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# DR. KARL LANDSTEINER

## NOBEL PRIZE LAUREATE IN MEDICINE, 1930[1]

By Professor STANHOPE BAYNE-JONES
THE UNIVERSITY OF ROCHESTER

IT would be fitting to dedicate a meeting of the American Association of Immunologists to the celebration of the award of an honorable international prize to a great immunologist. It is especially appropriate for us to dedicate this meeting to the celebration of the award of the Nobel Prize in Medicine for 1930 to one of our former presidents—Dr. Karl Landsteiner. The association takes this first opportunity it has had, since he gained that well-deserved recognition of his accomplishments, to give him from this group what he has received from his individual friends—admiration for his scientific achievement, confidence in the power of his methods of research to solve many of the problems of specificity, gratitude for the useful facts which his discoveries have placed in our hands, and an expression of affectionate personal esteem.

Dr. Landsteiner has done his work in several cities which could take pride in his achievements. A glance at his *curriculum vitae* and the list of more than 200 of his publications will show why this is so. It will be seen that no claim can equal that of Vienna, his birthplace and the place where all his fundamental work was begun and much of it completed. But for many years he has been at work at the Rockefeller Institute in New York, and the contributions from his laboratory there have formed a most significant part of the immunological literature of this country.

[1] Presidential address delivered at the annual meeting of the American Association of Immunologists, April 1, 1931, at Cleveland, Ohio. From the Department of Bacteriology, School of Medicine and Dentistry, University of Rochester, Rochester, New York.

He was born in Vienna, Austria, on June 14, 1868. He is the son of Leopold Landsteiner, doctor of laws, and his wife Fanny, née Hess. He followed the medical course at the University of Vienna, receiving the degree of doctor of medicine in 1891. Of great importance for his future work was the chemical training he received in the laboratories of Hantzsch in Zurich, Emil Fischer in Würzburg and E. Bamberger in Munich. In 1896 he became assistant under Dr. Max von Gruber in the Institute of Hygiene at the University of Vienna. From 1898 to 1908 he was an assistant under Professor A. Weichselbaum at the Pathological Institute of the University of Vienna. From 1909 to 1919 he was pathologist to the Wilhelminspital in Vienna. After the disruption caused by the war, he went to Holland, where he was pathologist at the R. K. Ziekenhuis, at the Hague, from 1919 to 1922. In 1922 he came to the Rockefeller Institute for Medical Research in New York and has been a member of the institute since that time.

During the thirty-six years since Landsteiner's first paper on cholic acid, his contributions have expressed the numerous interests of an original and versatile mind. They form a vast accumulation of facts discovered by an industrious and extraordinarily skilful investigator. It is undoubtedly true also, as Zinsser has said, "that the amount of work that he has inspired probably far exceeds in number of titles the communications which stand in his name." With a violation of chronological sequences, Landsteiner's contributions to knowledge can be grouped into reports of his investigations in chemistry, in pathological anatomy, in experimental infectious diseases, and in serology and immunology. It would be instructive to review them all. But time and this occasion require passing over with only a brief mention many papers which remain sources of fundamental information in their field.

Of these the chief are the records of studies of two infectious diseases—syphilis and poliomyelitis. In 1906, in collaboration with Finger, Landsteiner succeeded in transmitting syphilis to apes, repeating and extending the earlier work of Metchnikoff and Roux. During the investigation important observations were made upon immunity in syphilis, and at this time Landsteiner had much to do with the perfection of methods for the recognition of *Treponema pallidum*, particularly in the recommendation of the use of the dark field microscope. Three years later, in 1909, he succeeded with Popper in producing poliomyelitis experimentally in monkeys. This was the first time this mysterious disease had been reproduced experimentally. In the first observations and the studies continued during 1910 and 1911 with Levaditi and others, Landsteiner worked out the filterable nature of the virus, its localization in tonsils and other organs and disclosed many facts about the disease.

These studies, however, seem to have been interludes in the pursuit of the major quest of his life—the investigation of processes of immunity and of mechanisms of serological reactions and above all the problem of the specificity of antigens. His method of attack was derived from his thorough training in the fundamental science, especially chemistry. In the days of the beginning of his investigations, it seems to have been as clear to Landsteiner as it is to us today that the secrets of serology will be unlocked by the biochemist. He had the chemical faith of Ehrlich, and, if I may make a comparison which Landsteiner's modesty never would permit, he had a surer footing than Ehrlich in chemical rationalization. With fidelity to ascertain chemical facts, he has never wandered into the mysticism of some of Ehrlich's receptor-visualizations.

