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Stanley Cohen's and Rita Levi-Montalcini's Discoveries of Growth Factors Lead to 1986 Nobel in Medicine

Number 17 April 27, 1987

The 1986 Nobel Prize in physiology or medicine was jointly awarded to Stanley Cohen, professor of biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee, and Rita Levi-Montalcini, Institute of Cell Biology, Rome, Italy, for their discoveries of two growth factors that regulate cell development. Levi-Montalcini is one of only eight women to receive a Nobel Prize in science.[1] She is credited with the 1953 discovery of the nerve growth factor (NGF), while Cohen identified the epidermal growth factor (EGF) in 1962. These two early discoveries set the stage for the identification and characterization of additional growth-regulating substances that have increased our understanding of many disease states including neoplastic diseases and the neurological degeneration associated with senile dementia.

In the second issue of *The Scientist*™, Alexander Grimwade, director of ISI®'s *Atlas of Science*® division, observed that there are "at least 20 distinct areas of research that owe a heavy debt to Cohen and Levi-Montalcini's work."[2] Table 1 lists the 1986 *Science Citation Index*® research fronts involving growth factors; some of the topics covered by these fronts include cell division, cancer studies, and neural development.

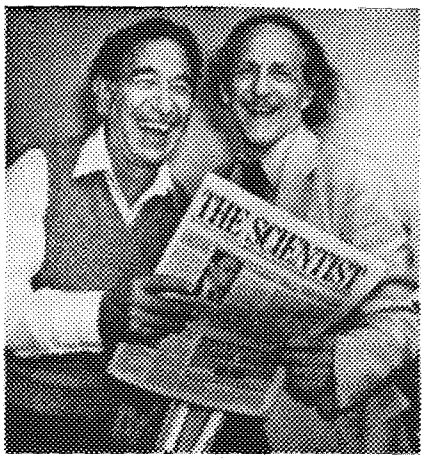
I had the opportunity to meet Dr. Cohen the day after the awards were announced, since I was in Nashville to observe the printing of the inaugural issue of *The Scientist*. It was fun to be able to congratulate Stan Cohen and at the same time present to him and his colleagues at Vanderbilt the first copies of *The Scientist*.

Nerve Growth Factor

Levi-Montalcini was born in 1909 in Turin, Italy, where she received her medical degree in 1936 from the University of Turin. During World War II, Levi-Montalcini was forced to leave the university by Italy's Fascist government. She persisted in her research by setting up a laboratory in her bedroom at home. When the Nazis occupied Italy, she and her family fled to Florence and lived underground for the rest of the war. After the war, she was reinstated at the University of Turin and resumed her research there.

In 1947 she came to the US to work in Viktor Hamburger's laboratory at Washington University in St. Louis, Missouri. Her early work in the US concerned the influences of peripheral tissues on the growth and maintenance of nerve cells.[3] In later studies Levi-Montalcini found that when a mouse tumor was transplanted into a chick embryo it produced a diffusible agent that stimulated nerve cell growth.[4,5] Using tissue culture, a technique that in the 1950s was not yet the standard tool it is today, Levi-Montalcini and coworkers were able to demonstrate that a chemical tumoral factor induced nerve cell growth,[6,7] and they named this chemical the nerve growth factor. As the Nobel Assembly noted, Levi-Montalcini's discovery of the NGF "is a fascinating example of how a skilled observer can create a concept out of apparent chaos."[8] The paper describing the NGF isolation technique is Levi-Montalcini's second most-cited paper (Table 2).

In 1953 biochemist Stanley Cohen joined Levi-Montalcini's lab at Washington Uni-



Stanley Cohen and Eugene Garfield

versity to identify and characterize the active agent. Born in Brooklyn, New York, in 1922, Cohen attended James Madison High School and Brooklyn College. He received a master's degree in zoology in 1945 from Oberlin College in Ohio and his PhD in biochemistry from the University of Michigan, Ann Arbor, in 1948.

