A Comparative Study of the Effects of Brain Extracts and Mesodermal Membrane Extracts on Nerve Cell Differentiation

Brain extract, prepared from 8-day-old chick embryos including the covering mesodermal membranes, has been shown to stimulate the differentiation of cerebral hemisphere cells[1]. The question arose whether brain tissue alone, which is of epithelial origin, produces factors that promote the maturation of its own cells, or whether the mesodermal layer influences the differentiation of these nerve cells. Interactions between mesodermal and epithelial cells have been demonstrated in various kinds of tissue[2-8]. Mesenchyme influence the rate of proliferation of epithelial cells and affect their morphological and biochemical differentiation. It has already been shown that neural induction is influenced by mesodermal cells[9] and that further organogenesis of the nervous system is dependent on the tissue environment[10].

This work presents the effects on nerve cell differentiation produced by brain extract prepared with and without the covering membranes, as well as the effects of extract prepared only from the surrounding mesodermal tissue.

Materials and methods. The cerebral hemispheres from 5- and 7-day-old chick embryos were dissociated by using a 48 µm pore size nylon sieve as previously described[11] and the dissociated cells were cultivated in Rose chambers on a reconstituted collagen layer. The standard nutrient

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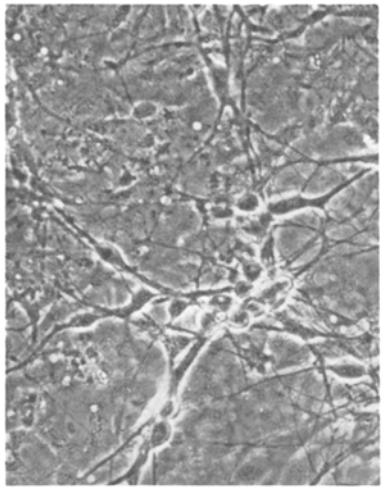
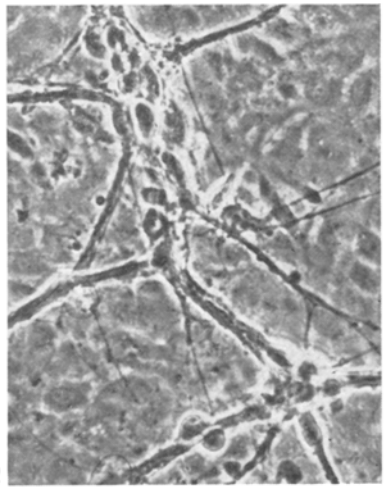




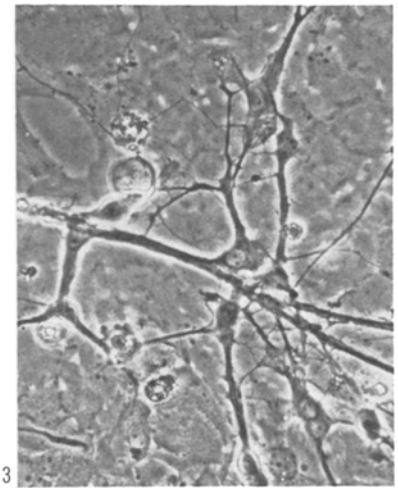
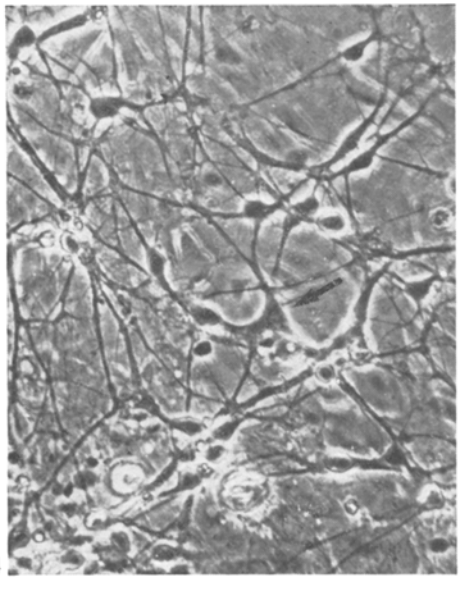


Fig. 1–3. Dissociated cerebral hemisphere cells from 5-day-old chick embryos cultivated during 2 weeks. Phase contrast; ×250. 1. In minimal nutrient medium. 2. In presence of brain mesodermal extract. 3. In presence of mesodermal extract.



