the growing zone in this series reached 4000μ and an increase of their total number was found. The effect of supernatant of somatic muscle was less pronounced, particularly in relation to the number of axons (Fig. 5). Thus, the addition of BSHUC (20%) to the nutrient medium significantly stimulates the growth of axons and dendrites of embryonic spinal neurons.

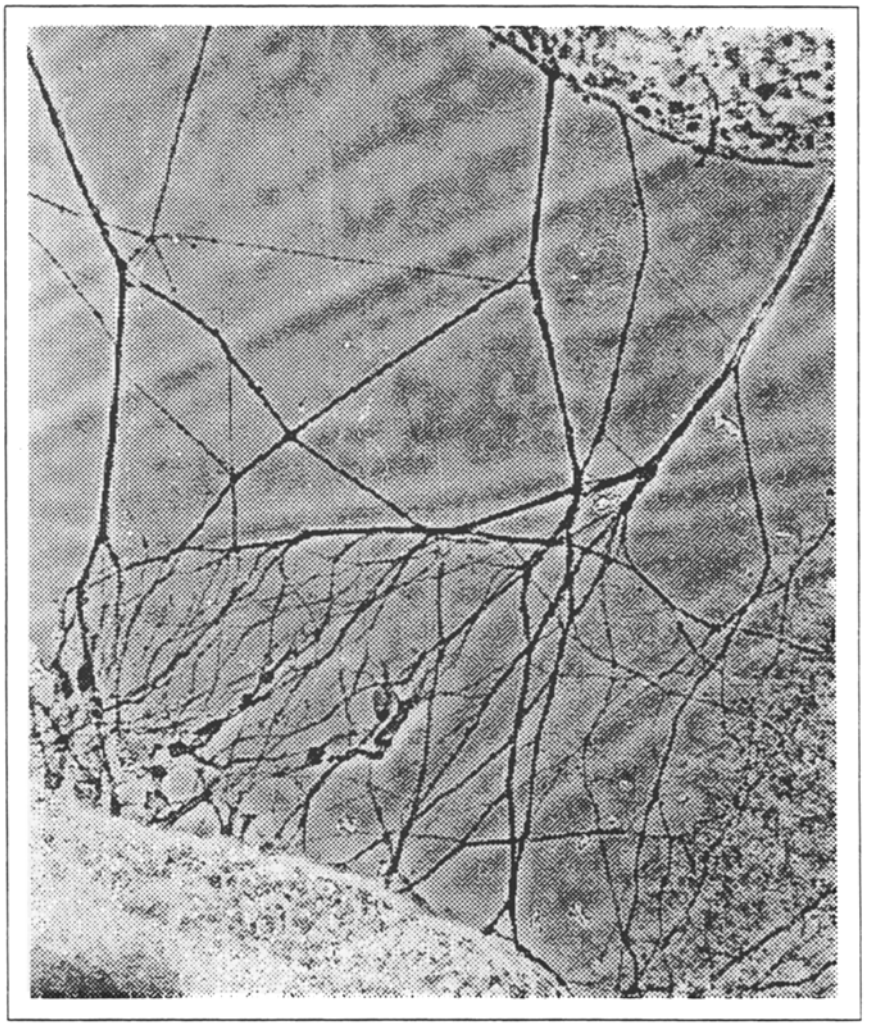

Fig. 4. Formation of a neuritic network between two spinal explants, cultured in nutrient medium with the addition of supernatant of somatic muscle tissue. Phase contrast, ×150.

The difficulties associated with the use of stimulating substances for restoration of the damaged spinal cord in mature animals are primarily due to the relatively low regenerating capacity of axons in these animals and to the different reaction of glial cells in the case of damaged axons. The formation of a glial-connective-tissue scar prevents the growth of axons, also in experiments with tissue cultures [4]. At the same time it is known that astrocytes synthesize a factor stimulating the growth of axons [8], and Schwann cells are necessary for restorating the contacts between the proximal and distal parts of the axon and its further myelinization.

The factors stimulating the growth of axons of embryonic spinal neurons can probably be used as stimulators of the differentiation of embryonic tissue transplanted to the damaged region of the spinal cord of mature animals.

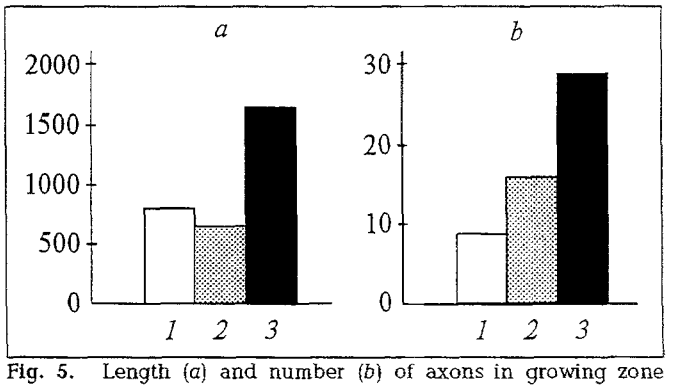

Fig. 5. Length (*a*) and number (*b*) of axons in growing zone of spinal explants after the addition of stimulating factors to the nutrient medium. Abscissa: *1*) explants of the control series (nutrient medium with 10% BSHUC); *2*) explants in nutrient medium containing supernatant of somatic muscle of chick embryos; *3*) explants in nutrient medium containing 20% BSHUC. Ordinate: *a*) length of axons, μ; *b*) number of axons.

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Histochemical Detection of Biogenic Monoamines in Developing Amphibian Embryos in Health and during Exposure to a Static Magnetic Field

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The regulation of biological processes is still one of the greatest mysteries in biology. Embryologists long ago discovered a clear-cut relationship between certain successive morphological signs and processes unfolding with the appearance of these signs in the course of amphibian development. The following chain of events is considered: formation of an animal-vegetal axis in the ovum, site of spermatozoon penetration on the ovum surface, formation of a gray falx on the side of the ovum contralateral to this site, the plane of the first cleavage furrow determined by the above two structures, and bilateral symmetry and anteroposterior axis of the animals determined by the first cleavage furrow. It is evident that this chain of events has a direct bearing on the fundamental notion of the structural plan of an animal. The causes of these events, however, have not been adequately studied. The mechanism of orientation of the first cleavage furrow is one example. It is usually linked to the site where the sperm penetrates the ovum, that is, to a random event. At the same time, a magnetic field is known to have an effect on mitosis [2].

The first series of our experiments was devoted to assessment of the degree of randomness of the localization of the first cleavage furrow in zygotes of the Asia Minor frog (*Rana macrocnemius*) under different ecological conditions of the

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