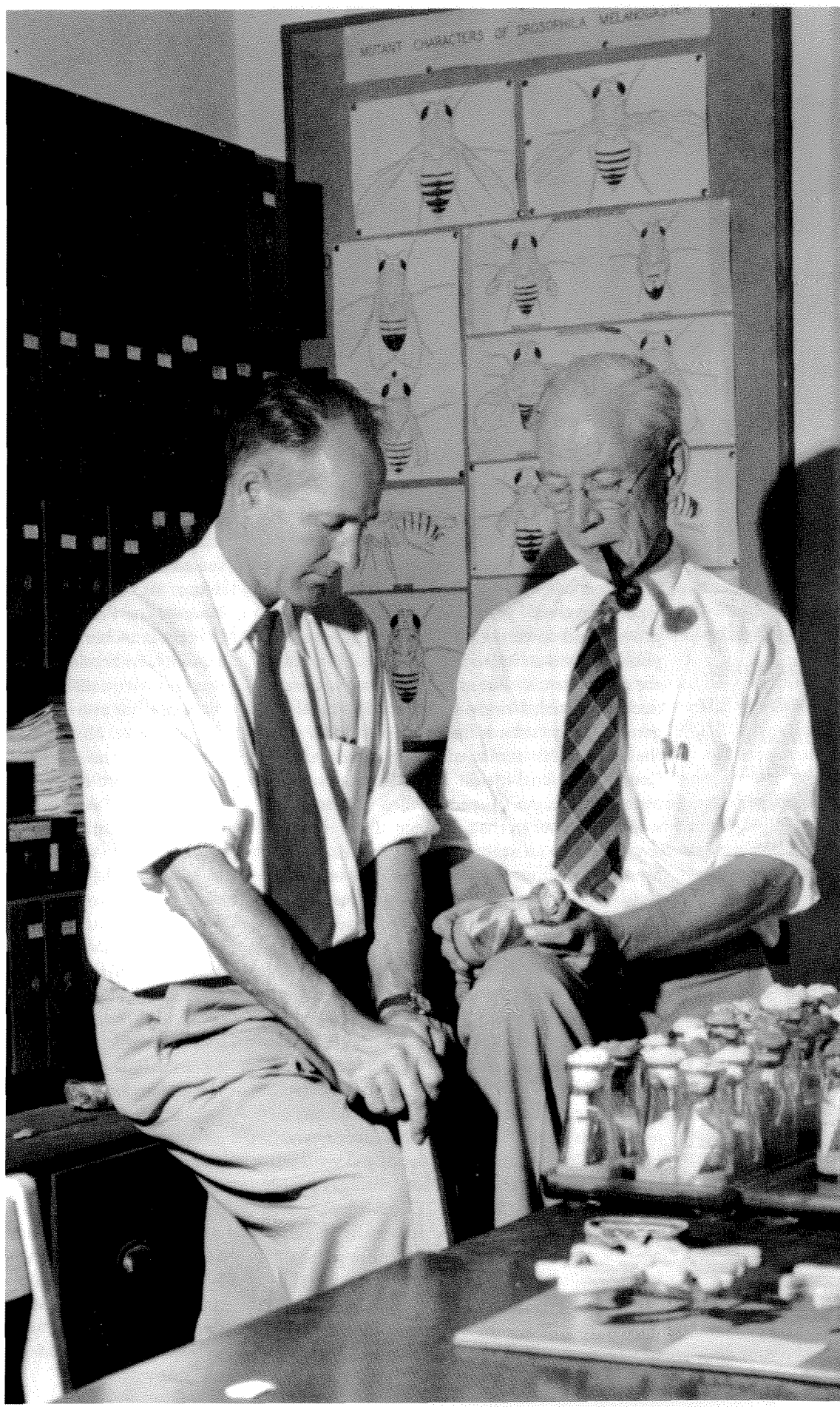
MUTANT CHARACTERS OF DROSOPHILA MELANOGASTER

