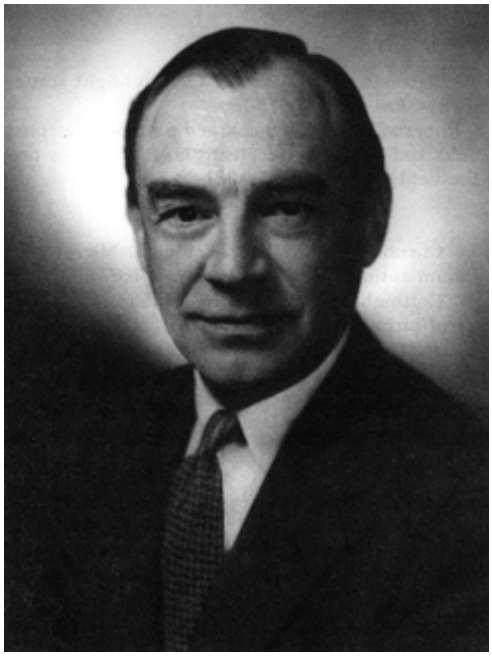

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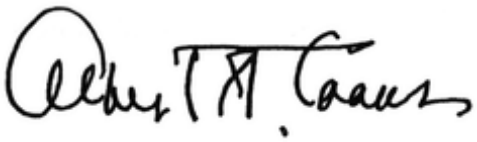

Albert Hewett Coons

June 28, 1912 — September 30, 1978

By Hugh O. McDevitt

ALBERT COONS, PROFESSOR IN the Department of Bacteriology and Immunology at Harvard Medical School and a member of the National Academy of Sciences since 1962, died September 30, 1978, at the age of sixty-six. He was born in Gloversville, New York, on June 28, 1912, the son of Albert S. and Marion (Hewett) Coons. He was educated in Gloversville public schools, graduated from Williams College in 1933, and received the M.D. degree from Harvard Medical School in 1937.

He initiated a major revolution in immunology and cell biology that continues to this day by developing the immunofluorescent technique for labeling specific antibodies with fluorescent dyes, thus permitting the detection of antibodies, antigens, and virtually any antigenic protein in cells and tissues. Fluorescent and immunohistochemical localization of foreign and self-proteins through the use of labeled antibodies is now an indispensable research tool in almost every field of biomedical research and continues to contribute to major new discoveries in all of these fields. The development of the fluorescent antibody technique in the early 1940s was a technical tour de force that required Dr. Coons to use the techniques of protein chemistry and organic chemistry. However, the impetus for the development of this technique came from his medical training and his interest in the pathogenesis of rheumatic fever.

Following his graduation from Harvard Medical School, Dr. Coons was a house officer on the Medical Service at the Massachusetts General Hospital from 1937 to 1939. In 1939 he joined the Thorndike Memorial Laboratory at Boston City Hospital as an assistant resident in medicine. Having completed his clinical training, Dr. Coons began a research fellowship in the Department of Bacteriology and Immunology at Harvard Medical School, where he remained from 1940 to 1942. His research studies were interrupted by service as a captain, and later as a major, in the Army Medical Corps, where he served as a pathologist and director of laboratory services with the 105th General Hospital in the southwest Pacific.

He returned to the Department of Bacteriology and Immunology as a research fellow in 1946, became an instructor in 1947, and finally a visiting professor of bacteriology and immunology and a career investigator of the American Heart Association in 1953. He was appointed a professor of bacteriology and immunology in 1970 and became a professor in the Department of Pathology in 1971.

As is true of many biomedical researchers, Dr. Coons's interest in research and immunology was originally stimulated by exposure to a bright, dynamic, and articulate teacher and researcher--in this case, Hans Zinsser, who was professor of bacteriology and immunology at Harvard Medical School from 1925 to 1940. Dr. Coons took Dr. Zinsser's course in immunology, which stimulated him to work during the summer of 1935 with John Enders, who was then an assistant professor in the department. His research project was to attempt to determine the blood levels of passively administered antibody before and after the induction of anaphylactic shock in the guinea pig. The precipitin reaction was used to measure the levels of passively administered rabbit and horse antibodies in guinea pig blood. The aim of the experiment was to find out why horse antibodies, in contrast to rabbit antibodies, were incapable of sensitizing guinea pigs for anaphylactic shock. The problem was never finished, and no clear results emerged from these studies. Nonetheless, Dr. Coons had become intimately acquainted with the

use of antibodies to detect foreign proteins in host tissue fluids. As he said much later, "Somehow, though, it bent the twig." This knowledge of the use of antibodies to detect foreign proteins in host fluids and tissues lay dormant through the next two years of medical school and through two years as a house officer at the Massachusetts General Hospital.

