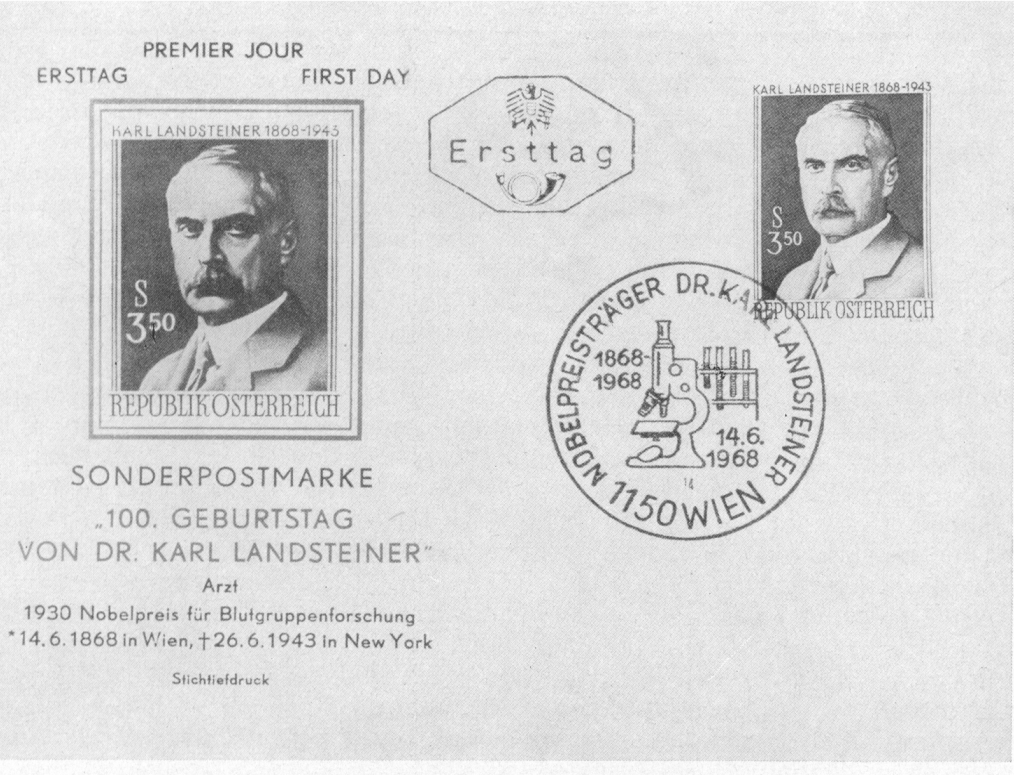


# Karl Landsteiner

*Father of Blood Grouping and Immunochemistry*

**A. S. Wiener**

It was on June 25, 1943 that the world lost a great mind and a great medical scientist, when Dr. Karl Landsteiner succumbed to the effects of a heart attack that had overtaken him two days before while he was actively at work in his laboratory. He had just passed his seventy-fifth birthday of a lifetime devoted to fundamental research. Shortly before his death he had completed the second edition of his classic book, " The Specificity of Serological Reactions ", and he had also published almost 350 scientific papers describing the results of his pioneering investigations. This year is the centenary of the birth of Karl Landsteiner, and it seems fitting at this time to pay tribute to this genius and benefactor of mankind by reviewing some of his great discoveries.



