A decisive role in regulation of the ER level in the rat liver is played by the pituitary, for its removal gives rise to a sudden fall of the ER level in the female liver and a significant decrease in the ER concentration in males after 3 weeks. Sex differences in the ER content in the liver disappear under these circumstances. Considering that functional castration of the males, developing against the background of prolonged absence of the pituitary, ought to have produced the opposite results, namely an increase in the ER content in the liver (Fig. 3), the possibility cannot be ruled out that pituitary secretes a certain factor (or factors) regulating the ER level positively in both female and male rats.

It must be emphasized that the present [3] and other writers [6, 12] have demonstrated a stimulating role of somatotrophic hormone in relation to ER in the liver of female and male rats.

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GROWTH OF AXONS IN ORGANOTYPICAL SPINAL CORD CULTURE

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KEY WORDS: culture; axons; growth bulb; glial cells

Growth of axons and selective establishment of synaptic contacts are important stages in the recovery of function of various parts of the nervous system. Sprouting of axons has been described in the central and peripheral nervous system in adult animals. Particular attention is currently being paid to the study of growth of axons in organotypical and dissociated cultures [4, 15, 7]. The mechanism of growth of axons and formation of selective contacts is contained in the membrane receptors of the cone of growth. The study of the structural organization of the cone of growth has shown that this part of the axon has a complex organization, and the microtubules which constitute its main components are not only responsible for the mechanical structure of the lengthening neurite [6, 9, 11], but are also involved in the function of transporting material to the growing end of the nerve fiber

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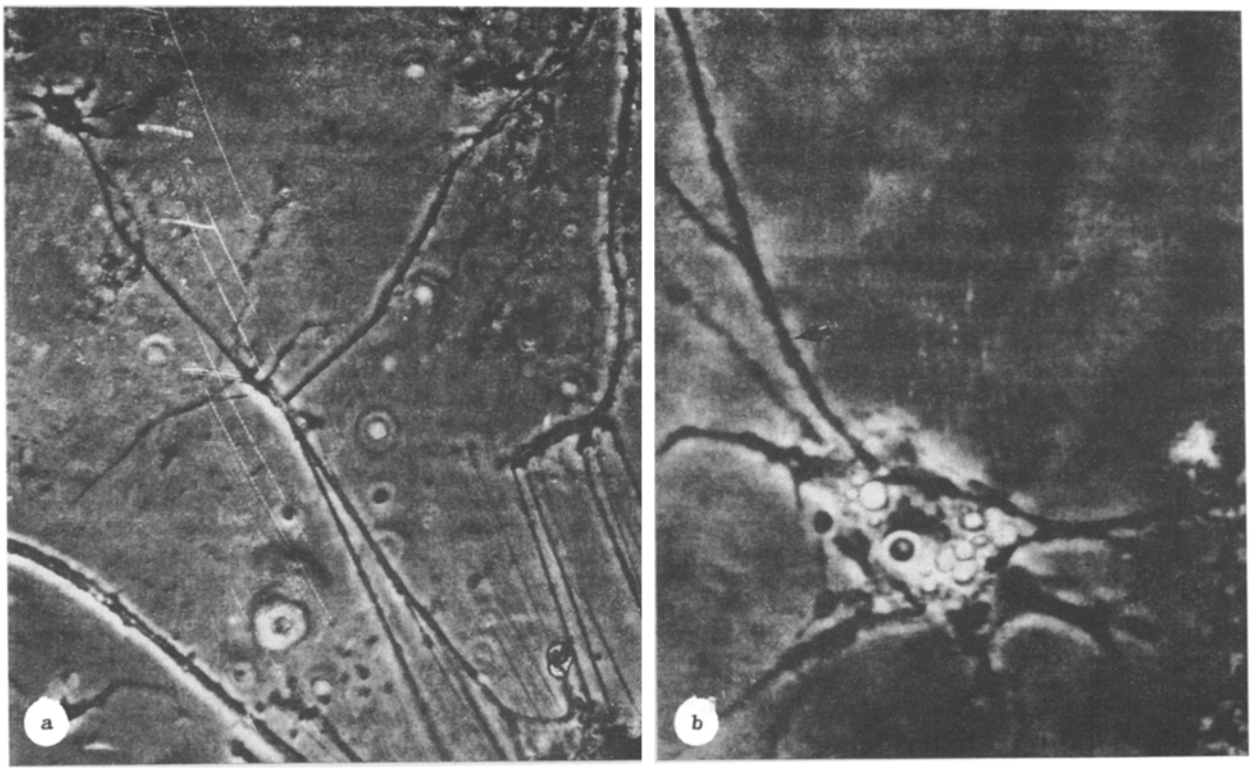

Fig. 1. Growth of axons in organotypical culture of spinal cord. a) Zone of growth of spinal cord explant of 14-day chick embryos after culture for 5 days. Arrow indicates astrocyte, making contact with growing end of axon. 50×; b) The same astrocyte, under magnification of 400. Arrow indicates axon. Phase contrast.