Cohen was able to localize the growth factor in a fraction from tumor cells that contained protein and a trace of nucleic acid. To determine if NGF was located in the protein or the nucleic acid portion of the tumor cell, Cohen treated the material with snake venom, which contains enzymes that degrade nucleic acids. Unexpectedly, Cohen found that the snake venom itself contained NGF in larger concentrations than the mouse tumors. Cohen and Levi-Montalcini were then able to extract NGF from the venom and demonstrate that it is a protein.[9] Pursuing the theory that snake venom is secreted by a modified salivary gland, Cohen isolated a more active form of NGF from mouse salivary glands, which provided larger quantities of the material.[10]

With an abundant source of NGF available, new studies on the biologic characteristics of NGF began. In the last 30 years, researchers have established that NGF is necessary for the development and maintenance of sympathetic and certain sensory neurons. NGF is secreted by innervated tissue cells and binds to specific receptors on these nerve cells. It is then transported to the nerve cell body where it spurs the growth and elongation of the nerve fiber.[11] A 1968 paper describing the characteristics of NGF is Levi-Montalcini's most-cited paper, with over 790 cites.[11]

Epidermal Growth Factor

In 1959 Cohen left Levi-Montalcini's lab and moved to the Vanderbilt University School of Medicine. As a direct outgrowth of his work on NGF, he found that giving mice daily injections of salivary gland extracts containing NGF resulted in their eyelids opening earlier and their teeth growing in sooner.[12] Since these anatomical changes did not occur with injections of pure NGF, Cohen suspected a new factor was responsible. He isolated this new factor, and the 1962 paper describing the isolation technique is Cohen's fourth most-cited paper, as shown in Table 3. The paper is also core to the research front on "Identification and metabolism of epidermal growth factor" (#86-1756). Later studies confirmed that this substance has a stimulatory effect on the growth and differentiation of the cells of the epidermis; thus Cohen named the substance the epidermal growth factor.[13]

In the years that followed, Cohen was able to purify EGF and determine its amino acid sequence.[14] Cohen and his coworkers found that EGF stimulates glucose and amino acid transport, activates protein synthesis, and initiates DNA synthesis and cell replication.[15] Cohen also identified the protein on cell surfaces that acts as a receptor. Receptors bind EGF, and the EGF-receptor complex is taken into the cell.[16]

The study of cell receptors also played a role in the work of last year's Nobel Prize winners in medicine or physiology. Michael S. Brown and Joseph L. Goldstein, University of Texas Health Science Center, Dallas, were honored for their work in identifying the low-density lipoprotein (LDL) receptor pathway—the mechanism controlling how the body's cells obtain cholesterol.[17] Cohen, Brown, and Goldstein collaborated

Rita Levi-Montalcini

growth-factor research. Additional growth factors are being discovered and new applications are being explored.

To show how the field of growth factors has changed and advanced in the last seven years, Figure 1 presents a historiograph tracing research on EGF and NGF from 1980 to 1986. Each box includes the research-front name, the number of core articles, and the number of citing papers. The fronts that are included are determined by the continuity of the core literature from year to year. If the same core documents are cited at the required thresholds in two adjacent years, then a "string" is established. By continuing this procedure, cluster strings are identified and expanded to a historiograph. While Figure 1 begins only with 1980, we have research fronts dating back as far as 1970 that contain core documents from Cohen and Levi-Montalcini. Those of you interested in receiving a historiograph from 1970 to 1980 should contact me.

Table 1: The 1986 *SCI*® C1 research fronts involving nerve and epidermal growth factors. A = research-front number. B = research-front name. C = number of core papers. D = number of published papers. Asterisks (*) indicate research fronts that appear on the historiograph in Figure 1.