The next, and crucial, stage in the development of the concept that led to the development of the fluorescent antibody technique is best described in Dr. Coons's own words, since they offer fascinating insights into how important concepts develop in the mind of an investigator:

> At the end of my internship I had a six months' gap before my next appointment as an Assistant Resident and I was lucky enough to spend them in Berlin. This was the summer of 1939, a period of great international excitement, just before the outbreak of the war. I did not go there as a student but as a tourist. However, I had an entrée into the pathological institute at the Charite Krankenhaus where a friend, Kurt Apitz, was the Oberartz. I spent my mornings watching autopsies, and my afternoons wandering around talking to people in cafés and trying to improve my halting German. I also had many talks with Apitz, who was an exceptional young pathologist interested in leukemia and the Schwartzman reaction.
>
> In strange cities, visitors have many hours alone. I found myself walking in the streets or sitting in my room reading or brooding. One afternoon I was thinking about rheumatic fever and about the Aschoff nodule, the microscopic lesion characteristic of it. It was at that time and I think probably in many circles still is, thought to be the result of a local hypersensitivity reaction involving components of the group A hemolytic streptococcus and circulating antibodies or hypersensitive cells. It struck me that this theory had never been tested and indeed could not be tested without the demonstration of antibody or antigen, preferably both, in the local lesions. I considered that it might be easier to find the antigen than the antibody, for a start anyway, and that what was required was a visible microprecipitate. The notion of labelling an antibody molecule with a visible label was perfectly obvious in such a context. However when I tried this notion on my friend, Apitz, he was not enthusiastic. I think he thought it was not feasible and indeed, in the terms in which I initially thought of it, as a colored molecule, it wasn't.

Dr. Coons's remark that the idea of putting a visible label on an antibody molecule was perfectly obvious was perhaps too modest. Given the primitive knowledge of the structure, nature, isolation, and manipulation of antibodies in 1939, the concept of putting a visible label on an antibody molecule seems both bold and original, even if technically naive. The technical problems, which might have stymied many young researchers, were to occupy the next several years.

At this critical stage in his career Dr. Coons received both support and encouragement from Dr. George Minot, director of the Thorndike Memorial Laboratory, and from John Enders of the Department of Bacteriology and Immunology at Harvard. Both urged him to pursue the problem and to apply for a research fellowship instead of continuing with clinical studies, and argued that even if labeled antibodies did not solve the problems of rheumatic fever, they would provide a general procedure for locating proteins in tissues and cells that would obviously have wide application to countless problems.

Having received this initial support and encouragement, Dr. Coons was fortunate to receive further encouragement and expert assistance from a number of researchers at Harvard University. Louis Fieser, professor of organic chemistry at Harvard, introduced Dr. Coons to Hugh Creech and Norman Jones, who were working in his department on the conjugation of isocyanates of various carcinogens to protein molecules. Since Dr. Coons had already determined that coupling of dyes to antibody molecules resulted in immunoprecipitates that were only faintly pink even in solution, he urged Creech to help him couple a fluorescent dye, anthracene isocyanate, to some antipneumococcal antiserum. This antibody solution agglutinated specific pneumococci, and the agglutinated bacteria were brilliantly fluorescent in ultraviolet light. At this point, primarily because many tissues showed blue or red autofluorescence, Dr. Coons asked Dr. Fieser if he could obtain or synthesize fluorescein isocyanate. Fluorescein was chosen because it fluoresces with a brilliant apple green color not seen in any normal tissues. Dr. Fieser assigned a graduate student, Ernst Berliner, to this synthetic organic problem, and Berliner and Coons became fast friends. During this period Coons learned how to synthesize fluorescein isocyanate, knowledge that was invaluable in later years.

The next step was to visualize antigen in tissue sections. To do this, a fluorescent microscope was needed. Once again, by another stroke of luck, a colleague, Dr. Allan Grafflin, assistant professor of anatomy, was engaged in the assembly of an apparatus for fluorescence microscopy. Using his single fluorescent antipneumococcal antibody conjugate, Coons was able to find bacterial polysaccaride in the phagocytic cells of mice injected intravenously with large numbers of pneumococci and to carry out inhibition reactions for the establishment of specificity and to show that these reactions were reversible. Thus, by the beginning of 1942, Coons had succeeded in demonstrating the feasibility of putting fluorescent tags on antibodies and using them to localize foreign antigens in host tissues. His initial results were described in two brief papers (1941,1942) and the research was halted while he joined the army and spent the next four years in the South Pacific.

