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Dedicated to Rita Levi-Montalcini

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INTRODUCTION

Rita Levi-Montalcini and the nerve growth factor

With this special volume of Biomedical Reviews we bring to you an update of Nerve **Growth Factor in Health and Disease.**

In the early 1930's Rita Levi-Montalcini was a research associate working with the eminent neuroanatomist Guiseppe Levi in Turin, Italy. Viktor Hamburger, at Washington University, St Louis, Missouri, sent a copy of his 1934 paper to Guiseppe Levi, who shared it with Levi-Montalcini. She was inspired by the results but was dubious about the validity of the Hamburger's recruitment hypothesis. Together with Levi, she reexamined the effects of limb bud removal on spinal ganglia in the chick embryo. Levi-Montalcini and Levi postulated that after the loss of peripheral targets, neuronal cell death, not the failure of recruitment as suggested by Hamburger, correctly explained the results of the limb removal experiments. In historic perspective, results of Levi-Montalcini, obtained with Levi (in Turin, 1942) and with Hamburger (in St Louis, 1949), showed that many neurons normally die during development and that a major effect of target removal is the perturbation of this normal death process, which, since 1972, has been known as apoptosis, a specific mode of programmed cell death. Indeed, she did propose that both normal cell death and target removal-induced death result from a lack of target-derived signals necessary for growth and survival. In 1950, Levi-Montalcini and Hamburger introduced the term trophic or neurotrophic to designate these hypothetical signals. In this context, "neurotrophic" is distinct from "neurotropic", indroduced by Santiago Ramon y Cajal in his 1882 "hypothesis chemotaxica", and which refers to diffusible chemoattractant signals derived from target cells, to guide the migration of axons toward the target. Levi-Montalcini, Hamburger, and Stanley Cohen, using subsequently transplanted mouse sarcoma 180, snake venom, and male mouse submandibular glands, found that they all elicit, in a crescendo fashion, growth-stimulating effect from neurons. Despite this early conceptual breakthrough it would be more than 30 years before the accumulated convincing evidence in support of the neurotrophic theory was achieved. The discovery of NGF was marked by a rare combination of scientific reasoning, intuition, and chance, the latter "favors only the mind that is prepared", quoting Louis Pasteur. Here a table presents major events in the discovery of NGF. and recognition of its importance for neuronal life and death.

Table. Major events in the saga of the nerve growth factor*

1934-1939 Hamburger discovered that removal of the limb bud in the chick embryo resulted in reduced numbers of sensory and motor neurons in the spinal cord. He proposed that targets act on innervating neurons by providing signals that recruit undifferentiated cells to develop into sensory and motor neurons.

1942 Levi-Montalcini and Levi confirmed that limb bud removal reduced the number of sensory and motor neurons but proposed that the target-derived signals act to maintain the survival of differentiated neurons.

1948 Bueker, a former student of Hamburger, implanted mouse tumors into the region of the chick hind limb as a source of rapidly growing peripheral "target" tissue in an attempt to identify the cellular source of normal target-derived signals. One tumor, sarcoma 180, was found to produce Amodest but significant increase in the size of limb sensory ganglia, without effect on motor neurons. He concluded that a quickly growing tissue was furnished with enriched nerve supply.

1949 Hamburger and Levi-Montalcini repeated earlier limb removal experiments. In addition, they discovered that many sensory neurons undergo a period of normal or naturally occurring cell death. They proposed that removal of a limb bud acts to enhance the

Table *(continued)*

normal cell death by perturbing target-derived signals that promote neuronal survival. In the today's context, they proposed an antiapoptotic action of the hypothetical target-secreted signals.
1951-1954 Levi-Montalcini and Hamburger repeated the Bueker experiment using sarcoma 180 and discovered an even more striking effect of the tumor on both sensory and sympathetic ganglia. They proposed that the tumor cells produce a diffusible factor, they named firstly nerve growth-stimulating factor, later named nerve growth factor (NGF).
1956 Cohen and Levi-Montalcini, in attempt to purify the tumor-derived nerve growth-stimulating factor, used snake venom as a rich source of phosphodiesterase, a nucleic acid-destroying enzyme, for the separation of nucleic acids and protein fractions in the tumor material. To their great surprise, the tumor fraction containing the snake venom was several thousand-fold more potent that control tumor homogenates in promoting nerve growth, both *in vitro* and *in vivo.*
1960 Cohen examined the mammalian homologue of the snake venom gland, the salivary gland, and discovered that the male mouse submandibular glands were an even richer source of the same nerve growth-stimulating activity found in both the tumor and the snake venom. When an antiserum to the mouse submandibular gland factor was injected into newborn mice, all sympathetic neurons were lost, that is, immunosympathectomy.
1969 Bocchini and Angeletti described a method for purification of biologically active NGF from male mouse submandibular glands, the richest NGF source known. The activity is the beta subunit (beta-NGF, also know as 2.5S NGF) of an oligomeric macromolecule (7S NGF).
1971 Angeletti and Bradshaw identified the amino acid sequence of 2.5S NGF, as a homodimer, each chain being with molecular weight of 13 250 D, and containing 118 amino acids, connected *via* three disulfide bridges.
1983 Korsching and Thoenen developed a sensitive two-site immunoassay, allowing for the first time the detection of NGF in target tissues. With this method it was possible to demonstrate a strong correlation between the density of sympathetic innervation and target levels of NGF, a finding consistent with the neurotrophic theory.

* Levi-Montalcini R. *The Saga of the Nerve Growth Factor. Preliminary Studies, Discovery, FurtherDevelopment.WorldScizntific,* Singapore, 1997.
The present table is a modification of Table 20.1, page 583 in *Funtamental Neuroscience,* MJ Zigmond, FE Bloom, SC Landis, JL Roberts, LR Squire, editors. First edition. 1998. Academic Press, San Diego, London, Boston, New York, Syndey, Tokyo, Toronto.

Rita Levi-Montalcini may also be appreciated as a writer. She recently wrote five books. The first, "In Praise of Imperfection" is a very inspiring autobiographic work where she tells of her success, but also of the delays and distractions and potential mistakes researchers can make and their tendency to underestimate difficulties. A second one entitled // *tuo Futuro* is dedicated to young people who during their early lives often face the need to make vital choices. In the third book, entitled *Senza Olio e contra Vento,* she tells 10 true stories of well-known or unknown people united by the courage and honesty they showed in very difficult moments of their lives. In the most recent book *"L 'asso nella Manica a Brandelli",* she discusses the capacity and creativity of famous elderly people and how to maintain or improve cognitive abilities in later life. From a scientific point of view the most important book is perhaps "The Saga of the Nerve Growth Factor". This is a scientific book reporting her experience as a medical scientist during her early postgraduate years, her later studies on the NGF discovery and her findings concerning recent developments in NGF studies, including the hypothesis of NGF's role in homeostatic, or, as suggested more recently, homeodynamic responses.

The neuroscientist AD Smith wrote in 1986 that Levi-Montalcini is "one of the chief protagonists of the most glorious episode in the modern history of neuroscience". It is therefore a rather difficult task even for a disciple, Luigi Aloe, of some 30 years standing and a close scientific collaborator of this famous scientist to report all her significant contributions. Therefore, for the readers who find our attempt'incomplete, we suggest reading the books mentioned above.

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