FEATURE ARTICLE

Rebecca Craighill Lancefield, Pioneer Microbiologist

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In 1943, Rebecca C. Lancefield became President of the Society of American Bacteriologists. She was the second woman to be President of the Society. In 1961, she became President of the American Association of Immunologists, the only woman to be President of that Association. In 1970 she was elected to the National Academy of Sciences, being honored for her outstanding research on streptococci and their relation to rheumatic fever, a field for which she early won respect for erecting guideposts in a thicket of confusion which existed when she started working with streptococci, work which she continues to this day.

Rebecca C. Lancefieid

Dr. Lancefield was born Rebecca Price Craighill in 1895 at Fort Wadsworth, Staten Island, New York, on the Army post where her father was stationed. A West Point graduate like his father before him, her father spent his career in the Army moving the family to his assigned posts and providing his daughters with a varied geographic and educational background. His own family, the Craighills, derived from Virginia where his forbears had settled in the early 1700s. Rebecca Craighill's maternal forbears had also settled in Virginia, but moved as pioneers to Mississippi. Her mother, Mary Wortley Montague Byram, married William Edward Craighill, a West Point classmate of her brother, assigned to the Corps of Engineers.

Dr. Lancefield's early education was obtained from many schools: public schools when satisfactory and convenient to the Army base, otherwise, governesses or private schools. She entered Wellesley College in the fall of 1912 with vague intentions of majoring in French and English, but her interest was aroused by her roommate's freshman course in zoology. She, then changed her major to zoology and took as many other courses in biology as could be fitted in with the college requirements for the B.A. degree. This included an elementary course in bacteriology, the only bacteriology course offered at the college at that time. When she discovered that she should have been obtaining a really good grounding in chemistry, it was almost too late. However, she devoted her last two years at Wellesley to remedying this deficiency as much as possible. She graduated from Wellesley in 19 16.

At that time the expectation was that graduates not immediately getting married would teach. Rebecca followed the conventional pattern and started out teaching science and mathematics at a girls' boarding school (Hopkins Hall) in Burlington, Vermont. The science turned out to be physical geography, but this did not disconcert her. Her annual salary was a splendid $500 plus room and board. Out of this she saved $200 to go to graduate school.

She had been offered a scholarship with graduate tuition at Teachers' College at Columbia University. This scholarship was established by the Daughters of the Cincinnati for daughters of Army or Navy officers. She wished to do her graduate work in either bacteriology or genetics. On studying the catalog, she could not find courses suited to her program. However, her adviser assured her that the scholarship did not require that she take courses at Teachers' College; it was only necessary to matriculate there; she might take any course offered at Columbia.

So while registered at Teacher's College, she studied bacteriology in Zinsser's department. Graduate and medical bacteriology were then given in an old ramshackle building at 59th Street and Amsterdam Avenue, the College of Physicians & Surgeons of Columbia University. A year was spent there studying all the bacteriology and allied subjects available. Zinsser was then (1918) away in Europe involved with World War I, and A. K. Balls, an enzyme

chemist, was serving as head of the department. Following the Zinsser tradition, Balls expected his students to spend all of their waking hours in class or in the laboratory. At the end of the year, Rebecca Craighill received the degree of Master of Arts from Columbia and then married Donald Lancefield whom she met as a graduate student at Columbia in the Drosophila genetics "Fly Room" of T. H. Morgan. Donald Lancefield was called into service at this time during World War I. At the beginning of his service, while still a private, he was stationed with the Sanitary Corps Unit at the Rockefeller Institute for Medical Research to attend a special course conducted in part by 0. T. Avery and A. R. Dochez.' These two eminent investigators found time in their pneumonia research program at the Rockefeller Institute Hospital to lend their services to a study of streptococci isolated from bronchopneumonia which was rife in military camps.