Much work remained to be done before the fluorescent antibody method could be generally applicable to a wide variety of biological problems. When Dr. Coons returned to the Department of Bacteriology and Immunology in 1946, he found that no one in Fieser's Department of Organic Chemistry was interested in synthesizing fluorescein isocyanate. He therefore decided to synthesize his fluorescent compounds himself, a process that was slow and painful. With sufficient fluorescein isocyanate available, many of the other technical problems were tackled. With his colleagues Gene Connolly and Melvin Kaplan, Coons began studying frozen sections and discovered the problem of nonspecific staining of tissues by fluorescent-labeled antibody solutions. This led to the use of acetone-dried tissue extracts as an absorbing agent to remove nonspecific staining.

With the availability of frozen sections and antibody solutions that did not bind nonspecifically to tissues, the fluorescent antibody technique became widely applicable to many problems in immunology and cell biology. This led to a series of papers on the localization of a variety of antigens in animal tissues (1950,1-4;1951) and on the localization of viral antigens in infected tissues. These crucial papers had a major impact on immunology and related fields. The demonstration that virtually any protein could be localized in tissues by the fluorescent antibody method led to its use in many laboratories around the world by immunologists, microbiologists, pathologists, and cell biologists.

The fluorescent antibody technique permitted the study of the fate of antigens in tissues, the expression of many different cell proteins in tissues, the identification of cells producing specific antibodies, detection of immune complexes and complement in lesions of serum sickness and in glomerulonephritis, the location of viral antigens in infected cells and of tumor antigens in malignant cells, and the use of the fluorescent antibody method in a wide variety of experimental settings.

At the same time, Dr. Coons embarked on a study of mechanisms of antibody production and developed methods for detecting specific antibody in cells producing that antibody in tissue sections of spleen and lymph nodes (1955,1). This led to a series of studies on antibody production (1955,2) and on specific inhibition of antibody formation during immunological paralysis (1959). These studies, showing sharply localized clusters of plasma cells all producing antibody of the same specificity, both foreshadowed and supported the clonal selection theory of antibody formation.

Dr. Coons was among the first to perceive that a detailed understanding of the mechanisms of antibody formation would require the development of techniques for the study of in vitro antibody production. This led to a series of papers from his laboratory on the establishment and analysis of in vitro secondary antibody responses. These studies were among the first to establish reproducible secondary antibody responses in vitro, to demonstrate the critical role of early cellular proliferation in the in vitro secondary response, and to permit analysis of the effect of a wide variety of drugs, including chloramphenicol and colchicine (1963,1-3).

As the importance of the immunofluorescent technique became apparent, Dr. Coons was widely honored by his colleagues. He received the Lasker Award in 1959, the Paul Ehrlich Award in 1961, the Passano Award in 1962, the Gairdner Foundation Annual Award in 1963, and the Emil von Behringer Prize in 1966, as well as honorary Sc.D. degrees from Williams College, Yale University, and Emory University. He is survived by his wife, Phyllis (Watts); his son, Albert H., Jr.; and four daughters, Elizabeth, Susan, Hilary, and Wendy.

A shy, bright, articulate, and gentle man with a wonderful, if private, sense of humor, Dr. Coons began his career with the idea of becoming a clinician and perhaps a teacher in a medical school. Stimulated by Zinsser and Enders to study immunology, and pondering the problems of rheumatic fever in a small hotel room in Berlin, he developed the bold and elegant idea of putting a sensitive visible label on antibody molecules. Given encouragement by mentors and help from colleagues, he carried through all of the technically difficult steps required to take an elegant but impractical idea into the realm of everyday application. Although acutely aware of the endless possibilities opened up by his technique, he nonetheless remained a little amazed and perhaps a bit embarrassed at how widely successful his method became. He left a legacy that will only grow with the passing years, as well as advice to his colleagues that is also a description of his own research methods:

> Imaginative approaches and the fruitful association of apparently unrelated phenomena are the result of indirection, brooding, indolence. In the beginning a store of facts and methods--in the end the free hand. (1961)

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