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Viktor Hamburger (1900–2001): Journey of a Neuroembryologist to the End of the Millennium and Beyond

In Memoriam

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But will there be anyone to remember us in another thousand years? Surely it's not possible that not a single molecule of memory will be found for us, like a yellowing manuscript at the bottom of a forgotten drawer, whose very cataloguing guarantees its eternity even if not a single reader ever discovers it. But will the catalog itself survive?

—A.B. Yehoshua, 1998

Introduction

Oblivious to the voyeuristic-like attention of the two scientists peering at it from outside, within the protected environment of a temperature- and humidity-controlled plexiglass chamber, a chicken embryo, after many hours of preparation, had begun its final embryonic performance—hatching—a one act drama lasting less than an hour, for which the scientists had coined the term *climax*, which was defined as the process of opening and escaping from the shell, although admittedly the use of this term as a double entendre hadn't entirely escaped their attention.

One of the observers was Viktor Hamburger, age 66, and the other one was me, a 27-year-old Ph.D. student, still somewhat awed to be sitting shoulder to shoulder with the doyen of neuroembryology, even after 3 1/2 years of collaborative research with him on several projects (see below), including this one on the prehatching motility and hatching behavior of the chick (Hamburger and Oppenheim, 1967). The many hours and days we spent together in the 1960s observing the acrobatics of the developing embryo remain as vivid and cherished memories, even overshadowing many of the events occurring in the outside world at the time, such as racial issues, the Bay of Pigs, President Kennedy's assassination, the Vietnam War, and the Beatles. For it was a result of our conversations during that period that I first began to grasp the beauty and joy of science. In thinking back on those days, I am reminded of Göethe's comment that "Überall lernt man nur van dem, den man liebt" (In general, one only can learn from someone one loves).

Our time together with the chick embryo also captured for me aspects of Viktor's character and personality that helped to explain his success as a scientist and the qualities that have inspired students, colleagues, and friends for more than 80 years. For me, the chick embryo served the role of a *Shadchen* (Yiddish for a "matchmaker"), initiating a close friendship and an affair of the mind and the heart that lasted for almost 40 years.

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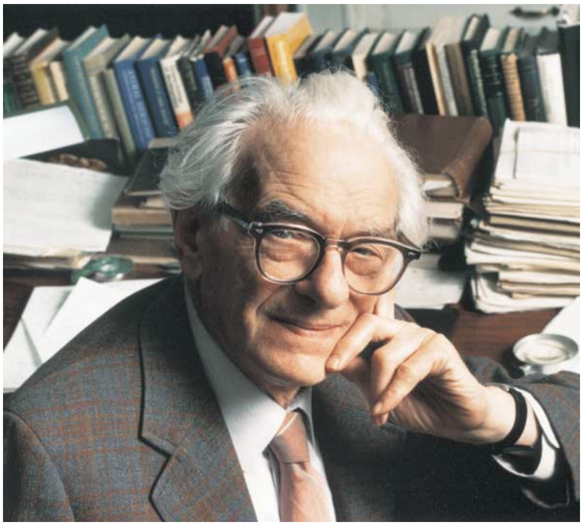

Viktor Hamburger in his office at Washington University in St. Louis in 1987.

Born on July 9, 1900, Viktor was conceived in the 19th century, lived for the entire 20th century, and died on June 12, 2001, in the 21st century. Notwithstanding our 40 years of friendship, having not participated in his first 60 years, I often felt like a relative newcomer in his life. For most of us, the first half of our lives is often more interesting and productive than the second half. However, because Viktor was an exception to this rule, I was able to directly observe and participate in events during the second half of his life that to a large extent define his scientific career. In trying to understand events prior to 1960, I have had to rely on the published literature, aided immensely, however, by conversations with Viktor and others who witnessed much of this period in his life. With this in mind, my primary goal here is neither strictly biographical nor hagiological but rather is an attempt to evaluate objectively his major scientific contributions.

A Synopsis of a Life in Two Centuries

Germany, 1900–1932

Childhood. By 1900, the year of Viktor's birth, Germany had existed as a unified country—the German Empire—for more than a quarter century following the union of 18 separate German states in 1871, created under the influence of Bismark and the Prussians. As a monarchy led by the first emperor, Wilhelm I, and then by his son, Wilhelm II, Germany prospered during the Wilhelmine era, and, by the beginning of World War I in 1914, it was the third leading industrial power in the world (Craig, 1978). Following the end of World War I in 1918, the monarchy ended and was replaced by the democratic Weimar Republic, which persisted until the victory of the National Socialists (the Nazis) in the election of 1930 and the subsequent rise of Hitler and the Third Reich.

Viktor was born in the small town of Landeshut (population 12,000) in the Prussian province of Silesia, which, following World War II, became part of Poland when the town was renamed Kamienna Gora. Landeshut was a textile manufacturing center, and Viktor's father owned one of the many factories in town, employing 300–400 workers. His parents were upper middle class Jews with wide-ranging cultural, artistic, and political interests. They fostered Viktor's life-long interest in music, art, dance, literature, and philosophy, and they encouraged and supported his early interest in nature. Almost from the time he could walk, he started collecting plants, animals, rocks, and fossils and brought home frog and salamander eggs and watched them develop and metamorphose. He was further encouraged to pursue his interest in nature by two high school biology teachers and by an older friend, Walther Arndt, with whom he later coedited a comprehensive two-volume book about the Landeshut area. Viktor's childhood and adolescence occurred in the security and optimism of an upper middle class home during a period of prosperity and peace in Germany. All of that would soon change.