Among the first of Landsteiner's immunological studies were those which led to his discovery of human isoagglutinins and the blood groups of man. Before 1900 he was investigating the agglutinating and lytic actions of various sera to test whether individuals like species were serologically recognizable. In a foot-note to a paper on this subject, published February 10, 1900, he stated that the sera of some normal men agglutinated the red corpuscles of other men. The results of more numerous observations soon allowed Landsteiner to state explicitly that isoagglutination was a normal occurrence after the mixing of certain bloods, and by 1901 he had enough data to substantiate a biological conception of individual differences in blood. In that year he published an account of three blood groups and correctly recognized the existence of the two major agglutinogens in the corpuscles and the two agglutinins in sera which constitute the basis of this type of serological differentiation of individuals. At the same time he pointed out the significance of isoagglutination for transfusion. The next year, 1902, his associate, Sturli, in cooperation with Decastello, continuing the work, described the fourth blood group. In 1902–1903, Landsteiner suggested the use of blood group determination for the identification of individual human blood for medico-legal purposes. It is to be regretted that Landsteiner, in agreement with the findings of Ehrlich and Morgenroth and Todd in animal blood, did not seem to think it necessary to summarize this knowledge in an orderly four-group classification in some paper in 1902 or 1903—or at least long before his first four-group classification in the manuscript which he sent to the publisher on August 11, 1908, for printing in the 1909 edition of Oppenheimer's "Handbuch der Biochemie." Such a publication would have kept the literary atmosphere clear of the swarm of papers on questions of priority and nomenclature which have vexed this subject. But it seems now entirely plain to me that with the exception of the later discoveries of von Dungern and the Hirschfelds on the mode of inheritance of the factors determining the blood groups and the racial distributions of these groups, these original observations and deductions of Landsteiner and his associates contain all the fundamental discoveries and suggested applications of blood-grouping reactions. An immense benefit to humanity, coupled with an advancement of science, has flowed from these discoveries. The Nobel Prize in medicine for 1930 was awarded to Landsteiner in recognition of the importance of his investigations in isohemagglutination. It is one token of a just reward.

To continue the review of Landsteiner's studies in isoagglutination requires another rupture of chronological sequences, because his experimental investigations of agglutinable substances in the corpuscles have been episodes in his main theme—the search for cell-antigens conditioning individual specificity and finally the specificity of antigens.

A brilliant series of experiments in collaboration with Donath, published in 1904, clearly elucidated the nature of paroxysmal hemoglobinuria through the demonstration of an autolytic antibody which combined at low temperature with the antigen in the corpuscles of the same individual. This was the discovery of a type of cold-lysin and cold-agglutinin which has assumed importance in later studies beyond its interesting connection with paroxysmal hemoglobinuria.

A distinct renewal of Landsteiner's investigations of agglutinable substances in red corpuscles occurred after his arrival in this country in 1922. Since then, in collaboration with van der Scheer, Levine and others he has published numerous papers on work centering around the principles of species specificity, the chemical nature of cell antigens, and the blood groups of man and other animals. Analyses of the bloods of species hybrids, especially the mule, have demonstrated the transmission of parental agglutinable factors, and analyses of the bloods of the higher primates have exhibited their relationship to man, and also significant differences from man. By the use of human sera, animal sera, and especially by the use of absorbed sera of rabbits immunized by injections of human blood, Landsteiner has shown that there are enough differences between cells to justify the surmise "that almost every blood has peculiar serological features." He has coordinated this astonishing individual specificity with the known results of transplantation experiments, in which grafts as a rule succeed only with tissue from the same individual. Several of the newly discovered agglutinogens, notably those designated M and N, are inheritable in accordance with Mendelian laws. As a consequence of the additional agglutinogens discovered by Landsteiner the number of recognizable blood groups has been greatly increased. But Landsteiner has pointed out at the same time that while this result has a bearing upon the general problem of biochemical individuality, it has minor practical consequences, "since the main application of blood grouping, namely for transfusion, has, broadly speaking, reached a satisfactory stage." Fortunately for the increase of knowledge, Landsteiner seems to have an abiding interest in the phases of minor practical consequence!