A	B	C	D
*86-0222	Mode of action of human insulin-like growth factor	35	716
*86-0259	Neuronal growth factor	56	1155
86-1179	Ionic signaling by growth-factor receptors	30	697
*86-1756	Identification and metabolism of epidermal growth factor	10	333
86-2927	Growth-hormone-releasing-factor—studies in humans and rats	11	234
*86-4303	Epidermal, insulin-like, platelet-derived, and transforming growth factors—mode of action and relation to oncogenes	13	694
86-5724	Effects of epidermal growth factor on growth and development of human cultured cells	11	165
86-7573	Mechanisms and consequences of growth-factor stimulation of cultured cells	2	69
*86-8096	Platelet-derived, transforming, and epidermal growth factors *in vivo* and *in vitro*	3	120

on a paper studying the characteristics of the cell receptor, using both EGF and LDL.[18]

Recent History

When NGF and EGF were first discovered, their potential importance was not immediately recognized. In an interview Cohen noted that "this had the advantage that people left you alone and you weren't competing with the world. The disadvantage was that you had to convince people that what you were working with was real."[19] Today, however, new recombinant DNA techniques have caused a burgeoning interest in

Applications

NGF has recently been found to be active not only in the peripheral nervous system but also in the brain. The brain cells that respond to NGF are also the ones that degenerate in Alzheimer's disease. Franz Hefti and William J. Weiner, Department of Neurology, University of Miami School of Medicine, Florida, are considering the possibility that this disease may result from a failure to produce or respond to NGF.[20] We have discussed Alzheimer's disease earlier.[21]

EGF is linked to oncogenes, which are derived from normal cellular genes that have

Table 2: Rita Levi-Montalcini's eight most-cited papers, based on the *SCI®*, 1955-1986. A=number of citations. B=bibliographic data. The *SCI* research fronts to which the paper is core are included in parentheses.

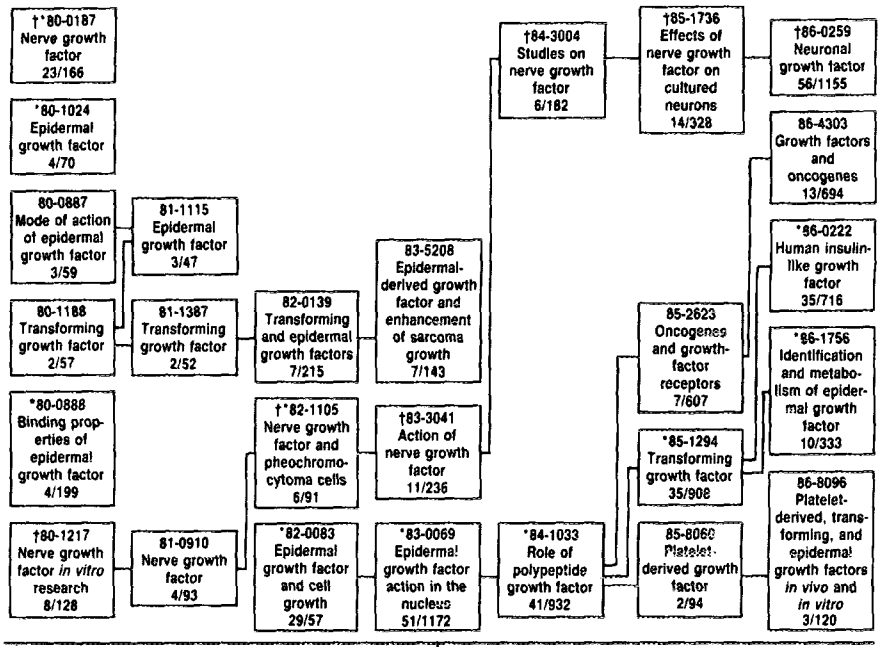
A	B
793	**Levi-Montalcini R & Angeletti P U.** Nerve growth factor. *Physiol. Rev.* 48:534-69, 1968. (70-0469, 71-0577, 73-0979, 74-0415, 75-0151, 76-0068, 77-0394, 79-1942, 80-1217, 81-0378, 84-3004, 85-1736, 86-0259)
267	**Levi-Montalcini R, Meyer H & Hamburger V.** *In vitro* experiments on the effects of mouse sarcomas 180 and 37 on the spinal and sympathetic ganglia of the chick embryo. *Cancer Res.* 14:49-57, 1954. (70-0415, 78-0190, 79-1941, 80-0187)
254	**Levi-Montalcini R.** The nerve growth factor: its mode of action on sensory and sympathetic nerve cells. *Harvey Lect.* 60:217-59, 1966.
210	**Levi-Montalcini R & Booker B.** Destruction of the sympathetic ganglia in mammals by an antiserum to a nerve-growth protein. *Proc. Nat. Acad. Sci. USA* 46:384-91, 1960. (82-1105, 86-0259)
186	**Levi-Montalcini R & Angeletti P U.** Essential role of the nerve growth factor in the survival and maintenance of dissociated sensory and sympathetic embryonic nerve cells *in vitro*. *Dev. Biol.* 7:653-9, 1963.
180	**Levi-Montalcini R & Booker B.** Excessive growth of the sympathetic ganglia evoked by a protein isolated from mouse salivary glands. *Proc. Nat. Acad. Sci. USA* 46:373-84, 1960.
160	**Levi-Montalcini R & Cohen S.** Effects of the extract of the mouse submaxillary salivary glands on the sympathetic system of mammals. *Ann. N.Y. Acad. Sci.* 85:324-41, 1960.
156	**Angeletti P U & Levi-Montalcini R.** Sympathetic nerve cell destruction in newborn mammals by 6-hydroxydopamine. *Proc. Nat. Acad. Sci. USA* 65:114-21, 1970.

