

Historical Review

RED CELL AGGLUTINATION: THE FIRST DESCRIPTION BY CREITE (1869) AND FURTHER OBSERVATIONS MADE BY LANDOIS (1875) AND LANDSTEINER (1901)

The safe practice of blood transfusion is dependent on the agglutination reaction, the visual recognition of the clumping of red cells as the result of antigen–antibody reactions. It is a simple technique which requires no more than a Pasteur pipette and a test tube to perform but which has led to the recognition of 26 major blood group systems and at least 270 different red cell phenotypes, and it is the basis for the successful transfusion of blood. Two books bear witness to both of these developments, namely *Blood Groups in Man* (Race & Sanger, 1975) and *Blood Transfusion in Clinical Medicine* (Mollison *et al.*, 1997).

The description of the phenomenon of red cell agglutination and its development as a tool in elucidating blood groups took place in the last 30 years of the 19th century in Germany and Austria and was largely the work of three people: Adolf Creite, a medical student in Göttingen, Leonard Landois, Director of the Physiological Institute at the University of Greifswald, and Karl Landsteiner, working in the Pathological Anatomy Institute in Vienna.

ADOLF CREITE

Creite's almost unknown contribution was published in 1869 in the *Zeitschrift für Rationelle Medizin* under the title of 'Versuche über die Wirkung des Serumeiweisses nach Injection in das Blut' (Investigations concerning the properties of serum proteins following intravenous injection) (Creite, 1869a). Creite was a medical student in Göttingen at the time of the study and in the text of the paper he states that the work was suggested by and carried out under the guidance of Professor Georg Meissner in the Physiological Institute at the University. This work of Creite's is quite remarkable in that it showed that serum proteins had the property of both 'dissolving' and bringing about 'clustering' of red cells, that is lysis and agglutination in present day terms, anticipating the discovery of antibodies by a quarter of a century. According to Creite, the stimulus for his investigations lay in an observation made by Stokvis (1867) that egg white protein injected into animals resulted in the appearance of protein in the urine. Claude Bernard had made a similar observation (Bernard, 1859) but in addition had found that the injection of dog serum into a rabbit also caused the urine to become 'bloody' (*blutig*) and to contain protein.

In order to follow-up Bernard's observations, Creite injected about 8 ml of serum obtained from calf, pig, dog, sheep, cat, chicken, duck and goat into rabbits. The first three had little or no effect on the recipient but the sera of the latter five almost always resulted in the appearance of 'blood-stained urine' (*blutgefärbter Harn*), general malaise and the death of the animal. He noted that the urine was free of intact red cells which 'excluded the possibility that damage to the vessels in the kidney had occurred as a result of the increased arterial pressure'; moreover, he had measured the blood pressure following the injection of serum and found it to be unchanged. It was the blood-stained urine that particularly caught Creite's attention and it is clear from the text that this finding sent him back to the library where he found that similar observations had been made by others and cited Panum (1863) and also Sheel (1803), who had quoted Ippolito Magnani, Denis and Gaspard de Gurye. All these authors had reported blood-stained urine, although not in exactly the same circumstances as they had injected whole blood rather than serum. The haemoglobinuria in their experiments was presumably mainly due to lysis of the donor cells and not the recipient's. Nevertheless, Creite concluded from his own experiments and from these reports that constituents of serum had certain 'chemical properties' (*chemische Eigenschaften*) which affected foreign red cells directly. He thought it very unlikely that it was the water content of the foreign serum that had caused the cells to burst and concluded that 'we are left with the explanation that serum contains agents which are able to dissolve red cells directly'. He was not certain which substance in the serum was responsible for this effect but suggested that the most likely active ingredient was serum protein (hence its appearance in the title of the paper). He based this conclusion on an experiment in which he removed the protein from serum by heat coagulation and then injected the filtrate. He states that 'all the urine samples examined until the evening of the following day are normal. They contain neither haemoglobin nor protein...the experiment proves that the non-protein components of the serum do not play a part in the effect; therefore one must assume that it is the proteins which are responsible. However I cannot say how they function.'