University. In the spring of 1918, Viktor graduated from high school and was drafted into the German army, where he served until the end of World War I in November of that year. In the winter of 1918, he enrolled at the University of Breslau (now the city of Wroclaw, in Poland), studying zoology, botany, and geology. Based on his early interest in nature, he has noted that from age ten "there was never any doubt in my mind that I would become a naturalist" (Hamburger, 1989). In 1919, at the urging of his parents, Viktor moved to the University of Heidelberg, where his aunt, Dr. Clara Hamburger, one of the first women in Germany with a Ph.D. in Zoology, worked in the Zoological Institute as an assistant to Otto Bütschli. In Heidelberg, he took an advanced seminar in experimental embryology with Curt Herbst and a course in the history of philosophy with one of the founders of experimental embryology, Hans Driesch. It was these courses that influenced his decision to become an embryologist. On the recommendation of his aunt, in the spring of 1920 he transferred to the zoology department at the University of Freiburg to begin his Ph.D. studies. Under the direction of its new head, the eminent experimental embryologist Hans Spemann, the zoology department was dominated by embryological research. In keeping with his love of nature, Viktor has noted that his decision to move to Freiburg was motivated as much by the superior hiking and skiing, compared to Heidelberg, as by the presence of Spemann.

Two of Viktor's fellow students in Freiburg were Hilde Proescholdt, the codiscoverer with Spemann of the organizer, and Johannes Holtfreter, who was one of the most imaginative embryologists of his generation (Hamburger, 1996a). In 1921, Hilde Proescholdt married Otto Mangold, who, in 1924, was appointed director of the Division of Experimental Embryology at the Kaiser Wilhelm Institute in Berlin-Dahlem. In September of that year, just as the organizer paper was published (Spemann and Mangold, 1924), Hilde Mangold died, at age 26, from severe burns following the explosion of a gasoline heater in her kitchen. In 1938, Holtfreter voluntarily left Nazi Germany, and, after working at Cambridge University and McGill University, he ended up, in 1946, at the University of Rochester, where he remained until his death in 1992. Throughout their lives, Holtfreter and Viktor were close friends (Holtfreter, 1968; Hamburger, 1996a).

The Ph.D. and Postgraduate Work. Graduate education in Germany was vastly different then than today. According to Holtfreter, "There were no prescribed study programs, no advisors, no examinations of any kind, no records kept of academic performance and no pressure to publish preliminary reports in the race for priority" (Holtfreter, 1968). In 1923, in an apparent effort to provide him with a field of research independent of his own, Spemann assigned Viktor a Ph.D. project on the development of the nervous system that launched his subsequent career in neuroembryology (see below). Ross Harrison, one of the founders of modern experimental biology and a pioneer neuroembryologist, was a friend of Spemann and a frequent visitor to Freiburg, and he also helped foster Viktor's interest in neural development. Viktor received his Ph.D. (summa cum lade) in 1925 and then spent 4 months working at the Zoological Station in Naples to learn marine biology. On Spemann's recommendation, he then moved to the University of Göttingen, where he worked for several months in the winter of 1925–1926 with Alfred Kühn, studying color vision in fish, which involved training fish to jump for food rewards when paired with color stimuli (Hamburger, 1926), a harbinger of his later research on the development of behavior in the chick embryo. It was in Göttingen that he met his future wife, Martha Fricke, a biology teacher; they married in 1928 and had one daughter, Doris, in 1930 and a second daughter, Carola, in 1937. In the spring of 1926, Viktor accepted a position at the Kaiser Wilhelm Institute in Berlin-Dahlem with Otto Mangold. Here, he returned to studies of nervous system development, extending the research begun during his Ph.D. work in Freiburg. It was also during this period in Berlin, under the influence of the geneticists Richard Goldschmidt and Curt Stern, that he became seriously interested in developmental genetics, and he collaborated briefly with Stern in the study of *Drosophila* mutants.

Berlin and the Weimar Republic. At this time, Berlin was the cultural and intellectual center of the Weimar Republic. By 1925, the crises that followed Germany's military defeat, including the payment of war reparations, the financial collapse of 1923, and catastrophic inflation, had finally receded (between 1920 and 1923, the dollar value of the German mark went from 1:4 to 1:1,000,000, and values changed so rapidly that many workers were paid twice a day). The economy was now stabilized, and a feeling of well being and prosperity gradually returned. Erich Maria Remarque's popular novel, *Der Schwarze Obelisk* (1957), captures the hopeless atmosphere of the crisis period between 1918 and 1925, whereas the period after 1925 is perhaps best reflected in the musical *Cabaret*, based on the play *I Am a Camera*, adapted from *The Berlin Stories* (1946), by Christopher Isherwood, who lived in Berlin from 1929 to 1933. In *Cabaret*, one finds the uninhibited gaiety, vibrancy, and freedom of the democratic Weimar Republic being increasingly interrupted by the injustices, repression, and brutality of the Nazi dictatorship of Adolf Hitler. Who can forget the moving performance in *Cabaret* of the song, a thinly veiled lament for tolerance, "If they could see her through my eyes, (she wouldn't look Jewish at all)," juxtaposed with the refrains of the Hitler Youth song "Tomorrow belongs to me." Dark and evil forces were emerging in Germany that would dramatically influence Viktor's future and that of a whole generation of German-Jewish scientists and scholars (Medawar and Pyke, 2000).

Freiburg, 1927–1932. In 1927, Spemann offered Viktor a faculty position back in Freiburg (which he accepted) to teach and supervise the department laboratory courses and to continue his research. Although most embryologists of this period had no interest in the role of genes in development, Viktor began genetic studies with salamanders by creating species hybrids and then examining the growth rate of the limbs. This research was influenced by Viktor's interactions in Berlin with Goldschmidt and Stern. This research program was to be followed by cross-species limb transplants, but this plan never came to fruition, owing to the unique opportunity to do a 1 year sabbatical in the United States. As I relate below, however, once established in the United States, Viktor would return to the study of developmental genetics.

