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Renato Dulbecco and the New Animal Virology: Medicine, Methods, and Molecules

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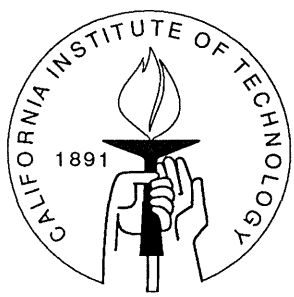

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Renato Dulbecco and the New Animal Virology: Medicine, Methods, and Molecules

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Animal virology -- the study of viruses that prey on animals and human beings -- deserves historical treatment if only because since the 1950s it has become one of the most important fields in the biomedical sciences. Nowadays, it is central to the understanding of many infectious diseases, including AIDS, and the non-infectious scourge of cancer. Yet the development of the new animal virology -- "new" because it was a biological science as distinct from an arm of clinical practice in medicine -- is richly suggestive not only because of its salient importance to medicine but also historiographically. It provides an opportunity to examine the role of several important issues in the development of modern biology, not least the interplay between medical goals and the practice of basic science, the influence of patronage on scientific development, and the role of methods, techniques, and research schools in the advancement of a field.*

The research school from which animal virology derived was the phage school, the informal group that coalesced in the United States during World War II and was devoted to the study of bacteriophage, viruses that prey on bacteria. Historiographic interpretations of the phage group's approach to its task have been strongly colored by the outcome to which it contributed -- the development of molecular biology, particularly the conclusively reductionist identification of DNA as the material of heredity and as a molecule with the genetically-functional structure of a double helix. A key feature of historiographic debate has been whether, like molecular biology in general, the phage group's program expressed an intentionally reductionist drive to reduce life processes to the laws of physics and chemistry or whether it exemplifed the anti-reductionist inclination of its guiding spirit, Max Delbrück, to find laws of life that would be consistent with those of physics and chemistry but not reducible to them.[1]

The interpretive debate has also shaped historiographic attempts to locate the phage school in social context: Much of this analysis has focused on the patronage provided by the Rockefeller Foundation, pointing in particular to the influence of Warren Weaver and his eagerness to foster a reductionist program in the life sciences.[2] However, the development of animal virology suggests that what counted most in the development of molecular biology was not so much reductionism or anti-reductionism as the type of laboratory tools and methods that particular investigators found themselves able to employ in practice for the analysis of life processes. At the least, the phage school is cast in a different perspective by an examination of the career of Renato Dulbecco, who

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was its product and who in the 1950s, as something of its agent, contributed mightily to the transformation of animal virology into a biological science.

In a recent autobiography, Dulbecco recounts the personal circumstances and professional events that led him from his native Porto Maurizio, a small town in Liguria, on the northwestern coast of Italy, to a career in biological research in the United States and a share in the 1975 Nobel Prize for his work on animal viruses, genetics, and cancer. He grew up with the shadowy presence of an older brother who had died not long before his own birth, in 1914, leaving an empty place in the family to which Dulbecco succeeded. In his recollection, the lost sibling, whom his mother frequently mentioned, became "like an invisible companion bigger than I, whom I could count on." Dulbecco adds that having begun life "not by a debut, but rather by a continuation," he found that his parents always treated him as though he was older and entrusted him with greater responsibilities. He developed considerable faith in his capabilities and "confidence in my future, even though belonging to a modest family."[3]

Dulbecco's father, a native Ligurian, was a civil engineer and an avid follower of scientific developments in fields far beyond his metier, including genetics and hybrid corn. Dulbecco felt himself a hybrid of sorts, a product of his mother's expressive Calabrian culture as well as of his father's northern devotion to work. He was bookish and socially withdrawn yet possessed of a romantic streak, inspired by the town's vistas of the sea to imagine himself like the sailors of antiquity, who confronted perils to find new worlds, sometimes disappearing beneath the waves. He recalls contemplating the modern adventurers of thought, who, though not at risk of life, might become so lost in the pursuit of their unknown as to destroy themselves.[4]

Like many boys of the 1920s, Dulbecco built a crystal radio set and delved into electronics. He had his first taste of research in a project that he carried out at a local seismological observatory; he recounts that this success encouraged him to be open to new ideas, to recent technologies, and to adopt a progressive attitude and be ready to refuse the habits of the past. In 1930, when the time came to enter university, his inclinations ran to medicine, partly because an uncle was a surgeon, partly because the premature deaths not only of his brother but of a close school chum made him want to contribute something to the understanding and cure of disease. In any case, medicine was a discipline that "seduced him more than others because he knew it least."[5]

Enrolling at the University of Turin, where his father had gone before him, Dulbecco was drawn to professor Giussepe Levi, a tall, disheveled man with piercing, defiant eyes who was the dominant personality on the medical faculty. Levi was a demanding teacher, an exacting medical scientist, and a member of the Academy of the Lincei. The students revered and celebrated him not only for his scientific distinction but also for his short-lived rages and recklessly outspoken

antifascism.[6] Dulbecco's father was a liberal with socialist leanings, and Dulbecco had embraced his father's dislike for aristocracy. But it was a new experience to know someone who openly rejected fascism. Although Levi was in the anatomy institute, he was an anatomist at the cellular level, specializing in studies of the morphological and cytological characteristics of the nervous system. Each year, he took several second-year students as interns in his institute to pursue medical research. Dulbecco, an academic standout, applied and was accepted, thus entering what he has recalled as "the holy of holies," where the spirit of research dominated all other considerations and "where everyone was equal without regard to age, experience, or social status."[7]

Among the other second-year interns was the brilliant young biologist Rita Levi-Montalcini, whom Dulbecco got to like "both for her great intelligence and her innate and very feminine elegance" but not to know well at the time because she was five years his senior and he maintained a shy reserve. Less roseate than Dulbecco in her memory of life in Levi's laboratory, Levi-Montalcini has recalled being assigned the virtually impossible task of determinining how the convolutions of the human brain are formed and the utterly tedious one of counting nerve cells to test one or another theory of Levi's. Dulbecco did score a research success (his second since the seismograph project) in a dissection and counting of the cells in nerve ganglions, confirming Levi's theory that a deficiency of cells in one ganglion was compensated for in a neighboring one. However, Levi-Montalcini recalls his confiding to her that he was bored and was resolved to leave his internship for the institute of physiology in the third year, which he did. (Levi-Montalcini, who was eventually assigned a new and highly stimulating project -- it started her toward the line of research that would lead to her own share in a Nobel Prize -- remained with Levi, developing, as she has written, "a Master-disciple relationship, characterized by ever-increasing affection and reciprocal esteem which lasted until his death thirty-one years later.") Dulbecco passed his medical examinations with kudos from the examination commissioners and went off to practice medicine in a hospital.[8]

Although deriving deep satisfaction and a sense of importance from helping others, he remained eager for a life in research; but, having no laboratory of his own, he decided on a career in surgery, hoping to be an innovator in operations on the chest or brain. To follow such a career, he would first have to be taken on as an assistant to the professor of surgery. His hopes were dashed when the professor told him that many applicants were ahead of him, that he would have to wait years for his turn, and that he would need money to live on in the interim. When he told the professor that he had no such money, the professor declared, Surgery is not for you, Dr. Dulbecco.[9]

Dulbecco was devastated. What counted was neither all he had learned in medical school nor his merits but only money and seniority. A friend wisely counseled him to forget clinical medicine in favor of basic scientific research, for which he was well suited anyway. Although weighed down by his rejection, Dulbecco came to feel less pessimistic, consoling himself that medical advances depended upon scientific progress. While fulfilling his regular military service, in 1936-1938, he kept his hand in research, working over the observations that he had compiled during his medical studies and publishing the results. Afterward, he bided his time, hoping to become an assistant in the Institute of Pathological Anatomy in Turin. When the war broke out, he was mustered back into the army and stationed in San Remo, but he managed to prepare for his agregation, the diploma that would qualify him to become a professor, obtaining it by examination in Rome, in 1941.[10]

Some months later, Dulbecco, now married and the father of an infant son, was sent with his regiment to the Russian front. On the way, his troop train stopped for a few minutes at a station in Poland where he saw a gang of men and women maintaining the track; they were dressed in work clothes adorned with yellow stars. The station master told him that they were Jews and that, when they finished their job, they would be shot. Back on the train, Dulbecco, shocked, wondered why he was aiding "these assassins," thinking that "it was my duty" but that he was "unable to participate in this insanity." He reflected how the work gang might have included Jews whom he knew, particularly Levi-Montalcini or a young woman named Anita with whom he had once had a romantic relationship. The images grew searing and indelible in his memory, he writes -- "the Polish station, the work clothes with the yellow star, the station master, Rita, Anita." He had been accustomed to accepting the world, asking no questions, thinking that nothing could be done to change it. Now his cast of mind began to change.[11]

Wounded in action, Dulbecco was returned to Italy in 1943; after his release from hospital, he moved with his family to the small village of Sommariva, where he opened an office as doctor and dentist -- he taught himself the intricacies of dental care -- relinquishing science to serve the community and work in the underground. After the war, he returned to Turin, finally joining the Institute of Pathological Anatomy and becoming involved with a left-wing anti-fascist group. He says that although he was not displeased to think that the battle against fascism, once won, would lead to some type of communist regime, he withdrew from the group when it decided to enter the communist party. Politics, he realized, did not suit him, while science emphatically did.[12]

While at the Insitute, Dulbecco renewed his acquaintance, which now began to develop into a fast friendship, with Levi-Montalcini. Having been excluded from the university by the racial laws, she had pursued research in a small laboratory space in her bedroom in her family's house in Turin early in the war and then, driven from there by Allied bombing, in a small house

in the highlands an hour away from the city. In 1943, she had fled south to escape the advancing Nazis, establishing herself in Florence, where she frequently saw Levi, who was also living there, and busied herself distributing false identity cards. In 1945, Levi was back as head of the Anatomy Institute and Levi-Montalcini had been reinstated as his assistant. Learning from Dulbecco that he still wished to pursue research, she arranged for him to become an assistant to Levi, too, who needed someone to help in his work on experimental embryology.[13]

