# CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Study</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Introduction</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>Pyrexial Reactions</td>
<td>Study A</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Study B</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Study C</td>
<td>28</td>
</tr>
<tr>
<td>III</td>
<td>Transmission of Disease</td>
<td>Study D</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Study E</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Study F</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Study G</td>
<td>76</td>
</tr>
<tr>
<td>IV</td>
<td>Mechanical Reactions</td>
<td>Study H</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Study I</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Study J</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Study K</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Study L</td>
<td>129</td>
</tr>
<tr>
<td>V</td>
<td>Haemolytic Reactions</td>
<td>Study M</td>
<td>149</td>
</tr>
<tr>
<td>VI</td>
<td>General Conclusions</td>
<td></td>
<td>200</td>
</tr>
</tbody>
</table>
CHAPTER I

INTRODUCTION

The evolution of Medicine from the fears and superstitions of primitive man with his healing gods and disease demons to the ideals of a clear-eyed rationalism has been of a slow and painful character. The development of Blood Transfusion from vague references in ancient literature to a scientifically established and invaluable therapeutic procedure reflects the historical development of Medicine. In the particular case of Blood Transfusion, stern opposition to progress has been forthcoming not only from Church and State, but at times from eminent members of the medical profession. The chief hindrance to the development of Blood Transfusion has been the inherent difficulties and dangers of the operation. In a study on "The Hazards of Blood Transfusion" it is important to review the History of Blood Transfusion, as this reveals the dangers of the operation and the attempts to overcome these difficulties.

It is difficult to establish the antiquity of the concept of blood as a therapeutic agent. The Egyptians, the Hebrews and the Syrians are supposed to have practised "transfusions", and Greek and Roman writers claim to have witnessed it. The references (Kilduffe and De Bakey 1942) are however obscure and contradictory. This confusion probably arises from the fact that during the Middle Ages the drinking of human blood was popularly considered a health restorative measure, and in the older literature the ingestion and transfusion of blood were frequently confused. There is considerable controversy regarding the first performance of the
Priority is frequently given to the "transfusion" of Pope Innocent VIII, in the year 1492. According to Villari (1863) the Pope "had for some time fallen into a kind of somnolency, which was sometimes so profound that the whole court believed him to be dead. All means to awaken the exhausted vitality had been resorted to in vain, when a Jewish doctor proposed to do so by the transfusion, by a new instrument, of the blood of a young person; an experiment which hitherto had only been made on animals. Accordingly, the blood of the decrepit old Pontiff was passed into the veins of a youth, whose blood was transferred into the veins of the old man. The experiment was tried three times, and at the cost of the lives of three boys, probably from air getting into their veins, but without any effect to save that of the Pope". There are several different versions of this story (Brown 1917, Mathews 1912, and Ore 1876), and in the account given by Mathews (1912) it is stated that the blood was administered as a draught. All versions agree that the three boys as well as the Pope died, and the Jewish physician quickly disappeared.

It is difficult to conceive how blood transfusion, as we understand the procedure to-day, could have been practised at a time when the circulation of blood was not recognised. An outstanding historical landmark in the evolution of the modern practice of blood transfusion was the announcement of the theory of the circulation of the blood by Harvey (1628), a theory first propounded in his lectures in 1616. All references to the operation of blood "transfusion" prior to Harvey's epochal exposition must be regarded with considerable doubt.
operation of blood transfusion in the modern sense.

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Harvey's immortal contribution stimulated investigators throughout the world to observe the effect of infusing various substances into the bloodstream. However, it is not until after the middle of the seventeenth century that authentic references to blood transfusion are to be found. Sir Christopher Wren (British Medical Journal 1923), the astronomer and architect, while at Oxford in the year 1656, experimented with dogs by injecting various medicaments such as ale, wine, scammony, and opium into the veins. Stimulated by these investigations to develop a new method of administering blood, Richard Lower, a Cornish physician and anatomist, began his experiments of direct transfusion of blood from one animal to another. To Lower (1665) must go the credit for having performed the first successful transfusion of blood.

