

Long, fulfilling career focuses on research

By Philip S. Norman, MD, FAAAAI

Pittsburg, Kansas (pop. 18,000) in the twenties and thirties had two main industries: Coal mining and the Kansas State Teachers College (KSTC). I went the laboratory school of the College for the first six grades. Science as an interest started early; I would go to the school library and read whatever the encyclopedias could tell me about astronomy. In the fifth grade, a gifted science teacher reinforced my interest.

By high school, however, this interest was overlaid by a developing interest in debate and dramatics. I was excited to be a member of the debate squad and act in school plays. In 1940, I started college intending to become a speech and dramatics teacher. The depression was still on and the only place my family could afford was KSTC (tuition for Kansas residents was a remarkable \$26 per semester).

After two and a half years, World War II interrupted education. I was taken into the Army and assigned to the Air Corps. Being too near-sighted for flight duty, I was sent to school to become a weatherman. Meteorology was then and still is a highly mathematical subject and I discovered that mathematics wasn't as bad as I thought in high school. A weatherman's duty is far from battle, and I had some leisure to read and think. I particularly remember Paul de Kruif's "Microbe Hunters" and René Dubos's biography of Pasteur as being influential. So the interest in science and specifically, medicine, returned.

After three years in the service, I returned to KSTC determined to become a physician and do research. Financial constraints on education were gone due to the GI Bill of Rights. Pre-medical education required a major in Biology. The head of the department was J. Ralph Wells, who had obtained his PhD at Washington University in St. Louis in immunology. I took his graduate level course in immunology and was hooked. It seemed the best possible field for research. After the course, Wells recommended that I go to medical school at Washington University.

I started in 1947 in a class that was largely World War II veterans. Many of us were older than the Senior class, who were the last of the wartime accelerated MD program. The School of Medicine placed great (and inspiring) emphasis on research. Most of the department chairmen were young and actively engaged in research. Carl Cori, the chairman of biochemistry, won the Nobel Prize with his wife Gerty the year I was a freshman. Years later, four of the young faculty I had been exposed to won the Nobel Prize.

The summer between my second and third year, I worked in the laboratory of Oliver H. Lowry, chairman of Pharmacology, who was beginning to use microchemical techniques that could perform analyses on minute pieces of tissue. The next summer, I teamed up with my medical school roommate, Richard Mason, to study the immunology of mismatched transfusion reactions. A study in dogs showed that aspects of the reaction resembled anaphylaxis and resulted in my first publication.

I interned in Medicine on W. Barry Wood's service at Barnes Hospital and then transferred to Vanderbilt Hospital for two years as an Assistant Resident. There I met my wife, Marion, who was working as a research laboratory technician. Marion has always encouraged me in an academic research career, not the least by never, in 50 years of married life, mentioning that practicing physicians make more money.

After three enjoyable years in clinical medicine, I was anxious to return to research and obtained a fellowship with **Merrill W. Chase, PhD, FAAAAI** at the then Rockefeller Institute for Medical Research. Merrill has recently discovered the first practical example of immune tolerance (although he didn't call it "tolerance") and had also discovered that delayed immune reactions can be transferred from one animal to another by lymphocytes. I had an interest in anaphylaxis, however, and we decided that I should test the hypothesis that activation of serum proteases plays a role.

Since no one knew how to measure such an enzyme accurately, the first task was to develop an assay. The only protease we know about was the clot-lysing enzyme, plasmin, so I set out to learn about plasmin. After many months, I developed a spectrophotometric assay (my experience with Lowry came in handy here) and then found that it would be necessary to understand the interaction of plasmin with naturally occurring serum inhibitors. This was complicated and consumed the

rest of my two-year fellowship. The work resulted in two publications in The Journal of Experimental Medicine about enzyme chemistry but no insights into anaphylaxis. It was a valuable lesson for me, nevertheless. Research results come only after hard work in long hours.

I didn't want to give up clinical medicine, so I looked around for a job at a hospital or medical school that included research time. I met Ivan L. Bennett, Jr., who had been commissioned to start an allergy and infectious diseases division in the Department of Medicine at Johns Hopkins. An interview with Ivan led to an offer to become an Instructor in Medicine and set up my own lab. So, in 1956, Marion and I moved to Baltimore for what has become a 50-year stay.