In the course of the embryological research, Dulbecco observed with surprise that chicken embryos exposed to radiation developed only male gonads. The result, which Levi enthusiastically communicated to the Academy of the Lincei, stimulated Dulbecco to embark on a program of general research into the effects of radiation on cellular development. At the time, he knew nothing about radiation or its biological effects. At Levi-Montalcini's urging, he enrolled in the University of Turin to study physics, at which he had demonstrated considerable talent and about which he had already learned enough to skip the first year's curriculum. After two years, he had learned enough additional mathematics and physics to imagine himself, he says, "performing experiments based on solid mathematical analyses, modifying properties of cells or embryos with radiation to penetrate to the mystery of genes and the control of life." But he had no precise plan about which class of cells or of organisms he would use. Indeed, he knew little about genes or genetics, having heard nothing about them during all his years at the university.[14]

To simplify the radiation research, Dulbecco had the idea of using cell cultures -- that is, the generation *in vitro* of living and reproducing cells. Levi had helped develop the method to study changes in nerve cells and he encouraged Dulbecco to exploit it. Dulbecco learned how to prepare cultures of chicken embryos, taking a fragment of embryo, enclosing it with a drop of chicken plasma between two strips of glass, and finding to his satisfaction that after incubation, the cells appeared to be truly living and ready for studies on the effects of radiation.[15]

However, just as Dulbecco was getting down to the radiation work, Salvador Luria visited Turin, where he, too, had done his medical studies before the war, matriculating a year ahead of Levi-Montalcini and Dulbecco and becoming a part of Levi's institute. Luria, a Jew, had emigrated to the United States in 1940 and he returned to Italy periodically to visit his family. During the war he had helped establish the phage school together with Max Delbrück, who was now a professor at the California Institute of Technology, and Alfred D. Hershey, a professor at Washington University, in St. Louis. The group's scientific hallmarks were an emphasis on the use of simple, uniform biological systems -- for example, bacteria and phage isolated and bred to have standard characteristics -- and the study of these systems with quantitative experimental techniques. Their fundamental experimental tool was a uniform culture of bacteria that revealed

the action of infective phage by the presence of countable clusters of dead cells, called plaques, readily observable on the culture's surface.[16]

Dulbecco and Luria had known each other only vaguely when they were medical students, but Levi-Montalcini had known Luria well and, as Dulbecco has remembered it, she reintroduced the two men. Dulbecco recounts the lengthy meeting:

> I told him of my interest in the action of radiation on cells, explaining to him that I had studied physics in order to comprehend these effects. He told me that he was concerned with bacteriophages. . . . He was interested in genes and he used these viruses because they had few genes, which simplifed the research. He also used radiation, had also studied physics during a period with Enrico Fermi and used mathematics for his experiments and to analyse the results. It was an agreeable surprise to discover that we had so many interests in commmon, although he was already a master in this field and I was merely a beginner.

After returning to Indiana, Luria -- apparently in response to a suggestion in a letter from Levi-Montalcini -- invited Dulbecco to join his laboratory. Dulbecco promptly quit his post with Levi and in September 1947 sailed for the States on the Sobieski in the company of Levi-Montalcini, who was bound for Washington University in St. Louis.[17]

The University of Indiana was then a major center of genetics research, its faculty including H. J. Muller, Ralph Cleland, and Tracy Sonneborn, among others. Luria's laboratory, located in an immense room under the roofs, was lit by two large windows that opened on a garden of majestic trees. Dulbecco was given a table just opposite one of these windows, while further away, in a sort of alcove, was James D. Watson, whom Dulbecco remembers as, at the time, "a very intelligent student with peculiar qualities. He was tall, skinny, and wore a large long jacket which seemed to fall off his shoulders. He had an extremely mobile look; when he reflected, he rolled his eyes and grimaced in an unbelievable manner, moving his lips and grinding his teeth."[18]

Luria was then working on the phenomenon called "multiplicity reactivation," a line of work that had originated in a chance observation by Delbrück and a collaborator, in 1946, that exposure to ultraviolet light would inactivate phage -- that is, make them unable to produce viable offspring after they infected a bacterial cell. Luria had recently discovered that several phage particles so damaged could jointly produce viable phage after infecting a bacterium. He had formulated several interpretations of the reactivation phenomenon, including the hypothesis that the exposure to ultraviolet light lethally mutated the individual phages but that viable phage were recreated in the bacterial cell by genetic recombinations among the damaged versions. Eager to test the hypothesis, Luria gave Dulbecco the task of defining its consequences in mathematical terms that could be measured in the laboratory. Dulbecco succeeded, the tests were performed,

and the effort produced a joint paper reporting the apparent confirmation of Luria's recombination hypothesis.[19]

In the course of making the calculations for Luria, Dulbecco realized that he had to consider as demonstrated a tacit assumption about phage behavior that had not been verified and that seemed improbable to him -- namely, that a limitless number of phage could mutually assist each other in the same bacterium. Working at nights and secretly for some weeks, he demonstrated that no more than 20 phage could aid each other in the same bacterium. The work impressed Luria, who later, in Dulbecco's presence, remarked to some friends, "My greatest contribution to biology is perhaps to have brought Renato into it."[20]

However, Dulbecco had also caught the attention of Max Delbrück, who in November 1948 offered him a senior research fellowship at the California Institute of Technology. The post, which was renewable, came with a stipend of $5,500 a year and no teaching duties. Dulbecco would be expected to conduct independent research in Delbrück's group. Delbrück told Dulbecco that he wanted him not only because of the "great contribution you could make to our scientific strength" but also because he could help increase the efficiency of the laboratory, explaining, "The laboratory is now running quite smoothly but with the increasing number of Fellows who work here only for a few months or a year I find it most desirable to have a reliable man on whose continued presence for a number of years I could figure."[21] Luria, made unhappy by Dulbecco's interest in Pasadena, renewed his contract and doubled his salary. Dulbecco, unsure about what to do, turned for advice to Watson, who stared wide-eyed at him, then rolled his eyes and declared, "Caltech has the best school of biology in the world. You have to accept."[22]

Accept he did, in a letter to Delbrück dated November 22, 1948, which hurried through the formalities ("the position and the kind of work are of the type I like") and then reported at length on a remarkable new phenomenon that he had just discovered by accident: Phage inactivated by ultraviolet light could be reactivated by visible light, so long as they were involved with bacteria. Luria was made glum by Dulbecco's discovery, wondering whether it undermined his recombination theories of multiplicity reactivation. In his autobiography, Dulbecco remembers that they soon convinced themselves that the two phenomena are independent. However, his letter to Delbrück indicates that even before stumbling onto photo reactivation, he had begun to doubt that multiplicity reactivation could be accounted for by genetic exchange alone, since just a single particle could be reactivated.[23] In January 1949, Dulbecco sent a letter to Nature announcing his detection of photoreactivation, noting that it confirmed a parallel observation by Albert Kelner, a bacteriologist at the Cold Spring Harbor Laboratory, made about the recovery of spores of Actinomycetes that had been treated with ultraviolet light then exposed to light in the visible spectrum. But Dulbecco was by no means finished with photoreactivation or with its bearing on

Luria's reactivation theories, and he told Delbrück that he wanted to pursue work on the phenomenon, which he found obscure, at Caltech.[24]

The Dulbeccos bought a tent and sleeping bags and camped their way from Bloomington to Pasadena, arriving late in the summer of 1949.[25] It was a tonic time to be in biology at Caltech: The Biology Division was headed by the distinguished biochemical geneticist George W. Beadle, and during the several years beginning in 1949 Delbrück's group counted among its visitors and regular staff many rising stars of phage genetics, including Luria, Watson, Seymour Benzer, Gunther Stent, Jean Weigle from the University of Geneva, and Elie Wollman from the Pasteur Institute. The group partied with musicales -- at which Dulbecco might play the piano, Benzer the violin, and Delbrück the recorder -- took their biological discussions with them on trips into the California desert and to summer sojourns at Cold Spring Harbor.[26]

Dulbecco, exhilirated, threw himself into further investigation of the interrelationship of multiplicity reactivation and photoreactivation. He soon found that multiplicity reactivation did not occur in one of the smaller phages, called T2. More important, by the middle of 1951, he had demonstrated, as Delbrück wrote in a report on the research, that "the rough agreement between theory and more limited earlier data was fortuitous" and that "the principal support for Luria's hypothesis has thus vanished, and a new explanation for multiciplicity reactivation has to be looked for."[27] However, Dulbecco was disinclined to do the looking, since by mid-1951 he was well embarked on research with animal viruses.

* * *

While the phage researchers' ultimate goal was, of course, to understand genetics, in establishing their model system -- phage and bacteria -- they inevitably had to deal with the interactions of virus and cell in their own right. For Dulbecco, the virus-cell system in and of itself was a compelling topic. Indeed, it prompted a type of patronage for some of the phage group that was concerned partly with the physics and chemistry of life but also with analyses of viral-cell interactions as such because they were taken to be fundamental to the understanding of disease and, by extension, to its prevention and cure.

