NGF MAY HOLD THE KEY—BUT TO WHAT?

Does the mysterious growth-triggering chemical figure in the cancer process? Is it some type of hormone that promises new medical benefits? Researchers say maybe, but they still don't know

BY JOAN AREHART-TREICHEL

During the late 1940s two Washington University scientists—Viktor Hamburger and Rita Levi-Montalcini—came up with some provocative research results. Specifically, if chick embryos were grafted onto mouse tumors, nerves in the embryos grew eagerly into the tumors. In fact, the nerves were more prone to innervate the tumors than embryonic tissues, and they thrust their way into the tumors' blood vessels as if in search of something.

But what was it? One bright spring day in 1951 Levi-Montalcini arrived at an exciting suspicion—that the young, developing nerves were attracted by some growth factor in the tumors. Yet how could she demonstrate the existence of such a factor with the usual time-consuming embryological experiments? What she needed to quickly prove the existence of such a chemical were tissue-culture tests. So, she go in touch with a friend in Rio de Janeiro who was in charge of a tissue-culture unit and asked whether she might fly down to Rio to culture some tissues in the unit. The answer was yes. In 1952, Levi-Montalcini was on her way to Brazil.

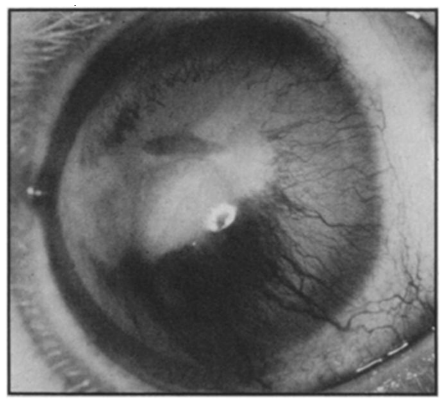
As soon as she reached her friend's lab she dissected tumors into small pieces, cultured the pieces in chick blood and embryonic extract, then incubated chick embryo nerves in the medium. Within 12 hours the nerve fibers reached eagerly toward the tumor pieces, then spread rapidly around them like rays of the sun. This halo effect was virtually indisputable proof that the pieces possessed some nerve growth-promoting chemical. Levi-Montalcini returned to St. Louis brimming with delight and the keen desire to isolate this singular molecule.

Indeed, it was isolated in 1954, thanks to the efforts of Levi-Montalcini, Hamburger and Stanley Cohen, a biochemist who had just arrived in their department at Washington University. They christened the molecule "nerve growth stimulating factor," or simply "NGF," and published their findings in the PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES.

This discovery of a quarter-century ago has turned out to be a landmark achievement in the field of neurobiology. NGF was, and still is, the only chemical known that stimulates the growth and differentiation (specialization) of immature nerve cells. Thus it is a powerful tool for better understanding how nerves grow and develop, and investigators throughout the world, including Levi-Montalcini, are now using it for this purpose. On the other hand, the more researchers learn about NGF's actions and origins, the more remains to be learned, it seems. Still, scientists studying NGF are convinced that they will eventually unmask its secrets and fully appreciate its value to life. They are also confident that as NGF comes more into focus, it will benefit clinical medicine, very possibly in ways that they could never have foreseen.

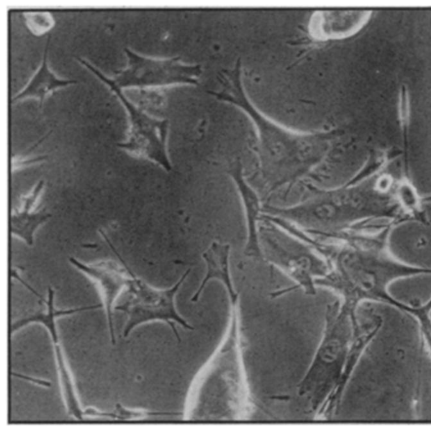
What has NGF divulged about nerve growth and development these past 25 years? One of the most striking observations is that only select immature nerves make use of NGF. For instance, only two kinds of healthy immature nerve cells in the embryo use NGF. They are sensory and sympathetic nerves, nerves that conduct sensory impulses and nerves that are involved in the contraction of blood vessels and the secretion of glands. Yet all slightly differentiated embryonic nerves that are cancerous—neuroblastoma cells—employ NGF. In newborn and adult mammals, in contrast to the embryos, only one kind of immature nerve uses NGF. It is the sympathetic nerve cell. No other kinds of immature nerves, including those of the brain and central nervous system, appear to engage NGF.