Dochez and Avery, returning from a trip with a medical commission sent by the Surgeon General to visit Texas army camps, brought back with them to the Institute about 120 cultures of streptococci. Rebecca Lancefield, having just finished her year of graduate work in Zinsser's department at Columbia, applied to the Institute for a position. She was assigned in June 1918 as a technician for the streptococcus study at the Rockefeller Hospital. So, for a time, both Lancefields worked at the Institute.

Streptococcal classification and related pathogenicity were in a very confused state at that time. However, using the most difficult, and at the same time the most trustworthy, method for typing, that is, mouse protection tests, the group studying streptococci at the Rockefeller Institute were able during the ensuing winter to identify at least four immunologically specific types of streptococci among their 120 or so cultures. This finding was accepted at the time and has been subsequently confirmed in numerous laboratories by several different approaches. These studies, published in 1919, were interrupted soon after by termination of funds from the Surgeon General's office. Avery and Dochez returned wholeheartedly to research on *Pneumococcus,* partly interrupted by the war.

In the summer of 19 19, the Lancefields went to Woods Hole with the Columbia Zoology group for their annual three-month stay at the Marine Biological Laboratory. On their return to New York, Rebecca C. Lancefield was engaged as a research assistant by C. W. Metz to work in his genetics laboratory at Columbia. He wished to add genetic and cytological studies of ***Drosophila willistoni*** and of other species he was already studying to the findings of the Morgan laboratory. Three publications on ***Drosophila willistoni,*** both cytological and genetical, resulted from Lancefield's work with Metz in the next two years in his laboratory at Columbia.

In 1921 Donald Lancefield had finished the work for his Ph.D. degree and was offered a position at the University of Oregon. His home had been in Amity, Oregon, in the Willamette Valley, and he was a member of the first class to graduate from Reed College in Portland, Oregon. Thus, after careful consideration he readily accepted the position in Oregon. Rebecca Lancefield also found a position at the University of Oregon, teaching bacteriology. During that year she also became acquainted with the state where Donald Lancefield's parents had been pioneers, his mother having arrived by covered wagon at the age of 10. At the end of the school year, the Lancefields were ready to return to New York and Columbia University, Donald Lancefield to join Morgan's department and Rebecca Lancefield to finish her degree with Zinsser.

She has said that Zinsser didn't much care for women working around his laboratory, but he knew of her previous work and that she had taken almost all the courses offered in his department. So he agreed to accept her as a candidate for the Ph.D. and suggested she might like to consider an opening then available at the Rockefeller Institute Hospital, where she had been before. Homer Swift was starting a study of rheumatic fever at the Institute, and Rebecca Lancefield accepted a position with him.

She remained at this Institute for the rest of her professional life. Thus, it was her great good fortune to see the Rockefeller Institute in one of its great periods and also to live through the heyday of Morgan's research group in the Department of Zoology at Columbia, where her husband remained on the faculty for many years prior to becoming Chairman of the Department of Zoology at Queens College. Now retired, he is well remembered as a teacher whose students regarded him highly and have kept in touch with him for years.

At that time (1922), rheumatic fever was associated by the medical community with streptococcal infection, but the wrong strepto***coccus, Streptococcus viridans,*** was suspected. Lancefield worked in Dr. Swift's department with the "green" streptococci for two years, attempting to get a satisfactory polysaccharide or other antigens from them that would react specifically with rheumatic fever patients' sera for a diagnostic test. Her three publications on

S. viridans bear witness to the frustration she felt after working for two years and finding that the "nucleoprotein" antigen finally decided on as a laboratory test in rheumatic fever was unsuitable. It exhibited a broad range of activity in tests with sera of animals immunized with streptococcal preparations, but this antigenic fraction reacted strongly not only with sera from rheumatic fever patients, but equally well with 50% of the sera from control subjects.

Her other objective, that of distinguishing specific types of *S. viridans,* could be accomplished in much the same way that the pneumococcus investigators had found so successful for pneumococcal types, but Lancefield had only one strain that reacted the same as one of Swift's four original individual strains, hardly sufficient to create a type! It seemed that each strain of S. *viridans* was a law unto itself, quite unlike the hemolytic streptococci from the Texas army camps previously studied with Avery and Dochez. Many serologically similar or identical strains had occurred in each of the four types identified in this epidemic during wartime.