Salient among such patrons was the National Foundation for Infantile Paralysis, whose annual operating budget was close to $20 million in 1945, and more than $50 million in 1953 and which used its funds primarily to explore the nature of poliomyelitis and to develop defenses against it.[28] The Foundation's medical and scientific advisers were well aware that polio was caused by an animal virus that attacked the cells of the nervous system, but that little was understood about the virus itself or how to proceed in dealing with the disease. They apparently

advised its officials to mount a two-pronged attack: award research grants to advance knowledge of the polio virus in particular and of viruses in general; and give postdoctoral fellowships to promising young scientists so as to increase the number of trained practioners in the field. The magnitude of its activities is suggested by the fact that even in 1953, when the National Institutes of Health (NIH) made microbiology an explicit commitment of its external grants program, providing some support for work in polio, the National Foundation spent more than twenty-five times as much on polio research as did the federal agency, which then devoted the largest share of its grant money to cancer research. Between 1938 and 1956, the National Foundation awarded 322 postdoctoral fellowships in virology and other fields related to polio, including 97 in microbiology. An official at the foundation estimated in 1956 that no fewer than one third of the virologists under 45 in the United States had been trained under National Foundation fellowships.[29]

Dulbecco was brought to Caltech with funds that Delbrück took from a multi-year grant that the National Foundation had inaugurated, in 1947, for the support of his viral group and that by 1949-50 totaled almost $90,000.[30] The Foundation did not seek to shape the Delbrück group's phage research program; its patronage was intended to foster basic research into viral behavior in general, and, in any case, nothing in phage research could be turned directly to bear upon the problem of polio. However, Delbrück's group was not immune to the influence of patrons with medical interests; indeed, its responsiveness to one such patron provided the impetus for Dulbecco's shift into animal virology.

The patron was James G. Boswell, a wealthy Los Angeles cotton broker who was a member of the Caltech board of trustees and had the misfortune to suffer from bouts of shingles, a painful disease caused by the herpes zoster virus. Boswell learned from his physician, Dr. Lawrence A. Williams, that no effective therapy was known for the disease -- but that one might eventually be found by extending the basic research on bacterial viruses then underway at Caltech into the area of animal viruses. By October 24, 1949, following a meeting with George Beadle, Boswell had pledged $100,000 to Caltech to establish the James G. Boswell Foundation Fund. The money was "to be used exclusively for basic biological, chemical, and physical studies on the fundamental nature of viruses," particularly "viruses that cause disease in man and more specifically viruses of herpes zoster and pneumonia."[31]

By mid-January 1950, Boswell had turned over half the pledged sum to Caltech -- the other half arrived in mid-March -- and Max Delbrück found himself wondering how to employ the gift so that Caltech might contribute to animal virology in a way that no other laboratory could. Caltech's comparative advantage lay in the distinction of its phage research, whose powerful techniques had so far had slight impact on animal virologists. Leading from strength, Delbrück made his first charge against the Boswell Fund the support of a symposium on viruses

that was held at Caltech in March 1950 and that brought together twenty outside specialists in plant, animal, and bacterial viruses together with fifteen Caltech scientists to compare notes.[32] The symposium was stimulating for the phage workers, most of whom were part of Delbrück's international circle, but was disappointing for its main purpose. Delbrück later told Lawrence Williams:

> The principal impression we derived from this symposium was that it would be very difficult for us, or for anybody else working at the Institute, to make a radically new contribution. The best we could then see to do was to bring one of the younger and brighter animal virologists to the Institute and hope that with our cooperation he would be able to improve upon the methods now used in the field. The prospects did not seem very bright, though, and we were reluctant to commit ourselves for a long term by adding a new man to the faculty without a concrete goal.[33]

What apparently made the prospects appear so dim was the methodological crudeness of a great deal of animal-virus research. It had long been recognized that viruses would not grow outside the living cell, which meant that the most convenient place for growing them was live animals. The best live animals for the purpose were those that, like mice or rabbits, were small and reproduced relatively quickly. However, many animal viruses could not be cultivated in mice; for example, polio virus had long appeared to be cultivatable only by injecting them into the brains of living monkeys, which had been employed early in the century to demonstrate that polio was a viral disease of the central nervous system. Even if small animals were used, the *in vivo* constraints made studies of animal viruses in the laboratory expensive, time consuming, and cumbersome.

In 1931, A. M. Woodruff and E.W. Goodpasture had reported that they had grown viruses on the sheets of uniform cells of the whole developing chicken embryo, that is, inside the fertilized egg (which, it has been noted, "can be seen as a particularly cheap and convenient experimental animal"). The method was comparatively successful and widely used during the 1930s. For example, in Australia, F. Macfarlane Burnet, one of the leading pioneers in animal virology, succeeded in growing the influenza virus in the developing egg. For all their utility, chicken embryos were not a suitable host for all animal viruses of interest and, more important from Delbrück's point of view, they did not lend themselves to precise assessments of viral action.[34]

An alternative to relying on eggs or animals was tissue culture. The first successful method of culturing tissue -- called the "hanging drop" -- had been invented in 1907 by the biologist Ross G. Harrison, then at The Johns Hopkins University, to study how nerve fibers developed. The hanging drop was formed by placing a bit of tissue on a flat piece of glass, then covering it with a small quantity of liquid nutritive medium, which would hold the tissue to the glass by surface

tension; the assembly would then be inverted to rest on a glass microscope slide with a shallow hollow in its surface so that the tissue-medium drop hung down into the cavity. It was the potential of Harrison's method for studying nerve cells in vitro that stimulated Giuseppe Levi to embark on research with tissue culture, a line of investigation that Levi and his students, including Levi-Montalcini, exploited to excellent effect. However, since the volume of the hanging drop medium was small, the useful lifetime of the culture was at most a few days, too short for extended studies of virus.[35]

All types of tissue culture suffered from one paramount problem -- the threat of bacterial contamination, which would kill any culture after only a few days. During the years bracketing World War I, Alexis Carrell, then at the Rockefeller Institute for Medical Research, had devised protective flasks -- eventually called "Carrel flasks" -- comprising a flattened glass container fitted with a long sloping neck that prevented particles of dust from falling in when the culture was manipulated or its medium changed. Carrel also introduced elaborate prophylactic measures into the laboratory environment, including the wearing of black gowns and masks and complex procedures for handling the cultures. He thought that tissue culture might be adaptable to the *in vitro* cultivation of animal viruses.[36] Although Carrel's methods were highly successful, they were so intricate that few scientists ventured to use or develop them. In 1954, a professor at the Royal Caroline Institute in Sweden would note that Carrel's was "a complicated ritual," continuing, "Tissue culture developed almost into a tissue cult, a mystery the secret rites of which were revealed only to a narrow circle of inaugurates with Carrel as their high priest."[37]

During the early 1930s, another method of culture became available for the cultivation of animal viruses. Devised in 1928 by H.B. Maitland and his wife Mary Cowan Maitland, at the University of Manchester, it involved suspending fragments of tissue in a salt mixture to which serum or plasma could be added. The suspension kept cells viable long enough to allow the multiplication and study of certain viruses. However, cell viability was too short and cell growth too minimal for close study of viral behavior. Indeed, none of the three methods -- the hanging drop, Carrel flasks, or the Maitlands' -- was, in its original form, adequate for the sort of controlled precision experiments, like those characteristic of phage research, that Delbrück regarded as necessary to advance animal virology.[38]

Following the Caltech virus conference, Delbrück resolved to explore the matter further. During the summer of 1950, he visited several animal virus laboratories, including, at the suggestion of Harry M. Weaver, the medical director of the National Foundation for Infantile Paralysis, that of John Enders, of Harvard Medical School. He was decidedly impressed by what he found there.

Enders had become interested in the viral culturing problem during the 1930s when he joined the Harvard medical faculty. At the end of the decade, together with Thomas H. Weller, who was a Harvard medical student, he sought to appropriate the so-called roller-tube method to the cultivation of virus. An idea first advanced in 1913 by Alexis Carrell, the roller-tube was basically a test tube arranged to lie horizontally in an apparatus that would slowly rotate it around its cylindrical axis. The method had recently caught the interest of George O. Gey, a young research physician at the Johns Hopkins Medical School who was interested in exploiting tissue culture to study malignancies. He had adapted the roller-tube method to obtain prolonged maintenance of tissues in an active state, which overcame the major shortcomings of the Maitlands' cell suspension. Gey's method involved imbedding tissue fragments in a plasma that was distributed evenly over the wall of a test tube, adding nutrient fluids, and then rotating the tube while keeping it at 37° C. -- body heat -- and replacing fluids and gas mixtures at frequent intervals.[39]

Such advances in tissue culture were as much the product of art and experience as of science. Enders and Weller could only speculate why the roller-tube maintained the activity of cells for longer periods than did classical methods, guessing that the rotation permitted the cells to respirate better because it alternately exposed the cells to the fluids and gases in the system. They added that the rotation possibly also permitted a more uniform distribution of harmful metabolic products and nutritive substances. Whatever the reason, the increased vitality of roller-tube cells suggested to Enders and Weller -- as it had, unknown to them, to Gey and his young collaborator Frederic B. Bang, who had just received his M.D. from Johns Hopkins -- that the method might be useful for the extended study of virus in culture.[40]

Indeed, Enders and Weller not only successfully cultivated vaccinia virus in roller tubes but found that the quantity of virus early increased to high concentration and remained at that level for as long as nine weeks. All the while, the cells in the tube continued to undergo division. Reporting their results in 1940, Enders and Weller cautioned against drawing general conclusions about the cultivability of viruses in roller tubes, but they did note that while they had been at work on their project, Gey and Bang had reported the roller-tube cultivation of the virus of lymphopathia venereum.[41]

In 1947, his research having been interrupted by the war, Enders, now dividing his time between Boston Children's Hospital and Harvard Medical School, resumed work on the cultivation of virus in tissue culture in collaboration with Weller and Frederick C. Robbins, who had been Weller's medical school roommate. Their attention was focused on the mumps virus. Their strategy was to continue to experiment with cultures of the Maitland type, but, apparently in light of the success in 1940, to provide the cultures with a turnover of nutrients and gases. They also

proposed to look for detectable levels of hemagglutinin, a clumping of red blood cells that had been used to estimate the concentration of viruses in infected materials, but that had not been tried in cell cultures.