Ample information is also emerging about how NGF promotes growth and development at the target-cell level. First, NGF hooks up with receptors on the membrane of a burgeoning nerve-cell body. (It may also attach to receptors at the end of the nerve cell's budding axon, speculates Piero Calissano, one of Levi-Montalcini's current colleagues at the Laboratory of Cell Biology in Rome.) NGF then enters the nerve-cell body, rallies a protein in the cell cytoplasm called tubulin and organizes it into rows of tiny tubules. The massive, highly structured organization of these microtubules is then rapidly followed by growth of the nerve-cell's axon, and then the nerve starts functioning as a full-fledged nerve, conducting nerve impulses.



Gospodarowicz

Tiny blood vessels in cornea of eye grow in response to epidermal growth factor.



Todaro

NGF antibodies could kill melanoma cells (above) and provide anticancer treatment.

There are still some gaps in information about this cascade of events, however. Ruth Hogue Angeletti and her colleagues at the University of Pennsylvania Medical School are trying to find out what happens to NGF after it interacts with membrane receptors. And is there really a casual relationship between the organization of microtubules and nerve growth and differentiation? Calissano is convinced that there is, but neither he nor other NGF researchers have yet proven it. And do other biochemical events triggered by NGF trigger nerve growth? For instance, Branislav Nikodyevic of the University of Skopje Medical School in Yugoslavia has found that NGF increases the concentration of the intracellular messenger cyclic AMP in a target-nerve cell. Yet Michael Young and his co-workers at Harvard Medical School report that cyclic AMP does *not* mediate NGF's effects on nerve growth (SN: 10/13/73, p. 229). So what is NGF

Levi-Montalcini: A determined inquirer.

doing "frisking around" with cyclic AMP, then? And how about NGF's demonstrated ability to increase the synthesis of two enzymes—tyrosine hydroxylase and dopamine hydroxylase—that sympathetic nerves use to make their nerve transmitter noradrenaline? Is this enzymatic influence linked with NGF's ability to make nerve cells grow? Unanswerable questions still.

If these gaps in knowledge about NGF's impact on nerve cells are bothersome, though, there is one that is even more nettling. Why is it that NGF is present in so many and diverse tissues and especially in tissues that seem to have little connection with nerve growth? Back in the late 1950s, for example, Levi-Montalcini and Cohen found that NGF is present in snake venom and mouse salivary glands in incredibly large amounts—far greater than in tumors. Studies by other investigators during the 1970s have revealed that glial cells, the supportive cells of nerves, also contain NGF. So do cancerous glial cells, cancerous nerve cells and cancerous skin cells. NGF even seems to be present in low concentrations in blood and urine.

What is NGF doing in all these tissues? Researchers can only speculate at this time. Still another big challenge, Angeletti points out, is determining whether these tissues make NGF or if they get it from some other source. For instance, mouse salivary glands make NGF, or they may get it from sympathetic nerves or glial cells that innervate it.

And if these questions are unsettling, still other tough ones face NGF scientists—determining whether NGF has any relationship to other growth factors that have been discovered in its wake. In 1962, Cohen (after he left Washington University to take a post at Vanderbilt University) discovered an epidermal growth factor in mouse salivary glands that stimulates the growth of epidermal skin cells. Still other growth factors have been discovered since the late 1960s by other

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Rita Levi-Montalcini: The woman who started it all

A modern six-floor office building can be found on the corner of Via Romagnosi and Via Francesco Carrara in downtown Rome. Surrounded by traffic snarls, impassioned Latin drivers and deafening building construction, this edifice is a far cry from the proverbial Ivory Tower. Yet it houses the Laboratory of Cell Biology—Italy's second-largest biological research institute.