It will be recalled that in 1906 and 1907 Landsteiner was interested in experimental syphilis, immunity in syphilis and the demonstration of spirochetes. It is natural that he, as a serologist, should have been interested also in the Wassermann reaction, which at that time was beginning its oracular progress. It is natural also that, as a scientific serologist, Landsteiner should have submitted this reaction to a searching analysis with a skilful technique in broadly conceived experiments. The results of these experiments laid the foundation of all modern sero-diagnosis of syphilis, since they introduced the use of a lipoidal antigen. Landsteiner, with his associates Müller and Pötzl, found that extracts of normal organs would fix complement in the presence of syphilitic serum, and finally that the alcoholic extract of normal heart muscle was a more effective antigen than any of the saline extracts of either normal or syphilitic organs. These studies were not the result of a sudden diversion of Landsteiner's attention to a new field of clinical serology. They were a rational consequence of previous investigations of the ability of colloids, and especially lipoids, to absorb complement. In a paper in 1921, Landsteiner comments also on the possible relationship of these alcoholic extracts of heart muscle to the lipoidal-like body described by Bang and Forssman—the so-called heterogenetic antigen—and expresses the opinion that the Wassermann "antigen" is a hapten. Thus the investigation which led to the introduction of an alcoholic solution of lipoidal substances as an antigen for the very practical procedure of the Wassermann reaction is seen to be in line with his fundamental theme of the chemical nature of antigens. It brings Landsteiner a step further in his researches on the complex-antigens and the fruitful study of the substances he has called "haptens."

The great work of Landsteiner's life has been his experimental investigation of the specificity of antigens. These studies have had such brilliant results in his own hands and have been the basis of such remarkable discoveries by others that most immunologists regard them as even more worthy of the Nobel Prize than his discoveries of the blood groups.

Landsteiner has for a long time recognized that of the two kinds of substances set in the inner mysteries of immunology and serology—the antibodies and the antigens—the latter are more susceptible than the former to chemical attack. Antigens, available in bulk and in relative purity, can be manipulated chemically. Antibodies, on the other hand, present in much smaller amounts in almost indissoluble mixtures, are surrounded with difficulties as impenetrable as those which until recently have impeded the approach to enzymes. At one time, Landsteiner studied models of antigen-antibody reactions with inorganic colloids. The great value of this investigation was to convince him that while colloidal phenomena conditioned the course and part of the mechanism of these reactions, they could not account for their specificity. As the problem of specificity has engaged his thought and effort for many years, he wisely decided to apply his chemical knowledge and philosophy through his original methods to the investigation of antigens in an attempt to find the correlation between serological specificity and chemical constitution. With the enthusiasm of an investigator who has a vision of one of the secrets of natural phenomena, Landsteiner has said that "the knowledge of specific chemical differentiation of animal and bacterial organisms is one of the most beautiful fields of theoretical serology." This knowledge also has become of the greatest practical importance.

The nature of the immunizing mechanism, which remains unexplained, seems to require that the start in these investigations be made with the highly complicated soluble proteins. Landsteiner himself, and others, have attempted repeatedly to cause the production of antibodies in animals by the injections of lipoids, polysaccharides, cleavage products of proteins and synthetic peptides. In some of his experiments with lecithin, with the specific fraction of the Forssman heterogenetic antigen, and with azoalbumoses, Landsteiner has obtained some evidence that antibodies may be formed when these substances are injected into an animal. In general, however, for the present, the results of the work of Wells, Osborne and many others may be summarized in a sort of negative law—stating that no substance other than a complete protein has been conclusively shown to be a functional antigen, capable of inciting the formation of antibodies. It seems, however, that this question is not absolutely settled.

The proteins with which Landsteiner has dealt have been the so-called simple proteins, like those of the blood serum, and the complex proteins extractable from the cells of animals and bacteria. It is generally accepted that the simple proteins are large aggregations of amino acids. The complex proteins are more or less firm combinations of proteins with lipoids or proteins with polysaccharides. Species specificity has been shown to reside chiefly in the simple proteins, from the precipitin reactions of which the zoological tree can be roughly constructed. Individual specificity of cells, on the other hand, is most closely bound up with the characteristics of the complex proteins. Reactions with these substances disclose the presence of similar antigens in totally unrelated species. The basis of the specificity of the natural simple soluble proteins remains undisclosed, although the work of Wells, Osborne and many others has pointed out differences in proteins which might account for their different serological reactions. Landsteiner's work, as will be shown, has provided many facts which might be used by analogy to account for the specific behavior of unaltered simple proteins. But the proof has not been brought as in his experiments with chemically altered and complex proteins. He has expressed the opinion that the method of partial synthesis of antigens which he has used can not be substituted for the chemical study of natural antigens and that it is still impossible to say whether small parts of the antigenic molecule are sufficient to determine specificity in the natural antigens. "It is possible that in natural antigens several complicated factors may be involved."