Lancefield received her doctorate in bacteriology at Columbia University in 1925 with this work used for her dissertation. Frederick P. Gay had by that time succeeded to the chairmanship of the Columbia department. Her thesis, based on the *S. viridans* study made in Dr. Swift's laboratory at the Rockefeller Hospital, was entitled "The Immunological Relationship of *Streptococcus viridans* and Certain of its Chemical Fractions." Two publications in the *Journal of Experimental Medicine* (Vol. 42) resulted from this work.

She returned to the Rockefeller Institute to work full time with hemolytic streptococci, evidently of such importance in rheumatic fever and so much more rewarding for her work in rheumatic fever than *S. viridans.* With Avery and Dochez, she had obtained proof of the existence of immunologically specific types of hemolytic streptococci. By then it was becoming clear that hemolytic streptococci were highly pathogenic for man. Avery and his group, located at the opposite end of the floor from Lancefield, were at the height of their research on pneumococcus polysaccharides, and frequent discussions took place among them.

Lancefield found a polysaccharide common to all streptococci studied at that time from acute human infections. She later found this polysaccharide characteristic of the serological group A; this "C" carbohydrate has been employed to classify all group A strains. Separate type-specific substances isolated from the individual and serologically specific types found within group A were discovered to be proteins, a distinct so-called M substance for each type. The finding that antibodies to the M antigen determine the immunity of the host against infection with streptococci of each individual type led to a. general agreement that this M antigen can designate the group A types. The nomenclature of these group A types is therefore referred to their M antigens.

In 1928 she published a series of five papers in the *Journal of Experimental Medicine* on the antigenic complex of *Streptococcus haemolyticus.* In these publications she described and characterized the type-specific M substance (J. Exp. Med. **47**:91), its chemical (protein) and immunological properties (J. Exp. Med. **47**:469), and the chemical and immunological properties of the species-specific (now recognized as "group-specific") C substance, a polysaccharide (J. Exp. Med. **47**:481). She also reported that the nucleoprotein fraction P, widely cross-reactive among gram-positive cocci, is a true antigen capable of stimulating antibody production in rabbits and producing anaphylactic shock in guinea pigs actively or passively sensitized, whereas the purified polysaccharide C substance did not give rise to antibodies in rabbits but did produce passive anaphylactic shock in guinea pigs sensitized with antibacterial sera against whole organisms. Similar results obtained with the type-specific M substance suggested both were haptens (J. Exp. Med. **47**:843, 857). However, in collaboration with George K. Hirst, she found the M protein in better preparations to be a true antigen (J. Exp. Med. **69**:425).

Two additional publications (1928), in which Dr. Lancefield collaborated with E. W. Todd, were concerned with the correlation of cultural variants of streptococci with virulence. Matt colonies of these variants contained the M antigen and were capable of being virulent. During the year 1926, which Todd spent in the Rockefeller Rheumatic Fever Department as a British Medical Research Council Fellow, he and Lancefield found that their independent interpretations of his matt and glossy colonies on the one hand and of her type-specific M antigens (named for his matt colonies) meshed with extraordinary precision. A delightful collaboration and life-long friendship ensued.

In 1933 Lancefield published one of her most significant papers "A Serological Differentiation of Human and Other Groups of Hemolytic Streptococci" (J. Exp. Med. **57**:571-599). In this paper she detailed her methods of producing streptococcal antigens and antisera for use in precipitin tests, and she noted that "the results of this study are of interest not only from

the theoretical viewpoint of establishing an orderly grouping of these microorganisms but also from an epidemiological aspect in providing means of identifying the probable origin of a given strain." She discussed the results of the biochemical (metabolic) tests then available and mentioned differential tests in use, such as hydrolysis of sodium hippurate, which were consistent with her serological classification. Using 106 strains of hemolytic streptococci from various sources (humans, other animals, dairy products), she demonstrated that the streptococci could be classified by precipitin test into five distinct groups. The first streptococcal strains studied earlier with Avery and Dochez were all of human origin. These she designated as group A and later distinct groups were assigned succeeding alphabetical letters.