Table 3: Stanley Cohen's seven most-cited papers, based on the *SCI®*, 1955-1986. A=number of citations. B=bibliographic data. The *SCI* research fronts to which the paper is core are included in parentheses.

A	B
733	**Carpenter G & Cohen S.** ^{125}I-labeled human epidermal growth factor: binding, internalization, and degradation in human fibroblasts. *J. Cell Biol.* 71:159-71, 1976. (78-0214, 79-0056, 80-0888, 82-0083, 83-0069, 84-4091, 85-3736)
688	**Savage C R & Cohen S.** Epidermal growth factor and a new derivative. *J. Biol. Chem.* 247:7609-11, 1972. (77-1029, 78-0214, 79-0056, 83-0069, 84-4091, 85-1294)
585	**Carpenter G & Cohen S.** Epidermal growth factor. *Annu. Rev. Biochem.* 48:193-216, 1979. (82-0083, 83-0069, 85-1294)
558	**Cohen S.** Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the new-born animal. *J. Biol. Chem.* 237:1555-62, 1962. (75-0169, 76-0204, 77-1029, 78-0214, 79-0056, 80-1024, 82-0138, 85-1294, 86-1756)
429	**Ushiro H & Cohen S.** Identification of phosphotyrosine as a product of epidermal growth factor-activated protein kinase in A-431 cell membranes. *J. Biol. Chem.* 255:8363-5, 1980. (81-0108, 82-0083, 83-0069, 84-1033, 85-1294)
352	**Cohen S.** Purification of a nerve-growth promoting protein from the mouse salivary gland and its neuro-cytotoxic antiserum. *Proc. Nat. Acad. Sci. USA* 46:302-11, 1960. (70-0469, 76-0099, 78-0190, 80-0187, 82-1105)
330	**Cohen S, Carpenter G & King L.** Epidermal growth factor-receptor-protein kinase interactions. *J. Biol. Chem.* 255:4834-42, 1980. (81-0108, 82-0083, 83-0069, 84-1033, 85-1294, 86-0222)