The United States, 1932–2001

Chicago, 1932–1935. In the fall of 1932, Viktor received a prestigious Fellowship from the Rockefeller Foundation to spend 1 year working in the laboratory of Frank Lillie, a pioneer in the use of the chick embryo, at the University of Chicago. Although there existed a rich literature on descriptive aspects of chick development, experimental research was limited, with the exception of chorioallantoic grafts and hormone injections. At Spemann's suggestion, in Chicago, Viktor tried to see whether the simple but elegant tools developed by Spemann for manipulating amphibian embryos could be used with the chick. Lillie suggested that Viktor try to resolve a discrepancy between experiments by Harrison's student, Samuel Detwiler, and a former student of Lillie's, Marian Shorey. Shorey (1909) had reported that unilateral limb bud ablation using electrocautery in the chick resulted in severe hypoplasia of both the ipsilateral sensory ganglia and spinal cord, including the motor column in the ventral horn, whereas, in salamanders, Detwiler (1933, review) found profound sensory hypoplasia but little effect on the spinal cord or the motor column. Because Shorey's results could have been an artifact of the electrocautery technique, Viktor repeated her study using the less invasive methods of Spemann (glass needles). Tutored by Lillie's colleagues, the embryologists Benjamin Willier and Mary Rawles, on how to handle chick embryos, within a few months, Viktor was able to extirpate and transplant limb buds in the chick and to confirm and extend the results of Shorey. In doing so, Viktor established a conceptual framework that led to the discovery of nerve growth factor (NGF) and to the recognition of programmed cell death (PCD) in the nervous system. Furthermore, by demonstrating for the first time that the chick embryo was just as amenable to experimental perturbations as amphibian embryos, he almost single-handedly established the chick as a valuable model in experimental embryology. The chick embryo stage series published in 1951 (see below) further solidified the chick as an experimental model.

In addition to his interactions with Lillie, in Chicago, Viktor was also influenced by several other prominent biologists and psychologists, including Paul Weiss, Sewall Wright, Charles Child, Warder Allee, Ralph Emerson, C. Judson Herrick, David Bodian, Karl Lashley, and Heinrich Klüver. In January of 1933, shortly before he was due to return to Germany, Viktor received a letter from the rector of the University of Freiburg, the eminent phenomenological philosopher Martin Heidegger, a Nazi sympathizer (Inwood, 1997), informing him that, owing to the new "Law for the Restoration of the Professional Civil Service," which was anti-Semitic legislation enacted by the Nazis in 1933, barring Jews from University faculties, he was being dismissed from his position in Freiburg. In 1935, the even more draconian Nuremberg Laws were passed, depriving all Jews of German citizenship, and, during this period, over 1000 Jewish university professors and scientists were exiled (Medawar and Pyke, 2000). Supported by emergency funds from the Rockefeller Foundation, Viktor was able to remain in Chicago for 2 more years, and, in 1934, he obtained a faculty position in the Zoology Department at Washington University (his only other job options were in Russia and Brazil). He returned briefly to Germany in 1934 to get his wife and daughter, and in September, 1935, the whole family moved to St. Louis. Viktor would not return to Germany again for two decades and would never return to his hometown, Landeshut. During the 1930s, his father's textile factory was confiscated by the Nazis, and the family was forced to emigrate, first to China and then to the United States. His aunt, Dr. Clara Hamburger in Heidelberg and her twin sister, Anni Hamburger, were deported to a concentration camp in 1939 but were released in 1941 following the purchase of their freedom by a wealthy American relative. Clara died in 1944 after emigrating to the United States.

St. Louis, 1935–2001. The chairman of the zoology department at Washington University was Dr. Caswell Grave, an ascidian biologist, who retired in 1939 and was replaced by a young biophysicist in the department, Dr. Frank Schmitt, who later founded the Neurosciences Research Program (NRP) (see Schmitt, 1992). Schmitt moved to MIT in 1941 and was replaced by Viktor as departmental chairman—a position he held until 1966. Although space does not permit me to describe Viktor's administrative and teaching contributions or his role as a research mentor, these aspects of his life have been admirably summarized in autobiographical essays (Hamburger, 1989, 1996b) and in essays by his colleagues and students (see Cowan, 1981; Holtfreter, 1968; Oppenheim and Lauder, 2001). In 1936, on the recommendation of Grave, who was a member of the Board of Trustees at Woods Hole, Viktor began teaching the summer embryology course and, in 1941, became its director—a position he held for 10 years and from which he helped to train and influence a generation of experimental embryologists. This position also provided the impetus for his writing *A Manual of Experimental Embryology* (Hamburger, 1942a), the first such guide (revised in 1960), which had sold over 10,000 copies before it finally went out of print in the 1980s.

As described in more detail below, Viktor's 67 year tenure at Washington University is notable for the many research and scholarly contributions he made to diverse fields of biology, embryology, neuroscience, and the history of science. He was one of the founding fathers of developmental neuroscience, a pioneer in the study of PCD, a driving force in the discovery of NGF, a leader in the study of neuron-target interactions and the development of innervation patterns, an important contributor to our understanding of the developmental origins of neuronal activity and behavior, and a leading scholar and historian of experimental embryology and developmental neuroscience. These efforts have been repeatedly recognized by his colleagues in the form of many awards and honors, including honorary degrees, membership in the National Academy of Science and the American Academy of Arts and Sciences, the F.O. Schmitt Prize, the Fidia Award, the Horvitz Prize, the Wakeman Award, the Lifetime Achievement Award from the Society for Developmental Biology, and the National Medal of Science.

Highlights of a Research Career

Ph.D. Work, Nerveless Limbs, and the Ontogeny of Innervation Patterns

By 1920, the descriptive comparative approach that had characterized the field of embryology in the 19th century was being rapidly replaced by experimental embryology (Maienschein, 1991; Horder et al., 1985). Founded by Hans Driesch and Wilhelm Roux in the last decade of the 19th century and embraced by the new generation of biologists, such as Spemann and Harrison, students of embryology were enthusiastic about extirpating, transplanting, and perturbing otherwise normal development as a means for understanding developmental mechanisms. It was in this context that Viktor began his research career. His Ph.D. research was an attempt to confirm an earlier report by B. Dürken (1911) that, following eye extirpation and the resulting hypoplasia of the optic lobes in frog larvae, a cascade of neural deficits extended to the spinal cord, affecting limb innervation and ultimately structural development of the limbs. After many hundreds of eye extirpations, Viktor failed to replicate Dürken's report and concluded that the leg abnormalities reported by him were most likely the result of inadequate rearing conditions of the partially blind frog larvae (Hamburger, 1925). Because his study was not conclusive regarding the role of innervation in limb development, however, when he moved to Otto Mangold's laboratory in Berlin, in 1926, he did the definitive experiment of extirpating the limb innervating segments of the spinal cord in frog larvae. As predicted, with the exception of muscle atrophy, limb morphology was normal, unequivocally disproving Dürken's hypothesis that development of the legs depends on innervation (Hamburger, 1928).

