IMMUNOHEMATOLOGY

Second Edition

Immunohematology

SECOND EDITION

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PREFACE

The past twenty years have witnessed a veritable explosion in our knowledge and understanding of immunology. Significant advances have been made in explaining the nature and probable mechanisms of the immune response, the structure and formation of antibody molecules, the possible autoimmune origin of certain diseases, and the importance of hypersensitivity as a biologic phenomenon. At the same time, it has become apparent that immunology and serologic methods are useful tools to investigators interested in fields other than those centered around a study of the body's defense mechanisms against infection and disease. In most major universities today, we may find experts in immunology in almost every major scientific discipline. Therefore, it has become necessary for any student interested in even the most remote of the biologic sciences to acquire at least a rudimentary knowledge of immunologic phenomena.

A study of immunology in the abstract can be very complex and unrewarding except for those who wish to make it their major field of endeavor. We have always felt that this science can be best understood if the student has some concrete and very practical skeletal framework upon which to hang the facts. The human blood groups seem to fulfill all of the criteria to make them ideal in this respect. Risking criticism from many of our clinical colleagues, we like to think of immunohematology as the study of immunology in relation to the elements of the blood, rather than the study of pathogenic phenomena involving blood due to immunologic mechanisms. It is with this point of view that this work has been written. The basic premise is that certain lessons of immunology can be learned from each of the blood group systems.

This is a textbook for students in all areas of the biologic sciences who are interested in acquiring a working knowledge of the immunology of human blood group systems. To emphasize this point, many of the discussions involving the genetics of each of the systems have been deliberately omitted. The references included are limited to those which, in our opinion, are classic works in the field and which will be of some help to the student in gaining a better and richer understanding of the subject matter. Of course, references can be of value only if the student can procure the necessary journals in his own library. For this very reason, review articles or secondary articles in readily accessible journals have sometimes been substituted for original contributions published in works with a limited degree of availability.

Students in the medical sciences may at first feel somewhat disappointed, since the book begins with an historical account of man's somewhat futile attempts at transfusing blood, yet very little else is mentioned in the subsequent chapters regarding this specific technique. This is not really the case. The science of blood transfusion at the immunologic level requires the matching of isoantigens between donor and recipient and a thorough search for antibodies. A deep-rooted understanding of the immunology of blood group antigens and antibodies is a prerequisite for anyone embarking on a career in transfusion therapy. Numerous texts and technical manuals have been written regarding the clinical aspects of transfusion therapy; and therefore, it seems that once a fundamental immunologic foundation has been laid in this subject, the student can refer to those other works with a more prepared mind.

It has not been possible to answer all of the questions that still remain a mystery in the field of human blood groups, and even if it had been, it is doubtful that we would have. In some controversial areas, degrees of doubt have been deliberately interjected merely to stimulate interest. One outstanding problem that was not even mentioned is related to the biologic function of the blood group antigens Doctor Scott Swisher of Michigan State University, once said that he was sure that they were not made merely for the edification and amusement of serologists and geneticists. We cannot agree with him more, and it is our fondest hope that the next few years of research, perhaps by a student who is inspired by this text, will bring an answer to this and many other yet unsolved questions.

September 1, 1971

CHESTER M. ZMIJEWSKI
JUNE L. FLETCHER

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CONTENTS

CHAPTER

1

THE HISTORY OF BLOOD TRANSFUSION

Man's centuries-long desire to perform blood transfusion as a therapeutic procedure forms the cornerstone of the modern science of immunohematology. At the present time, the use of whole blood is a well-accepted and commonly employed measure without which many modern surgical procedures could not be carried out. One who is thoroughly familiar with this now routine technique and studies its historic development cannot help but wonder why so many of the difficulties encountered, with seemingly simple solutions, were not resolved more quickly. It is only by transposing ourselves into a world that had no knowledge of immunology, genetics, modern biochemistry, statistics, and many other basic disciplines that we can truly appreciate the efforts of the pioneers to whom we owe our heritage.

Blood has held a mysterious fascination for man since the dawn of time. We can imagine the awe of the cave men as they watched this peculiar red fluid oozing from the wounds of one of their fellows. If the wound was severe enough to allow a great deal of the vital humor to escape, the man would grow cold and die; less serious wounds would leave him weak and almost lifeless. It is no wonder that traditionally blood was thought of as being the living force of the body and the seat of the soul. . . .