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Of Dr. Landsteiner's discoveries, the one that has captivated the popular imagination the most is his discovery of the A-B-O blood groups, frequently called the Landsteiner blood groups in his honor. At the beginning of the present century, it was recognized that bloods from animals of different species could be distinguished from one another by their serological reactions. For this reason, the use of animal blood for transfusions to human beings was abandoned, but the frequent serious reactions that followed transfusions of blood from one human being to another still remained unexplained. Landsteiner then tested the blood of workers in his laboratory by mixing serum taken from one individual with the red cells of others. He found that instead of the weak or negative reactions that might have been expected, in some combinations strong agglutination of the red cells occurred, while in other combinations no effect was apparent. He then demonstrated that the phenomenon he had observed could be explained by postulating the existence in the red cells of two different agglutinable substances, which he called A and B, and in the serum of two corresponding isoagglutinins, anti-A and anti-B. Depending on whether one, the other or neither or both of these agglutinogens were present in the red cells, four blood groups could be defined, namely, A, B, O and AB, while, according to the rule discovered by Landsteiner, those agglutinins and only those are present in the serum for which the corresponding agglutinogens are absent from the red cells. Application of A-B-O blood grouping for the selection of blood donors, as suggested by Landsteiner in his very first paper on the subject, has made blood transfusion the safe procedure it is today; it has saved the lives of millions of persons, many of whom have never heard of this famous scientist.

Early in his investigations, Landsteiner became convinced that an individuality of human blood exists, comparable to that of the fingerprints. Therefore, when in 1922 he was invited to become a Member of the Rockefeller Institute in New York City, he resumed his studies by immunizing rabbits with human red cells, and (with Philip Levine) he discovered the three M-N types and the agglutinogen P. Additional investigations of Landsteiner and his associates disclosed further individual blood differences, and then, in collaboration with A. S. Wiener, a different approach was used, namely, the immunization of rabbits and guinea-pigs with the red cells of rhesus monkeys. Previously it had been found (Schiff and Adelsberger) that the injection of rabbits with sheep red cells frequently elicited agglutinins of anti-A specificity for human red cells, while immunization with rhesus red cells (Landsteiner and Wiener) stimulated the formation of anti-M agglutinins. Continuing this line of study, Landsteiner and Wiener found that rabbits (and, more easily, guinea pigs) injected with rhesus red cells produced an antibody that clumped the red cells of about 85% of Caucasians, who were therefore labelled as Rh (rhesus) positive, while the red cells of 15% of individual failed to react (Rh negative). When A. S. Wiener and H. R. Peters then found that incompatibility with respect to the Rh factor was the usual cause of intragroup hemolytic transfusion reactions in repeatedly transfused individuals, Landsteiner and Wiener announced their discovery in 1940. Shortly thereafter P. Levine and his associates showed that Rh isosensitization is also the basis for

the bulk of the cases of erythroblastosis fetalis, a hitherto mysterious blood disease of the newborn. These discoveries led to the routine use of Rh testing as well as A-B-O blood grouping for the selection of donors for blood transfusions, not only to prevent hemolytic transfusion reactions, but also to avoid isosensitizing girls and women of the childbearing age resulting in severely affected or stillborn erythroblastotic babies.

Some confusion has arisen from the discovery that guinea-pig antirhesus sera strongly agglutinate the red cells of cord blood and newborn babies, both Rh negative and Rh positive and by the fact that anti-Rh sera from sensitized human beings fails to agglutinate rhesus red cells at all (an example of an areciprocal reaction, as discussed by Landsteiner in his book), so that recently an attempt has been made to assign a different symbol LW to the original rhesus blood factor. However, the fact remains that anti-rhesus guinea-pig (and rabbit) sera distinguish sharply between Rh-positive and Rh-negative blood of human adults (and also of newborn after suitable absorption or dilution of the antisera), and that for a number of years this was the only reagent readily available and used for typing donors for blood transfusions, so that Landsteiner and Wiener's priority for this important discovery and the use of the symbol Rh factor (for rhesus) remain unassailable. (In recent unpublished experiments, reagents having anti-$Rh_0$ specificity were readily obtained by immunizing guinea pigs with rhesus or baboon red cells, while, paradoxically, injections of Rh-positive (or Rh-negative) human red cells into guinea pigs failed to produce usable reagents.) Following the discovery of Rh blocking antibodies by A. S. Wiener, he developed methods of producing potent anti-Rh sera in human volunteers, and also developed techniques of Rh typing that circumvented the interference of the Rh blocking antibodies. Wiener also introduced the presently used successful method of treating erythroblastosis fetalis by exchange transfusion. All these subsequent developments were the natural sequelae of the original discovery of Landsteiner and Wiener of the Rh factor with the aid of anti-rhesus rabbit and guinea-pig sera. The end result has been the increased safety of blood transfusion, a successful method of treating erythroblastosis fetalis, and a marked reduction in the incidence of erythroblastosis fetalis, which before the discovery of the Rh factor was largely a iatrogenic disease resulting from isosensitization of females by injections of blood not typed for the Rh factor.