This line of research was also important for focusing Viktor's attention on general issues of neuron-target interactions and the development of limb innervation. In 1927, he published an influential review in which he discussed three of the major research areas that have come to be identified with him: (1) effects of the nervous system on limb development; (2) effects of the limb on nervous system development; and (3) the origin of nerve patterns (Hamburger, 1927). Regarding the issue of nerve patterns in the limb, in typical Hamburger fashion, he systematically reduced the issue to five fundamental processes, reviewed the evidence for each one, and then provided a framework for future studies: (1) what guides axons out of the spinal cord; (2) what controls the formation of the mixed nerve and its initial projection toward the limb; (3) what regulates plexus formation; (4) how do the nerves sort out in the plexus and form the typical limb pattern; and (5) what controls terminal connections with muscle and sensory receptors. This is a framework still followed today (e.g., Landmesser, 2001) and one that guided Viktor's own later research in this field (Hamburger, 1928, 1929, 1939, 1961; Hamburger and Waugh, 1940; Narayanan and Hamburger, 1971; Hollyday and Hamburger, 1976; Hollyday et al., 1977).

As alluded to above, this highly systematic approach to scientific issues characterized Viktor's entire career. As he has described it, "Some investigators are adventurous and enjoy exploring uncharted territory. They make bold forays and get their deepest satisfaction from the unforseen surprises that await them. The best are endowed with a green thumb [Viktor included Rita Levi-Montalcini in this group]. Others, with a different temperament, start with some general idea that may be nothing more than a dim hunch. They conquer their territory by patient step-by-step analysis, guided by the inner logic of their pursuit. They get rewards when the story unfolds and has a happy ending.…I belong to this second type" (Hamburger, 1989). A former student of both Viktor and Rita, Bob Provine, has provided an entertaining and insightful comparison of their personalities and distinct approaches to science (Provine, 2001).

Developmental Genetics

As noted above, an early interest in developmental genetics that was instilled by Fritz Baltzer, a geneticist in Freiburg during the early years of Viktor's Ph.D. work, was later fostered by the prominent geneticists Curt Stern and Richard Goldschmidt during his stay in Berlin. This interest in developmental genetics was unusual in that most embryologists of this period, including Spemann and Lillie, tended to ignore genetics (Allen, 1978; Gilbert, 1991). In Spemann's lab, Viktor, together with his first Ph.D. student, Salome Gluecksohn (later Gluecksohn-Schoenheimer and still later Gluecksohn-Waelsch), were exceptions. While in Stern's lab, Viktor had begun to study genetics using *Drosophila* mutants and later, as a faculty member back in Freiburg, he had begun genetic studies of limb development in different species of salamanders (Hamburger, 1935, 1936). It was not until the 1940s in St. Louis that Viktor returned to this field. Motivated by the availability of chicken mutants, through his friend from Herbst's lab in Heidelberg, the geneticist Walter Landauer, who was now at the University of Connecticut, Viktor began to study limb (phocomelia) and eye (micropthalmia) abnormalities in the Creeper mutant by transplantation of the anlage of these structures between mutant and wild-type embryos. In this way, he was able to demonstrate that when transplanted to normal hosts, the mutant phenotype was rescued (Hamburger and Rudnick, 1940; Hamburger, 1941; Hamburger and Gayer, 1943). Although these studies, together with Gluecksohn-Schoenheimer's pioneering work begun at about the same time (Gluecksohn-Waelsch, 1981; Gilbert, 1991), established developmental genetics and teratology as viable research areas for experimental embryologists, Viktor recognized already what is now commonplace, that the "complete story of the mode of gene action must be written jointly by geneticists, embryologists and physiologists" (Hamburger, 1942b) and that the most promising approach to genetic and other developmental anomalies is an embryological analysis (Hamburger, 1947; Scott, 2000).

The Stage Series of the Chick Embryo

I have heard it argued that the most enduring legacy of Viktor Hamburger will be the chick stage series created together with Howard Hamilton (Hamburger and Hamilton, 1951). Although arguably the least intellectually challenging of his many contributions, this prediction may turn out to be correct. Motivated by the need to prepare a revised third edition of Lillie's popular and classic book *Development of the Chick* (1919), which included a crude and inadequate stage series based on chronology (hours and days of incubation), Hamilton and Hamburger agreed that a first-rate stage series based on external morphological criteria was a high priority. Because of variability in the incubation conditions used in different laboratories, as well as genetic variations between chicken strains and other uncontrolled variables, a chronological-based series is not reliable. With his extensive previous experience in developing a morphological-based stage series for frogs and salamanders (Hamburger, 1989), Viktor was the ideal person to undertake this enormous job. Because development is a continuum and all stage series are only snapshots of a dynamic process, the goal became which snapshots to use to characterize each stage. Hamburger and Hamilton used three ground rules to guide them: stages must be identifiable unequivocally by external features; successive stages should be spaced as closely as possible; and, whenever possible, objective measures, such as toe or beak length, should be employed. The resulting product was an enormous success and has been adopted universally by developmental biologists who use the chick embryo. It was republished in 1992 and remains one of the most frequently cited papers in the field (a "citation classic"). As Viktor has noted with considerable pride, "For me, the greatest reward is the fact that in all these years nobody has suggested to me a change or improvement" (Hamburger, 1996b).