somehow been altered so that they make abnormal products or make their products in an uncontrolled fashion. In 1984 J. Downward, M.D. Waterfield, E. Mayes, G. Scrace, N. Totty, and P. Stockwell, Protein Chemistry Laboratory, Imperial Cancer Research Fund, London; Y. Yarden and J. Schlessinger, Department of Chemical Immunology, Weizmann Institute of Science, Israel; and A. Ullrich, Genentech Incorporated, San Francisco, found that the EGF receptor is similar to a part of the sequence of the oncogene v-*erb-B* transforming protein. This study indicates that the v-*erb-B* gene encodes a truncated version of the EGF receptor that may be locked in the "on" position, which may cause a continuous growth signal to the cells containing it.[22] Downward's paper was the fourth most-cited paper in our study of the most-cited 1984 life-sciences articles.[23] It is core to the front on "Epidermal, insulin-like, platelet-derived, and transforming growth factors—mode of action and relation to oncogenes" (#86-4303) in Table 1. Figure 2 shows a higher-level multidimensional-scaling map for research fronts included in "Growth factor and oncogenes" (#85-0121), showing how a variety of higher-level fronts from this field are linked by co-citation.

Figure 1: Historiograph tracing research on epidermal and nerve growth factors, 1980-1986. Numbers of core/citing papers are indicated at the bottom of each box. An asterisk (*) indicates that Cohen is a core author in that research front; a dagger (†) indicates Levi-Montalcini is a core author.



Recent studies have focused on the role EGF may have in promoting wound healing. Gregory L. Brown, Luke Curtsinger, Joseph R. Brightwell, Douglas M. Ackerman, Gordon R. Tobin, Hiram C. Polk, and Gregory S. Schultz, University of Louisville, Kentucky, and Carlos George-Nascimento and Pablo Valenzuela, Chiron Corporation, Emeryville, California, found EGF very effective in accelerating epidermal healing in cuts and burns. This study suggests the potential clinical use of EGF for the healing of burns, wounds from trauma, diabetic ulcers, and skin graft donor sites.[24]