In the lab I started where I left off at the Rockefeller, but I also wanted to learn to be an allergist, as the only immunologic specialty. Formal training programs were unknown, so I went to the Hopkins allergy clinic two mornings a week to learn to be an allergist. Walter Winkenwerder, who had recently been President of the AAAAI and had a private practice in Baltimore, took me under his wing. I would see a new patient, discuss diagnosis and management with "Wink" and then carry out what we had decided. After a couple of years, without any formal declaration, I was more and more on my own, and consulted Wink only when I felt the need. In another year or two, post-doctoral fellows in the division were learning about allergies from me.

Wanting to extend my research into allergies, I tried an analysis of ragweed pollen extract, separating components by electrophoresis in starch and testing the eluted fractions in the skin of hay fever patients. The results were equivocal, but I used a trip to New York to show **Merrill W. Chase, PhD, FAAAAI** my results. Merrill, with characteristic frankness, told me that I was a lousy protein chemist, but had a good test system. He said that T.P. King at the Rockefeller, a skilled protein chemist, was working on the proteins of ragweed but was using an animal test system that had nothing to do with human allergies. Since T. P. and I had been good friends while post-doctoral fellows, it was easy to arrange a collaboration. Soon, T. P. was mailing me vials of lyophilized powders that represented fractions from his protein separations. The most successful separation came from a 3 meter Sephadex G-75 column that T. P. had constructed at the Rockefeller. Since ceilings at the Rockefeller were not quite 3 meters high, the column had been constructed in three 1-meter sections connected together with plastic capillary tubing.

The most active fractions came from an elution peak that was mostly one protein. Since it was the fifth of a series of antigens in ragweed that rabbits made precipitating antibodies to, T. P. called it Antigen E. Absorption experiments indicated that Antigen E accounted for 90% or more of the skin test activity of ragweed. In many patients, its biologic activity resided in as little as 10^{-12} gm. Later, Antigen E, rechristened Amb a 1 by a nomenclature committee, became a standard adopted by the FDA, whose regulations name a minimum amount to be present in ragweed extracts. Standardizing extracts by content of specific proteins continues to be a goal for many national and international regulatory agencies because it provides a consistent way to describe doses in immunotherapy.

About this time, Lawrence M. Lichtenstein was earning a PhD to add to his MD in the laboratory of Abraham Osler in the Department of Microbiology. Under Osler's tutelage, Larry had developed quantitative system for measuring histamine released from suspensions of leukocytes donated by allergic people. They were anxious to see if it contributed to the understanding of human disease. We decided that Larry's test would help in a projected study of "desensitization" with ragweed extract. The extract and Larry's test would be standardized for Antigen E.

Our first study, published in 1964, showed that histamine release measurements predicted ragweed symptoms upon natural exposure. Furthermore, desensitization altered histamine release, and caused the development of antibodies that inhibited histamine release. Finally, the dose we had used for treatment, which was based on common clinical practice, produced at best trifling relief of symptoms. After several more studies showed that larger doses of ragweed extract (and purified Antigen E by itself) produced solid results, we proposed that "immunotherapy" was a better name for allergy shots.

In 1964, I was approached by the Merck Co. to try a dexamethasone nasal spray they had developed for hay fever. Walter Winkenwerder and I organized a blinded trial during the ragweed season, which showed remarkable improvement in symptoms compared to placebos. Two subsequent studies showed that the effect was local and was accompanied by minimal suppression of adrenal function. These studies were sufficient to obtain FDA licensure and "Turbinaire" went on the market, the first of a series of nasal steroids for allergic rhinitis. Several years later, we showed that another steroid,

flunisolide, was helpful not only for seasonal hay fever but also perennial rhinitis and could be used safely over several years.

Around 1968, A.M. Harvey (Chairman of Medicine at Hopkins) offered Larry and me the chance to form a separate Clinical Immunology Division to be housed in laboratories at the newly constructed Good Samaritan Hospital in Baltimore. The move was accomplished in 1970 and included **Martin D. Valentine, MD, FAAAAI** and David G. Marsh, PhD. Hopkins also recruited **Kimishige Ishizaka, MD, FAAAAI** and **Terukuo Ishizaka, MD, FAAAAI** to move into adjacent laboratories.

In 1972 I asked a newly arrived fellow, **Richard R. Rosenthal, MD, FAAAAI**, to help develop a safe method for bronchial challenge. We reported a technique for quantitative graded aerosol inhalation through a "Dosimeter" that could be used with allergens or bronchoconstrictors such as methacholine. The endpoint was called the "PD20" representing the cumulative dose required for a 20% drop in FEV1. We and others have extensively used the technique for the study of lung physiology of asthmatics after allergen or bronchoconstrictor challenge. It has also been used for diagnosis and to quantify responses to immunotherapy.