Even more remarkable, this 80-scientist lab is headed by a handsome woman who, in her elegant black dress, gold brooch and rings, looks more like a fashion plate than a scientist. She is Rita Levi-Montalcini, codiscoverer of nerve growth factor, one of Italy's leading scientists and recipient of numerous awards for her NGF achievements, notably membership in the U.S. National Academy of Sciences and the Papal Academy of Rome (the only woman ever elected).

How did Rita Levi-Montalcini come to make such contributions to 20th century science? "I'm from an intellectual Jewish family in Turin, Italy," she explained to me during a recent interview in her Rome lab. Even with this background, though, she had to overcome her father's Victorian attitude that women should not have careers, in order to graduate as a neurologist from the University of Turin Medical School in 1936. And then from 1940 to 1944, her growing interest in neuroembryological experiments had to be conducted secretly as she and her family attempted to survive the anti-Semitic campaign and slaughter of Fascist and Nazi Italy. In 1947, however, Viktor Hamburger of Washington University at Saint Louis, Mo., offered her a position so that they could work together on neuroembryological research. She accepted, and thus began the most productive and exhilarating span of her scientific career—the discovery of nerve growth factor—from 1948 to 1954.

Since 1954, she and various colleagues have learned much about NGF and are attempting to learn still more. She conducts her research both at Washington University, where she fulfills her duties as professor part of the year, and at the Rome lab, where she has directed various research activities, including NGF studies, since 1969.

During the day I spent in Levi-Montalcini's Rome lab, I came to know not only one of the world's leading neurobiologists but an exceptionally fine human being. The first thing that struck me was her demeanor. She walks with grace and has a rich, musical laugh that hints at spiky cedars, the scent of pines and other Roman delights. Renato Dulbecco, a 1975 Nobel laureate at the Imperial Cancer Laboratory in London, and a classmate of Levi-Montalcini back in their medical school days in Turin, agrees that she has "tremendous poise. A mutual friend of ours," he told me, "calls her 'La Regina,' which means 'the Queen' in Italian." Thus, she is not only a topnotch scientist but a very womanly woman.

The next thing that impressed me was her vivaciousness, her zest for life. I lunched with her and Pietro Calissano, a young neurochemist who is both her current NGF collaborator in Rome and a close friend. As Levi-Montalcini ate her zucchini, she laughed with gusto at the stories Calissano was telling. "Rita has the enthusiasm of a 25-year-old," Calissano later confided.

Even more striking is Levi-Montalcini's kindness. In spite of her 12-hour work day (she starts her NGF research at home at 5 a.m.), she found time after my visit to shepherd me through maddening Rome traffic to get a cab. "I have seen her give her staff both moral and financial help when they were in need," Calissano says.

"She is a marvelous person to work with," asserts Lenore Friedman, Levi-Montalcini's secretary at Washington University for 14 years. "She is considerate, appreciative and warm. I remember once I was working late and she had a lot of letters to get out. She stopped to help me fold and insert them in envelopes, which is extraordinary. I have worked for a lot of people, but I have never really enjoyed a job like this."

But probably the greatest commentary on Levi-Montalcini's phenomenal generosity is her "hobby." During my Rome visit I had the honor of dining with Levi-Montalcini and her twin Paola, a well-known Italian artist, at their apartment on Viala di Villa Massimo. Toward the end of dinner, Levi-Montalcini excused herself to take a phone call from a young American mathematician. As she explained on her return, she helped place this young woman with one of Italy's finest mathematicians in Pisa. "Helping young people is my hobby," she smiled, and her smile was as scintillating as a day in Rome.

Nor does Levi-Montalcini's "hobby" know any national boundaries. She has helped some 50 young Italian scientists find positions in the United States as well.

—Joan Arehart-Treichel

opposition—the low-flying cruise missile and B-1 bomber, which would be harder to track.