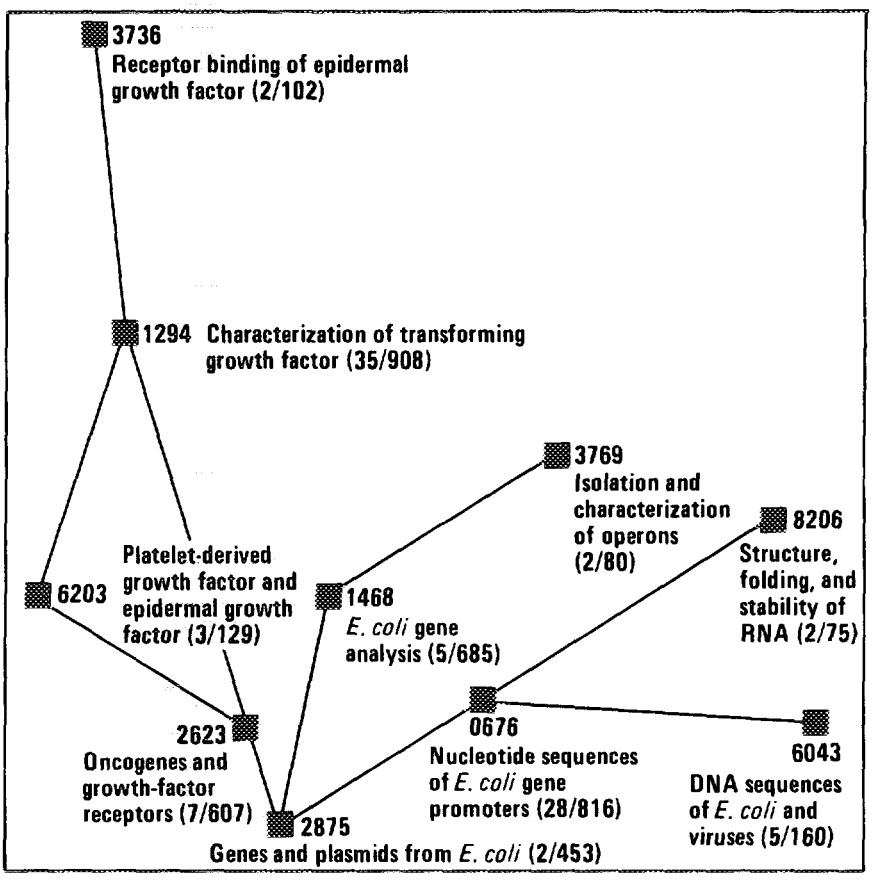
Other Growth Factors

In recent years, additional growth factors have been isolated and characterized. The Nobel Assembly noted that "all research groups who discovered 'new' growth factors have followed in the tracks of Levi-Montalcini and Cohen."[8] Reviewing the list of growth-factor-related research fronts in Table 1, you'll notice that substances other than NGF and EGF are mentioned. For instance, the research front on "Growth-hormone-releasing-factor—studies in humans and rats" (#86-2927) contains 11 core papers characterizing this new factor. The core paper cited most frequently in this front is by 1977 Nobel laureate Roger Guillemin and colleagues, Laboratories for Neuroendocrinology, Salk Institute for Biological Studies, La Jolla, California. This paper describes the isolation and subsequent amino-acid-structure analysis of a peptide with growth-hormone-releasing activity isolated from a human tumor of the pancreas.[25]

Conclusion

While the value of growth factors was not immediately understood, today the scientific community is convinced of their importance. As a result, Levi-Montalcini and Cohen have been recipients of some very prestigious awards other than the Nobel Prize.

Figure 2: Higher-level multidimensional-scaling map for research front #85-0121, "Growth factor and oncogenes." Numbers in parentheses indicate the numbers of core/citing papers in each research front.



They were corecipients of the 1986 Lasker Awards, presented by the Albert and Mary Lasker Foundation. In 1985 Cohen received the Gairdner Foundation International Award of Merit, and in 1983 Levi-Montalcini was awarded the Louisa Gross Horwitz Prize. Sociologist Harriet Zuckerman, Columbia University, New York, calls these awards "premonitory prizes" because they often anticipate the Nobel Prize.[26]

As the Nobel Assembly noted, "the discovery of NGF and EGF has opened new fields of widespread importance to basic science."[8] The persistence and creativity demonstrated by Levi-Montalcini and Cohen are excellent examples of Nobel-class science. In the near future we will discuss the Nobel Prize winners in literature, economics, physics, and chemistry.

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My thanks to C.J. Fiscus and Lisa Holland for their help in the preparation of this essay.