[5]. Meanwhile data have recently been published according to which growing axons establish contact with astrocytes [8], which synthesize a factor stimulating axonal development [10]. It has been shown that during combined culture of brain structures with neither anatomical nor functional connections between them, glial cells can participate in the directing of axon growth [1].

To study the character of growth of axons and the role of glial cells in this process, we investigated the growth of axons of spinal motoneurons of chick embryos cultured under ordinary conditions and in combination with explants of somatic muscle tissue. It was postulated that since myocytes are target cells, development of axons under these conditions ought to take place on account of growth bulbs, which possess the appropriate receptor apparatus.

EXPERIMENTAL METHOD

Spinal cord explants of 14-day chick embryos were studied after culture for 24 and 48 h and 3, 5, and 7 days in nutrient medium containing Eagle's medium (60%), chick embryonic extract (20%), horse serum (20%), and glucose (0.1%). The explants were cultured in Maximow's chambers at 37°C. There were three series of seedings: in the first series fragments of spinal cord were cultured in ordinary nutrient medium, in series 2, spinal cord explants were cultured together with explants of somatic muscles, and in series 3 supernatant obtained after centrifugation of a suspension of the thigh muscles of 14-day chick embryos was added to the nutrient medium (the supernatant accounted for between 20% and 40% of the total volume of the nutrient medium). Observations on the explants were made in phase-contrast (Jenaval, East Germany) and interference (MPJ-5, Poland) microscopes, using a Nomarski optical system. The preparations were impregnated with silver by Bielschowsky's method.

EXPERIMENTAL RESULTS

An essential feature of differentiation of neurons is growth and arborization of their processes. Investigation of differentiation of nerve cells of the spinal cord explants revealed two types of axonal growth: straight, characteristic of motoneurons

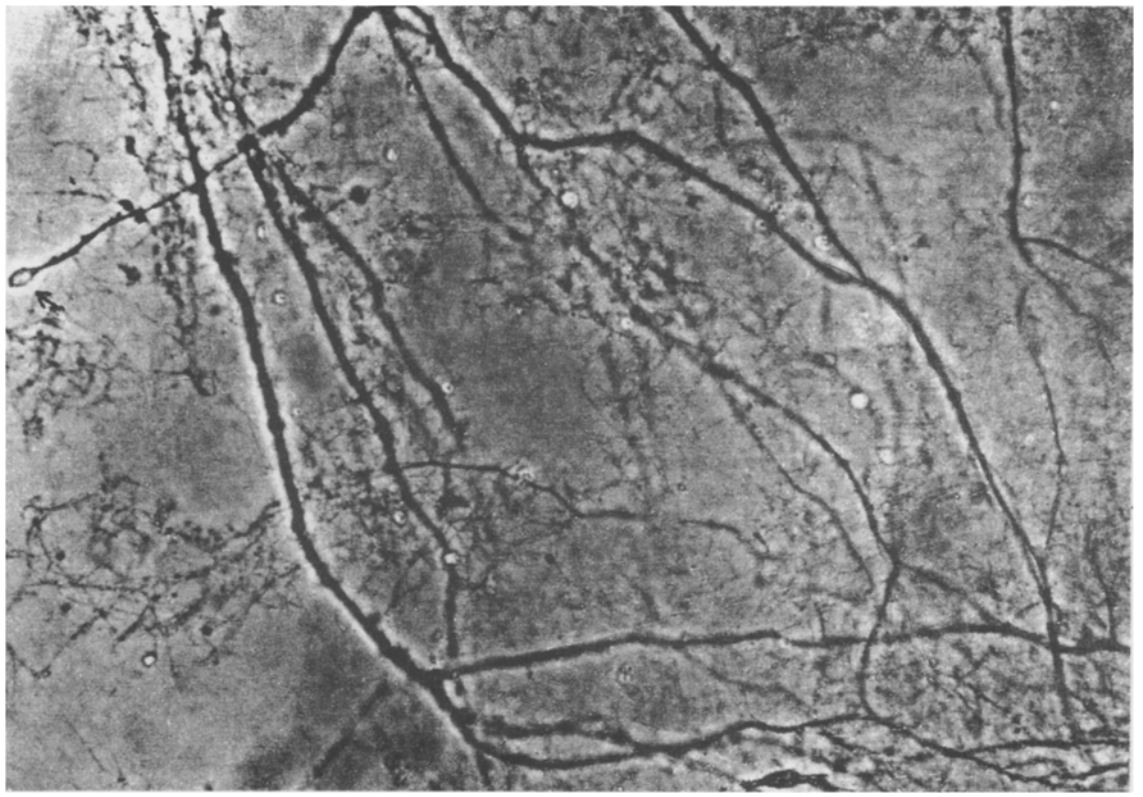

Fig. 2. Axon with cone of growth at periphery of spinal cord explant after 8 days in culture. Arrow indicates bulb of growth. Magnification 50. Phase contrast.

of the ventral horn, and backward, with growth of the axons into the substance of the explant. It is suggested that the second type of growth is specific for association neurons of the spinal cord [3, 13].