Historians tell us that the ancient Egyptians, cognizant of the beneficial and lifegiving properties of blood, used it for baths to resuscitate the sick and rejuvenate the old and incapacitated. As early as 76 to 100 A.D. Pliny the Elder and Celsus, two scholarly Roman authors, described the custom of spectators rushing into the arena to drink the blood of dying gladiators. The people felt that such blood was especially beneficial since the athletes were strong and brave, qualities that certainly were seated in and transmissible by their blood.

In the middle ages, the drinking of blood was advocated as a tonic for rejuvenation and for the treatment of various diseases. In the summer of 1492 the blood of three youthful and robust boys was given to the ailing Giovanni Battista Cardinal Cibo who was then Pope Innocent VIII (Fig. 1). Apparently the procedure was not successful since it is recorded

FIG. 1. *Pope Innocent VIII, a recipient of a blood draught.* (*From Brusher.* Popes Through the Ages. *Courtesy of Borden Publishing Company.*)

that the Pope died on July 25, 1492. Interestingly enough, this particular therapeutic regimen was even more devastating since the three youths also died as a result of their donation.[14]

According to Keynes [9] a similar series of events is related in Malory's *Morte d'Arthur*. Although these procedures involved the transfer of blood from one individual to another, they cannot be considered transfusions in the same sense as we regard modern transfusions, since the intravenous route was probably not used. This is not surprising in view of the fact that widespread knowledge of the circulation of the blood and the vascular system did not occur until after 1628. In that year, Harvey published his immortal monograph, *Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus*, which described the theory of blood circulation. Nevertheless, as early as 1505 and 1576 individuals with the foresight of Hieronymus Cordanus and Magnus Pegelius suggested the possibility of transfusing blood from one individual to another.

Traditionally it is accepted that Andreas Libavius was the first to advocate a blood transfusion in 1615.[11] The method he described was essentially a direct transfusion, but most historians seriously doubt that he actually attempted his experimental procedure. Libavius wrote, "Let there be a young man, robust, full of spirited blood; and also an old man, thin, emaciated, his strength exhausted, hardly capable of retaining his soul. Let the master of the art have two silver tubes fitting one into the other. Let him open the artery of the robust young man and into it insert one of the tubes, fastening it in; immediately after, let him open the artery of the old man and put the female tube into it. And then when the two tubes are joined together, the hot and spirited blood of the young man will pour into the old one as if it were from a fountain of life, and all of his weakness will be dispelled." This is truly a monument to man's daring ingenuity, and reaffirms the idea that thoughts of the scientific community in those days regarding the value of blood revolved about the central theme of it being a tonic. The concept that blood had a certain critical volume within the body, below which life could not be sustained, was not yet realized.

There is no question that Harvey's treatise added impetus to the investigation of intravenous therapy. Even as today, we often encounter minor skirmishes regarding the priority of a great discovery, so also many scientists of the seventeenth century claimed to be the originators of the idea of blood transfusion. One of these, Francesco Folli,[5] published a pamphlet in 1680 stating that he invented the procedure 26 years before and demonstrated it to Ferdinand II, Grand Duke of Tuscany. Later on, however, he casually confessed that he had never perfected the experiment and that he had stated his views merely to teach, inspire, and spare others expense in their own investigative efforts.

An interesting account of attempted transfusions between chickens

by a certain Vicar of Kilmanton, Francis Potter, in 1652, has been cited by Keynes.[9] It should serve as an inspiration to all students of the fine art of phlebotomy, who experience failure at their first few attempts. The

FIG. 2. *Sir Christopher Wren. (Courtesy of Trent Collection, Duke University Medical Center Library.)*

Vicar wrote, "I am as yet frustrated, *in ipso limine*, but it is only by my owne inexpertness, who never attempted any such thing upon any creature before; for I cannot, although I have tried divers times, strike the veine so as to make him bleed in any considerable quantity." Some historians [15] interpret this passage as meaning that there existed an overwhelming fear of venesection especially on the part of the operator.