The Regulation of Neurogenesis (Proliferation)

In his original studies on the effects of limb extirpation and transplantation on the development of sensory and motor neurons in the chick (Hamburger, 1934, 1939), Viktor pointed out that the hypoplasia in these neuronal populations could be the result of a perturbation of proliferation, migration, or differentiation (he tended to favor proliferation, see Hamburger, 1939). He hypothesized that pioneer nerve fibers might be the means for transmission of a retrograde signal from the target to the central nervous system (CNS) that would act on one or more of these events to induce or recruit optimal numbers of innervating neurons. Subsequently, this experiment was repeated, and, when the number of mitotic figures in the brachial spinal cord were counted, no differences were detected between the operated (wing ablation) and control sides of the spinal cord (Hamburger and Keefe, 1944). In contrast, in a later study with Levi-Montalcini, they discovered that sensory neurons in the peripheral spinal ganglia exhibited a reduction in mitotic figures following limb removal and an increase in mitotic activity following peripheral overloading by transplantation of a supernumerary limb (Hamburger and Levi-Montalcini, 1949). Together, these studies indicated that the proliferation of sensory but not motoneurons was regulated by target-derived signals. However, it was later shown that it was very likely the proliferation of glial cells in the ganglia and not sensory neurons that were responding to limb-derived signals (Carr and Simpson, 1978a, 1978b; Calderó et al., 1998). Finally, by surgically isolating the brachial segments of the chick embryo spinal cord, Viktor demonstrated that the proliferation of CNS cells proceeds normally in the absence of signals from either adjacent segments or longitudinal fiber tracts (Hamburger, 1946).

The possibility that the periphery might regulate proliferation in the CNS motivated Viktor to undertake a detailed analysis of mitotic patterns in the spinal cord of the chick (Hamburger, 1948). With the exception of Coghill's related work on salamanders (Coghill, 1924, 1933), at the time, almost nothing was known about the onset or duration of mitotic activity in different parts of the CNS, and, consequently, Viktor's study represents a milestone in which rostralcaudal and dorsalventral patterns of proliferation were first described. This study together with the comprehensive analysis of mitotic activity in spinal ganglia (Hamburger and Levi-Montalcini, 1949) and a later study using thymidine autoradiography to determine the birth dates of motoneurons (Hollyday and Hamburger, 1977) establish Viktor's pioneering role in our understanding of neurogenesis and the relationship between proliferation, induction, and early pattern formation (Hollyday, 2001; Holtfreter and Hamburger, 1955). Although the early studies using mitotic figures as an index of proliferation were criticized as methodologically flawed (Jacobson, 1970), later studies using modern techniques have confirmed the original results (Carr and Simpson, 1978a, 1978b; Hollyday and Hamburger, 1977; Calderó et al., 1998; Gould et al., 1999).

Nervous System Regionalization and Cell Strain Specificity

As discussed above, the studies of neurogenesis by Viktor and his students were considered important not just for their insights into the regulation of neuronal proliferation but also as a means of addressing the broader issue of the origin of regional differences in the nervous system. For example, in his 1946 paper, Viktor attempted to address the issue of whether rostralcaudal propagated signals within the neural tube regulated proliferation or regional differentiation (Hamburger, 1946).

The general issue of pattern formation in the nervous system was widely recognized by neuroembryologists in the first half of the 20th century as being fundamentally important for understanding neuronal development (e.g., Weiss, 1955). Beginning as early as 1942, Viktor repeatedly drew attention to this issue in numerous reviews and essays (Hamburger, 1942a, 1952, 1956, 1962, 1977, 1988a; Holtfreter and Hamburger, 1955), often formulating the problem as one of "cell strain specificity, rostral caudal regionalization and dorsoventral regionalization" (Hamburger, 1977). Despite the rather formidable technical problems involved in addressing this issue during most of the last century, Viktor and his students were nonetheless able to make substantial contributions. In addition to examining regional differences in neurogenesis (proliferation), they also examined the origins of rostralcaudal and dorsoventral differentiation in the spinal cord.

In 1950, one of Viktor's Ph.D. students, Eleanor Wenger, published the results of her dissertation research in which she examined dorsoventral regionalization in the chick spinal cord (Wenger, 1950). In 1951, two other Ph.D. students, Byron Wenger (Eleanor's husband) and Paul Shieh published reports examining rostral caudal regionalization (Wenger, 1951; Shieh, 1951), and, later, Narayanan and Hamburger (1971) extended this line of investigation by examining the origins of morphological and functional differences between brachial and lumbar segments of the spinal cord. The general conclusion from these studies was that the specification of dorsoventral and rostralcaudal polarity occurs shortly after neural tube closure. These studies reflect Viktor's influence, as indicated by the combination of careful morphological description, experimental perturbation, and well-reasoned interpretation. Collectively, these papers represent one of the first attempts to address these questions experimentally, and they also established a conceptual and empirical framework that has led to the recent progress in our understanding of the cellular and molecular mechanisms involved in pattern formation in the developing nervous system (Ensini et al., 1998; Yamada et al., 1991; Liem et al., 1995).