This report of Creite's was almost entirely devoted to intravascular lysis. Nevertheless, at the end of the paper he described *in vitro* experiments that he had carried out and gave an account of what is probably the first description of agglutination. In order to support his belief that the appearance of blood-stained urine resulted from chemical agents present in the serum, he stated that he carried out

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experiments of which he said that 'although not fully conclusive, they are worth mentioning'. The final paragraph of his paper runs as follows: 'If you add blood serum from any of the animals with which I have carried out my experiments to a drop of fresh rabbit blood, then you observe under the microscope that in the regions where the foreign serum mixes with the rabbit red cells, the cells suddenly flow together in a peculiar way forming different-shaped drop-like clusters with irregular branches' (*plötzlich in einer eigentümlichen Weise zu tropfenartigen verschieden gestalteten Anhäufungen mit unregelmässigen Ausläfern zusammenfließen*). 'In cases where the reaction was strong, as for instance when cat serum was added, the contours of the cells were no longer apparent but reappeared after the addition of salt solution. Since I carried out the first experiments with cat serum, I believed that I had found an explanation for the appearance of blood in the urine, as it was possible that some blood cells had dissolved completely. However since the same phenomenon was observed with dog serum, this explanation was no longer tenable (dog serum did not bring about the appearance of blood-stained urine in his *in vivo* experiments). Clustering (*Anhäufung*) of red cells also occurred after the addition of lamb, chicken, duck and goose serum. I did not carry out experiments with calf and pig serum. The above mentioned sera had the same effect on human red cells as on rabbit cells. However, no change was observed when rabbit serum was added to rabbit blood cells.'

The description of 'drop-like clusters with irregular branches' occurring where foreign serum and red cells came into contact suggests that it was a true agglutination reaction, resulting from antibody activity and not non-specific rouleaux formation; he only commented on the clarification of the red cell contours on the addition of saline but made no mention of any dispersal, which he would almost certainly have noticed had they been rouleaux. Moreover, he specifically stated that there was no clustering following the mixing of rabbit serum and rabbit red cells, also indicating that the clustering was not the result of rouleaux formation.

Biographical note concerning Adolf Creite. The 1869 publication of Creite is almost unknown, although it was cited by Landsteiner (1910) who linked it with the experiments of Landois as follows: 'It was some time ago, in the course of blood transfusion studies, when it was observed that blood corpuscles in the serum of a foreign animal species can be precipitated out of solution or even made to form clumps'. The recent recognition of Creite's work resulted from the investigations of the science historian, Peter Voswinckel, who has published a book, *Der schwarze Urin* (Voswinckel, 1992) which is concerned with the historical aspects of those diseases that are characterized by the excretion of haemoglobin and related compounds in the urine; this book also contains a photograph of Creite taken in the last year or two of his life (Fig 1).

There is very little information concerning Creite's career. The archives of the Georg-August Universität at Göttingen state that he was born in Helmstedt, Niedersachsen, that he was the son of a merchant and that he matriculated in the



Fig 1. Adolf Creite, about 1920. Obtained from Voswinckel (1992). ©1993 Blackwell Wissenschafts-Verlag, Berlin.

University in 1871; his dissertation was published in 1874. Although Creite states in his paper on agglutination that he worked in the Physiological Laboratory in Göttingen (Figs 2 and 3), his name does not appear in the list of Professor Georg Meissner's research fellows. The Royal Society Catalogue for Sciences states that he published two other papers. The experiments for one of them was also carried out in Meissner's laboratory and were concerned with measuring the inosinic acid content of muscles of various animals (Creite, 1869b). The other work, also carried out at Göttingen, was on the physiological activity of alcoholic extracts from *Cynoglossum officinale*, a plant used in the preparation of an astringent ointment (Creite & Marmé, 1870). Voswinckel (1992) quotes an obituary from which he gives the following additional information: Creite was born in 1847 and died in 1921. His academic studies in Göttingen were brought to an end by the declaration of war by France in 1870 and he served as a hospital physician (*Lazarettartz*) during the military campaign of 1870–1871. He then finished his medical studies in Vienna and for the last 47 years of his life he was as a physician in the town of Schöninggen specializing in public health.