Lower recorded his experiences, and writing (Lower 1669) of his first attempts at transfusion he "connected the jugular vein of one dog with that of another by means of a cannula. However, the comparatively slow-flowing venous blood immediately coagulated and obstructed the lumen of the tube. Then I decided to try another plan, one that would more closely follow nature, and convey the blood of one animal into the vein of another". Early in the year 1665 several unsuccessful attempts to transfuse blood were made at a meeting of the Royal Society. However, in February 1665, Richard Lower, together with R. King, performed the operation of transfusion successfully (Lower and King 1665, 1667). Quills were employed, and later a silver tube to convey blood from the carotid artery of one dog to the jugular vein of another dog. The cure of a mamy dog in ten days after transfusion from a healthy one was reported by Lower.
These experiments of Richard Lower are mentioned by Pepys (1666) in his diary. On November 14, 1666 Pepys writes: "Here Dr. Croone told me that at the meeting at Gresham College to-night, which, it seems they now have every Wednesday again, there was a pretty experiment of the blood of one dog let cut, 'til he died, into the body of another on one side, while all his own run out on the other side. The first died upon the place, and the other very well and likely to do well. This did give occasion to many pretty wishes, as of the blood of a Quaker to be let into an Archbishop and such like; but, as Dr. Croone says, may if it takes, be of mighty use to man's health, for the amending of bad blood by borrowing from a better body". Again the diarist makes reference to the transfusion by Lower of Mr. Arthur Coxe, "a mildly melancholy insane man" using the blood of a lamb: "November 30, 1667. But here above all, I was pleased to see the person who had his blood taken out. He speaks well, and did this day give the Society a relation thereof in Latin, saying that he finds himself much better since, and as a new man, but he is cracked a little in his head, though he speaks very reasonably, and very well: the first sound man that ever had it tried on him in England".

At the same time as Lower was making his observations, similar experiments were being performed in Paris by Jean Baptiste Denis (1667, 1668), physician to Louis XIV. While credit for having performed the first successful transfusion of blood from one animal to another must be awarded to Richard Lower, the first authentically recorded successful transfusion on human beings was performed by Denis in June, 1667. The patient was a boy of 15 or 16 years of age who was suffering
from a fever of uncertain origin. Treatment had consisted of repeated venesections with the result that the youth was in a state of extreme exhaustion. The boy made a remarkable recovery following the administration of nine ounces of blood from the carotid artery of a lamb. Following a second successful transfusion, Denis' renown as a transfusor brought on a storm of opposition, hatred, and jealousy. A subsequent transfusion ended fatally, apparently because of an incompatible reaction. Denis was arrested and tried for murder, but after a prolonged legal battle was exonerated. However, the Faculté de Médecin, in Paris, and the French Parliament decreed as criminal the procedure of transfusion, and prohibited the operation in France. The procedure was also abolished by the Royal Society, and prohibited by the magistrates of Rome. Accordingly, interest in transfusions rapidly abated throughout Europe.

In these early transfusions the complete ignorance of asepsis, of immunology, of the process of coagulation, of blood groups, and of incompatibility inevitably caused severe and fatal reactions. But in addition to the inherent hazards, ecclesiastical disapproval and state interference resulted in blood transfusion being relegated to almost complete desuetude, for nearly a century and a half following the experiments of Lower and Denis.

It was James Blundell (1818), a London physiologist and obstetrician, who "rediscovered" blood transfusion. He was stimulated by the appalling helplessness of the medical profession when faced with severe and often fatal puerperal haemorrhage. Blundell attempted transfusion in the human being, and after
initial failure he was successful later in saving the lives of three women in whom death from postpartum haemorrhage seemed inevitable.

Several surgeons and obstetricians attempted to follow Blundell's example but three obstacles, namely, agglutination and haemolysis following the transfusion of incompatible blood, infection, and blood coagulation hindered the application of a therapeutic procedure which occasionally yielded such brilliant results.

The publications of Blundell (1818, 1827, 1828, 1829) stimulated interest in blood transfusion in America where hitherto little interest had been shown in this subject. The early interest and discussion instigated by Blundell's publications led to a clear understanding of the indications for and rationale of blood transfusion (Jones and Macknull 1928).