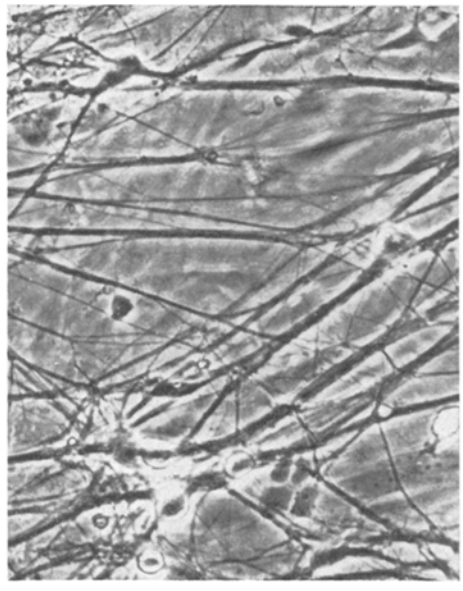


Fig. 4 and 5. Dissociated cerebral hemisphere cells from 7-day-old chick embryos cultivated during 2 weeks in presence of brain extract. Phase contrast; ×250. 4. A large multipolar neuron (arrow). 5. Numerous fibre bundles.

medium consisted of Eagle's basal medium (Institut Pasteur, Paris) supplemented with 20% fetal calf serum (GIBCO). In addition, experimental medium contained 10% of one of the following extracts: a) brain-mesodermal extract prepared from brain including the surrounding covering membranes as used in previous studies[1,12]; b) brain extract prepared from the brain tissue after removal of the surrounding membranes; c) mesodermal extract prepared from the covering membranes. All these extracts were prepared from 8-day-old chick embryos at a concentration of 20% in Tyrode solution and centrifuged 1 h at 105,000 *g*. The experimental medium was added to the cells after 48 h incubation and renewed every 2 days.

Results. The development of 5-day-old chick embryo brain cells under minimal nutritional conditions and the stimulatory effect of the total brain extract (brain-mesodermal extract) on the differentiation of these cells have previously been described in detail[1]. After 2 weeks cultivation, bipolar and multipolar neurons had developed and were dispersed upon a monolayer of flat polygonal astroblasts (Figure 1). Under the effect of total brain extract, the size of the cell body was increased; the nerve fibres were thicker, longer, possessed many ramifications and formed bundles as compared to the control cultures (Figure 2). The brain extract, prepared without the covering membranes, was observed to have no significant effect on the young neuroblasts from these 5-day-old chick embryos. However, under the effect of the mesodermal extract large multipolar neurons developed rich in Nissl bodies and large fibre bundles were observed after 2 weeks in culture (Figure 3).

The relative amount of large multipolar neurons were obtained by analysis of the cultures and by a visual quantification. Detailed measurements of length and number of nerve fibres was not made due to the often tortuously ramified fibres and the difficulty in estimating accurately the number of fibres in nerve bundles. In control cultures, an average amount of 3 to 5 large multipolar neurons for a total of 20 neurons were seen, while in cultures treated with mesodermal extract an average of 10 to 12 large neurons had developed.

It has previously been demonstrated that total brain extract stimulates the differentiation of nerve cells from 7-day-old chick embryo with the same intensity as it influences the cells from the 5-day-embryo[1]. The differentiation of these nerve cells is also stimulated by brain extract prepared without the covering membranes. Compared to cultures without the added extract, more large multipolar neurons develop as well as neurons with ramified fibres after 2 weeks cultivation (Figure 4). Thick bundles of nerve fibres appeared in several areas of the culture (Figure 5). In contrast, the mesodermal extract has a very low influence on these brain cells.

Discussion. These results showed that the young neuroblasts from 5-day-old chick embryo, which are still morphologically undifferentiated respond differently to the various extracts studied. While brain-mesodermal extract and mesodermal extract influenced the maturation of these neuroblasts, the brain extract had no effect. Therefore, at this stage of the embryonic development, the maturation and the differentiation of the neuroblasts seem to be influenced only by the mesodermal covering membranes. Later on, when the neuroblasts became pyriform and have already started the differentiation process (7-day-old chick embryo), they are mainly stimulated by factors produced by the brain-cells and respond only slightly to mesodermal influence.

It can be concluded that the surrounding mesenchyme of the brain stimulates the differentiation of the morphological undifferentiated neuroblasts. Later on the maturation of the neuroblasts is influenced by factors produced by the brain cells themselves.

Summary. Extracts prepared from the mesodermal tissue surrounding the brain stimulate the differentiation of morphologically undifferentiated neuroblasts, while the differentiation of more mature neuroblasts is influenced by brain extracts.

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Muscular Respiratory Receptors in Self-Regulation of Normal Breathing in Man

Vagal blockade in a normal man evokes no change in the pattern of respiration. Thus the lung receptors do not take part in self-regulation of normal breathing in man[1]. As a consequence the respiratory muscle receptors were brought into focus of interest[2,3]. But to establish the role of the afferents from the respiratory muscles in selfregulation of breathing one must investigate the effect of posterior rhizotomy of the respiratory muscles. Unfortunately the results of such investigations in animals, as well as in man, are contradictory[4-6]. This probably depends on the difficulties of the operation which may involve a damage of the ventral roots of the spinal nerves, as is supported by the following example. Although the diaphragm has a very scanty supply of spindle muscles[2,3], even paralysis of the diaphragm

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Fig. 1. Respiratory discharges of the chest muscles recorded during relaxation are replaced by a tonic activity as soon as the dog stands up and begins to walk. Calibration, 100 μV and 1.5 sec.