NATIONAL ACADEMY OF SCIENCES

ALWIN MAX PAPPENHEIMER, JR.
1908–1995

A Biographical Memoir by

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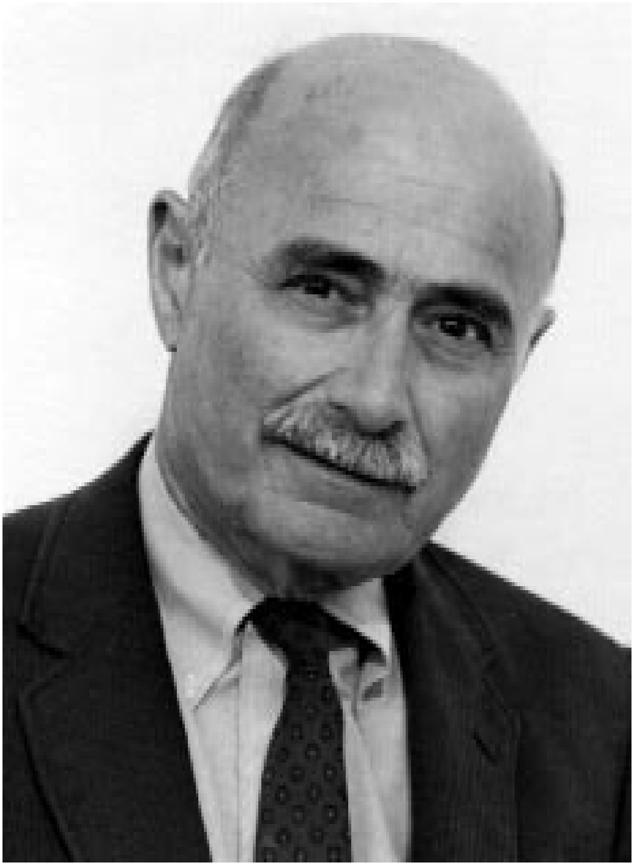
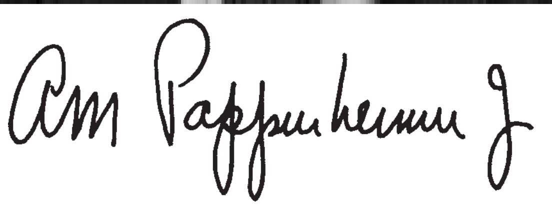
Biographical Memoirs, VOLUME 77

PUBLISHED 1999 BY
THE NATIONAL ACADEMY PRESS
WASHINGTON, D.C.





ALWIN MAX PAPPENHEIMER, JR.

November 25, 1908–March 21, 1995

BY H. SHERWOOD LAWRENCE

THERE IS ALWAYS A lingering sadness as we remember and sorely miss our dear friend Pap and, although we mourn our loss, it is also a time to celebrate his life and accomplishments. For he experienced an unusually gifted and full life and felt that he was the most fortunate man on Earth, as he often avowed. It was indeed a life studded with high achievement, surrounded by a proud loving family, devoted friends and colleagues, and successive generations of bright, eager, admiring, and appreciative students and fellows. In each he took the greatest pride and with each he maintained a strong bond over the years.

This is the saga of diphtheria toxin, which is the saga of Alwin M. Pappenheimer, Jr. The science itself has such a stunning and artistic symmetry it can only excite pleasure and admiration to behold. And even more so, since the whole pursuit of this idea encompassed Pap's greatest adventure and tells us so much about this unique man. As a young investigator Pap deliberately set himself the daunting task of unraveling the intricate mechanism of an infectious process in precise biochemical terms. Over the years, despite recurrent vicissitudes and occasional detours, he succeeded triumphantly in doing just that. Of the spate of innovative scientific contributions Pap made during his distinguished and highly productive career many are ranked as major advances that have illuminated the path to our current understanding of the pathogenesis of infections in general and the unique mode of action of microbial toxins at the molecular level in particular.