Early in his career, Karl Landsteiner also became fascinated by the problem of the basis for the specificity of serological reactions, which, for example, made possible the easy differentiation by a simple precipitin test of proteins from different animals, e.g., beef and horse meat, a problem which had defied the best efforts of organic chemists of his period. In an extensive series of esperiments carried out at the Rockefeller Institute in collaboration with J. van der Scheer, Landsteiner demonstrated that the specificity of serological reactions was based on the chemical structure of so-called combining or determinant groups within the antigen molecule. He produced artificial antigens with determinant groups of known simple chemical structure, by diazotization moreover in order to eliminate as much as possible overlapping reactions caused by the protein-carrier part of the antigen, Landsteiner immunized

his rabbits with azoprotein prepared with horse serum, while for the reactions in vitro he used azoproteins prepared with chicken serum. He introduced the term " hapten " for substances lacking antigenicity but capable of reacting specifically in vitro with immune sera, and he also introduced the inhibition test with simple chemical compounds for testing the specificity of antisera produced in response to his conjugated antigens. (Later, the inhibition technique was applied by others, notably Watkins and Morgan, for elucidating the chemical structure of the A-B-O and Lewis blood group substances).

These results as well as the results of his contemporaries Landsteiner summarized and crystallized in his classic book on the Specificity of Serological Reactions. Some of his more outstanding results regarding the specificity of antigen-antibody reactions are so profound that they have escaped the attention and understanding of many workers in the field, even at the present time. As he points out in his book, " the high specificity of many serum reactions led Ehrlich to the view that each antibody is sharply adjusted to one particular structure (receptor), and that accordingly overlapping reactions must depend on the presence in them of identical substances or chemical groupings ". This erroneous concept has survived to the present day in the naïve one-to-one correspondence between antigen and antibody implicit in such symbols as the C-D-E and 1-2-3 notations for the Rh-Hr blood group system. Instead, Landsteiner's experiments, for example, with cross reactions of immune sera for conjugated o-amino benzoic acid, demonstrated that " even a small determinant structure can combine with quite different antibodies ". Landsteiner's concept that each antigenic substance (or even each small determinant chemical grouping) can elicit and combine with a multiplicity (theoretically unlimited in number) of antibodies of differing specificities was later to be applied by Landsteiner's protégé, A. S. Wiener, when he worked out the serology of the complex Rh-Hr blood group system and devised his nomenclature for the Rh-Hr phenotypes, genotypes and blood factors. The profound nature of Landsteiner's concept is evident from the fact that now, as long as 25 years after his death, Wiener's explanation of the serology, genetics and nomenclature of the Rh-Hr system which makes use of Landsteiner's ideas, is still not universally understood. Landsteiner's concepts also find important practical applications to the complex Gm system, the leucocyte blood groups, the histocompatibility groups of mice, etc, but no worker in those fields has yet come up with an acceptable terminology that takes Landsteiner's concepts into account.

Landsteiner later became interested in the problems of drug allergy and contact dermatitis, a field of considerable importance in industrial medicine. Here the exciting agent is usually a simple non-antigenic chemical or even an element like the metal nickel. First working with Dr. John Jacobs, Dr. Landsteiner devised methods of inducing sensitivity in animals to some of the chemical substances causing contact dermatitis in man. Then, together with Dr. Merrill W. Chase, he adduced considerable evidence to show that here also an antigen-antibody reaction was involved, and that the noxious agent acquired its antigenic properties by combining with body proteins to form a complex antigen.