They prepared a culture of minced chick embryos, suspended the fragments in a nutrient medium that included ox blood serum and a balanced salt solution, then placed drops of the suspension in 25 cc. Erlenmeyer flasks containing 3 cc. of the nutrient medium. The turnover, a departure from the usual procedure, was accomplished by replacing the nutrient fluid at intervals (the flasks were tilted, allowing the tissue to settle; the supernatant fluid was then removed as best as possible and new fluid was added.) The results were decidedly satisfying: Mumps virus increased rapidly and also produced measurable amounts of hemagglutinin in the tissue culture. In their first paper on the work, published in 1948, Enders, Weller, and Robbins concluded that their innovative technique -- continuous culture, with periodic replacement of the nutritive medium while leaving the virus culture intact -- provided "a swift and convenient means of following the rate and degree of multiplication of the mumps and influenza viruses in a system which, compared with a medium such as the chick embryo or the mouse, is far less complex."[42]

Enders, Weller, and Robbins promptly extended the technique to the cultivation of varicella (chicken pox) virus in cultures of its natural host, human embryonic skin and muscle tissues, and it then occurred to them to try it with polio virus. Although experiments in the mid-1930s had indicated that polio virus would grow only in neural tissue, they were aware of accumulating recent evidence that it might not be a strict neurotrope. Along with others, they found it difficult to see, for example, how the nervous system alone could produce the abundant quantities of polio virus found in the feces of many patients. They also had in a laboratory freezer a sample of the Lansing strain of polio virus that had been sent them some time earlier by the National Foundation. In 1948, appropriating some of the cultures that they had prepared for the chicken pox experiment, they managed to cultivate the polio virus, thus demonstrating that it could be cultivated, and not only in neural tissue.[43]

Now focusing on the polio virus, they developed and refined their technique in a variety of ways. Among the most significant, they obtained usable polio virus from feces or spinal cord suspensions by suppressing the bacterial contamination of these sources with the newly available antibiotics, penicillin and streptomycin, then centrifuging the sample. They thus eliminated the necessity of obtaining polio virus via the laborious and time-consuming procedure of inoculating the brains of living monkeys.[44] They also managed to cultivate polio virus in both hanging drop and roller-tube cultures and to obtain cytopathogenic effects in both those as well as suspended cells. In a 1950 report on their work, they noted that the results were significant in two important respects:

> "First, they leave no doubt that poliomyelitis virus *in vitro* can multiply in cells other than those of the nervous system and cause profound injury of such cells. Secondly, they provide criteria by which the presence of the virus can be recognized *in vitro* and hence may afford a basis of techniques for isolating virus from patients or animals, for the quantitative assay of virus, for serologic typing and possibly for the screening of chemotherapeutic and antibiotic substances."[45]

In 1954, the achievement of Enders, Weller, and Robbins would be recognized by the award of the Nobel Prize in physiology or medicine. However, in 1950 Max Delbrück already appreciated the significance of their work, later noting:

> This line of attack appealed to me because it brings the study of animal viruses a little closer to the cellular level, and also because it seems to hold out the hope of improving the quantitative precision of the work without running into gigantic expenses for vast numbers of experimental animals, embryonated eggs, technical personnel and laboratory equipment. Moreover, technically it seemed to be relatively simple, so that any person, after a short period of training, should be able to do the work.[46]

Probably some weeks after his trip to Enders' laboratory, in the fall of 1950, Delbrück invited Seymour Benzer and Dulbecco to his office to ask whether either of them would like to interrupt his work on phage for a few months to make a thorough study of the new approaches to viral culture. Dulbecco, perhaps nostalgic for his forays into tissue culture in Levi's laboratory, jumped to say yes before Benzer could respond (Benzer was not interested anyway).[47] Delbrück wanted Dulbecco to visit Enders' laboratory in particular, but Enders, short on space and unable to accommodate him right away, suggested that Dulbecco first spend time at the laboratory of Gey and Bang, at Johns Hopkins University, a sojourn that Delbrück arranged during a trip there in December. On January 20, 1951, Dulbecco, with a travel advance of $500. from the Boswell Foundation Fund in hand, boarded a train that would take him for a month with Gey and Bang in Baltimore, a few days with Enders, plus a few more days in each of several other virus laboratories between Boston and Denver.[48]

Arriving in Baltimore on January 29, Dulbecco promptly got in touch with Gey and Bang, who urged him to follow their work closely for a while to familiarize himself with the details and difficulties. In a letter to Delbrück at the end of the first week, Dulbecco reported that he was beginning to understand the troubles of people in the animal virus field. While Bang had been very friendly, Gey appeared to think he knew everything but was not much use, except in questions confined to the techniques of tissue culture. In that area, Dulbecco judged him "really competent" but like other people in it "vague and frustrated, because this field of great promises has given in the end very little," adding:

> His main work is to culture different types of cells, and look at them, and describe fine differences between, for example, a normal and a cancerous cell; but beyond that he is impotent, and the weakest point is that he has very little opportunity of experimentation, except slightly influencing velocity of growth. Now the possibility of putting a virus into a culture has given him a big push because [it] is an intelligent way of experimentation. But still things are very confused also in this particular point, and this, I think because these people have not tried to study the elementary things, like how many cells in a culture are infected, if the whole cell is infected, differences in infectabilities of different cells with different viruses, but have jumped to the most complicated things like the difference in effect of a given virus . . . on a cancerous and a non-cancerous strain of some cellular type.

Dulbecco regarded the "things" as of such complexity as to be worse than putting together multiplicity reactivation and photoreactivation.[49]

The more he learned about viruses and cell cultures, the more Dulbecco became convinced that the work would be much improved by a more accurate system of assay -- that is, counts of viruses, infected cells, time for infection. Later in February, following a seminar on the assay problem that his hosts organized for him with assistance from statisticians in the School of Hygiene, Dulbecco wrote to Delbrück that "the method of assay presently used is unbelievably coarse." Estimates of relative viral concentration commonly differed from each other by a factor of seven in either direction, allowing ratios as high as 50 in estimates of maximum and minimum values.

Still, to Dulbecco, neither Bang nor Gey seemed much interested in the assay problem or in using tissue culture for well-designed experiments concerned with viruses. At one point, he had infected chicken embryo cultures with Newcastle virus, which preys on chickens, and found a few patches of dead cells -- perhaps something to count -- but Bang, to whom he showed the results, appeared unconvinced that the patches were significant.[50]

A few days before leaving Baltimore on February 27, Dulbecco wrote to Delbrück that his stay there had been instructive and -- in confirmation of Delbrück's advice to forget viruses at night and concentrate on pleasant readings -- also made enjoyable by his discovery of the Peabody bookshop, which housed piles of dusty books, including a number in Latin and Greek, and a small place to drink a good beer and meditate over which volumes to purchase. He had learned a little about tissue culture and a lot about viruses and had become increasingly convinced that tissue culture might be made into a valuable tool for virus research. Now he was off to Philadelphia, New York, and Boston, though he had been compelled to cancel a planned visit to Canada because the Immigration Service had refused to permit him to cross the border. Having

served in the Italian Army, he was considered a fascist, he told Delbrück, adding that the change in plan was no great loss because he found the work of the people he had wanted to visit there unexciting.[51]

Two weeks later, on March [??], Dulbecco started for home, writing Delbrück just before his departure that the last week of his trip had been the most successful because of his visits to two places where he had obtained "the best information connected with our purpose." The first was the laboratory of Enders, whose viral culture techniques struck Dulbecco as impressively useful and whose hospitality brought him to spend an excellent Saturday afternoon and evening playing a considerable amount of Mozart and Hayden on a marvelous Steinway, in a four-hand mode with his host. The second was the laboratory of Wilton R. Earle, a cytologist and authority on tissue culture at the National Cancer Institute. Earle had recently devised techniques that permitted him to obtain two results of interest to Dulbecco: suspensions of apparently individual cells, alive, at high concentration; and large uniform sheets of cells of uniform nature. Dulbecco told Delbrück that the techniques were "very easy," adding that Earle had also grown cultures from a single cell, a feat that ten years earlier had been regarded as "a dream."[52]

The import of Earle's techniques were made clear in a 13-page typed report that Dulbecco wrote -- it was dated March 23, 1951, four days after his return to Pasadena -- on what he had learned from his exploration of, to quote from the report's title, "modern trends in animal virus research and the possibility of using tissue cultures as hosts for animal viruses."[53] Crafted from the perspective of an acolyte of the phage school, the report stressed that in key areas even the most advanced methods in animal virology lacked exactitude and specificity. The inaccuracies began with the common methods of assay, Dulbecco pointed out, formally elaborating on his session with the Johns Hopkins statisticians. Titration -- that is, comparative measures of viral concentrations -- were generally made by what were called "end-point methods" -- that is, by estimating how much a given viral solution had to be diluted to kill 50% of inoculated sensitive elements such as animals, embryonated eggs, or culture in flasks. Statistical analysis revealed that with successive dilutions by a factor of ten "any one estimation of a given virus activity is subjected to uncertainty in a range whose ratio between upper and lower limits is about a factor of 40!!" Even with a dilution factor of two the ratio was still seven. The uncertainty in titration made it impossible to determine biologically the absolute number of active virus particles in a sample. Non-biological determinations -- for example, with electron microscopic observations of the number of virus particles or analysis of the light-scattering properties of virus suspensions -- were also "highly inaccurate."[54]

Further uncertainties arose from relying on cell cultures that derived from embryos as they were usually prepared, by mincing: the cells in a given culture might differ from each other in

type since they might come from different cells in the original embryonic tissue. This ambiguity undercut studies of fundamental viral-cell interactions -- adsorption of the virus as well as its genetics and reproduction. It also played havoc with analysis of a virus' impact on the cell. When phage reproduced in a bacterium, the cell would burst -- phage researchers said "lyse" -- but electron microscope observations in the laboratory of Gey and Bang had indicated that animal viruses did not always behave the same way. While in some cases the cells could be seen to burst, liberating virus, in others newly produced virus were observed with no visible cell destruction or, in still others, with partial cell destruction.[55]