Neuron-Target Interactions, Cell Death, and the Neurotrophic Hypothesis

As noted above, in his 1927 review, Viktor posed three questions regarding neuron-target interactions, one of which was whether the normal formation of the CNS depends on peripheral, target-derived signals. After reviewing the available literature on this topic (including the paper by Shorey, 1909), he concluded that the answer was a qualified yes, in that the relationship appeared to be mainly a quantitative one affecting cell numbers but not CNS patterning. It was in this context that, after moving to Chicago in 1932, he began the first of a career-long series of studies of this issue. By removing the early wing bud of the chick embryo, he was able to confirm Shorey's (1909) experiments demonstrating a substantial quantitative hypoplasia of the brachial spinal cord and sensory ganglia (Hamburger, 1934). In her original study and in a subsequent paper (Shorey, 1911), Shorey suggested that "metabolic products" from the muscles that diffuse into the lymph provide the stimulus that regulates the differentiation of innervating neurons. Although Hamburger accepted the notion of target-derived substances, he rejected the idea of nonspecific diffusion in favor of retrograde signals mediated selectively via nerve fibers and proposed that separate signals, produced by muscle versus sensory end organs, are transmitted to the CNS over their respective nerve fibers (sensory versus motor axons). In this way, each peripheral field was thought to control the quantitative development of its own neuronal population. The remaining unresolved issue then became whether the signals were regulating proliferation, migration, or differentiation. Although the peripheral regulation of neuronal survival (and death) was not considered, he came remarkably close to recognizing this possibility when he postulated that "in the case of poorly developed peripheral fields some fibers may even undergo *partial resorption* [my emphasis] and their respective cell bodies be blocked in their further differentiation" (Hamburger, 1934). However, because he was conceptually unprepared for the idea that neuronal death could be involved (Hamburger, 1992), Hamburger, more in keeping with his heritage from the Spemann lab, proposed a recruitment or induction hypothesis, whereby signals transmitted via pioneer nerve fibers induce optimal numbers of innervating neurons to proliferate or differentiate relative to the size of the periphery. Even without having considered cell death, however, he concluded the paper by proposing a three-point paradigm that would provide the basic framework for all subsequent research in this field for the next 60 years: (1) the peripheral targets, musculature and sense organs, generate two specific agents: one controlling the spinal ganglia and the other controlling the lateral motor columns; (2) the agents travel retrogradely in the nerves to their respective nerve centers, the lateral motor columns and the spinal ganglia; and (3) the agents regulate the development of the nerve centers in a quantitative way.

It remains a mystery how Viktor as well as others that were studying the effects of target removal on innervating populations of neurons (e.g., Shorey, Detwiler, Barron; see the review by Piatt, 1948) missed both the phenomenon of naturally occurring cell death and the increased occurrence of cell death following limb ablation. This is all the more surprising when one considers that, at least in the case of the chick embryo, at the ages they analyzed (as is now recognized), there are many frankly degenerating motor and sensory neurons, especially following limb removal. I have previously discussed some possible conceptual and methodological reasons for this failure (Oppenheim, 1981), but, in the final analysis, one is forced to conclude that because of a zeitgeist that did not recognize cell death as a developmental phenomenon, investigators were blinded to its occurrence even when the evidence was obvious. I can think of no better example of how, often, only the "prepared mind" is able to reveal the true nature of biological events. It is to their lasting credit that somehow Levi-Montalcini and Levi (1942, 1943, 1944, see below) were able to escape this inhibition and recognize that, in the absence of the limb, nerve cells initially develop normally but then degenerate. (One explanation for their prescience in this matter may stem from the fact that during this period in Italy (the early 1940s) Rita was also conducting two other important studies in which deafferentation of the cochlear nuclei and the ciliary ganglion early in chick development led to initially normal development followed by atrophy and cell loss in both populations [Levi-Montalcini, 1949, Levi-Montalcini and Amprino, 1947]. Collectively, these studies may have sensitized Rita to the role of regressive events in response to perturbation of neuronal interactions.)

Because most of the embryos in his 1934 study were killed 4–6 days after the surgery, which was done on the second day of incubation, Viktor recognized that determining what aspects of neuronal development were being perturbed by limb bud removal would require "a study of what happens in the nervous system during the first days after wing bud extirpation" (Hamburger, 1934). After reading a reprint of the 1934 paper sent by Viktor to her mentor, the Italian neuroanatomist

Guiseppe Levi, Rita Levi-Montalcini together with Levi performed just such an investigation examining embryos between 2 to 17 days following surgery (Levi-Montalcini and Levi, 1942, 1943, 1944) and found that sensory and motor proliferation, migration, and differentiation appeared normal following limb removal but that the subsequent survival of sensory and motor neurons was affected, resulting in atrophy and degeneration. At this same time, Donald Barron, who was examining neuron-target interactions in the sheep fetus, proposed (without any evidence, however) that "if only those neuroblasts survive which establish peripheral connections, then the balance between the number of sensory neurons in the ganglion and the size of the peripheral field its processes supply would be achieved" (Barron, 1944). By demonstrating experimentally that the periphery acts to maintain neuronal survival and not to induce the differentiation of neurons as proposed in Hamburger's recruitment hypothesis, the studies of Levi-Montalcini and Levi confirmed Barron's suggestion and set the stage for a line of investigation that led to the discovery of NGF, to the recognition of the significance of developmental neuronal cell death, and to the concept of the neurotrophic hypothesis (Hamburger and Oppenheim, 1982). Although Viktor's recruitment hypothesis was proven wrong, the three-point paradigm derived from his 1934 paper established the basic framework for these breakthroughs, and this, together with his many subsequent contributions to each of these issues (Hamburger and Levi-Montalcini, 1949; Hamburger, 1958, 1975, 1992, 1993; Brunso-Bechtold and Hamburger, 1979; Hollyday and Hamburger, 1976; Hamburger et al., 1981; Hamburger and Yip, 1984) has assured for him a lasting place in history as one of the outstanding pioneers and leaders in this field.

In 1950, Hamburger and Levi-Montalcini (1950) proposed that "the trophic relations between the nerve cell and its nonnervous milieu are of fundamental importance for the integrity of the nerve cell..., the medium in which the neurite grows provides the latter with some substance necessary for its further growth" (Hamburger and Levi-Montalcini, 1950). Between 1949 and 1954, mouse sarcomas had been discovered to be a rich source of a diffusable agent influencing the growth and survival of sensory and sympathetic neurons, and by 1958, Stanley Cohen and Rita had isolated NGF from the male mouse submaxillary salivary gland (Hamburger, 1993, Cowan, 2001; Levi-Montalcini, 1987). Although it would be several more years before limiting amounts of endogenous target-derived NGF was shown to be the driving force behind sensory and sympathetic neuronal death and survival (Oppenheim, 1996a), the studies of Hamburger and Levi-Montalcini in the 1930s, 1940s, and 1950s were indispensable steps to this discovery. Without those early studies, the discovery of NGF would likely have been delayed by a quarter of a century, and our current understanding of neurotrophic factors and programmed cell death would not have been possible. Although Viktor did not participate in the final studies leading to the actual biochemical isolation and characterization of NGF, his earlier contributions did not go unheralded. (Although it has been stated [Levi-Montalcini, 1988, 1994] or implied [Cowan, 2001] that Viktor may have sometime coauthored papers in which he made little, if any, contribution [e.g., some of the NGF studies], in fact, his modus operandi in this regard was just the opposite. He was always scrupulously honest in only coauthoring papers to which he contributed directly either experimentally or intellectually by formulating the problem, analyzing and interpreting the results, or helping to write the manuscript. This is especially clear in the case of the Ph.D. research of many of his students who published their work alone, despite Viktor's role as mentor, advisor, and often as the source of the research question [e.g., Wenger, 1950, 1951; Shieh, 1951; Oppenheim, 1966; Bekoff, 1976; Noden, 1975].)