Pap, as he encouraged all his friends to call him, was born in Cedarhurst, New York, on November 25, 1908, the eldest child of Dr. Alwin Max Pappenheimer and Beatrice Leo Pappenheimer. His father was a distinguished pathologist on the faculty of the College of Physicians and Surgeons at Columbia University. Pap was raised with his sister Anne and brother John amidst a scholarly, academically oriented, and musically accomplished family environment. He admired his father greatly and sought his advice in many matters. As Pap has stated proudly elsewhere:[2]

> I cannot remember a time, since giving up my early ambition to drive a locomotive or a fire engine, when I did not plan a career in science. This was unquestionably because of the influence of my father and his academic friends . . . All three of my father's children pursued careers in science and all three became professors at Harvard University.
>
> Among my earliest memories are summers spent at Woods Hole before and during World War I. T. H. Morgan, the geneticist, lived nearby and I used to play with his children and later went to the same school with them in New York. I remember Jacques Loeb the physiologist; Gary Calkins, professor of biology at Columbia University; Alfred Redfield, who took us sailing and later became professor of biology at Harvard; and Michael Heidelberger, who lived next door, played the clarinet, and who years later stimulated my early interest in immunology and immunochemistry. There was a summer school at Woods Hole where I learned to catch and mount butterflies and moths and to raise them from caterpillars. Many years later this early interest led to my collaboration with C. M. Williams at Harvard University.

Pap had discovered joy in nature as a lad and it lasted a lifetime. He was happiest when regaling in its bounty—planting and reaping—and in its beauty and variety and even in the pursuit of its derangements to which we give names and call diseases.

When the family moved to Hartsdale in 1919 Pap went to the Lincoln School of Teachers College where he enjoyed learning calculus, physics, and chemistry. In 1925 he entered Harvard as a seventeen-year-old freshman and despite weighing only 99 pounds he coxed the freshman crew, as well as the crew that later defeated Yale in New London. From that time on he remained a dedicated sculler and engaged daily in the sport he loved in all weather until his early eighties, ultimately becoming a member of the Board of Directors of the Cambridge Boat Club.[2]

In 1926 Harvard inaugurated a new tutorial field of concentration (biochemical sciences), and Pap was the first student to enroll in this area, which was his main interest. Prophetically, he was later to return to Harvard in 1958 to succeed John Edsall as chairman of the Board of Tutors in the same biochemical sciences program.

Pap was faced with a dilemma during his senior year at Harvard: whether to go on to graduate school or to medical school, since the latter was the only place that biochemistry was taught at that time. He was intent on preparing for the future in biological research, where he was convinced an intimate knowledge of chemistry and physics were indispensable. For advice, he went to his father, who suggested that he see his friend the biochemist Hans Clarke, who in turn referred him to James B. Conant, professor of organic chemistry at Harvard. Conant readily accepted Pap as a graduate student despite his frank avowal that he was not interested in becoming a chemist but wished to acquire the training to enter biological research![1,2]

Pap received his Ph.D. in organic chemistry in 1932 in the midst of the Great Depression and held a part-time appointment as instructor and tutor in biochemical sciences, which fortunately was extended for another year of post-doctoral study. For the latter he went to work in Hans Zinsser's laboratory with Hugh Ward and John F. Enders on pneumococcal polysaccharides. The choice of this problem was fortuitous in that, although the results remained unpublished, Pap's significant contribution was brought to the favorable attention of O. T. Avery at the Rockefeller Institute. As we shall see some years later, Avery, who thought very highly of Pap and his work, would recommend to Colin MacLeod that he recruit Pap to the new Department of Bacteriology that he was forming at New York University School of Medicine.

Following his work on the pneumococcal polysaccharides, at Zinsser's suggestion Pap applied for and was awarded a National Research Council Fellowship to work in Sir Henry Dale's laboratory at the National Institute of Medical Research in London. There he spent the next two years attempting to isolate a bacterial growth factor "sporogenes vitamin" in collaboration with B. C. J. G. Knight and Sir Paul Fildes. Although he was able to concentrate a gram of oil with specific activity, he was unable to crystallize it. He took this disappointment philosophically, knowing that he had learned a great deal and had made a lasting coterie of staunch friends.[1]

Pap returned to Cambridge, Massachusetts, in 1935 without a job but happy nonetheless that he had finally chosen a problem he wanted to solve, in his own words, simply "to isolate a pure potent bacterial toxin and to find out what made it so toxic."[1] So the pursuit of this idea was begun. He approached the isolation of diphtheria toxin reasoning that if he provided a simple medium for culture that contained only known low molecular weight nutrients, then the supernatant should contain only those proteins secreted by the bacteria. He discussed this strategy at length with

Howard Mueller of the Department of Bacteriology at Harvard Medical School, who thought it a good idea and helped Pap get a Bradford Fellowship at Harvard to provide him with living expenses, while Elliot Robinson, director of the Jamaica Plains Antitoxin and Vaccine Laboratory, provided space and the technical assistance of Sylvia Johnson to support the project.[1]

It was here that Pap showed that minute quantities of iron added to the cultures of diphtheria bacilli resulted in a significant increase in toxin production, and he was on the first step to his goal. The next step was taken when he produced large quantities of pure diphtheria toxin by this means. This feat was further improved by his subsequent finding that media that supported toxin production best did so after the excess iron present had been precipitated with calcium phosphate and removed from the culture. Of this period in his career Pap wrote:[2]

> As a graduate student and even as a postgraduate fellow, it worried me a great deal that I did not have an important problem in mind. However, with the discovery of the importance of iron in controlling the production of diphtheria toxin, this insecurity left me and I have never since had to worry about finding something to work on. One thing always leads to another.