Despite this understanding, the use of blood transfusion in America increased slowly. The technical difficulty in the performance of transfusion was one of the most important hindrances to its general use. Thomas (1878) stated that "transfusion of blood into the human system holds the position of an operation the plausibility and theoretical advantage of which all admit, but the absolute utility and practical results of which amount to very little indeed..... The reason for the unfortunate fact that the transfusion of blood is rarely resorted to, and that it to only a very limited degree enjoys the confidence of the profession, is to be found, I think, in the inherent difficulties and dangers of the operation, almost all of which arise from the tendency to coagulation". It was suggested by Thomas (1878) that some other "vital animal fluid"
not possessing the disadvantages of coagulation should be used, and he advocated the use of cow's milk. Fortunately, the procedure did not gain favour and promptly disappeared from the literature.

About this time there was a revival of interest in the use of animal blood. Several reports of cases in which lamb's blood was employed appeared in British and American literature (Barnes 1874, Gradle 1874, Hotz 1875, Sittel 1874, Winants 1872). The consequent severe and even fatal reactions which occurred prevented its popular use, and a number of investigators emphasised its dangers. Hotz (1875) employed lamb's blood in nine cases and stated that it was a failure in six and caused death in one patient. Cohnheim (1882) also discussed the dangers of heterogenous transfusion, and emphasised the fact that "serum of one species is a direct poison for the corpuscles of another".

Towards the beginning of the last quarter of the nineteenth century there was an extraordinary departure from previously established fundamental principles regarding the indications for blood transfusion (Kilduffe and De Bakey 1942). The indications began to comprise various vague infections and debilitating diseases including phthisis, uraemia, syphilis and insanity. As a result of its indiscriminate use, and of the lack of knowledge of blood compatibilities, the number of severe reactions and fatalities increased. This again resulted in blood transfusion being considered a hazardous and disreputable procedure, to be used only as a last resort. It was about the same time that physiological saline solution was introduced, and its safety and efficacy were demonstrated. The result was that blood transfusion was virtually abandoned during the last quarter.
of the nineteenth century. Then occurred what was probably the
greatest achievement and the most important single discovery in
the history of blood transfusion, namely, the recognition of
human blood groups by Landsteiner (1900, 1901). It was in the
year 1900 that Landsteiner demonstrated the presence of
isoagglutinating and isoagglutinable substances in human blood.
In the following year Landsteiner divided human blood into three
main groups by virtue of their agglutinating reactions.
De Castello and Sturli (1902) added the fourth main blood group.

This knowledge of the human blood groups was first
applied to human transfusions by Ottenberg (1911), and during the
first decade of the twentieth century interest in blood
transfusion was greatly stimulated. One of the most important
causes of fatalities had been discovered, and the methods of
prevention had been demonstrated. This revival of interest
resulted in many attempts being made to facilitate the transfer
of blood. But although the cause of haemolytic reactions had
been elicited, there remained the difficulty of coagulation.

Attempts to overcome coagulation had been made
prior to the twentieth century, and Bischoff (1835) overcame
this hindrance by utilising defibrinated blood. While some
workers (Boisnot 1874, Hoehling 1869, Morton 1874) staunchly
supported the use of defibrinated blood, others (Gesselius 1868,
Kuester 1874) objected to its use. The procedure soon lost
its popularity chiefly on account of bacterial contamination
which was almost inevitable in the whipping of blood obtained
from the donor. Prevention of coagulation is achieved by
permitting the blood to come in contact only with intima, and
this fact was the basis of the techniques of Carrel (1907),
of Crile (1907), and of Bernheim (1909) in artery-to-vein anastomosis. The techniques were so difficult however, that relatively few transfusions were performed in comparison with the need.

Then in 1914 sodium citrate was introduced as an anticoagulant by Hustin (1914) in Belgium, Agote (1915) in Argentina, and Lewisohn (1915) and Weil (1915) in the United States. This advance was perhaps next in importance to the discovery of the four blood groups, and it permitted a much needed simplification of the procedure of transfusion. The "delayed transfusion" was now a possibility in that the collection of blood from the donor could be separated from its injection by minutes or even hours. The procedure immediately became popular and was employed extensively in the treatment of the wounded in World War I.