REFERENCES

1. **Opfell O S.** *The lady laureates.* Metuchen, NJ: Scarecrow Press, 1986. p. vi.
2. **Grimwade A.** Nobel winners stimulate research. *The Scientist* 17 November 1986. p. 4.
3. **Hamburger V & Levi-Montalcini R.** Proliferation, differentiation and degeneration in the spinal ganglia of the chick embryo under normal and experimental conditions. *J. Exp. Zool.* 111:457-501, 1949.
4. **Levi-Montalcini R.** Effects of mouse tumor transplantation on the nervous system. *Ann. N.Y. Acad. Sci.* 55:330-43, 1952.
5. **Levi-Montalcini R & Hamburger V.** A diffusible agent of mouse sarcoma, producing hyperplasia of sympathetic ganglia and hyperneurotization of viscera in the chick embryo. *J. Exp. Zool.* 123:233-88, 1953.
6. **Levi-Montalcini R.** In vivo and in vitro experiments on the effect of mouse sarcoma 180 and 37 on the sensory and sympathetic system of the chick embryo. *Proceedings of the XIV International Congress of Zoology*, 5-12 August 1953, Copenhagen, Denmark. Copenhagen: Danish Science Press, 1956. p. 309.
7. **Levi-Montalcini R, Meyer H & Hamburger V.** *In vitro* experiments on the effects of mouse sarcomas 180 and 37 on the spinal and sympathetic ganglia of the chick embryo. *Cancer Res.* 14:49-57, 1954.
8. **Karolinska Institute. Nobel Assembly.** Press release. No. 1445N. 13 October 1986. 7 p.
9. **Cohen S & Levi-Montalcini R.** A nerve growth-stimulating factor isolated from snake venom. *Proc. Nat. Acad. Sci. USA* 42:571-4, 1956.
10. **Cohen S.** Purification of a nerve-growth promoting protein from the mouse salivary gland and its neuro-cytotoxic antiserum. *Proc. Nat. Acad. Sci. USA* 46:302-11, 1960.
11. **Levi-Montalcini R & Angeletti P U.** Nerve growth factor. *Physiol. Rev.* 48:534-69, 1968.
12. **Cohen S.** Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the new-born animal. *J. Biol. Chem.* 237:1555-62, 1962.
13. **Cohen S & Elliott G A.** The stimulation of epidermal keratinization by a protein isolated from the submaxillary gland of the mouse. *J. Invest. Dermatol.* 40:1-5, 1963.
14. **Savage C R, Inagami T & Cohen S.** The primary structure of epidermal growth factor. *J. Biol. Chem.* 247:7612-21, 1972.
15. **Cohen S, Carpenter G & King L.** Epidermal growth factor-receptor-protein kinase interactions. *J. Biol. Chem.* 255:4834-42, 1980.
16. **Carpenter G & Cohen S.** Epidermal growth factor. *Annu. Rev. Biochem.* 48:193-216, 1979.
17. **Garfield E.** The 1985 Nobel Prize in Medicine—Michael S. Brown and Joseph L. Goldstein have revolutionized our knowledge about cholesterol metabolism and heart disease. *Current Contents* (38):3-13, 22 September 1986.
18. **Carpentier J-L, Gorden P, Anderson R G W, Goldstein J L, Brown M S, Cohen S & Orci L.** Co-localization of ^{125}I-epidermal growth factor and ferritin-low density lipoprotein in coated pits. A quantitative electron microscopic study in normal and mutant human fibroblasts. *J. Cell Biol.* 95:73-7, 1982.
19. **Marx J L.** The 1986 Nobel Prize for Physiology or Medicine. *Science* 234:543-4, 1986.
20. **Hefti F & Weiner W J.** Nerve growth factor and Alzheimer's disease. *Ann. Neurol.* 20:275-81, 1986.
21. **Garfield E.** Senility: a major health problem in need of a solution. *Essays of an information scientist.* Philadelphia: ISI Press, 1983. Vol. 5. p. 138-42.
22. **Downward J, Yarden Y, Mayes E, Scrace G, Totty N, Stockwell P, Ullrich A, Schlessinger J & Waterfield M D.** Close similarity of epidermal growth factor receptor and v-*erb-B* oncogene protein sequences. *Nature* 307:521-7, 1984.
23. **Garfield E.** The most-cited 1984 life-sciences articles highlight AIDS research. *Current Contents* (49):3-17, 8 December 1986.
24. **Brown G L, Curtsinger L, Brightwell J R, Ackerman D M, Tobin G R, Polk H C, George-Nascimento C, Valenzuela P & Schultz G S.** Enhancement of epidermal regeneration by biosynthetic epidermal growth factor. *J. Exp. Med.* 163:1319-24, 1986.
25. **Guillemin R, Brazeau P, Bohlen P, Esch F, Ling N & Wehrenberg W B.** Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. *Science* 218:585-7, 1982.
26. **Zuckerman H.** *Scientific elite.* New York: Free Press, 1977. p. 33.