Fifty Years Ago: The *Neurospora* Revolution

This brief paper, revolutionary in both its methods and its findings, changed the genetic landscape for all time.

by Norman H. Horowitz

This year marks the 50th anniversary of the publication of George Beadle and Edward Tatum's first *Neurospora* paper—a pivotal work of modern biology. This brief paper, revolutionary in both its methods and its findings, changed the genetic landscape for all time. Where previously there had existed only scattered observations (albeit with some acute insights) on the relation between genetics and biochemistry, this paper established biochemical genetics as an experimental science, one where progress would no longer be limited by the rarity of mutants whose aberrations could be understood biochemically, but rather where such mutants would be generated at will, and findings could be repeated and hypotheses explored as in other experimental sciences. This paper was the first in a series of fundamental advances in chemical genetics that by 1953 had bridged the gap between genetics and biochemistry and ushered in the age of molecular biology.

George Beadle (left) first came to Caltech as a National Research Council fellow in 1931 and stayed until 1936. He returned in 1946 to succeed Thomas Hunt Morgan as chairman of the Division of Biology. Here, shortly before he left to become president of the University of Chicago in 1961, he discusses *Drosophila* with Alfred H. Sturtevant, who came to Caltech with Morgan (see previous article).

I first heard of biochemical mutants in *Neurospora* at a memorable seminar given by George Beadle in the fall of 1941 at Caltech, where I was a postdoc at the time. (Beadle had come to Pasadena to recruit a couple of postdoctoral fellows to join him and Tatum at Stanford, and I ended up being one of them.) In his lecture Beadle presented their results with *Neurospora* that would shortly thereafter be published in the *Proceedings of the National Academy of Sciences*. The talk lasted only half an hour, and when it was suddenly over, the room was silent. The silence was a form of tribute. The audience was thinking: Nobody with such a discovery could stop talking about it after just 30 minutes—there must be more. Superimposed on this thought was the realization that something historic had happened. Each one of us, I suspect, was mentally surveying, as best he could, the consequences of the revolution that had just taken place. Finally, when it became clear that Beadle had actually finished speaking, Frits Went—whose father had carried out the first nutritional studies on *Neurospora* in Java at the turn of the century—got to his feet and with characteristic enthusiasm addressed the graduate students in the room. The lecture proved, said Went, that biology is not a finished subject—there are still great discoveries to be made.

The methodological innovations of the 1941 Beadle-Tatum paper were twofold. First, the authors introduced what was for most geneticists a new kind of experimental organism—a microorganism that was ideally suited for classical genetic studies, but which differed from the classical organisms in that its nutritional requirements were explicitly known—that is, it grew readily on a medium of defined chemical composition. This novel creature was the red bread mold *Neurospora crassa*. Most of the investigations that led to the development of molecular genetics employed microorganisms, but the *Neurospora* discoveries first described in the 1941 paper were crucial for making bacteria genetically useful.

Beadle had learned of *Neurospora* at a lecture by Bernard O. Dodge given at Cornell University in 1929, when Beadle was a graduate student. Dodge, a mycologist (one who studies fungi) at the New York Botanical Garden, was a strong advocate of *Neurospora* as an organism for genetic

experiments. It was he who found that the mold's ascospores (which are the products of sexual fusion and recombination) require heat shock to induce germination. This made it possible to carry through the whole life cycle in the laboratory; *Neurospora* thus became domesticated. (Dodge had originally made this discovery with another fungus by accidentally setting down some plates of its ascospores in a sterilizing oven that he thought was turned off.) He worked out the basic genetics of *Neurospora*, investigating among other things the inheritance of mating type, albinism, and other single-gene characteristics. He showed that the ascospores, which come in sets of eight, each set descended from a different fertilized egg cell, display a 4:4 ratio for single-gene traits—just what Mendelian genetics predicts. By isolating and culturing the ascospores in the linear order in which they are found in the organism, he discovered the patterns of first- and second-division segregations (4:4 and 2:2:2:2, respectively). These patterns result from crossing over, or the lack of it, between the trait being studied and a point in the chromosome called the centromere; the relative frequencies of these patterns are important for gene mapping.