The material of most of Landsteiner's creative investigations has been two sorts of complex antigens: (1) Artificially altered proteins conjugated with nonantigenic substances of slight or great complexity, and (2) the naturally occurring complex proteins, compounds of protein and lipoid or protein and carbohydrate. In all the cases studied, the protein part of the compound has been the functional antigen. The attached compound has conferred specificity upon the complex. These attached substances, while unable to stimulate the production of antibodies, were found to have the remarkable capacity to enter into specific union with the antibodies elicited by the injection of the complex. To these non-antigenic but specifically acting substances, Landsteiner has given the name haptens. His investigations of altered and complex proteins and the reactions of haptens have greatly advanced the knowledge of many fundamental phenomena of immunology and serology.

The pioneer experiments in this field were made in 1906 by Obermayer and Pick, who found that the specificity for species was altered or lost when proteins were iodized, nitrated or diazotized. They assumed that the substitution of hydrogen atoms in the benzene ring of the aromatic amino acids in the protein by I, $NO_2$ and N=N brought about these changes. Subsequently Landsteiner and his associates, especially Wormall, have shown that the serological properties of the antigens prepared by Obermayer and Pick are not simply dependent upon the nature of the substitutes. In addition, later experiments with the Forssman antigens, the haptens of pneumococci and with simple compounds have shown beyond doubt that the groupings necessary for specificity may be entirely devoid of aromatic radicals. In the reactions employed by Landsteiner a common feature has been the production of alterations in the salt-forming groups of the protein molecule. Furthermore, the experiments of Obermayer and Pick gave no basis for expecting the precise results of the specific inhibition reactions and other serological phenomena produced by the isolated simple compounds and haptens. While the investigation of Obermayer and Pick must be honored as a pathfinding research, with heuristic influence, it is necessary to make this clear distinction between the altered protein antigens studied by them and the synthetic antigens prepared in an original manner by Landsteiner.

About ten years after the work of Obermayer and Pick, the further step was taken by Landsteiner when he prepared complex antigens whose specific groups were composed of substances of known chemical composition coupled in a definite manner to otherwise relatively unaltered proteins. The chief chemical processes used by Landsteiner in the preparation of these compounds have been the formation of acyl complexes by treatment of proteins with acid anhydrides and acid chlorides and the production of a great variety of diazo-compounds by the application of Pauli's reaction. In this way, Landsteiner opened up the field of investigation of the relationship between chemical constitution and serological specificity. Out of a long catalogue of synthesized antigens and their reactions only a few can be chosen to illustrate some of the general principles of specificity which Landsteiner has established.

One of the first principles established by Landsteiner is that the specificity of the artificial complex antigen depends largely on the attached substance. The species specific character of the protein fraction is not always entirely annihilated, since strongest reactions are obtained with homologous antigen-antibody mixtures, even when the isolated native protein gives no reaction with the antibody to the complex antigen. The experiments showed further that the ability of these antigens to react with antibodies is not conditioned by a specific chemical group, such as the aromatic portion of the protein molecule, but is a general property of the attached chemical group. The acid groups have the greatest influence upon the "electivity" of the reactions. In fact, the important action of acid groups upon specificity suggests that the part of the protein bearing free carboxyl groups may have a special significance for the specificity of proteins. Immune sera against these compounds act strictly specifically or show group reactions. The immune sera for the azoproteins prepared with para-aminophenylarsinic acid are good examples of sera which react in different degrees with a series of antigens containing the arsinic acid radical. More strictly specific reactions were obtained with compounds containing diazotized para-aminobenzoic acid, exhibiting the overwhelming effect of acid radicals upon specificity.

Another general principle established by Landsteiner from the results of these experiments is that the spatial configuration of the specific group in the antigen molecule plays a most important rôle. This effect was noted in studies of antigens composed of protein coupled with d- and l-phenyl-p-aminobenzoyl-acetic acid, and was established beyond question by Landsteiner's more recent experiments with dextro, levo and meso tartaric acid-protein compounds. It was easy to differentiate by precipitin reactions between the d- and l-tartaric acid antigens in dilutions of 1:100 and upward. From the results of these experiments published in 1928, Landsteiner postulated that since steric isomers, differing only in the relative positions of H and COOH groups around an asymmetric carbon atom, are acted upon selectively by immune sera, stereo-isomerims may be expected to play a significant part in the serological specificity of carbohydrates such as those discovered in bacterial antigens. This prediction has been substantiated in the results of the protein-glucose and protein-galactose compounds successfully synthesized by Avery and Goebel and by their brilliant studies of the synthetic pneumococcus antigens.