Greater uniformity of cell types could be achieved with Carrel-type flask cultures than with Maitland cultures, but, in both, assessments of viral behavior were further clouded by the use of tissue fragments. Introduced into the culture, the virus would infect the peripheral cells in the fragments, then diffuse slowly toward the center, where it might or might not destroy cells -- which, in any case, would grow more slowly than those on the periphery -- and where its progeny might be hidden. Both the production of virus and estimations of cell death could only be made indirectly: for example, in the former case by hemagglutination; in the latter, by tracing the change in pH, which was modified by cell death, in the medium during the life of the culture. Whatever the type of culture in use, the number of cells could not be determined accurately, and neither could the number of virus nor the number of cells infected. In Dulbecco's summary, these techniques of tissue culture, "although presenting advantages for quantitative studies over the use of whole animals or embryos, are still subject to very serious limitations."[56]

It was all these inadequecies that made Dulbecco think that the techniques recently devised in Earle's laboratory "allowed the formulation of a project" that might render the cultivation of animal viruses comparable in experimental value to that of phage. What Dulbecco expected the technique to permit was the production of a flat layer of culture of a relatively pure cellular type. The method of detecting virus activity would then be "an unmistakable action of the virus on the cells, like lysis," and with the help of this effect, techniques would be worked out to determine the number of active viral particles.[57]

What Earle had done was to accomplish the growth of a uniform layer of cells from a tissue fragment that was introduced on the bottom of a Carrel flask and then covered with a sheet of perforated cellophane under a blanket of liquid nutrient medium. A relatively pure tissue type could be obtained by washing the cellophane of cells that had adhered to it, then recovering them as a suspension of isolated cells, fractions of which could be used to start new cultures of uniform cell layers on the bottom of a flask. Still, there remained the problem of how to put a virus suspension on the cell layer so that, as in phage, infections could appear as isolated plaques -- which was to say, as foci of infection that were discreetly countable. The trick was to arrange the

culture so that newly produced virus would not diffuse much over the layer, away from their own infective neighborhood.[58]

Dulbecco, who had discussed how to accomplish the trick with Earle and Enders, among others, concluded his report with a proposed line of attack. Place a layer of agar (i.e., nutritive medium) at the bottom of the flask, then try to grow a suspension of cells, isolated by Earle's technique, and virus on top of it. The virus produced by infection might be kept to their own neighborhoods by covering the entire cell layer with another layer of nutrient. Foci of cell destruction might be detectable and countable if the virus used was highly destructive. The best qualified virus appeared to be equine encephalitis virus. The preferable tissue seemed to be mouse rather than chicken embryos, because connective fibers in chick embryos made it difficult to separate cells from each other. Dulbecco added that his project had the support of virologists, and that if it worked, "it would bring a tremendous improvement in the research techniques for animal viruses." He would need only modest equipment -- a room with two cubicles, one with a hood for handling virus, the other without a hood for tissue cultures; a small incubator for eggs, for control titrations; an electric deep freeze, for nutritive media; a tissue grinder; glass flasks; and a microscope.[59]

Delbrück thought that the project would work: Dulbecco was told to go ahead, and by April 1951, laboratory space was being fixed up for him. Dulbecco recalled that he assured his colleagues that Western equine encephalitis virus was "not dangerous to human beings," although in reality, it was, explaining that he had to use it "at all costs" because it rapidly killed embryonic cells and thus offered a higher probability of success. "At Caltech, no one knew much about viruses and my proposal was accepted, but as a precaution, I was relegated to a laboratory far away in the sub-basement."[60]

Dulbecco spent some weeks attempting to grow cells as bacteria were grown, using tissue fragments on an agar layer. He failed. By then it was late summer, and he got an idea about how to proceed when he planted new dichondra lawn in his backyard. Dichondra lawns are grown from flats that are planted in the soil about a foot apart and that then spread to cover the entire ground. It occurred to Dulbecco to try to grow a cell sheet from uniform small jots of embryo planted on the agar. He devised mechanisms -- stacks of razor blades thinly spaced, for example -- to slice tissue into the right kind of bits and achieved results that were encouraging. However, Earle, knew that Dulbecco was trying to grow cells in a layer without cellophane. He invented a highly effective way to dice chicken embyros -- centrifuging them through wire gauzes of successively finer mesh -- and sent Dulbecco a description of the method along with proof that it worked, a photograph of what he called a "steak" of cells growing at the bottom of a flask. The method worked for Dulbecco, too, who arranged to free the chick embryo cells from each other

before culturing by digesting their connective fibers with trypsin (the technique had been suggested by Enders).[61]

Dulbecco recounts what happened next:

> When everything was ready, I performed the crucial experiment, infecting the culture with very diluted virus. Once the plaques were placed in the incubator, an anguished wait began for me, because I didn't know how much time the process would take. That night, I slept very poorly. I got up early, ran to the laboratory, but I saw nothing interesting; in the afternoon, still nothing. I passed yet another night without sleep and the next morning, again nothing. I began to be preoccupied; should I concede that I was blocked? I reexamined the plaques in the afternoon and noted some irregularities. While I manipulated one of them next to an electric light, I suddenly saw in the cellular layer about 50 round stains which appeared whitish in the weak light but which were not visible in sunlight. Examining them again several times, I assured myself that it was not a dream and that it was truly the plaques that I was looking for.

A few days later, he invited Delbrück downstairs and showed him a plate with white plaques. Delbrück wanted to know the date, remarking that it was one they should remember.[62]

Dulbecco did not note the date, but it was no later than January 1952. By then, he had sufficiently developed his viral culture technique for George Beadle to write to James Boswell that Dulbecco had succeeded in growing cells in thin layers, adding viruses, and getting centers of infection that could be studied carefully, noting that the new method of tissue culture "should make it possible to get the virus of Herpes Zoster out in laboratory culture and really learn something about it." Beadle added that Caltech had "the best group of workers in the virus field . . . anywhere in the world today" and that a supplement to the Boswell Fund -- enough, say, to provide $25,000 a year -- would ensure Caltech's future in the area. In March, Boswell, donated another $125,000 to the Boswell Foundation Fund at Caltech. That month, Dulbecco was promoted from senior research fellow to associate professor of biology with tenure, effective July 1, at a salary of $7,000 a year that would come from the Boswell Fund.[63]

In April, Dulbecco, joyful at the recognition, reported on his results at the annual meeting of the National Academy of Sciences and soon thereafter he published a full-scale paper on the work in the Academy's Proceedings under the title "Production of Plaques in Monolayer Tissue Cultures by Single Particles of an Animal Virus." The paper stressed that he had established a virus-cell system suitable for quantitative analysis. He had obtained the growth at the bottom of a Carrel flask of chicken-embryo cells in a uniform layer -- that is, generally consisting of only one cell type and being mostly one-cell thick. After three days of incubation with Western equine encephalitis virus, round necrotic areas about 2 to 4 mm. in diameter appeared as bright, discrete areas -- the plaques -- against the dark background of the living cell culture.[64]

Dulbecco also contended, no less important, that each plaque represented the production of a single original viral particle and their progeny. The contention was grounded empirically in the fact the number of plaques produced depended linearly on the dilution of the virus concentration, and theoretically on an analysis, using the Poisson distribution, that such a linear relationship would hold only if each plaque was produced by a single infecting particle. The one particle-one plaque relationship was what qualified Dulbecco's technique of viral culture as suitable for quantitative analysis in the mode of the phage group. As he noted, it indicated that "the plaque count is an efficient assay technique" and it established "a basic concept concerning animal virus action, namely, that infection of an embryo is produced by one virus particle."[65]

In February, proposing the communication from Dulbecco for the meeting of the National Academy of Sciences, Delbrück had written, "I believe that this paper marks a turning point in research on animal viruses and that it will be of interest to many people in biological and medical research."[66] So it did, and so it, at least eventually, it was.

* * *

In the late spring of 1952, shortly after the National Academy meetings, Harry M. Weaver, the medical director of the National Foundation for Infantile Paralysis, approached Dulbecco, offering him the foundation's subvention to apply his techniques to the study of polio. With the enthusiastic support of Beadle and Delbrück, Dulbecco embarked on a program of research on the polio virus in a laboratory established for him away from the campus -- because of fears concerning the infectiousness of the virus -- at the Huntington Hospital, in Pasadena. (Dulbecco has written of the arrangement, "I had to laugh about it a bit because the equine encephalitis virus with which I had begun my experiments were much more dangerous.") The laboratory was well equipped thanks to the foundation, and thanks to Caltech he had a valuable collaborator named Marguerite Vogt, a research fellow in biology who was the daughter of a celebrated German neurologist, a dedicated scientist, and a fine adept at tissue culture. Delbrück had thought that she would greatly like the work and be excellent at it.[67]

Dulbecco and Vogt soon demonstrated that the techniques of culture and assay that had worked with encephalitis virus also worked with polio virus. Their research yielded improvements in both types of techniques, including simpler and quicker methods for obtaining polio virus cultures and the trick of staining the cells with a red dye so that plaques would stand out. By 1955 they had exploited the plaque technique to investigate intracellular growth of polio virus, its release from infected cells, its genetic properties, and its inactivation by antibodies.[68]

In a recent interview, Dulbecco reflected that "it was the methodology that determined progress, as it always does," adding that the crucial method in the case of animal viruses was the quantitative assay. Clearly the patronage that shaped Dulbecco's work was, in the case of the Boswell gift, virtually determining and, in the case of the National Foundation's subvention, certainly infuential. Dulbecco observed, "This question of patronage in the selection of research, I think it plays an important role, because you know, at that time, polio virus to me was a virus just like any other. I didn't have any special reason to work or not to work on the polio virus. Since there was a need and there was good support, I said, Sure, why not?"[69]