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Dodge also understood the benefits that haploidy (having a single set of chromosomes, rather than two sets as in higher organisms) offered for simplifying and accelerating genetic studies. When combined with *Neurospora*'s other features, it convinced him that this fungus was the ideal genetic organism. He claimed that it was superior to *Drosophila*, as he frequently argued to his friend Thomas Hunt Morgan.

As its second methodological innovation, the Beadle-Tatum paper introduced a procedure for recovering an important class of lethal mutations—those blocking the synthesis of essential biological substances. These mutations were expressed in the organism as new nutritional requirements, and were crucial for understanding the biochemistry of gene action. They showed that each step in the biosynthesis of a vitamin, amino acid, purine, or pyrimidine is under the control of a particular gene. They displayed in a most convincing manner the central importance of genes in biochemistry and ended forever the idea that the role of the genes in metabolism was somehow a subordinate one. Genetics, which before the *Neurospora* revolution had been notably isolated from the physical sciences, now found itself in the mainstream of biochemistry. Or, more correctly, genetics and biochemistry were now seen to be different aspects of the same thing.

The fundamental character of the substances whose syntheses were affected in the *Neurospora* mutants suggested that similar mutations should occur in other microbial species. This proved to be the case. In 1944 it was shown that "biochemical mutations" could be induced in bacteria. This result solved a basic difficulty—the lack of suitable markers—that had long prevented progress toward a genetics of bacteria, and led directly to the demonstration of genetic recombination—the reshuffling of genes following mating—in *E. coli* by Tatum's student Joshua Lederberg. Biochemical mutations were induced later in yeast and other microorganisms.

Aside from its revolutionary methods, the Beadle-Tatum paper was remarkable for the results it reported. It described three x-ray induced mutants that grew on "complete medium" (a complex, undefined mixture containing yeast extract), but that failed to grow on "minimal medium" (a mixture consisting of the minimal nutrients capable of supporting the growth of wild-type, or unmutated, *Neurospora*). The presumption was that the mutations expressed in these cultures affected genes needed for the production of growth-essential compounds present in complete, but not minimal, medium. A systematic search revealed that each of the mutants required a different substance. The three substances were pyridoxine, thiamine, and p-aminobenzoic acid, and the loss of the ability to synthesize them was eventually shown in every case to be inherited as a single-gene defect.

The 1941 paper reported the genetics of only the "pyridoxineless" mutant—Number 299. This was, so to speak, the breakthrough mutant,