LEONARD LANDOIS

Red cell agglutination and lysis was put on a firmer basis by Leonard Landois working in the Physiological Institute in the University of Greifswald in Germany. He published an extensive monograph on the subject of transfusion (Landois, 1875), which included a section describing his *in vitro* experiments; these extended those of Creite, although he did not cite the latter. Landois' stated aim

Fig 2. An 18th century engraving of the street 'Göttinger Allee' in Göttingen. The Institute of Physiology was housed in the building on the right hand side of the picture, named Michaelishaus after the Orientalist, Johann Michaelis. Supplied by the Niedersächsische Staats- und Universitätsbibliothek, Göttingen.



Fig 3. A mid-19th century engraving of the main University building at Göttingen. Supplied by the Niedersächsische Staats- und Universitätsbibliothek, Göttingen.



was to determine whether the lysis of red cells, so frequently reported after transfusion between different species, were those of the recipient or the donor. In his experiments, Landois was successful in demonstrating both lysis *in vivo* and agglutination *in vitro*. (It is to be noted that the terms 'lysis' and 'agglutination' were not in use until the end of the 19th century. For 'lysis', both Creite and Landois used *auflösen* (dissolve); for agglutination, Creite used *Anhäufung* (accumulation) and Landois used *Zusammenballung* (ball formation) or *klebrige Klumpen* (sticky clumps). Landois also distinguished agglutination from rouleaux for which he used the term *geldrollenartig* or 'like rolls of coins'.

Landois describes his experiments as follows: 'I put 4–5 c-cm of clear serum into a test tube and then added fresh defibrinated blood with a glass rod until the mixture was no

longer transparent. Very little foreign blood is required since blood is not transparent even at great dilutions. I then incubated the mixture either at 37–38 degrees or at room temperature and observed the initiation of the red cell lysis (*auflösen*). The beginning of lysis is easily recognized because the mixture, which initially is matt and non-transparent, develops a "shine". Sooner or later the mixture becomes completely clear and transparent and the cells are no longer visible. I observe the whole process of the lysis and the changes in red cell shape under the microscope.'

He used serum and red cells obtained from eight different animals. The results of experiments he obtained using dog serum are typical. He reported that nearly all foreign red cells lyse in dog serum within minutes of being added and that those cells in close proximity to each other also stick together and form clumps. He was struck by the

great variation in the activity shown by different sera on different red cells. For instance, human serum dissolved lamb's cells quickly, but cells from cats and dogs were relatively stable for up to 2–3 d. He examined cat cells suspended in dog serum under the microscope and observed that the cells first developed spines, sometimes assuming a mulberry shape and then became spherical; lysis occurred after about 30 min. When there was a high concentration of red cells, clumping of the cells occurred rather than lysis; he attributed this to a substance in the serum which acted on red cells and made the membrane soft and sticky, so that when the red cells touched each other they aggregated.

Commenting on another experiment on the mixing of cells and serum, Landois described the changes in shape and added the following: 'during these changes, the cells... develop the ability to stick to neighbouring cells, form larger or smaller clumps...eventually settling down on the bottom... Under the microscope the cells are close together and cannot be separated by pressure on the cover-slip but they spread out as if connected by threads. If the pressure is released, the cells move together again.'

Landois did not believe that the lysis resulted from the foreign serum having a higher water content than the cells, but suggested that the lytic ability (*Lösungskraft*) of the serum 'resulted from a strange and unknown ratio of its constituent parts'. It is clear that Landois considered that lysis and agglutination resulted from some type of interaction between the serum and red cells but he was not as definite as Creite, who believed that there was a chemical reaction between serum proteins and red cells.

LANDSTEINER

The discovery of the immune system with all its ramifications unfolded in the 1890s, starting essentially with the description of antibodies specific for tetanus antitoxin by Emil von Behring & Kitisato in 1890. The discovery of bacterial agglutinins was followed by a renewed interest in the clinical aspects of red cell agglutinins and lysins; Eisenberg (1901) cited 37 references on these subjects, all published between 1898 and 1901. Eisenberg's account showed that there was a considerable amount of disagreement and confusion at that time about the occurrence and significance of agglutination in both health and disease. For instance, both Shattock (1900) and Grünbaum (1900) thought that the appearance of serum agglutinins were a manifestation of certain diseases, especially infections, and that they were specific for the particular disease. However, neither author presented any substantial evidence for these statements. Shattock did not give any account of the activity of serum from healthy people but only those with febrile illnesses and Grünbaum based his conclusion on evidence derived from only one case each of typhoid and scarlet fever.