Despite the power of the plaque technique, it was not promptly embraced by animal virologists. In 1955, Dulbecco published a review of the interactions of viruses and animal cells, thinking that the "historical moment" required a survey of the new methods and techniques to point out their superiority to many of the traditional approaches in the field. He noted "a kind of aristocratic trend" among some animal virologists, an inclination to remain insulated from developments outside their own field. They appeared unwilling to take seriously the utility of phage methods because at least some of them were wont to say that "'bacteriophages are not viruses,'" a position they tended to hold, Dulbecco later explained, because phage did not cause animal or human disease. However, the techniques of culture, methods of assay, and, above all, quantitative approach to viral behavior -- all of which Dulbecco reviewed in the 1955 survey -- took increasing hold among animal virologists, were further developed, and, by the 1960s, had become fundamental to the arsenal of the field, including its enlargement into a branch of molecular biology.[70]

Dulbecco himself turned to a molecular interpretation of virus-cell interactions once he embarked on research in tumor viruses, during the late 1950s, when the field was growing in interest and excitement. His own interest was prompted by Harry Rubin, a postdoctoral fellow at Caltech, and Howard Temin, a doctoral student, who pioneered the quantitative study of the tumorogenic Rous sarcoma virus in cell culture, detecting the viral transformation of cells by the foci they formed and using the foci for analysis in the manner of plaques. In 1960, Dulbecco and Vogt achieved tumorogenic transformation in hamster cell culture with the polyoma virus, which had been recently discovered and also shown to be a DNA virus, unlike the Rous, the core of which comprised RNA. They observed -- not unexpectedly, in light of the behavior of temperate phage viruses -- that the polyoma virus quit reproducing once it started to transform the hamster cells. Dulbecco hypothesized that the polyoma DNA was no longer available for viral reproduction because, in transforming the cell, it was integrated into the hamster DNA. In 1962, Dulbecco left Caltech for the new Salk Institute, in La Jolla, California, where, together with collaborators, he

proved his hypothesis of molecular integration, a demonstration that helped significantly to open the field of molecular tumor virology.[71]

However, what Dulbecco learned in the 1960s was not indicative of how he approached animal virology in the 1950s. To be sure, like his fellow phage researchers, he assumed that viruses and genes were something physical, but he did not know what they were and scrupulously avoided assumptions, reductionist or otherwise, about their nature. In the first paper on polio virus, published in 1954, he declared that what caused the infection was a single entity but that otherwise "we do not know its morphological or genetic properties." And in the 1955 review article, he flatly asserted that a preparation of virus was a population of particles, and quantitative study of them had to be based on the statistics of discrete distributions, adding, "The only meaningful quantities are the number of virus particles, and certain relations between number of virus particles and number of host cells."[72]

It should not be surprising to find a product of the phage school embracing such an outlook. To be sure, the leaders of the phage group and many of its members had been physicists or had at least, like Dulbecco, undergone serious study in that discipline, but to be acculturated to physics in the mid-twentieth century was not necessarily to be an evangel of reductionism in biology.[73] Physics had, after all, just passed through the revolution of quantum mechanics -- which at the atomic level was widely taken to imply that science could not discover tangible models of reality but could only deal with measurable phenomena and predict the results of experiment. If Dulbecco is any indication, what the phage research program emphasized in its heyday was not primarily an eventual reduction of phenomena to physical and chemical models but a quantitative positivism. Phage researchers focused on phenomena that, like the characteristics of spectra in quantum physics, were directly measurable -- for example, the number of virus, rates of infection, quantities of virus produced, frequencies of recombination.

This emphasis had nothing necessarily to do with Delbrück's quasi-religious commitment to the Copenhagen interpretation of quantum mechanics or with anti-reductionism or with reductionism.[74] It had everything to do with what phage researchers *could* measure at the time, with what was within the contemporary scope and reach of laboratory practice for phage replication. In a 1937 memorandum on the "Riddle of Life," Delbrück had proposed thinking of viruses as well-defined molecules. In point of time, the memorandum qualifies as a heuristic statement, but it implied a research program that inadequacies of knowledge and technique made unrealizable for some years. Luria, who proudly approached phage problems with a physicist's cast of mind, illuminated the point in his autobiography:

> We could by radiation experiments estimate the specific portion of a phage that was needed for reproduction; and we could by mixed infection with two phages define a limit to their individual ability to multiply in a bacterium. But we could not translate these observations into terms of function. The missing link between the hereditary material of any organism and the functions of that organism, including its ability to reproduce, was the nature and activity of the genetic material. These were essentially chemical problems, and their solution had to await the discovery, twelve years later, of the DNA double helix and the biochemical interpretation of protein synthesis under gene control.[75]

Similarly for Dulbecco, who moved to the molecular level of analysis only when it became plausible and practical to do so. In the years before then, having succeeded in extending the plaque technique to animal virus research, he exploited it as best and richly as he could. Just as he had done in the work on multiplicity reactivation and photoreactivation, he made the hallmark of his early research on animal virology a reliance on measurable variables, as well as on testable hypotheses mathematically incorporating them. Dulbecco has recalled that the assertion of morphological ignorance concerning animal viruses in the 1955 review amounted to a programmatic statement, explaining in the recent interview, "At the time, we felt that we don't have to see this particle, we know what they do, we can study their properties without seeing them." He added with a laugh, "Actually, we were kind of proud that we couldn't see them."[76]

Notes

1. An excellent overview of the reductionist issue is John A. Fuerst, "Reductionism in Molecular Biology," Social Studies of Science, 12(May 1982), especially pp. 242-44, 250-53.

2. Ibid., pp. 253-56. A different view of the Rockefeller Foundation's intentions and effectiveness in biology is Pnina Abir-Am, "The Discourse of Physical Power and Biological Knowledge in the 1930s: A Reappraisal of the Rockefeller Foundation's 'Policy' in Molecular Biology," Social Studies of Science, 12(1982), 341-82.

3. Renato Dulbecco, Aventurier du Vivant (Paris: Plon, 1990), pp. 14-15.

4. Ibid., pp. 39-40.

5. Ibid., 13-14, 21, 23, 39-42, 45-46.

6. On Levi's overall career, see Oliviero M. Olivo, "Giuseppe Levi," Dictionary of Scientific Biography, VIII, 282-3. His daughter, Natalia Ginzburg, provides personal glimpses of him in her Family Sayings (New York: E.P. Dutton, 1967) and Rita Levi-Montalcini, In Praise of Imperfection (New York: Basic Books, 1988), pp. 48, 50-2, 204-5 sketches the scientific personality with a filial affection. Additional bits are to be found in Salvador E. Luria, A Slot Machine, A Broken Test Tube: An Autobiography (New York: Harper and Row, 1984), pp. 16, 62.

7. Ibid., pp. 41-42, 48-49, 51; Levi-Montalcini, In Praise of Imperfection, pp. 49-50.

8. Dulbecco, Aventurier du Vivant, 45-50, 52-53, 56, 65-66; Levi-Montalcini, In Praise of Imperfection, pp. 52, 58-60, 65.

9. Dulbecco, Aventurier du Vivant, 45-50, 52-53, 56, 65-66.

10. Ibid., pp. 65-66, 70-71, 80-83.

11. Ibid., pp. 90, 107-108.

12. Ibid., pp. 112, 125.

13. Ibid., p. 126; Levi-Montalcini, In Praise of Imperfection, pp. 92-109. Levi-Montalcini's account of why she arranged for Dulbecco's appointment with Levi, departing from his, holds that he would have more time as Levi's assistant to pursue the physics course than he would have had in the Institute for Pathological Anatomy, where much time had to be spent supervising students' laboratory sessions. Levi-Montalcini, In Praise of Imperfection, pp. 112-13. However, Dulbecco places the initiative to study physics after he had returned to Levi's institute and under circumstances that seem more plausible. See below.

14. Dulbecco, Aventurier du Vivant, pp. 127-30.

15. Ibid.; Olivo, "Giuseppe Levi," p. 283.

16. Ernst Peter Fischer and Carol Lipson, Thinking About Science: Max Delbrück and the Origins of Molecular Biology (New York: Norton, 1988), pp. 148-66; Robert Olby, The Path to the Double Helix (London: McMillan, 1974), pp. 225-26, 238-40; Horace Freeland Judson, The Eighth Day of Creation: Makers of the Revolution in Biology (New York: Simon and Schuster, 1979), p. 70.

17. Dulbecco, Aventurier du Vivant, pp. 127-30; Levi-Montalcini, In Praise of Imperfection, pp. 113, 117; Luria, A Slot Machine, p. 42. What must be a typographical error in Levi-Montalcini's book mistakenly dates the sailing in September 1946.

18. Ibid., pp. 138-39.

19. S.E. Luria and R. Dulbecco, "Genetic Recombinations Leading to Production of Active Bacteriophage from Ultraviolet Inactivated Bacteriophage Particles," Genetics, 34(March 1949), 93-125.

20. R. Dulbecco, "The Number of Particles of Bacteriophage T2 that Can Participate in Intracellular Growth," Genetics, 34(March 1949), 126-132. Dulbecco recalled that also impressive to Luria was how he had tackled the problem: "Before everything, I had found the weak point of a fact that everyone accepted tacitly; in searching its experimental verification, I formulated a precise hypothesis and developed a mathematical theory for it, obtaining thus an exact solution; finally, on the basis of the theoretical predictions, I conducted experiments utilizing a technique that was very adequate technique but never utilized before." Dulbecco, Aventurier du Vivant, pp. 138-39.

21. Delbrück to Dulbecco, Nov. 11, 1948, Max Delbrück Papers, Box 6.22; Delbrück to George W. Beadle, Jan. 7, 1949, Biology Division Records, box 42, Dulbecco file, both in California Institute of Technology Archives, Pasadena, CA.

22. Dulbecco, Aventurier du Vivant, p. 149. Here Dulbecco mistakenly recalls that it was his discovery of photoreactivation that prompted the offer from Delbrück. The offer came earlier; indeed, Dulbecco first told Delbrück about photoreactivation in his letter accepting the senior research fellowship. See Dulbecco to Delbrück, Nov. 22, 1948, Delbrück Papers, folder 6.22. On the development of biology at Caltech, see Judith R. Goodstein, Millikan's School: A History of the California Institute of Technology (New York: W.W. Norton, 1991), pp. 193-212.