Growth of the overwhelming majority of axons of both types is regulated by growth bulbs, but at the same time it was found that glial cells may also play an active part in axon development. In these cases the axons had no bulbs of growth and their endings made contact with processes or bodies of migrating astrocytes (Fig. 1).

Evidence in support of a role for neuroglial cells in the growth of axons is given by the observed stimulating effect of conditioned medium obtained from mullerian cells [12] and on the possible synthesis in the astrocytes of a substance stimulating growth and controlling the development of processes of differentiating neurons [14].

Our observations showed that replacement of the growth bulb by neuroglial cells is temporary in character, although astrocytes characteristically form selective contacts, for example, specialized contacts with the aid of their feet with the surface of the neuron and the basement membrane of capillaries.

We used combined culture of spinal cord and somatic muscle tissue explants as a model in which growth of axons would be determined by the specific action of the target. Growth of axons, stimulated by muscle explants, was found to take place with the aid of bulbs (Fig. 2). Replacement of growth bulbs by astrocytes was not found. These results can be interpreted unambiguously: astrocytes do not possess positive taxis relative to somatic muscle tissue, which is the target for axons of motoneurons.

The development of axons by means of a bulb of growth also was observed when the effect of supernatant, obtained after centrifugation of a suspension of thigh muscles from chick embryos on the development of axons was studied. Addition of the supernatant to the nutrient medium stimulated growth of the axons (Fig. 3), especially in cases when the supernatant accounted for 20% of the nutrient medium. After its addition active growth of axons and of their collaterals and also grouping of axons into bundles of nerve fibers were observed.

These data show that the supernatant of embryonic somatic muscles possesses a specific stimulating action on spinal cord neurons, unlike opioid peptides, which have a wider spectrum of action and which stimulate not only growth of axons, but also migration of glial cells and fibroblasts [2].

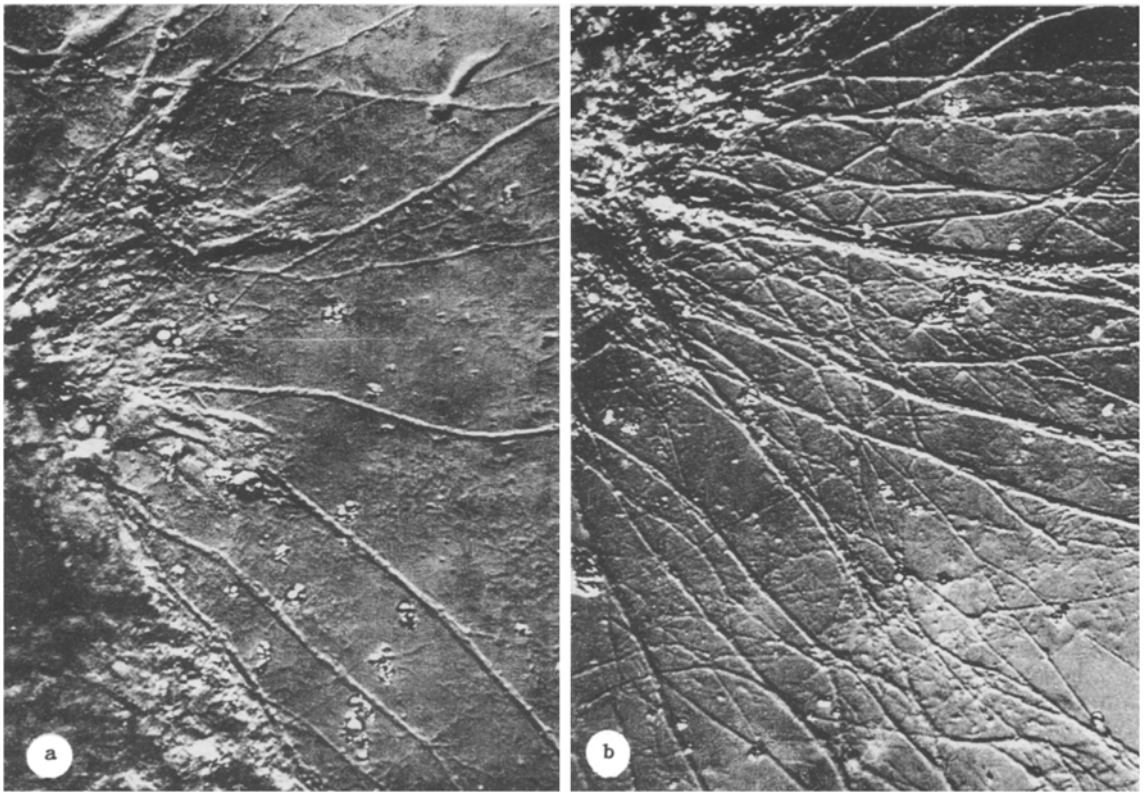

Fig. 3. Growth of axons in ordinary nutrient medium (a) and after addition of supernatant of muscle tissue to medium (b). Contrast by Nomarski's method.

With these data in mind it can be suggested that glial cells can perform the role of cells controlling growth of axons in the absence of the influence of the specific target factor.

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