The wisdom of this philosophy was amply illustrated in the next series of Pap's contributions, which resulted in the crystallization of the toxin; following that, he moved on to establish its purity by excluding contaminating proteins immunologically using the toxin-antitoxin flocculation reaction, which showed the toxin to be at least 95% pure. Pap then teamed up with Williams and Lundgren to determine the molecular weight of the toxin by sedimentation and diffusion in the ultracentrifuge and found it to be homogeneous.[1,2]

What had all started innocently enough in the Antitoxin and Vaccine Laboratory in Jamaica Plains when Pap isolated, crystallized, and characterized diphtheria toxin in the late 1930s became a tour de force for which he received the prestigious Eli Lilly award in 1942, along with international recognition for his achievements, since it was the first toxin to be obtained in pure crystalline form.

In 1938 Pap married Pauline Forbes, a gracious, gentle lady who was the light and lodestar of his life and with whom he was to live happily for fifty-seven years. They had three children, Ruth F. P. Brazier, Sarah, and John.

Pap remained at the Antitoxin and Vaccine Laboratory from 1935 to 1939, but, although his experimental work was most successful and rewarding, he wished to be at a university where he could teach as well. Hence, when an offer of an assistant professorship in the Department of Bacteriology at the University of Pennsylvania came, he accepted it; yet, after a two-year stint Pap found the restrictions of his teaching assignments and the limited future this position afforded were not encouraging.[1]

Fortune smiled on Pap once again at this critical time in his career. Colin MacLeod had been appointed chairman of the Department of Bacteriology at New York University School of Medicine in 1941, and was seeking to recruit a faculty with new and broader views of such a department's composition, scope, and mission. As noted earlier, O. T. Avery had come to know and admire Pap through his unpublished work on the pneumococcal polysaccharide, and he recommended that Colin recruit Pap as assistant professor in the department.[2] Needless to say, Pap accepted Colin's offer promptly and happily, because he and Colin shared the same ideas about what a new department of bacteriology should be like and because he was intrigued by the excitement of Colin's work on the pneumococcal transforming principle with Avery and McCarty at the Rockefeller Institute. The only stipulation that Pap had was that he bring his first fellow Alan Bernheimer along with him—an idea Colin was delighted to second.[1]

Pap joined Colin in the challenge of building the new Department of Microbiology. Soon new recruits were in place and new approaches to the boundaries and scope of the investigations, as well as the breadth and depth of the bacteriology curriculum, were enlarged and redefined by men such as Pap and Colin, Efraim Racker, Mark Adams, Royal Christianson, and Alan Bernheimer. Thus, a long and fruitful friendship and collaboration was launched with Colin, who became Pap's hero and his staunchest supporter.

In 1945, after service as an Army captain in the Pacific theater in World War II, Pap was delighted to return to the Department of Microbiology at New York University School of Medicine and to pick up where he had left off in his quest. It was in 1946 that I met Pap and had the privilege of becoming his first postwar fellow—studying the immunological responses of adults to immunization with diphtheria toxoid. Soon Pap's laboratory began to hum with fellows. Mel Cohn, Lane Barksdale, and then Jonathan Uhr, Matthew Scharff, Sam Salvin, and William Kuhns all were studying various phases of the immune response to diphtherial antigens, a task that resulted in a series of seminal contributions covering fundamental aspects of antigen-antibody reactions and mechanisms of delayed-type hypersensitivity. There was also a stream of visiting scientists, such as Ashley Miles, John Humphrey, Jacques Monod, Francois Jacob, and Andre Lwoff, who came to see Pap and discuss their individual scientific problems; on occasion, Monod and Pap would engage in heated debate.

During this period Pap was also back on the trail studying the effect of iron and the iron enzymes on diphtherial growth with Edelmira Hendee; phage-host relationships with Lane Barksdale and the metabolic effects of toxin on the

Cecropia silkworm with Carroll Williams at Harvard. Then in 1957-58 an exciting new approach in Pap's main pursuit to discover what makes diphtheria toxin toxic resulted from his urging Strauss and Hendee in his laboratory at NYU to test whether the toxin would inhibit protein synthesis in HeLa cells in culture. They found that the toxin indeed inhibited protein synthesis in the cells—a seminal finding that led to other findings of great significance that would soon unravel the mode of action of diphtheria toxin.