Lancefield's next publication (J. Exp. Med. **59:**441-458) dealt with group B streptococci. Most of those studied then were from bovine sources plus a series isolated by Hare and Colebrook from patients in a large London maternity hospital. The latter series pointed out the common occurrence of group B in human sources.

The next nine of Lancefield's publications refined and extended her basic discoveries and further characterized the streptococcal antigens. In 1936 at the 2nd International Congress for Microbiology in London, Dr. Swift, in collaboration with Lancefield, presented a paper on problems encountered in typing streptococci.

In the preceding five or ten years the results of her work concerned with classifying streptococci serologically had converged with that of Fred Griffith in London, who had approached the problem with different methods specially developed by him and with a somewhat different point of view suited to his epidemiological studies. Both investigators needed exact identification of the streptococci encountered in their studies. From 1926 to 1936, Griffith studied streptococcal epidemics in schools. By applying his famous slide agglutination technique and meticulous work to these studies, he was able to classify hemolytic streptococci into 30 accurately defined types. When this classification was published in 1934, he agreed at once to Lancefield's request to send representative type strains to her so that she could correlate the Griffith types identified by agglutination with those collected in her work and identified by the tests she employed, i.e., precipitin reactions, test tube agglutinations, and mouse protection tests. He supposed that his slide agglutination method of typing was probably dependent on the same M antigen prepared by Lancefield as the type-specific substance in her experiments. This was substantiated when they exchanged not only his type strains but also samples of his unabsorbed sera which could be tested by the M precipitin method. Both absorbed and unabsorbed undiluted anti-M sera crossed the Atlantic to Dr. Griffith from Dr. Lancefield's laboratory. With very few exceptions the agreement of types was so satisfactory that Lancefield then adopted Griffith's type numbers instead of the patients' names she had been using as type-identification. These types were restricted to group A.

The protein T antigen, studied in detail serologically and described (1940) chemically with Dr. Dole in connection with the correlation of Griffith's types with the results of M and T reactions, was considered inadequate as the basis of type classification because of cross-reactions both in agglutination and in the lack of protective properties. Griffith's antigens and antibodies for each type had been prepared in such a way that cross-reactions due to the T antigen-antibody system were eliminated except in special, unusual cases. In current usage, the slide agglutination reaction is used to advantage for preliminary, broader classification known to depend upon T reactions.

When the nomenclature subcommittee of the International Congress of Microbiology sanctioned this usage of the **M** antigen as the basis for the serological typing of group A streptococci, it avoided confusion in referring to them in research projects or in streptococcal epidemics.

In 1940 Lancefield presented the Harvey Lecture entitled "Specific Relationships of Cell Composition to Biological Activity of Hemolytic Streptococci." The lecture was an exposition and summary of her own contributions to the field. It began with a historical review from the earliest descriptions in 1879, by Pasteur who saw streptococci as a chain of beads in the blood of a patient dying of puerpural sepsis, to Schotmüller who pointed out (1903) that the various streptococci differ in their effect on blood, distinguishing hemolytic, viridans, and the indifferent or non-hemolytic streptococci. Lancefield points out that it was the so-called streptolysin (Besredka, 1901) that was first useful in classification and it has remained the most useful initial differential in characterization of the organism. Further work (Todd, 1928) differentiated and characterized streptolysin 0 and streptolysin S. Weld (1934) had also contributed her interesting studies on "serum soluble" streptolysin, studied as "streptolysin-S" by Todd.