In her Nobel lecture, published in *Science*, Levi-Montalcini stated, "I dedicate this article to Viktor Hamburger, who promoted and took part in this search, and to whom I am forever indebted for invaluable suggestions and generosity. Without him, the nerve growth factor would never have come to our attention" (Levi-Montalcini, 1987). The citation for the Fidia Award, which he received in 1987, states, "Specifically, this award recognizes his discovery of the significance of the role of cell death on brain differentiation and his leadership in fostering the innovative research leading to the discovery of the nerve growth factor." His failure to share the 1987 Nobel Prize with Rita Levi-Montalcini and Stanley Cohen, while a disappointment to his many friends and colleagues, was less so to him. In a private discussion in 1989 he stated: "Frankly, I think it is foolish to think about what happened or did not happen....I care very little about the Nobel Prize. I have won a great number of awards but, most of all, I have the esteem and affection of all of my colleagues. This, believe me, is a result which is worth as much as a Nobel in our profession."

The Embryology of Behavior

Following his withdrawal from the study of NGF in the 1950s, Viktor published two important neuroembryological papers: one on motoneuron cell death (1958) and one on the development of nerve patterns (Hamburger, 1961). Then, beginning in the early 1960s, at an age when many of us are anticipating retirement, he began an entirely new line of investigation on the embryonic origins of behavior. Although this abrupt shift of focus to behavioral studies surprised many of his colleagues, in fact, the stage had been set many years earlier. His behavioral studies on color vision in fish (Hamburger, 1926) had stimulated an early interest in behavior which, had he remained in Germany, he planned to pursue along the lines of the ethological studies of Konrad Lorenz. Discussions with the psychologist Karl Lashley in Chicago in the 1930s and with the pioneer in the field of embryonic behavior George Coghill in Woods Hole during the same period, together with his recognition that "neurogenesis and the genesis of behavior are inseparable" (Hamburger, 1968) and that "behavior patterns, like organs, have an ontogenetic development" (Hamburger, 1942a) further inspired him to pursue behavioral development in the chick embryo. As summarized below, these studies extended over a 15 year period and served to resuscitate a field that had flourished in the 1930s and 1940s (Hamburger, 1963; Oppenheim, 1982) but had subsequently languished and become moribund, and, in the process, they revolutionized our understanding of behavioral development. It is interesting that whereas Viktor turned to the study of behavioral development late in his career, Rita Levi-Montalcini had begun her career with pioneering investigations of behavioral development in the chick embryo (Visintini and Levi-Montalcini, 1939).

By a combination of careful behavioral analysis, detailed quantification of motor patterns, surgical ablations and transplantation, electrophysiology, and neuroanatomy, a comprehensive picture of behavioral development emerged, from the onset of the first embryonic movements and reflexes to complex hatching behavior (Hamburger, 1977; Bekoff, 2001). Not since the pioneering studies of neural and behavioral development in salamanders by Coghill (1929) had the ontogeny of behavior and its neural bases in a single species been examined in such detail and with such far-reaching implications for the entire field (Oppenheim, 1978, 1982; O'Donovan, 1999). Motility in the chick embryo was shown to be spontaneous, regulated by central pattern generators, as reflected in stereotyped CNS activity within spinal cord and neuromuscular circuits that are established prior to and are maintained in the absence of sensory input. Although previous studies in the adults of lower vertebrates (fish, amphibians) and insects had provided evidence for the role of central pattern generators in complex motor patterns (Weiss, 1941; Hoyle, 1984; Oppenheim, 1982), this work of Hamburger and his colleagues on the chick provided the first unambiguous evidence for the developmental origins of these mechanisms in the embryo. In reflecting on the philosophical implications of this work, Viktor has noted that "What has impressed me most in all phases of these investigations is the primacy of activity over reactivity or responses. This, to me, has become symbolic of animal life, and perhaps of life in general. The elemental force that embryos and fetuses can express freely in the egg or uterus, has perhaps remained, throughout evolution, the biological mainspring of creative activity in animals and man, and autonomy of action is also the mainspring of freedom" (Hamburger, 1977).

NGF, Cell Death, and the History of Science

By 1975, the investigation of behavioral development was drawing to a close, and, once again, Viktor changed the focus of his research to first return to studies of cell death and neurotrophic interactions and then, after closing his laboratory in 1985, he embarked on a series of scholarly historical studies of embryology, developmental neurobiology, and of individual pioneers in these fields, including Ramon y Cajal, Holtfreter, Roux, Spemann, Harrison, von Baer, and Hilde Mangold.

In 1975, Viktor published the first systematic analysis of PCD in avian spinal motoneurons (Hamburger, 1975). The impetus for that study was his earlier observation of an apparent normal loss of motoneurons contralateral to leg bud removal (Hamburger, 1958). That observation, together with the almost total loss of motoneurons that occurred on the ipsilateral, target-deprived side of the spinal cord, led him to conclude that "the quantitative relationship between the number of motor neurons and the size of the peripheral field of innervation is established by a selective survival of those neurons which find an adequate peripheral milieu and the degeneration of all others" (Hamburger, 1958). By carefully quantifying the number of degenerating and surviving cells, Hamburger (1975) confirmed a significant 40%–50% loss of motoneurons. This, together with his observations on motoneuron loss following limb bud removal and the effects of an enlarged periphery in rescuing motoneurons from normal cell death (Hollyday and Hamburger, 1976) extended the neurotrophic hypothesis from sensory neurons (Hamburger and Levi-Montalcini, 1949) to motoneurons and set the stage for the discovery of muscle-derived motoneuron trophic factors (Hamburger and Oppenheim, 1982; Oppenheim, 1996b).