23. Dulbecco to Delbrück, Nov. 22, 1948, Delbrück Papers, folder 6.22; Dulbecco, Aventurier du Vivant, pp. 147-49.

24. R. Dulbecco, "Reactivation of Ultra-Violet-Inactivated Bacteriophage by Visible Light," Nature, 163(June 18, 1949), 949-50; Dulbecco to Delbrück, Dec. 23, 1948, Jan. 4, 1949, Delbrück Papers, folder 6.22. The acknowledgment of Kelner's priority was a concession for Dulbecco that Delbrück had apparently urged him to make. Dulbecco's discovery had been made independently of Kelner's and concerned a different phenomenon, but Kelner had written to Luria about his observation with the Actinomycetes spores and had for that reason questioned the legitimacy of Dulbecco's precedence. Dulbecco hoped that the concession in Nature would be satisfactory to all concerned and that he would have "better luck next time." Dulbecco to Delbrück, Jan. 22, 1949, Delbrück Papers, folder 6.22.

25. Dulbecco to Delbrück, April 29, 1949, Delbrück Papers, folder 6.22.

26. The composition and activities of Delbrück's group can be followed in his semiannual reports to the National Foundation for Infantile Paralysis for 1949 to 1951, Delbrück Papers, folder 31.2. For the musical instruments, see Delbrück to Dulbecco, c/o Enders, March 5, 1951, ibid., folder 6.22.

27. Delbrück, "National Foundation for Infantile Paralysis Semiannual Reports," July 1-Dec. 31, 1949, p. 1; Jan. 1-June 30, 1950, p. 1; Jan. 1-June 30, 1951, Delbrück Papers, folder 31.2; R. Dulbecco, "A Critical Test of the Recombination Theory of Multiplicity Reactivation," Journal of Bacteriology, 63(1952), 207. For a review of the issue, which became recognized as a general phenomenon, see Renato Dulbecco, "Photoreactivation," in Alexander Hollaender, ed., Radiation Biology, Vol.II: Ultraviolet and Related Radiations (New York: McGraw-Hill, 1955), pp. 455-86.

28. Jane S. Smith, Patenting the Sun (New York: William Morrow, 1990, pp. 82, 161.

29. In 1953, the NIH polio research budget was $72,000; the National Foundation's, $2 million. Ibid., p. 24. In the twenty years after 1938, National Foundation grants went for work of pathbreaking significance across a broad spectrum of microbiology. By 1956, 1,870 papers had been published that acknowledged its assistance: roughly 10% were in basic biochemistry, 14% in basic physiology, and 20% in viruses and viral diseases other than polio. The Foundation's grants included sizable subventions to Linus Pauling at the California Institute of Technology for research into the structure of proteins, nucleic acids, and their components, and to Wendell Stanley, at Berkeley, for inquiries into the physical and chemical properties of plant, animal, and bacterial viruses. Its postdoctoral awards included a fellowship to James D. Watson that supported him during the year he puzzled out the structure of DNA with Francis Crick. T. E. Boyd, n.d. [1956], "Memo to Basil O'Connor, "Contributions to Science in the Field of Poliomyelitis," March of Dimes Birth Defects Foundation Archives, White Plains, New York, pp. 19-24, 31-32; James D. Watson, The Double Helix (New York: Athenaeum, 1968), p. 132. The National Foundation awarded grants for work on the encephalitides virus at Berkeley; on human viral diseases at Harvard, some of which monies were given to John Enders and his group; on animal and plant viruses and biophysical properties of viruses at the University of Pittsburgh, where the program was stimulated by the arrival of Salk, in 1947. Boyd, n.d. [1956], "Memo to Basil O'Connor," pp. 20-22.

30. Boyd, n.d. [1956], "Memo to Basil O'Connor," p. 23; California Institute of Technology, Annual Report, 1947/1948, p. 29; 1948/1949, p. 20; 1949/1950, p. 32. The Foundation provided another $56,459 in 1950/1951 and 1951/1952, and $618,665 more for the years 1952/1953 through 1958/1959. See Ibid. for the years 1950/1951 through 1958/1959.

31. Renato Dulbecco, "The Plaque Technique and the Development of Quantitative Animal Virology," in J.G. Cairns, G. Stent, and J. D. Watson, eds. Phage and the Origins of Molecular Biology (Cold Spring Harbor: Cold Spring Harbor Laboratory of Quantitative Biology, 1966), p. 287 [hereafter, PATOOMB]; Beadle to Boswell, Oct. 13, 1949; Lee A. Dubridge to Boswell, Oct. 24, 1949; E[dith] B[aker] to Dubridge, Feb. 19, 1952, Lee A. Dubridge Papers, California Institute of Technology Archives, folder 124.3. The formal agreement of gift was dated Nov. 10, 1949. C. A. Ames to C. Newton, Feb. 7, 1964, Biology Division Records, California Institute of Technology

Archives, Box 1, Boswell Foundation Fund folder.

32. See Max Delbruck, ed., Viruses 1950: Proceedings of a Conference . . . Held at the California Institute of Technology, March 20-22, 1950, Sponsored by the Institute's James G. Boswell Foundation Fund for Virus Research (Pasadena, CA: Division of Biology, California Institute of Technology, 1950).

33. Delbrück to Lawrence A. Williams, April 30, 1951, Delbrück MSS, folder 30.9.

34. A. P. Waterson and Lise Wilkinson, An Introduction to the History of Virology (Cambridge: Cambridge University Press, 1978), pp. 76, 138-39.

35. A. Moscona, O.A. Trowell, and E. N. Willimer, "Methods," in E. N. Willmer, ed., Cells and Tissue in Culture: Methods, Biology, and Physiology (2 vols.; New York: Academic Press, 1965), I, 30-37. On Harrison, see Jane Maienschein, "Experimental Biology in Transition: Harrison's Embryology, 1895-1910," Studies in the History of Biology, 6(1983), 118; and Jane Maienschein, Transforming Traditions in American Biology, 1880-1915 (Baltimore: The Johns Hopkins University Press, 1991), pp. 278-91; Levi-Montalcini, In Praise of Imperfection, pp. 58-60.

36. J.A. Witkowski, "Alexis Carrel and the Mysticism of Tissue Culture," Medical History, 23(1979), 279, 284, 293.

37. In 1928, Alexis Carrel wrote in a chapter entitled "Tissue Culture in the Study of Viruses," in the classic text on filterable viruses that Tom Rivers edited: "Through the rudimentary techniques of the early days of tissue culture . . . it was demonstrated that tissues kept *in vitro* can be utilized in the investigation of the properties of viruses. Although fourteen years have elapsed since this work was undertaken, the method of tissue culture has not greatly increased our knowledge of the heterogeneous group of filterable pathogenic principles. . . ." Carrell continued, "This must be partly attributed to the fact that pathologists are far from having mastered the techniques for the cultivation of tissues. Most of them have used the comparatively crude procedure which was derived immediately from the experiments of Harrison." Waterson and Wilkinson, An Introduction to the History of Virology, pp. 68-73; "Physiology or Medicine 1954: Presentation Speech by Professor S. Gard," Nobel Lectures . . . Physiology or Medicine, 1942-1962 (Amsterdam: Elsevier, 1964), p. 444. In 1954, P.R. White wrote a manual on tissue culture partly, he said, "to strip from the study of this subject its former atmosphere of mystery and complication," adding, "The grey walls, black gowns, masks and hoods; the shining twisted glass and pulsating coloured fluids; the gleaming stainless steel, hidden steam jets, enclosed microscopes and huge witches' cauldrons of the 'great laboratories' of 'tissue culture' have led far too many persons to consider cell culture too abstruse, recondite and sacrosanct a field to be invaded by mere *hoi polloi*." Quoted in Witkowski, "Alexis Carrel and the Mysticism of Tissue Culture," p. 281.

38. Jane Maienschein, "Experimental Biology in Transition: Harrison's Embryology, 1895-1910," Studies in the History of Biology, 6(1983), 118; H. B. Maitland and Mary Cowan Maitland, "Cultivation of Vaccinia Virus without Tissue Culture," The Lancet, 2(Sept. 22, 1928), 596-97; A. E. Feller, John F. Enders, and T. H. Weller, "The Prolonged Coexistence of Vaccinia Virus in High Titre and Living Cells in Roller Tube Cultures of Chick Embryonic Tissues," Journal of Experimental Medicine, 72(1940), 367; "Physiology or Medicine 1954: Presentation Speech by Professor S. Gard," p. 445;

Waterson and Wilkinson, An Introduction to the History of Virology, p. 144.

39. A. McGehee Harvey, "Johns Hopkins -- The Birthplace of Tissue Culture: The Story of Ross G. Harrison, Warren H. Lewis and George O. Gey," The Johns Hopkins Medical Journal, 136(1975), 147-48; Feller, Enders, and Weller, "The Prolonged Coexistence of Vaccinia Virus in High Titre and Living Cells in Roller Tube Cultures of Chick Embryonic Tissues," 368, 369.

40. Feller, Enders, and Weller, "The Prolonged Coexistence of Vaccinia Virus," pp. 368, 369. Concerning the art involved in successful biological preparations, see the comments of Levi-Montalcini on histological preparations in her In Praise of Imperfection, p. 51.

41. Ibid., 383, 385-86.

42. Thomas H. Weller and John Enders, "Production of Hemagglutinin by Mumps and Influenza A Viruses in Suspended Cell Tissue Cultures," Proceedings of the Society for Experimental Biology and Medicine, 69(Oct.-Dec. 1948), 124-26, 128. Significant changes in the formulation of culture media was being brought about by increased knowledge of cell metabolism. Witkowski, "Alexis Carrel and the Mysticism of Tissue Culture," p. 294.