In the Harvey Lecture, Lancefield also referred to early attempts to bring order into classification of streptococci by means of fer-

mentation reactions and other biochemical tests. Although some progress had been made using such tests, there was always overlapping among the classes differentiated, and the results were difficult or impossible to interpret. She mentioned the first efforts at serological classification, using agglutination tests, and the technical difficulties encountered. Lancefield's publication with Avery and Dochez presented the first reliable methods of serological typing of streptococci using agglutination, precipitin, and mouse protection tests, followed in later studies by other immunological tests. By this time (1940), Lancefield had characterized, or been consulted about, groups A through H and K (later dropped), L, and M (1933-1936).

When antibiotics burst upon the scene (1936), one of her colleagues needled her by asking, "What are you going to work on now?" She replied that she was glad to be freed of the many practical responsibilities connected with streptococcal research and could pursue her principal objective, which was to determine the connection, if any, between group A streptococci and rheumatic fever before leaving this field.

One concern that she and her collaborators and others studying rheumatic fever encountered was the type specificity of streptococci found in recurring attacks of rheumatic fever. They came to the conclusion before the availability of antibiotics that a strain of different type specificity initiated each recurrent attack. After the introduction of antibiotics this was no longer true; the same type strain could be found again. Antibiotic therapy had interfered with antibody production by early elimination of the infecting streptococcus.

Closely, though informally, associated with the Rockefeller Hospital Rheumatic Fever Department was Ann G. Kuttner, who was much interested in the recurrent attacks of rheumatic fever patients and contributed greatly to laboratory and clinical investigations in this area of work. She was joint author with Markowitz and Gordis of the first edition (1965) of ***Rheumatic Fever-Diagnosis, Management and Treatment.*** To the second edition (1972), dedicated to Dr. Kuttner after her death, Lancefield provided the preface.

In 1943-44, as President of the Society of American Bacteriologists, Lancefield was spared the work and distractions of a national meeting; the National War Travel Act made it impossible for Society members to travel for that purpose. No such encumbrance hindered her performance of duties and rituals when she became President of the American Association of Immunologists.

Puzzling strains of streptococci were sent to Lancefield from far and near. Her study of streptococci from an epizootic in guinea pigs at Saranac Lake in the 1930s led to her characterization of group C streptococci and suggested to her that serological groups of this sort existed among strains from other sources.

In collaboration with Hook and Wagner (Am. J. Hyg. **72:**111) in 1960, Lancefield studied organisms from a strange epizootic in Swiss mice at Johns Hopkins University, and a similar epizootic occurring in Memorial Hospital mice at about the same time and reported by John B. Nelson. In both these cases, the important finding was that these animals were infected by a group A streptococcus of the newly designated type 50. This streptococcus was the same as that isolated in 1935 by Jacob Furth from his mouse colony at Cornell University. This strain was identified by Lancefield in 1960 from dried cultures **which** she had stored in her collection. The 1960 epizootics were all in animals obtained from a single breeder.

During the decade of World War II, several of Lancefield's publications dealing with problems still to be elucidated were collaborative efforts by most members of the Rheumatic Fever Department. Published in a series of five or six papers, these studies were an attempt to identify antigens in group A types which might be involved in preventing pharyngitis and associated sequellae in military personnel. This included supplying type-specific antisera to military laboratories and, at times, typing large numbers of strains for them. During these large-scale studies, it was found in many laboratories that the dominant strain of streptococcus varies from year to year, and that the streptococci found in complications following scarlet fever may differ from the strain initiating the disease. This was significant in studying persistence of type-specific antibody in relation to the possibility of reinfection with the same type. Other studies of the persistence of antibodies in patients and occurrence of various strains resulted finally in elucidating changes in cell wall polysaccharides, research carried out chiefly by McCarty and Krause in the Rheumatic Fever Department and John Swanson with expert electron microscopy. At the same time, McCarty did thorough chemical analysis of the group cell wall polysaccharide of group A, and Curtis and Krause (in St. 'Louis) performed the same service for the group B cell wall determinative polysaccharide. Other workers have examined group-specific substances of certain other streptococcal groups.