A remarkable discovery of far-reaching analytical significance made in the course of these experiments was the capacity of the simple substances in their natural state, un-diazotized and unattached to protein, to give reactions with the antibodies formed to the complex protein. These reactions have been of two sorts, positive phenomena of the anaphylactic type in sensitized animals, and specific inhibition phenomena in animals and in the test-tube mixtures of antibody and the particular non-antigenic substance. Undoubtedly these reactions, which can be classed in the special group of hapten reactions, have great significance in connection with idiosyncrasies and many of the phenomena of hypersensitivity. It seems also that they may assist in bringing order into the observations of anomalous serological reactions which have been thought by some to differ quantitatively from results expected from some of Ehrlich's assumptions. In the final estimation they are apt to prove of greatest value in the determination of the specifically active groups in natural as well as artificially altered proteins. Striking examples of this are specific inhibitive reactions of optical isomers and the discovery of Wormall, who applied this analytical method to halogenated proteins, that the active group in iodized protein is apparently 3:5 diiodotyrosin.

Landsteiner's investigations of the natural complex antigens have proceeded *pari passu* with the researches on the artificially prepared protein-chemical compounds. One phase of the work has been advanced by discoveries in the other phase in such a way that a separation of the two is somewhat forced. Landsteiner has studied chiefly the Forssman heterogenetic antigen, but has also investigated the complex antigens of bacteria, similar to those discovered by Avery and Dochez and Zinsser and Parker. It seems established from these researches that the individually specific and heterogenetic antigens of animal cells are chiefly compounds of protein and lipoid-like substances, while the specific cellular antigens of bacteria are proteins conjugated with polysaccharides. In both cases, the protein serves the function of immunizing, eliciting the formation of antibodies, while the lipoidal or carbohydrate fraction, the hapten, incapable of giving rise to antibodies, reacts in a number of ways with these antibodies in the test-tube and in the animal body. Aside from this important bearing upon specificity, these lipoidal and carbohydrate haptens have been shown to have important anaphylactic effects in actively and passively sensitized animals, effects which may explain the origin of shock following the systemic introduction of protein split products and decomposition products of various organisms. Their effects suggest numerous new investigations of phenomena of hypersensitivity. In addition, these haptens in themselves are substances of considerable biochemical interest. In attempts to determine the nature of the lipoidal fraction of the Forssman antigen, Landsteiner and Levene have discovered hitherto unknown lipoids, which yield on hydrolysis a reducing sugar and components of lipoids. From such experiments by Landsteiner it is inevitable that chemistry will be enriched by new compounds and that serology and immunology will advance both as a science and as a practically serviceable field of knowledge for man and animals.

In order to give the researches of Landsteiner upon the specificity of antigens their proper setting it would be necessary to review the many biological processes in which specificity is the inner mechanism, to recapitulate at least the evidences for a conception of the evolution of proteins correspondent with the evolution of species. Even without a detailed review of these subjects, it is obvious that his work may have a bearing upon such diverse processes as specific ferment action, resistance to infection, systematic zoological and botanical classifications, and the serology of heredity. Landsteiner, fully aware of the manifold implications of his discoveries, has pursued a course of rational experimentation, describing many facts, expressing generalizations when fully justified, and occasionally suggesting an hypothesis. He is entirely worthy of the name experimenter in the sense in which Claude Bernard used it when he said, "To be worthy of the name, an experimenter must be at once a theorist and practitioner. While he must be completely master of the art of establishing experimental facts, which are the materials of science, he must also clearly understand the scientific principles which guide his reasoning through the varied experimental study of natural phenomena."

Those who have known Dr. Landsteiner best have expressed their high appreciation of his rare personal characteristics of simplicity, sincerity, gentleness and charm. The writer of this review, who has had less opportunity than they to know him, can nevertheless join cordially in the universal expressions of admiration of his accomplishments and in the regard and veneration felt for him by his friends all over the world. This review itself is clearly an inadequate summary of his work. It is presented in the spirit in which Dr. James addressed his Medicinal Dictionary in 1743 to the great Dr. Meade, "Sir: ——— you are to consider this address, if it be agreeable to you, as one of the rewards of merit; and if otherwise, as one of the inconveniences of eminence."[2]

# OBITUARY

## LOUIS HERMANN PAMMEL

THE death of Dr. Louis Hermann Pammel marks the passing of a pioneer in the field of botany in the upper Mississippi Valley. Dr. Pammel served as the head of the department of botany at Iowa State College for forty years. He died on March 23, en route to Ames from California, where he and Mrs. Pammel spent the winter.

[2] In the collection of material for this review of Dr. Landsteiner's work I have been greatly aided by Dr. Peter K. Olitsky. I acknowledge with sincere thanks his constant willingness to assist me generously and am obliged to him for many helpful suggestions.