Regardless of the numerous claims published, actual credit for the introduction of intravenous therapy must go to Sir Christopher Wren (Fig. 2). As early as 1657, this English astronomer, architect, and physi-

FIG. 3. *Robert Boyle* (*Courtesy of Trent Collection, Duke University Medical Center Library.*)

FIG. 4. *Richard Lower.* (*Courtesy of Trent Collection, Duke University Medical Center Library.*)

cian injected various medications and other sometimes noxious potions into the veins of dogs. He used an apparatus which consisted of a needle fashioned from a slender quill to which was affixed a bladder. This formed an instrument much like the medicine dropper of modern times, but with a sharp point capable of penetrating the skin. Apparently the animals reacted to these injections by vomiting, being purged, intoxicated, killed, or revived according to the medication administered. Similar experiments were performed by the famed Robert Boyle (Fig. 3), and, as far as these authors can ascertain, he was the first to infuse soluble drugs into humans. His experimental subjects consisted of incarcerated volunteers in a London prison.

One of the pioneers of the authentic practice of transfusion was Richard Lower (Fig. 4), an English physician, who performed his experiments on dogs in 1665. His account of the procedure is the first description of a direct transfusion from artery to vein. According to Lower,[13] a small dog was exsanguinated from the jugular vein until he was almost dead. Then a quill was connected to the cervical artery of a large donor dog, and the blood allowed to flow until the recipient was "overfilled and burdened by the amount of the inflowing blood." The procedure was repeated several times, after which the recipient dog's condition returned to normal. In subsequent experiments Lower substituted specially designed silver tubes for the quills originally employed as a means of anastamosing

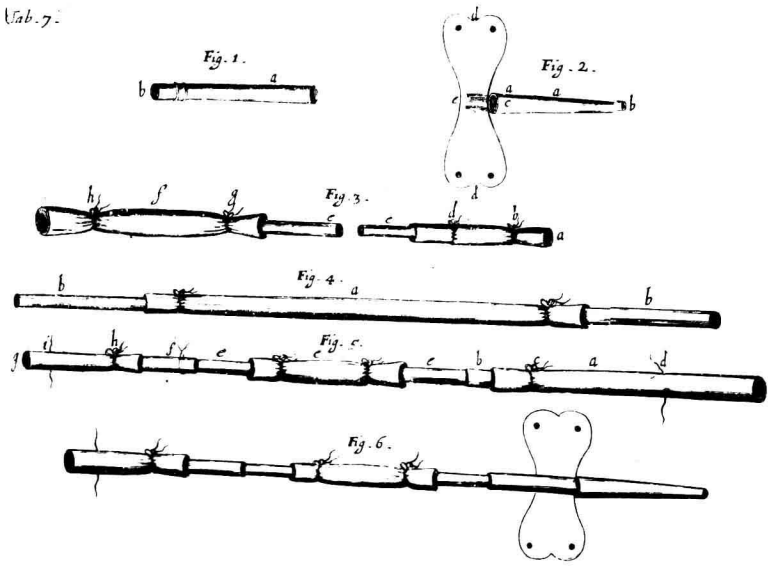

FIG. 5. *Cannulae designed by Lower for performing direct blood transfusions.* (*Courtesy of Trent Collection, Duke University Medical Center Library.*)

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A LETTER

Concerning a new way of curing ſundry diſeaſes by Tranſfuſion of Blood, Written to Monſieur de MONTMOR, *Counſellor to the* French King, *and Maſter of* Requeſts.

By J. DENIS *Profeſſor of* Philoſophy, *and the* Mathematicks.

Munday July 22. 1667.

SIR,

THE project of cauſing the Blood of a healthy animal to paſſe into the veins of one diſeaſed, having been conceived *about ten years agoe*, in the illuſtrious Society of *Virtuoſi* which aſſembles at your houſe; and your goodneſs having received M. *Emmeriz*, & my ſelf, very favorably at ſuch times as we have preſum'd to entertain you either with diſcourſe concerning it, or the ſight of ſome not inconſiderable effects of it: You will not think it ſtrange that I now take the liberty of troubling you with this Letter, and deſign to inform you fully of what purſuances and ſucceſſes we have made in this Operation; wherein you are juſtly intitled to a greater ſhare than any other, conſidering that it was firſt ſpoken of in your *Academy*, & that the Publick is beholding to you for this as well as for many other diſcoveries, for the benefits & advantages it ſhall reap from the ſame. But that I may give you the reaſons of our procedure & con-