In the meantime Pap had returned to Harvard. This move came about gradually after Colin MacLeod had been recruited to become professor of research medicine at the University of Pennsylvania in 1954 and Pap was appointed chairman of the Department of Microbiology at NYU in 1956 to succeed him. In 1957 John Edsall invited Pap to accept an appointment as professor of biology and to succeed him as chairman of the Board of Tutors in Biochemical Sciences at Harvard. This was an offer he could not refuse, and yet with characteristic gallantry Pap felt that NYU had been very good to him, and he would not leave until a new chairman was found to replace him. It was not until the autumn of 1958 that Pap and his family moved back to Cambridge, where he succeeded Edsall as chairman of the Board of Tutors in Biochemical Sciences and was appointed professor of biology.[2]

In 1961 another appointment that also meant a great deal to Pap ensued, namely, that of master of Dunster House, a position he held until 1970. A skilled and devoted clarinetist and violist himself, Pap fostered chamber music concerts at Dunster House, an innovation for which it became noted and attracted many talented musicians who were enrolled at Harvard during his tenure.

It was also during this period that pivotal research findings in Pap's laboratory accelerated and the gathering momentum yielded results that led ultimately to the denouement of the problem.

In 1963-64 John Collier and Pap showed that the inhibition of protein synthesis caused by diphtheria toxin in intact HeLa cells also occurred in cell-free systems of HeLa cells. Collier then in 1966-67 showed that elongation factor 2 (EF-2) was inactivated by diphtheria toxin in the presence of NAD, and in 1968-69 Michael Gill discovered the ADP-ribosylation reaction by which diphtheria toxin inactivated EF-2. Jack Murphy working with Pap in 1974-75 began studies on the transcriptional regulation of the tox gene, and Patrice Boquet studied the interaction of diphtheria toxin with detergents and cell membranes in 1975-76.

Later in 1978-80 John Collier and his student Gary Gilliand crafted the first generation immuno-toxin from diphtheria toxin independently—at the same time as did Vitetta and Uhr in Dallas and Uchida in Japan. Jack Murphy, as well as Vitetta and Uhr, then went on to design new generations of targeted toxins from diphtheria toxin, some of which are now in clinical trials. Also in 1980 Collier obtained X-ray grade crystals of diphtheria toxin and began a collaboration with David Eisenberg at UCLA that ultimately led to the solution of the 3D structure of the toxin in 1992 by Eisenberg and students in collaboration with Collier's laboratory. In 1983 Collier cloned and sequenced the gene for diphtheria toxin and subsequently in 1984 showed that glutamic acid is a key residue that is conserved in all ADP ribosylating toxins like those of cholera, pseudomonas, and pertussis. In 1992 the receptor for diphtheria toxin was determined by Eidel and coworkers in Jonathan Uhr's department in Dallas.

As I note in the dedication of his commemorative issue of *Cellular Immunology*:[3]

There is more to Pap's saga than just pure science and the poetic justice of bringing a daunting task to an incisive and clear solution. Along the way Pap was a powerful magnet that attracted generations of students and fellows at NYU and later at Harvard to a career in science. Pap's generosity was boundless; he gave of his talents, his vitality, his ideas, and his inspiration unstintingly. And he had a genius for bringing out the best in a long succession of students and fellows who came under his pervasive spell.

This resulted in the development of such first-rate scientists as Alan Bernheimer, Mel Cohn, Jonathan Uhr, and Matthew Scharff at NYU and John Collier, Ronald Goor, Mike Gill, Jack Murphy, and Patrice Boquet at Harvard. It also sparked in all of us a lifelong admiration and imitation of Pap's broad, imaginative, and rigorous approach to science and his chivalrous knack of gently nudging young investigators into the limelight while launching their careers. A reasonable assessment of Pap's success in this enterprise is that not only was he elected to the National Academy of Sciences (in 1973) but so have four of his former fellows and most recently the student of a former fellow as well, insuring the continuation of Pap's inheritance.