When highly potent lyophilized allergenic protein fractions had arrived from T. P. King's lab we had included a small amount of human serum albumin as a stabilizer when we made dilutions for intradermal skin testing. The same diluting fluid made commercial pollen extracts quite potent at extreme dilutions. David G. Marsh, PhD and I decided to study this effect quantitatively and found that extracts diluted in ordinary commercial buffers lost activity by as much as 1000 fold compared to the same extracts diluted in albumin buffer. We reported this at the AAAAI meeting and in the JACI in 1978. Very quickly, the FDA and commercial suppliers adopted the use of albumin in diluting buffers. It continues to this day and may be my most enduring contribution to the practice of allergy.

Marsh had invented "allergoid," pollen extract aggregated with formaldehyde, before arriving in Baltimore. He and I organized two studies of ragweed allergoid's clinical effects. They showed good efficacy in hay fever with fewer injections than commonly used with standard extracts. The FDA told the sponsoring company, Allergopharma, that multicenter studies would be needed to obtain licensure in the U.S. Allergopharma decided to forego the extra expense and chose to market the product in Europe, where it continues to be used extensively.

Robert M. Naclerio, MD, FAAAAI, trained as an ENT surgeon, came to our division as a post-doctoral fellow. Larry, Bob and I developed the notion that it might be possible to instill allergenic extracts into the nose and collect secretions for mediator measurement. We found that histamine appeared in nasal washings within minutes after a challenge. Naclerio demonstrated that there was a second wave of mediator release hours later, representing a late phase reaction. With **David Proud, PhD, FAAAAI**, the group showed that kinins and kininogens were generated.

A study headed by **Peter S. Creticos, MD, FAAAAI** also found peptide-leukotrienes. Subsequently, we used nasal challenge to demonstrate that the mediator mix suggests that the immediate reaction represents mast cell mediator release, whereas late phase mediators are more likely generated by basophils. Immunotherapy inhibits early mediator release partially but late phase mediators are inhibited more completely. Corticosteroids, both topical and systemic, also inhibit early mediator release. Alkis Togias, MD extended the technique to the study of responses to physical stimuli such as cold dry air.

While a post-doctoral fellow, Marek M. Pienkowski, MD, PhD, demonstrated that immunotherapy almost completely inhibits late phase reactions in the skin, an observation repeatedly confirmed. It has led to a continuing interest in the physiology underlying the late phase. Pienkowski used skin windows to collect not only mediators but also cells during allergic reactions. Prostaglandin D appears after the first hour but before the late phase reaction. **Ernest N. Charlesworth, MD, FAAAAI** refined the technique and demonstrated the action of several drugs. He described a late infiltration with basophils followed by eosinophils.

In 1989 our Clinical Immunology Division, grown to 25 faculty, 25 post-doctoral fellows and a total of 125 people, moved into the Johns Hopkins Asthma and Allergy Center at the Bayview Campus of Johns Hopkins.

My most recent research interest has been the use of T-cell reactive peptides based on allergen structure as a means of treatment for respiratory allergies. In collaboration with **John L. Ohman, Jr., MD, FAAAAI's**, group in Boston, we gave injections of a mix of two synthesized peptides based on the structure of the cat allergen Fel d 1 to cat allergic patients. Challenges in a room inhabited by cats showed a dose-related inhibition of immediate respiratory responses. Creticos found that peptides from Amb a 1 of ragweed gave partial amelioration of hay fever symptoms during natural exposure. The commercial sponsor, ImmuLogic Pharmaceutical Corporation, abandoned further development, but Larché and Kay in London have taken up the principle with different peptides.

After the peptide study, I retired as a primary investigator but continue to advise and help others on clinical studies. A recent occupation has been the steering committee of the Immune Tolerance Network (ITN). The ITN supports clinical studies of therapies designed to induce immunologic tolerance in autoimmune diseases, organ transplantation, asthma and allergies.

Research and teaching in allergic conditions has rewarded me with personal satisfaction and warm professional relationships with fellows and faculty at Johns Hopkins and elsewhere. I regret that I cannot name everybody I have worked with in a short memoir. The only people I value more are Marion and the three children we raised and now our five grandchildren.