- What is the significance of the "Rudakov connection"? Last year, a leading Soviet physicist, L. I. Rudakov, gave a talk on electron beams at four U.S. laboratories and one conference, and suddenly the *American* scientists were not allowed to discuss openly what he said. Speculation on why has run the gamut from warnings of an "Idi Amin bomb" (a nuclear weapon cheap enough to be built by small countries) to the "shopping trip" theory (the idea that Rudakov was telling a little, but wanting to find out a lot in return).
- Assuming the Semipalatinsk facility has anything to do with CPBs, why would the Russians spend so much (reportedly $3 billion) on a project whose applicability as a weapon appears so shaky from the outset? On the strength of this interest alone, some American physicists are trying to get funding to push similar work, which has languished in this country.
- Finally, what is likely to be the effect of this flap on high-energy physics, once the most open and cooperative of fields? That openness might well become the first and only victim of a CPB weapon. □

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. . . NGF

workers. These include a macrophage growth factor, made by fibroblasts (cells present in connective tissue); a fibroblast growth factor, made by the pituitary gland of the brain; and an ovarian growth factor, also made by the pituitary gland. Like NGF, these factors are proteins. However, their amino acid sequences differ from NGF's and from each other's. They also appear to exert different biochemical effects on their target cells. For instance, fibroblast growth factor induces DNA synthesis and cell division in a target cell, reports Denis Gospodarowicz of the Salk Institute for Biological Studies in San Diego. And while Cohen is still not sure how epidermal growth factor exerts its effects, he is sure that it does *not* act on microtubules as NGF does. Thus, "the relationship among these factors, if any, is still unknown," Young concludes.

Finally, should NGF and these other growth factors be classified as hormones, or should they be put in a tissue-enhancing category all their own? Gospodorowicz, who isolated the ovarian growth factor in 1974, reports that "it is distinct from known pituitary hormones." As for NGF, its actions are quite different from those of conventional hormones, Levi-Montalcini has found. And as Angeletti reported in the January BIOCHEMISTRY, the amino acid sequences of NGF from different sources are more similar to each other than they are to the protein hormone insulin, which they resemble to some degree. So are NGF and other growth factors hormones or not? "It's a very hazy area," Cohen concedes.

Meanwhile, more startling insights into NGF keep emerging from labs around the world, and they may well, like pieces of a jigsaw puzzle, finally bring NGF's true value to nerves and other tissues into focus and finally disclose its role in relation to other growth factors and conventional hormones. For instance, Young and his colleagues report in the April and May PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, that NGF is only a partial product of a parent molecule that is 10 times larger than it is, and whose amino acid sequence is grossly different from NGF. As soon as they isolate this compound, researchers may then be in a better position to understand the origin of NGF and what it does for the body. In other words, it may have an even larger role than nerve growth and development.

Also, before NGF's true impact on life is fully appreciated, emerging information about NGF may benefit medicine, and in some unexpected ways. A case in point: George J. Todaro, Robert N. Fabricant and Joseph E. DeLarco of the U.S. National Cancer Institute reported, in the February PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, that they have found receptors for NGF on cancerous pigment cells taken from several patients who died from malignant melanoma. Such receptors, they add, are not present on fibroblasts, epithelial cells and numerous other cell types. These findings suggest, as some past studies have, that cancer cells, especially melanoma, need NGF for some purpose. But the more provocative aspect of these results is that they might lead to better diagnosis and treatment of malignant melanoma.

In other words, if NGF receptors were found on a sample of pigment cells taken from a person, they might well indicate the presence of malignant melanoma, and possibly in its earliest stages. This sampling for NGF receptors might provide an early diagnosis for this form of cancer. In contrast, an antiserum to NGF or to its receptors might be devised, injected into a malignant melanoma patient and deprive cancer cells in the patient of needed NGF and lead to the cells' demise. Thus such an antiserum might make an effective form of treatment against malignant melanoma. In fact, the NCI researchers have reason to believe that such an antiserum might be even more effective against malignant melanoma after it has invaded the body than before because they have found even more NGF receptors on invasive cells than on noninvasive ones. So the first clinical uses for NGF may well emerge in the cancer arena rather than in the neurobiological one.

Thus, Levi-Montalcini, who watched with wonder the birth of her "miracle" molecule from the womb of malignant tissues, may well live to see NGF and NGF antibodies slay those very same tissues. The world of NGF is indeed baffling, but ripe with promise. □