It was at this point that Landsteiner entered the field; the first suggestion of the existence of serum agglutinins and red cell antigens within what would finally be known as the ABO blood group system is to be found as a footnote in

Landsteiner (1900). In the introduction to this paper, Landsteiner discusses the potential use of antibodies in the elucidation of protein structure. It had been established that antibodies had the ability to neutralize the effect of some enzymes and he quoted Morgenroth as hoping 'to conduct experiments using the effect of serum to show that several active groups can be found in rennet' and also von Dungern who attempted to 'utilize the anti-enzymatic effects of serum by immunizing animals with various microbes and showing that the resulting serum has a specific effect against the bacterial enzymes which he introduced. Therefore, in these experiments we are dealing with a kind of "serodiagnosis" of bacteria.' It is in his Nobel Prize lecture in 1931 that Landsteiner elaborates on this theme (Landsteiner, 1931), stating that the primary aim of the work was the 'goal of chemically characterizing single proteins', as it was clear that this could not be done with the known biochemical methods. Nevertheless, Landsteiner continues, 'the application of serological reagents led to an important general discovery in protein chemistry, namely, that proteins in various animals and plants are different and are specific for each species.' These observations on the action of animal antibodies resulted in Landsteiner speculating as follows: 'The discovery of biochemical species specificity prompted the question as to whether individuals within a species show similar, though presumably slighter, differences.' It was this speculation that led him to embark on the experiments which led to the discovery of the ABO blood group system. In his words, 'as no observations whatever were available pointing to such behaviour, I chose the simplest among the possible plans of investigation, and that material which gave promise of useful application. Accordingly, the investigation consisted in allowing blood serum and red corpuscles of different human individuals to interact.' The footnote in the 1900 paper hinting at the presence of antibodies and antigens of the ABO system reads as follows: 'The sera of healthy individuals not only have an agglutinating effect on animal red cells but also on human red cells from different individuals. It remains to be decided whether this phenomenon is due to individual differences or to the influence of injuries or bacterial infection. I observed this behaviour as being especially pronounced with blood obtained from severely ill patients. This phenomenon can be related to the dissolving capacity of serum for red cells as occurs in various diseases, as described by Maragliano (1892)'.

In the more detailed second paper (Landsteiner, 1901), *Agglutination Phenomena in Normal Human Blood*, Landsteiner no longer equates his observations with those of Maragliano because 'in Maragliano's case the serum only affects the blood cells from the same individual... However, my observations showed clear differences between the blood serum and the blood cells of apparently completely healthy persons.' Moreover, Landsteiner by this time had come to the view that the presence of agglutinins was not specific for certain diseases for the simple reason that in his experience they were also found in healthy people. Landsteiner obtained sera and red cells from 29 different people, including himself and four medical colleagues. We can see

in retrospect that the reason why Landsteiner was successful in elucidating the mechanism underlying intraspecies agglutination, whereas Eisenberg and others had failed, arose from the nature of Landsteiner's experimental design. Eisenberg only tested the sera against red cell samples taken at random but Landsteiner tested all the sera against all the samples of red cells, using 'checkerboard' blocks of five or six different sera and red cells in 144 combinations. He summarized his findings as follows:

'The sera in most cases could be separated into three groups. In many cases sera of group A react with red cells of another group, B, but not with group A; on the other hand, A red cells are affected in the same way by serum B. The sera of the third group (C) agglutinates red cells A and B, but C red cells are not affected by sera from A and B. Naturally the red cells must be considered insensitive to the agglutinins which are present in the same serum.' He also made the observation that 'the above mentioned agglutination... even occurred with a drop of blood which I dried on a piece of cloth and dissolved 14 d later... Finally I want to mention that the observations explain the varying consequences of therapeutic blood transfusions in humans.'

