Differentiation of Neuroblastoma Cells Induced by Nerve Growth Factor in vitro

Neuroblastoma is a fairly common malignant tumour in children. It has been found in autopsies of infants under 3 months of age over 40 times as often as its expected rate of occurrence[1]. Although neuroblastoma is considered a malignant tumour, observations have been reported suggesting that neuroblastoma may mature and form a benign tumour, ganglioneuroma[2,3].

Nerve growth factor (NGF), a macromolecular protein, is known to stimulate the neurite outgrowth of the embryonic sympathetic and sensory ganglia[4]. There are no earlier reports of the possible effect of purified NGF on human neuroblastoma cells in vitro. The effect of sera from normal and neuroblastoma patients have, however, been tested in the cultures of neuroblastoma cells without any significant differences[5]. No clinical correlations between the serum levels of NGF-like substance and the activity of neuroblastomas have been demonstrated[6]. In view of the obvious interest of the possible effect of NGF on the growth of neuroblastoma cells, the present study was carried out.

Neuroblastoma cells from a 4-year-old girl were cultivated in Falcon plastic petri dishes in a Tricine buffered Medium 199 completed with 10% heat-inactivated newborn calf serum and 300 mg/100 ml of glucose. This culture medium has been found suitable for culturing sympathicoblasts from chick embryos and it has been described in detail by HERVONEN and RECHARDT[7]. After preliminary culture for 2 days, small clusters of cells were subcultured in the same medium without and with 1 IU/ml of purified NGF (Burroughs and Wellcome Research Laboratories, England). The cells were cultured from 1 to 3 weeks, were observed daily by phase contrast microscope, and photographed.

Neuroblastoma cells were observed in the cultures as small round cells, which often formed small clusters. These cells seemed to be incapable of adhering on the surface of the plastic petri dishes. The neuroblastoma cells in the control medium without NGF remained round and failed to produce any processes (Figure 1). After an initial delay from some days to 1 week's period, several neuroblastoma cells were observed growing on the surface of the culture medium. In the medium containing NGF, several cells were observed to grow processes, as illustrated in Figures 2 and 3, in which long axon-like processes are seen.

The neuroblastoma cells were not cloned. Therefore, it is possible that other kinds of cells have originated in the

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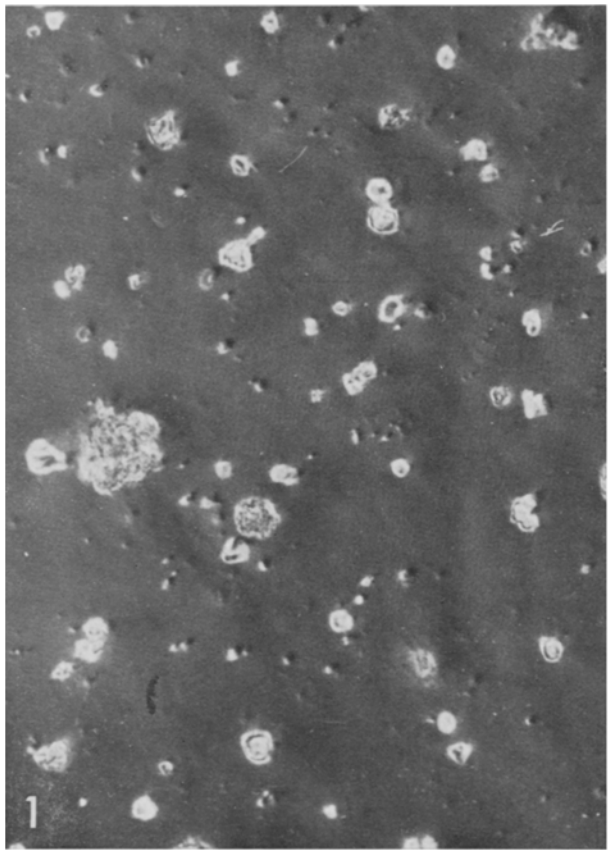

Fig. 1. Living neuroblastoma cells cultured for 1 week without NGF. No nerve fibre growth is seen. Dark field microscopy. Photographed through the culture dish. ×400.

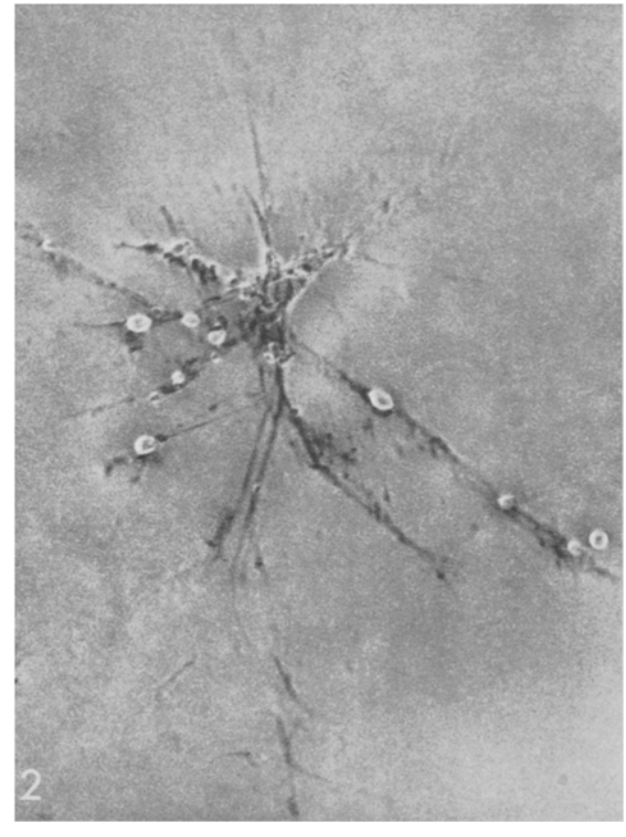

Fig. 2. Living neuroblastoma cells cultured for 1 week with 1 IU NGF in the culture medium. Several branching nerve fibres originate from the cells. ×400.