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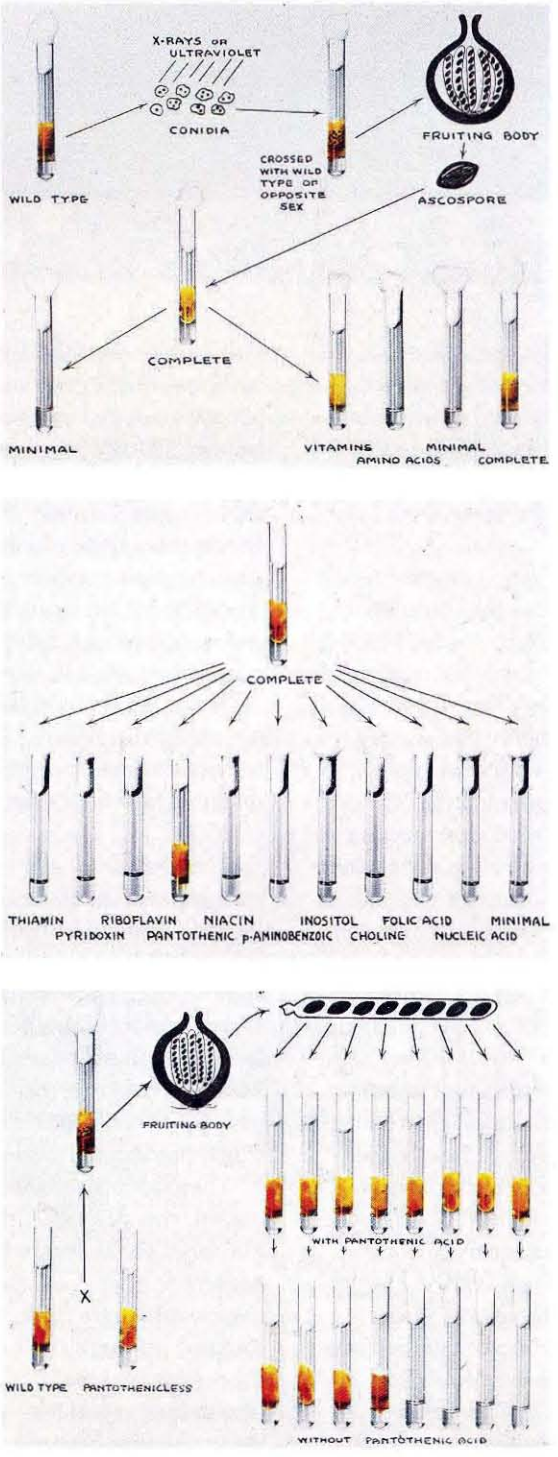
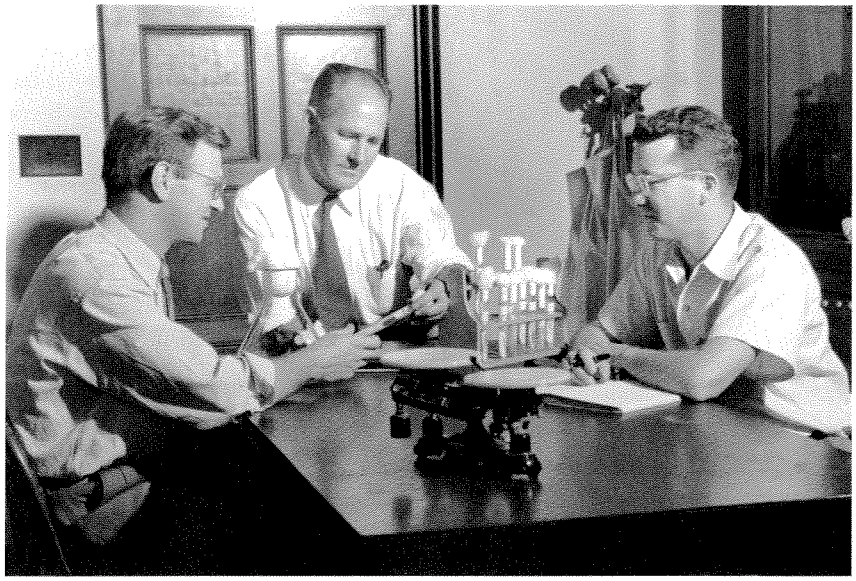


Beadle drew and lettered these diagrams himself; he used them as lantern slides to illustrate his talks during the 1940s. The top one shows the conidia (asexual spores) of wild-type (unmutated) *Neurospora* exposed to radiation, crossed with the opposite mating type, and producing ascospores in sets of eight. These then germinate in the complete medium (the reddish color indicates the presence of the mold), which has everthing they need to grow. But when a bit of the culture is transferred to the minimal medium, they don't grow, indicating that a mutation has affected genes needed to produce an essential growth compound—in this case vitamins (or nucleic acids). Further subcultures (center) show that pantothenic acid is the substance the mutant has lost the capacity to make for itself. Crossing this mutant with wild type and dissecting out the eight ascospores in order (bottom) shows that all grow on pantothenic acid, but without it four grow and four do not—a perfect Mendelian ratio, indicating a single-gene mutation.