Dr. Landsteiner made fundamental contributions to other branches of immunology and medicine, for example, the diagnosis of syphilitic infection. He showed that alcoholic extracts of normal tissues contained the active principle responsible for the Wassermann reaction, and that the use as antigen of fetal syphilitic liver teeming with spirochetes, as originally prescribed by Wassermann, is unnecessary. Landsteiner's observations are the basis for the current use of beef-heart lipids as antigen in the Wassermann test. Landsteiner also introduced the use of dark-field microscopy for the demonstration of spirochetes in primary syphilitic lesions. With Donath he devised an in vitro test for paroxysmal hemoglobinuria, and thus took the first step in the elucidation of the autohemolytic anemias. With Popper, Dr. Landsteiner succeeded for the first time in transmitting poliomyelitis to rhesus monkeys, and thus initiated the experimental study of that disease. He then proved that the causative agent of poliomyelitis is a filtrable virus, and his work eventually led, in the hands of later workers, to the development of vaccines so that at present poliomyelitis has been virtually eradicated as an infectious disease of any statistical importance.

Dr. Landsteiner was always actively interested in the practical implications of his discoveries. In his very first paper on the A-B-O blood groups, he not only pointed out the application in blood transfusion practice, but also for the grouping of dried blood stains in assault and homicide cases. By working out the mechanism of heredity of the M-N types that he had discovered with Levine, he increased the value of blood grouping tests for forensic problems of disputed parentage, and this application was advanced still further by his protégé A. S. Wiener who worked out the genetics of the complex Rh-Hr blood group system. Landsteiner studied the A-B-O blood groups in apes and monkeys with C. P. Miller, and later, with A. S. Wiener, he tested the blood of non-human primates for the M-N blood factors, and he concluded that the results of the blood tests " seem to agree with the theory that man and apes are descended of a common stock, rather than that man evolved from one of the apes ".

The fundamental significance of Landsteiner's discoveries was recognized only gradually by his fellow scientists, and his concept of the nature of serological specificity, as has been pointed out, is still not fully appreciated, so that honors came to him relatively late in life. In 1926 he was awarded the Hans Aronson Foundation Prize. It was not until 1930 that he received the Nobel Prize in Medicine for his discovery of the human blood groups made at the beginning of this century, and the same year he received the Paul Ehrlich medal for his chemical studies. At a citation in his honor read on the occasion of the award of the honorary degree of Doctor of Science at Chicago University, he was called " the world's greatest authority on the mechanism of immunity ", and a Harvard citation stated: " He founded a school of thought that has penetrated wherever immunologists are at work ".

This tribute to the genius and humanitarian Dr. Karl Landsteiner, only briefly skims over his many contributions and discoveries. It fails to take into account his influence on other investigators who worked with him and benefited from their con-

tact with him or from his publications. In fact, one well may state that Karl Landsteiner lives on, not only through the effects of his discoveries on the modern practice of medicine, but also in the living scientists who have benefited from their contact with him and are carrying on in his tradition, and transmitting that tradition in turn to their students and associates.

## References

LANDSTEINER K. (1945). The Specificity of Serological Reactions. Reprinted by Dover Publ. Inc., New York 1962.

SPEISER P. (1961). Karl Landsteiner, Entdecker der Blutgruppen. Biographie eines Nobelpreisträger aus der Wiener Medizinischen Schule. Brüder Hollinke, Wien.

WIENER A. S. (1943). Karl Landsteiner. Scientific Monthly, **67:** 280-283.

— (1968). Karl Landsteiner and the History of the Rh-Hr Blood Types. (In press).

Karl Landsteiner (1868-1943). J. Immun., **38:** 1-16, 1944.

Karl Landsteiner (1868-1943). Obituary Notices of Fellows of the Royal Society. **5:** 295-324.

*Selected Contributions to the Literature of the Blood Groups and Immunology.* Vol. I. The A-B-O System. Blood Transfusion Division, U. S. Army Medical Research Lab., Fort Knox, Kentucky, 1966.

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