Lancefield investigated the immunology of the confusing R antigen during the 1950s. She originally thought that this was the M antigen

of type 28 and conducted, in collaboration with Gertrude Perlman, a chemical study of type 28 R antigen. It developed in immunological studies that this antigen and its antibody had no effect on virulence or protection against streptococci when present in the system. At Colindale in London, Maxted early established the presence of R antigen, similar to 28 R protein in various groups as well as types of streptococci. The confusion caused by R antigen was due to its many characteristics similar to the M antigen of group A, type 28, with which it was first observed and confused.

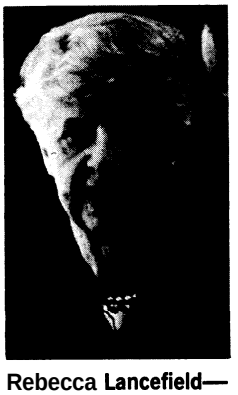
Lancefield has in recent years returned to studying group B streptococci, partly from purely theoretical considerations of the chemical and immunological nature of the type-specific antigens in this serological group, and partly to elucidate the occurrence and prevalence of group B streptococci in meningitis of newborn infants. This question has interested pediatricians and gynecologists who have been engaged in these perinatal problems. Dr. Lancefield has encouraged several younger investigators whose curiosity had been aroused about the prevalence of group B organisms and by their clinical implications. Her current publication with McCarty and Everly in the *Journal of Experimental Medicine* (142:165) is concerned with the broad theoretical question of certain bacteria, exemplified by group B streptococci containing both polysaccharide and protein antigenic determinants which induce antibodies capable of protecting challenged mice from becoming infected with a strain containing any one of the corresponding antigens which induce the antibody specific for that antigen.

In 1929 the Lancefields took sabbatical leave in England. They spent five to six months at Cambridge, and then traveled on the continent. Several other visits were made later to England, combining scientific meetings with pleasure. Customarily, their summers are spent at Woods Hole near their daughter and grandchildren.

Lancefield is supportive of women seeking equal rights, but she has said they sometimes expect too much. She feels that it is not easy to have both a scientific career and a family, without compromising one or both-despite the fact that she managed it quite well herself.

Rebecca Lancefield has been generous with her talents and time. She has trained young scientists and has long served as the Scotland Yard of streptococcal mysteries. A disappointment to her has been the current lack of definitive knowledge of the undoubted, but largely unexplained, role of group A streptococci in rheumatic fever. She has been a guest speaker throughout the land at home and abroad and she has received many honors. In 1960 she received the T. Duckett Jones Memorial Award. She was honored with the American Heart Association Achievement Award in 1964, and in 1965 she presented the T. Duckett Jones Memorial Lecture of the American Heart Association. Until its conclusion in 1973, she was a member of the Streptococcal Disease Commission of the U.S. Armed Forces Epidemiological Board. In 1968 she presented the second 'Griffith Memorial Lecture at the General Meeting of the Society for General Microbiology in England. Later, in the same year, she presented the Armine T. Wilson Memorial Oration. In 1970, she was elected to membership in the National Academy of Sciences, which up to that time had elected only 10 women to membership (there are now 27 women among 1,034 members). She received the New York Academy of Medicine Medal in April 1973 and Research Achievement Awards from *Medicine* and from the Alumnae Association of Wellesley College, also in 1973.

In June of 1973 she received the highest recognition from the institution where she has spent most of her professional life, The Rockefeller University, which awarded her a Doctor of Science (Honoris Causa) degree. Of her scientific contributions they have said, "That our knowledge of hemolytic streptococci has reached its present well-ordered state is due in large part to the work of Dr. Lancefield. The major portion of the conceptual schemes, methodology and detailed experimental analysis which form the basis for understanding the composition of this important group of organisms has come from her laboratory." The name Lancefield is associated throughout the world with the grouping and typing of streptococci.



Rebecca Lancefield—1973—on receipt of honorary degree from Rockefeller University