the one that vindicated Beadle and Tatum's ideas about a new kind of genetics. But its importance did not end there. Soon after the 1941 paper was published, Beadle received a letter from an acquaintance at the Merck Research Laboratory requesting a culture of Number 299 for the purpose of developing an assay method for pyridoxine. Beadle sent a transfer, as he invariably did once a mutant had been referred to in print. Beadle firmly believed that this policy was in the best interest of science, a belief that was certainly confirmed in this case because, in the course of their investigation, the Merck group discovered that Number 299 would grow without pyridoxine if the acidity of minimal medium was brought to a pH of 6 from its normal value of 5.

I recall first hearing of this unexpected result at an afternoon tea break in Beadle's Stanford lab. In the ensuing discussion we decided to learn whether other environmental variables—temperature in particular—might affect the characteristics of mutants in a specific way. The mutant hunt that ran more or less continuously in the lab was modified accordingly to include an incubation step at 35° C in addition to the usual one at 25°. Soon the first temperature-sensitive mutants were found—that is, ones whose nutritional deficiency was expressed only above (or occasionally below) some temperature in the normal temperature range of the organism. By modifying the gene in such a way that its activity was abolished only at certain temperatures, these mutations made it possible to identify genes that otherwise would be lost because their end



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product is, for example, too large to penetrate the cell (a nucleic acid, for instance); this product therefore cannot be restored to the organism by adding it to the medium. This attribute greatly extended the range of recoverable genes and made possible an early test of the "one gene-one enzyme" hypothesis.

Beadle published the one gene-one enzyme theory in 1945, developed from the cumulative results of the new approach to the study of biosynthetic pathways that the *Neurospora* mutants had opened, and for this he and Tatum won the Nobel Prize in 1958. This theory had already been foreshadowed in the first paragraph of the 1941 paper, where the authors suggested the possibility that genes may act "by determining the specificities of enzymes" as well as the further possibility of "simple one-to-one relations" between genes and chemical reactions. These ideas doubtless grew out of the authors' earlier work on *Drosophila* eye colors. In his Nobel lecture Beadle, in an oft-quoted passage referring to one gene-one enzyme, said: "In this long, roundabout way, first in *Drosophila* and then in *Neurospora*, we had rediscovered what Garrod had seen so clearly many years before." Beadle was without doubt sincere in this characteristically generous remark, but was he right? Was the one gene-one enzyme concept that forms one of the foundations of molecular biology really formulated decades earlier? I think the answer is no.

A. E. Garrod wrote his great work on human hereditary disease, *Inborn Errors of Metabolism,* in 1909, the same year that W. L. Johannsen introduced the word *gene* into the language. And although Garrod lived until 1936, recent writing on his work suggests that his understanding of genetics stopped around 1910 and concludes that he could hardly have had Beadle's one gene-one enzyme idea in mind at that time. The chromosome theory of inheritance was still in the future. Biochemistry was also in an embryonic state. In a monograph published in 1914, W. M. Bayliss considered it necessary to defend the idea that enzymes could be assumed to be definite chemical compounds, "at all events until stronger evidence has been brought to the contrary." The one thing that seemed clear in 1914 was that enzymes were not proteins, a belief that was not disproved until Sumner crystallized urease in 1926.

The most prescient of all writing about genes and enzymes are those of the French geneticist Lucien Cuénot. In 1903 Cuénot discussed his celebrated experiments on the inheritance of coat color in mice in terms of *mnémons* (genes), enzymes, and a chromogen, but he too at the time lacked the knowledge essential to putting the whole picture together. Unfortunately, Cuénot gave up genetics and discouraged his students from entering the field.