43. John F. Enders, Thomas H. Weller, and Frederick C. Robbins, "Cultivation of the Lansing Strain of Poliomyelitis Virus in Cultures of Various Human Embryonic Tissues," Science, 109(Jan. 28, 1949), 86; Thomas H. Weller, Frederick C. Robbins, and John F. Enders, "Cultivation of Poliomyelitis Virus in Cultures of Human Foreskin and Embryonic Tissues," Proceedings of the Society for Experimental Biology and Medicine, 72(Oct.-Dec. 1949), 153-55). In their Nobel address, they would recall, "Thereupon it suddenly occurred to us that everything had been prepared almost without conscious effort on our part for a new attempt to cultivate the agent in extraneural tissue." (According to an account by a member of the National Foundation staff only a short while later, Weller had prepared an excess of tubes of culture medium for the experiment with the chicken pox virus, so Enders suggested that he seed the cultures with some poliovirus from the laboratory freezer.) "John F. Enders," in Wasson, ed. 1987, pp. 300-302; Enders, Robbins, and Weller 1954, p. 457.

44. Smith 1990, pp. 135-37; Boyd 1956, p. 30; Enders, Weller, and Robbins later noted that the application of antibiotics had made it "possible to apply tissue culture to the routine isolation of viruses from materials heavily contaminated with micro-organisms" and "to use them under conditions and in numbers which in the past would have been quite unthinkable." Enders, Robbins, and Weller 1954, pp. 458-9.

45. Frederick C. Robbins, John F. Enders and Thomas H. Weller, "Cytopathogenic Effect of Poliomyelitis Viruses In vitro on Human Embryonic Tissues," Proceedings of the Society for Experimental Biology and Medicine, 75(Oct. -Dec. 1950), 374.

46. Delbrück to Lawrence A. Williams, April 30, 1951, Delbrück Papers, folder 30.9.

47. In 1966, Dulbecco recalled that "at that moment I became an animal virologist," and in his recent autobiography he writes that, seeing in the project a convergence of the scientific and medical interests that he had pursued to that point, he accepted without hesitation, "abandoning phage for the new objective of transforming the study of human viruses into a new true science," adding, "I was certain to succeed, it seemed to me almost as though destiny had led me to do all my preceding work to prepare me for that end." However, it is evident from Delbrück's contemporary description of Dulbecco's task -- an interruption for several months in his phage research to examine the new tissue culture approaches closely -- that the turn that Dulbecco's career took at the meeting was rather tentative and by no means so decisively in a new direction as he has later recalled it. Dulbecco, "The Plaque Technique and the Development of Quantitative Animal Virology," in PATOOMB, pp. 287-88; Dulbecco, Aventurier du Vivant, pp. 163-67. Delbrück to Williams, April 30, 1951, Delbrück Papers, folder 30.9.

48. Delbrück to Williams, April 30, 1951, Delbrück Papers, folder 6.22; Delbrück to Beadle, Jan. 3, 1951, Biology Division Records, Box 42, Dulbecco folder.

49. Dulbecco to Delbrück, Jan. 29, 1951, Feb. 4, 1951, Delbrück MSS, folder 6.22.

50. Dulbecco to Delbrück, Feb. 9, 1951; Feb. 23, 1951, Delbrück MSS, Delbrück MSS, folder 6.22; Dulbecco, Aventurier du Vivant, pp. 163-67.

51. Dulbecco to Delbrück, Feb. 9, 1951; Feb. 23, 1951, Delbrück MSS, folder 6.22.

52. Dulbecco to Delbrück, March 11, 1951, Delbrück MSS, folder 6.22. Methods of cell -- as distinct from tissue -- culture had been devised in 1916, by Peyton Rous and F.S. Jones, at the Rockefeller Institute for Medical Research, but cell culture was only beginning to come into common use among biologists in the early 1950s. Witkowski, "Alexis Carrel and the Mysticism of Tissue Culture," pp. 293-94

53. Dulbecco had visited the following: Bang, Gey and J.S. Murphy at the Johns Hopkins University Medical School; W. Henle and D.R. Coman at the University of Pennsylvania; R.E. Shope at Merck Research Laboratory, Rahway, NJ; G. K. Hirst and R.W. Schlesinger at the Public Health Research Laboratory in New York City; K. Porter, F.L. Horsfall, and I. Tamm at the Rockefeller Institute for Medical Research; Earle at Bethesda; Enders, Robbins, Weller, A. H. Coons, and J. H. Hanks at the Harvard Medical School; and V. Hamburger and J. Bronfenbrenner at Washington University in St. Louis. R. Dulbecco, "Report on a visit to animal virus and tissue culture laboratories to explore modern trends in animal virus research and the possibility of using tissue cultures as hosts for animal viruses," March 23, 1951, Delbrück MSS, folder 30.9, p. 1; Dulbecco, Travel Report, Biology Division Records, Box 42, Dulbecco folder.

54. Dulbecco noted that with eggs or animals, "it is only feasible to use the dilution of factor 10, because inoculation is a relatively slow procedure and an experiment always has a time factor which limits the number of inoculations possible." Ibid., pp. 2-4.

55. Ibid., pp. 4-8

56. Ibid., pp. 9-11.

57. Ibid., pp. 11-12.

58. Ibid., pp. 11-12.

59. Ibid., 11-13.

60. Dulbecco, Aventurier du Vivant, pp. 163-67; Delbrück to Williams, April 30, 1951, Delbrück Papers, folder 30.9. In the autobiography, Dulbecco says that he used rat cells, but probably he has misremembered that his plan called for mouse cells.

61. Dulbecco, "The Plaque Technique," PATOOMB, pp. 288-90.

62. Dulbecco, Aventurier du Vivant, pp. 163-67; Dulbecco, "The Plaque Technique," PATOOMB, pp. 288-89.

63. Beadle to Boswell, Jan. 28, 1952, Biology Division Records, folder 30.9; untitled document, Feb. 22, 1954, Biology Division Records, Box 1, Boswell Foundation Fund. In late February, Ernest C. Watson, the de facto dean of the faculty at Caltech, suggested that a report on Dulbecco's work might be given to the trustees and would be "much appreciated by Col. Boswell." Beadle to Dubridge, Feb. 22, 1952, Dubridge Papers, Box 1, folder 1.2. It is interesting to note that Dulbecco did not publish any work on the herpes zoster virus during his years at Caltech.

64. Dulbecco, Aventurier du Vivant, pp. 163-67; Renato Dulbecco, "Production of Plaques in Monolayer Tissue Cultures by Single Particles of an Animal Virus," Proceedings of the National Academy of Sciences, 38(1952), 747-752.

65. Dulbecco, "Production of Plaques," 747-752. According to Dulbecco, some biologists contested his claims, particularly the claim that the linearity in the relationship between viral dose and plaque production meant that a plaque derived from a single infecting particle. Dulbecco, "The Plaque Technique," PATOOMB, p. 290. For a formal proof of the single-particle claim, see Renato Dulbecco and Marguerite Vogt, "Plaque Formation and Isolation of Pure Lines with Poliomyelitis Viruses," Journal of Experimental Medicine, 99(1954), 176-77.

66. Delbrück to the Home Secretary, National Academy of Sciences, Feb. 29, 1952, Delbrück Papers, folder 6.22.

67. Beadle to W. B. Munro, June 2, 1952, Dubridge Papers, folder 1.2; Dulbecco, Aventurier du Vivant, pp. 168-69; Delbrück to Dulbecco, c/o Enders, March 5, 1951, Delbrück MSS, folder 6.22

68. Dulbecco, AVenturier du Vivant, pp. 168-69; Dulbecco and Vogt, "Plaque Formation and Isolation of Pure Lines with Poliomyelitis Viruses," Journal of Experimental Medicine, 99(1954), 167-182; R. Dulbecco and Marguerite Vogt, "Biological Properties of Poliomyelitis Viruses as Studied by the Plaque Technique," Annals of the New York Academy of Sciences, 61(1955), 790-800.

69. Author's interview with Dulbecco, Dec. 16, 1990.

70. Dulbecco, "The Plaque Technique," in PATOOMB, pp. 290-91; Renato Dulbecco, "Interaction of Viruses and Animal Cells: A Study of Facts and Interpretations," Physiological Reviews, 35(April 1955), 301-35; author's interview with Renato Dulbecco, Salk Institute, La Jolla, California, Dec. 16, 1990; [Report on Use of Boswell Foundation Fund], n.d. [1960s], Biology Division Records, Boswell folder.

71. Marguerite Vogt and Renato Dulbecco, "Virus-Cell Interaction with a Tumor-Producing Virus," Proceedings of the National Academy of Sciences, 46(1960), p. 369; Dulbecco, "Basic Mechanisms in the Biology of Animal Viruses: Concluding Address," Basic Mechanisms in Animal Virus Biology, Cold Spring Harbor Symposia on Quantitative Biology, Vol. XXVII(Cold Spring Harbor Biological Laboratory, 1962), p. 523; Dulbecco, Aventurier du Vivant, pp. 179-81, 190-92, 208-16. I intend to treat Dulbecco's work on molecular tumor virology in a succeeding paper.

72. Author's interview with Renato Dulbecco, Dec. 16, 1990; Dulbecco, "Interaction of Viruses and Animal Cells," pp. 301-35.

73. On the importance of physicists in the phage school, see Nicholas C. Mullins, "The Development of a Scientific Specialty: The Phage Group and the Origins of Molecular Biology," Minerva, 10(1972), 51-82.

74. John Fuerst notes that Delbrück's principal anti-reductionist statement, "A Physicist Looks at Biology," was made in 1949 and was more a swansong than a heuristic program for future research. Fuerst, "Reductionism in Molecular Biology," pp. 263-65.

75. Luria, A Slot Machine, p. 71.

76. Author's interview with Renato Dulbecco, Dec. 16, 1990.