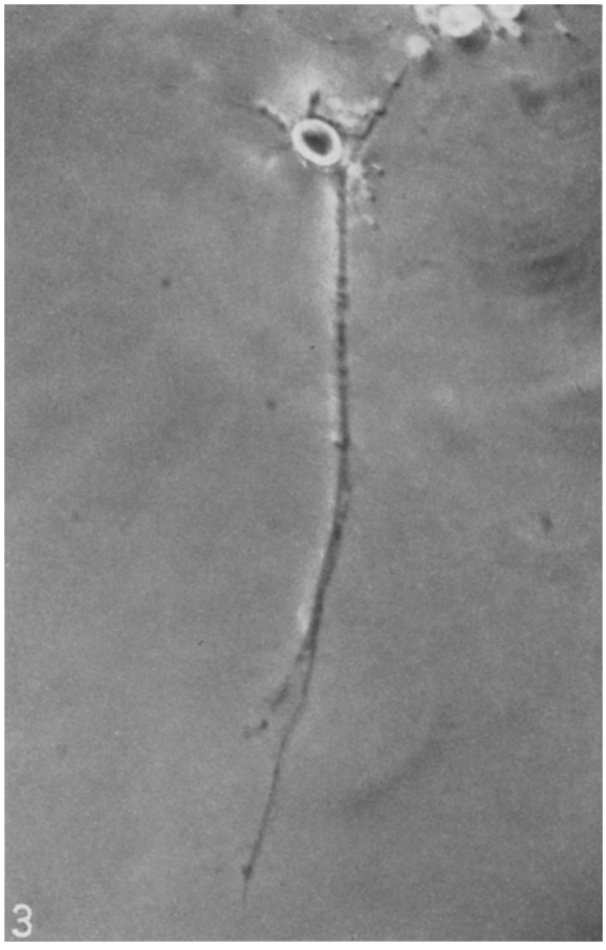

Fig. 3. Long axon-like process originates from a cell cluster. Phase contrast microscopy. Photographed through the culture dish. ×500.

tumour, like more mature cell types or still undifferentiated cells, which would behave like neural crest cells and be sensitive to NGF. On the other hand, no axon formation was observed in cultures without NGF, although the cells were taken from the same neuroblastoma. Several agents are known to induce the differentiation of neuroblastoma cells such as prostaglandins and cyclic AMP[8,9]. The promotion of neurite growth with NGF would be mediated through cyclic AMP, as has also been suggested, by ROISEN et al.[10].

Further studies on the nature and maturation stages of the processes produced by cultured neuroblastoma cells with NGF are needed, i.e. by fluorescence and electron microscopy. However, our observations show that purified NGF causes differentiation in vitro of the tumour cells and suggest that the spontaneous transformation of human neuroblastomas to ganglioneuromas may be due to the action of a NGF-like substance.

Zusammenfassung. Durch Züchtung in vitro wird gezeigt, dass Zellen eines menschlichen Neuroblastoms unter dem Einfluss eines Nervenwachstumsfaktors die Fähigkeit haben, sich in Nervenzellen zu differenzieren und somit die Zellen dieses bösartigen Tumors histogenetische Potenzen besitzen, welche nicht aktiviert werden.

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Development of the New Pacinian Corpuscles. Studies on the Foreign Innervation of Mesentery

Development of the receptors is one of the most important but unfortunately unsolved problems of sensory physiology. It is well known that the growth of afferent nerve fibres produces sensory receptors specific to the given tissue. However, it is yet to be seen what determines the development of such receptors: the properties of nerve fibres[1,2] or just the specification of tissue elements[3-5].

When studying the process of receptor development, using not only morphological but also physiological methods, it is very important to have a possibility of continuous observation of a single sensory element at various stages of development. So, the Pacinian corpuscles of cat mesentery, visible even to the naked eye, are well suited for a study of this kind. Preliminary report was published elsewhere[6].

The first series of experiments were performed to find out the regeneration ability of mechanoreceptors of the mesocolon. In 16 adult cats the Pacinian corpuscles were extirpated or simply the nerve fibres were severed. The data show that even 8 months after operation the regeneration of receptors from the remained nerve fibres could not be seen anywhere. These results confirm well with other data[7-11].

After finishing the first part of experiments, we investigated the effect of additional innervation of mesentery tissue by the somatic sensitive nerve (n. saphenus) foreign to this tissue, which ramifies in the hairy skin of an animal where Pacinian corpuscles are absent. To accomplish this task, the nerve was dissected on the level of the knee joint and then its central end was guided in to the abdominal cavity and led between the two layers of mesentery. Originally, there were no Pacinian corpuscles at all in the mesocolon of some cats. In other cats all Pacinian corpuscles were extirpated from

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