The discovery of both intravascular lysis and *in vitro* agglutination by Creite was serendipitous in that he was looking for one thing and found another. The initial aim of his work was to elucidate the observation of Bernard (1859) that the intravenous injection of serum from another species gave rise to proteinuria. In the experiments that he carried out, it was the unexpected 'blood-stained urine' that caught his attention and he argued that there must be some chemical reaction between the injected serum proteins and the host red cells which gave rise to intravascular lysis. In attempting to demonstrate lysis *in vitro*, he observed agglutination. Landsteiner on the other hand was looking for intraspecies antibody-antigen reactions and found the evidence that he wanted. In a sense, however, the discovery was also serendipitous in that the antibodies responsible for the observed agglutination were by chance part of the ABO system, a discovery which initiated the study of blood groups that had such significant therapeutic implications. The way in which Creite and Landsteiner pursued the physiological mechanisms underlying their observations illustrates the correctness of Pasteur's aphorism: 'In the field of observation, chance favours only the prepared mind.'

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REFERENCES

- Bernard, C. (1859) *Lecons Sur les Propriété Physiologiques et les Alterations Pathologiques Des Liquide de L'organism*, 2, 459–462.
- Creite, A. (1869a) Versuche über die Wirkung des Serumeiweisses nach Injection in das Blut. *Zeitschrift für Rationelle Medicin*, 36, 90–108.
- Creite, A. (1869b) Untersuchung der Inosinsäure im Fleisch verschiedener Tiere. *Zeitschrift für Rationelle Medicin*, 36, 195–199.
- Creite, A. & Marmé, W. (1870) Über die physiologische Wirkung des alkoholischen Extractes von *Cynoglossum officinale*. *Götttingen Nachrichten*, 17–21.
- Eisenberg, P. (1901) Isoagglutinins and Isolysins in Human Sera. *Wiener Klinische Wochenschrift*, 42, 1020–1024.
- Grünbaum, A.S.F. (1900) A Paper Given to the Liverpool Medical Institution and reported in the *Lancet*, April 28th, 1900.
- Landois, L. (1875) *Die Transfusion des Blutes*, Leipzig.
- Landsteiner, K. (1900) Zur Kenntniss der antifermentiven, lytischen und agglutinierenden Wirkungen des Blutserums und der Lymphe. *Zentralblatte für Bakteriologie*, 27, 357–366.
- Landsteiner, K. (1901) Über Agglutinationserscheinungen normal menschlichen Blutes. *Wiener Klinische Wochenschrift*, 14, 1132–1134.
- Landsteiner, K. (1910) Spezifische Bindung und Antikörper IV. Hämagglutination und Hämolyse. *Handbuch der Biochemie des Menschen und der Tiere*, 395–541.
- Landsteiner, K. (1931) Individual Differences in Human Blood. *Science*, 73, 403–409.
- Maraglio, (1892) Beiträge zur Pathologie des Blutes. *Verhandlung Des Congresses für Innere Medicin, Wiesbaden*, 11, 152–158.
- Mollison, P.L., Englefriet, C.P. & Contreras, M. (1997) *Blood Transfusion in Clinical Medicine* 10th edn. Blackwell Science, Oxford.
- Panum, P.L. (1863) Experimentelle Untersuchungen über die Transfusion, Transplantation oder Substitution des Blutes in theoretischer und practischer Hinsicht. *Virchow's Archives*, 27, 438–445.
- Race, R.R. & Sanger, R. (1975) *Blood Groups in Man* 6th edn. Blackwell Scientific Publications, Oxford.
- Shattock, S.G. (1900) Chromocyte clumping in acute pneumonia and certain other diseases. *Journal of Pathology and Bacteriology*, 6, 303–314.
- Sheel (1803) *Die Trasnsfusion des Blutes und Einspritzung der Arzeneyen in die Adern*. Copenhagen, I, 40 (as cited in Creite, 1869a).
- Stokvis, R.J. (1867) Recherches experimentales sur les conditions pathogeniques de l'albuminurie. *Journal de Medicine, de Chirurgie et de Pharmacologie*, 45, 221–225.
- Voswinckel, P. (1992) *Der Schwarze Urin* Blackwell Wissenschaft, Berlin.

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