In a final series of experimental studies completed before closing his laboratory in the 1980s, Viktor returned to an examination of the role of NGF in the survival of sensory neurons in the DRG of the chick embryo. Surprisingly, following the description of naturally occurring PCD in sensory neurons and the discovery of NGF, between 1960 and 1980, little progress had been made in our understanding of NGF as a target-derived trophic factor for sensory neurons (Oppenheim, 1996a). Accordingly, Viktor began this line of investigation by first demonstrating a selective retrograde transport of NGF from the leg to lumbar DRG during the period of normal PCD of sensory neurons (Brunso-Bechtold and Hamburger, 1979). Next, he demonstrated that NGF treatment in vivo rescued sensory neurons from PCD (Hamburger et al., 1981) and that, following limb bud removal, NGF also rescued target-deprived sensory neurons from PCD (Hamburger and Yip, 1984). Although it would be several more years before endogenous NGF was shown to be present in the peripheral targets of neurons and that the genetic deletion of NGF results in the death of sensory neurons (Snider, 1994), these studies with the chick embryo provided the first compelling evidence that NGF was the long sought after target-derived retrograde trophic signal for developing sensory neurons. In returning full circle to the confirmation of his original three-point paradigm proposed 50 years earlier (Hamburger, 1934), this provides a perfect example of his systematic step-by-step style of research.

When his experimental work came to an end, Viktor began a series of historical studies that continued to the end of his life. As he has pointed out, "I was aware of the fact that significant changes and innovations in the continuum of the history of biology are brought about by creative minds who combine intuition with profound thought, keen powers of observation and mastery of a particular methodology" (Hamburger, 1996b). This historical framework, together with his close personal acquaintance with many of the most creative minds in biology in the 20th century and as an eyewitness to some of the most important discoveries during that period, provided him with a unique perspective on the history of this field. In a series of 19 articles and a book published between 1981 and 1999, Viktor has left a historical record of embryology, developmental biology, and neuroscience that is unparalleled. The crowning achievement of these efforts is the book *The Heritage of Experimental Embryology. Hans Spemann and the Organizer* (Hamburger, 1988b). As one reviewer has noted, "There can be no one better qualified to give a personal account of this area and Dr. Hamburger has done a superb job, paying great attention to detail and yet making the book extremely readable and enjoyable...everything in it is from personal experience" (Gurdon, 1989). In view of the fact that Viktor has acknowledged that "Spemann's approach to the causal analysis of development has influenced me profoundly" (Hamburger, 1989), it is not surprising that in this book as well as in some of the other historical essays (Hamburger, 1969, 1985, 1996c, 1999), Spemann takes center stage. These essays are interesting not only for their insights into Spemann's work and personality but also because they, together with only a few other sources (Hamburger, 1977, 1957, 1962; Holtfreter, 1968; Cowan, 1981, 2001; Oppenheim and Lauder, 2001), provide a rare glimpse into Viktor's personality and philosophy of life.

Spemann's choice of the term "organizer" has sometimes been thought to have vitalistic implications along the lines of the undeniably vitalistic developmental concept of *entelechy* of Hans Driesch; entelechy refers to a nonmaterial organizing principle of life. Viktor argues persuasively, however, that Spemann, although sympathetic to some vitalistic notions, was one of the first to point out the weakness of Driesch's vitalistic arguments and to have shown that the developmental phenomena (e.g., embryonic regulation) that forced Driesch into vitalism were, in fact, amenable to experimental analysis (Hamburger, 1988b, 1999). Spemann, like most biologists and embryologists of that period, believed in a holistic, systems or organismic view of development, the idea that individuality and wholeness are manifested in the embryo no less than in the adult. According to this view, development proceeds from the beginning to end within the framework of the whole organism. This is a view that was also embraced by Viktor, in which the whole is more than the sum of the parts and in which cellular and molecular development only makes sense within the self-organizing context of the whole embryo (Hamburger, 1957). At the time, the opposing view, expressed most forcefully by Jacques Loeb, was that the embryo was no more than the sum of its parts as defined by physics and chemistry (Pauly, 1987). Although the holistic view has long been out of favor, there are recent signs that it is once again being resuscitated as a more plausible approach to developmental issues than a strictly molecular analysis of RNA, DNA and proteins (Kirschner et al., 2000). As Viktor has pointed out, "We found great satisfaction in handling living embryos, and no doubt the present generation finds equal satisfaction in handling molecules. But all of this should not obscure the fact that we all have a central concern in common. Whatever we explored with transplantations, and what you now explore with infinitely more sophisticated technology is actually played out in the living, developing embryo. It is a reassuring thought that the embryo which has already outlived the experimental embryologists, will still be around when their molecular successors have likewise become part of history" (Hamburger, 1989).

Finally, without in any way rejecting reductionism, Spemann and Hamburger both shared the philosophical view that efforts to reduce biological phenomena to physical and chemical processes will not answer all questions in nature. As Viktor has stated, "We had better realize that the scientific approach altogether opens only a small window to the universe. We cannot expect our intellect to fathom all depths" (Hamburger, 1969). If this sounds defeatist or anachronistic today, I suspect that the reader has not thought about these matters very deeply. In much the same vein, the Swiss molecular biologist Alex Mauron has recently argued that "genetics and biology enable us to wield increasing power over our destiny, but that does not mean that more traditional forms of inquiry about ourselves have been superceded by our greater understanding of human biology. More than ever, we need a richer account of the human condition" (Mauron, 2001). It is to the everlasting credit of Hamburger (and Spemann) that their achievements were based on both scientific acumen and a personal creed or philosophy of life that permitted them to pursue scientific issues wherever they led, constrained only by the techniques available to them, but, at the same time, with a clear understanding of the inherent limits imposed by the human mind.

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