All of us try in our own ways to pass on to our own fellows the best of what Pap passed on to us. This is the hope for the future of Science and its rarest treasure. At Jonathan Uhr's initiative we formed a Society of Pappenheimer Fellows, which met on regular occasions, the most notable of which resulted in the publication of a symposium composed of papers given by Pap's former fellows, which was dedicated as a "Commemorative Issue in Honor of Alwin M. Pappenheimer, Jr.," and was published in *Cellular Immunology*.[3] As the late Lewis Thomas aptly remarked in an appreciation of Pap at that symposium, "Pap had a powerful influence on a great many younger people, setting in place the very course of the whole scientific careers of many of them. But it needs remarking that he had an equally powerful influence on the people in his own generation, faculty colleagues like myself."[4]

An unpublished excerpt of the reply that Pap had intended to deliver at that symposium, which he later sent to me and to Jon Uhr, provides a touching insight to the man and his characteristic modesty, candor, and frankness:

> Nothing like this has ever happened to me before, and I find it impossible to avoid feeling emotional and sentimental. When Jon Uhr asked me to send him a list of students and postdoctoral fellows who had worked in my laboratory over the years, I had no idea that the list would be so long and distinguished. I can honestly say that I have never felt that my own personal contributions as a scientist have been exceptional. It seems to me that my greatest talent has been to have recognized the quality of others and to have had the good fortune to have interested a number of exceptional and very nice people in what I, myself, was interested in. They were able, over and over again, to show me how wrong my own ideas were and to steer me back on the right track.

Numerous other honors and awards have graced Pap's pioneering scientific discoveries, among which are the Eli Lilly Award, the presidency of the American Association of Immunologists, and election to the American Academy of Arts and Sciences and to the National Academy of Sciences. Most recently, together with his former student John Collier, he received the Paul Ehrlich Prize and Gold Medal.

We have some additional insights into Pap's view of his life and work from the following excerpts of his envoi:[1]

> On looking back over my scientific and academic career, I realize how much I owe to good fortune and to chance. I was fortunate from the very beginning in my choice of parents, who not only provided me with an excellent education but encouraged me to be interested in things for their own sake, rather than for what I might gain from them, and finally who instilled in me a sacred regard for the truth. I was fortunate, too, to have received my education and to have become established in my field at a time when the competition was not as severe or as frenzied as it appears to be today.

I have always enjoyed working in the laboratory and when administrative duties kept me away from the bench for any considerable length of time, I would begin to worry that I might have lost the ability to work with my own hands. Not only is lab work fun (especially when things go well) but it keeps one in closer and more intimate contact with one's students and one's coworkers. It also serves to remind one constantly of how much easier it is to sit in a chair and suggest experiments for others to carry out than it is to go out and do them oneself. It has always been my feeling that research should be fun, and I like to think that even in this day and age important and innovative contributions can still be made by individuals working in small groups. In hindsight, I believe that I have received as much satisfaction from the friendship and contributions made by my students as from any of the honors that may have come my way, and I like to think that I may have had something to do with starting them off on the road to success. If I had to do it all over again, I do not think I would wish my scientific life to be very different.

So, we salute our hero, Pap, illustrious son of a distinguished father, a born leader, a brilliant thinker, and an innovative and resourceful scientist who proved a wellspring for the good of science and humanity.

We all miss the warm presence and sparkling ideas of this unique gentleman of science who won the grateful admiration of us all and who has left so rich a legacy "to ages of children yet unborn who will speak in accents yet unknown."

IN PREPARING THIS MEMOIR I have been greatly assisted by John R. Pappenheimer and by R. John Collier. Additionally, I am indebted to Cambridge University Press for permission to quote from A. M. Pappenheimer, Jr.'s autobiographical paper, *The Story of a Toxic Protein 1888-1992*.[1] I am also most indebted to A. M. Pappenheimer, Jr.'s children Ruth F. P. Brazier, Sarah Pappenheimer, and John Pappenheimer for their permission to quote from unpublished portions of the original manuscript *Autobiographic Memoirs of a Scientific Career* by A. M. Pappenheimer, Jr., which had been submitted for publication to *Protein Science*, but which did not appear in the edited publication, and for permission to quote from a portion of Pappenheimer's unpublished remarks titled "Some Reminiscences

of Happy Bygone Days," which he had intended to deliver at the conclusion of the commemorative symposium.

NOTES

1. A. M. Pappenheimer, Jr. Recollections—The story of a toxic protein, 1888-1992. *Protein Sci.* 2(1993):292-98.

2. A. M. Pappenheimer, Jr. Autobiographical Memoirs of a Scientific Career. N.B. Original manuscript that formed the basis of the condensed edited publication cited in Note 1 above.

3. H. S. Lawrence. Dedication of commemorative issue in honor of Alwin M. Pappenheimer, Jr. *Cell. Immunol.* 66(1982):1.

4. L. Thomas. An appreciation of Alwin M. Pappenheimer, Jr. *Cell. Immunol.* 66(1982):41-42.

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