There were later antecedents of the one gene-one enzyme principle in the writings of Wright, Haldane, and others, where unfamiliarity with modern science does not enter in. But while these works were correct in deducing that genes must act through their effects on enzymes (and other proteins), none of them succeeded in persuading geneticists of the classical era that a direct relation between genes and proteins was

Left: Beadle (center), postdoc Harold Garner (left), and Norman Horowitz examine some *Neurospora* cultures in the early fifties. Right: Beadle bows to the Nobel assembly in 1958. Tatum is behind him to the left (with glasses).

real and important and was, in fact, the key to understanding the organization of living matter. Alfred N. Sturtevant (who came to Caltech with Morgan in 1928, eventually becoming the T. H. Morgan Professor of Biology) wrote in his *A History of Genetics* in 1965 that geneticists were disinclined to accept simple ideas of gene action because they were convinced that development was too complex a process to be explained by any simple theory. Not long before he died in 1970, Sturtevant told me that in particular E. B. Wilson's position on gene action had carried much weight. Wilson was one of the most influential figures in American biology. Although he died in 1939, the third edition of his monumental book, *The Cell in Development and Heredity*, published in 1925, is still in print. Usually very clearheaded, Wilson took what can only be described as an exceedingly murky view when, regarding the role of the genes, he wrote:

> In what sense can the chromosomes be considered as agents of determination? By many writers they have been treated as the actual and even as the exclusive "bearers of heredity"; numerous citations from the literature of the subject might be offered to show how often they have been treated as central, governing factors of heredity and development, to which all else is subsidiary. . . . Many writers, while avoiding this particular usage, have referred to the chromosomes or their components [Wilson rarely used the word *gene*] as "determiners" of corresponding characters; but this term, too, is becoming obsolete save as a convenient descriptive device. The whole tendency of modern investigation has been towards a different and more rational conception which recognizes the fact that the egg is a reaction-system . . . and that (to cite an earlier statement) "the whole germinal complex is directly or indirectly involved in the production of every character."

In an obvious and not very interesting sense, the foregoing statement is correct; but in another and much more important one, it is altogether wrong. With the *Neurospora* revolution, musings of this sort on the nature of gene action faded away. The evidence for a one-to-one relation between genes and enzymes (actually proteins, later modified to polypeptides) now became clear, abundant, and undeniable. The individual gene in some way determined the specific enzyme, although it was not yet seen how. The efforts of the pre-*Neurospora* workers to understand gene action had been made with systems often not suited for both biochemical and genetic studies. Beadle and Tatum changed this by founding a new science based on an organism and an experimental protocol designed to be maximally useful for the purposes of biochemical genetics. In doing so, they transformed biology, and that is the reason we remember this 50th anniversary. □

Norman Horowitz, professor of biology, emeritus, first arrived at Caltech as a graduate student after earning his BS from the University of Pittsburgh in 1936. Caltech's Division of Biology, under Thomas Hunt Morgan, was not even a decade old, and George Beadle was just leaving for 10 years at Harvard and Stanford. Horowitz worked with embryologist Albert Tyler, earning his PhD in 1939. After a National Research Council fellowship for a year at Stanford, he was back at Caltech as a research fellow from 1940 to 1942, when he witnessed Beadle's historic presentation, recounted above. This began a long collaboration on Neurospora, *first at Stanford and then back at Caltech, where Beadle returned in 1946, bringing Horowitz as associate professor. Horowitz was full professor of biology from 1953 until he reached emeritus status in 1982. He was acting chairman of the biology division in 1973 and chairman from 1977 to 1980.*

Besides his work on Neurospora, *Horowitz has long been interested in the biochemical aspects of the origin of life and the possibility of life on other planets. As chief of the bioscience section at JPL from 1965 to 1970, he sent biological experiments to Mars on Mariners 6 and 7 and the Viking landers; his book,* To Utopia and Back: The Search for Life in the Solar System, *was published in 1982. This article was adapted from one that first appeared in the April 1991 issue of* Genetics.