Ccc vince

FIG. 6. *The first page of the communication by J. Denis. (Courtesy of Trent Collection, Duke University Medical Center Library.)*

the circulation of the two animals (Fig. 5). During the next several years similar studies were being repeated in England and in France. The investigators, however, began to vary their techniques somewhat; they attempted exchanges of small amounts of blood between animals of different species. Eventually, of course, their thoughts turned to man.

FIG. 7. *Transfusion of a patient with animal blood.* (*From Scultetus. Courtesy of the National Library of Medicine.*)

In 1667, Jean Denis (Fig. 6), physician to Louis XIV, transfused 9 ounces of blood from a lamb into the vein of a young man suffering from luetic madness. The technique was successful, and he wrote that following the transfusion, the patient passed urine as black as soot, but apparently there was little effect in either disturbing his good physical state or mending his poor mental one. A short time later, Lower and King transfused sheep blood into man. The manner in which these experiments were performed was often diagrammed in textbooks of the day. One such illustration from Scultetus, 1693 [17] (Fig. 7), shows a man being transfused with the blood of a dog. A puncture had been made in the subject's arm from which the blood rushed geiser-like into a basin, no doubt to make room for the blood flowing in from the dog. This concept was in keeping with the original method used by Lower when transfusing dogs.

After his initial success, Denis continued his experiments on two other patients. Unfortunately, the fourth patient in his series died. Denis' [4] description of this particular case indicated that the patient in question was a luetic who had been transfused twice before. The first infusion of blood produced no detectable symptoms. The second time, however, "his arm became hot, the pulse rose, sweat burst out over his forehead, he complained of pain in the kidneys and was sick at the bottom of his

stomach. The urine was very dark, in fact, black." After the third transfusion the patient died. This description is probably the first recorded account of the signs and symptoms of what is recognized today as a hemolytic transfusion reaction. The most likely explanation for its absence in the previous instances is that the volumes of blood were relatively small and the symptoms benign enough to go unnoticed. As a result of this unfortunate outcome, the patient's wife charged Denis with murder. A long legal battle ensued. Eventually, Denis was exonerated of the murder charge, but the court decreed that transfusions were to be prohibited except with the sanction of the Faculty of Medicine of Paris. Several years later, an edict of the British Parliament similarly prohibited transfusions and drew an official close to the first phase of man's desire to replace the vital force of the body.

The actual practice of transfusion lay dormant for nearly 150 years, although the basic idea did not escape the minds of the scientific community. As medical knowledge advanced, physicians began to understand the real importance of blood, not merely as a mysterious tonic or rejuvenating potion, but as an essential material with a physiologic function that made it a requirement for life.

Although some claim that a Dr. Philip Syng Physic of Philadelphia performed a transfusion as early as 1795,[16] many stories have been written about one particular English obstetrician, James Blundell (Fig. 8), who had a great deal of compassion for his patients. It has been said that he was appalled at his own helplessness at combating fatal hemorrhage during delivery. Therefore, in 1818, he revived the procedure of blood transfusion. His contributions were great enough to earn him the title of "Father of Modern Blood Transfusion."

It is apparent that Blundell's motivation stemmed from an idea that the loss of blood with a resultant diminution of total circulatory volume could be detrimental or even fatal. This becomes clearer when his research efforts into investigating the effects of the withdrawal of various amounts of blood are studied. Certainly the approach taken by this physician was founded on a new concept. No longer was the procedure based on mysticism and the ancient desire to replace old blood with new blood or to rid a suffering patient of evil humors. Instead, it was subjected to astute scientific investigation. A considerable number of experiments were performed by Blundell and his collaborators. They demonstrated the shocking effects of withdrawing large quantities of blood from an animal, much in the same manner as Lower did many years before. However, they also showed that these effects could be reversed with relatively small quantities of blood that were in no way injurious to the donor animal. This was an important observation for it was certainly of no use to proceed with the development of a technique that might bring harm to a potential donor of blood.