Two years after the introduction of sodium citrate as an anticoagulant, Rous and Turner (1916) of the Rockefeller Institute published their pioneer studies on the preservation of blood for transfusion. Almost immediately the results were applied by Oswald H. Robertson (1918) who conceived and operated the first Blood Bank. He collected stores of blood in casualty clearing stations of the British Army, and transfused wounded soldiers.

The practice of Blood Transfusion developed rapidly in the early years of the twentieth century, but despite the great advance, and an increased understanding of the dangers of transfusion, an experienced transfusionist, Pemberton (1920), expressed his serious attitude towards this valuable therapeutic measure - "the procedure is very often
considered only a simple intravenous medication or a minor operation while in reality its potential dangers place it with major operations". Since the time that Pemberton expressed these views two important discoveries properly applied have increased the safety with which blood may be administered.

The first of these discoveries was announced by Florence Seibert (1923) who reported the finding of heat-stable products from bacteria growing in distilled water. These substances named Pyrogens caused febrile reactions in the recipient when injected intravenously. By taking certain precautions against the contamination of fluids with pyrogens, injections of solutions or of blood can be given to human beings with a minimal incidence of chills and fever.

The second important recent advance was the discovery of the Rhesus blood group antigens by Landstainer and Wiener (1940). In addition to explaining the cause of haemolytic disease of the newborn this discovery clarified many hitherto unexplained transfusion reactions, and increased the safety of blood transfusion.

This discovery of the Rhesus blood group coincided with the greatest stimulus to the development of blood transfusion as a therapeutic weapon that the world has ever known, namely, World War II. The principle of the blood bank had been temporarily forgotten after World War I. The idea was revived in Russia by Yudin (1933) who used cadaver blood. Then in 1936 came the Spanish Civil War in which the Loyalist forces developed the transportation of preserved blood (Jorba 1939). During World War II Transfusion Services became an integral part of the medical organisations of the
forces of Russia, the British Empire, France and the United States, while in Great Britain a National Blood Transfusion Service was organised for the treatment of civilians injured in air raids.

The experience gained in war has had a profound influence on blood transfusion in civilian practice. Many physicians and surgeons who learned the value of blood transfusion in war have applied their knowledge to civilian medicine. In Great Britain the need was so great that the war-time organisation of The National Blood Transfusion Service became a permanency. Blood Transfusion is now commonly employed in the treatment of a wide variety of medical and surgical conditions.

The obvious popularity of blood transfusion as a therapeutic weapon, and the undoubted success which has attended its use might obscure the attendant complications which still exist. The relative infrequency of serious consequences may lead the physician to consider that blood transfusion is an innocuous procedure. However, Hardin (1949), an eminent authority on Blood Transfusion has recently stated that transfusion is attended by a very real morbidity and a definite mortality. The probable benefits must always be weighed against the infrequent but possible untoward results. Kilduffe and De Bakey (1942) in an analysis of transfusion reactions have shown that blood transfusion in the past has proved fatal in 0.14 per cent of cases. Even if no other fact is considered, this is sufficient warning. The death rate following the surgical treatment of uncomplicated, acute appendicitis is not appreciably higher. Yet considerably more time and thought are commonly devoted to
advising operation in appendicitis than are employed in considering transfusion.

The present study is an attempt to assess the nature, the incidence and the gravity of transfusion reactions which occur in modern blood transfusion practice. The experimental and clinical observations are the result of experience of Blood Transfusion work during the past decade, first in a highly organised teaching hospital, then in the British Army Blood Transfusion Service, and more recently in a modern Regional Transfusion Centre.

It is important at the outset to differentiate the various types of transfusion reactions. When a patient presents clinical manifestations of disease during or after transfusion, the physician must attempt to answer two related questions: (i) Is the manifestation due to the primary disease or is it caused by blood transfusion? (ii) If the abnormality is caused by transfusion what type of reaction is it?

Many attempts have been made by various authors to classify transfusion reactions (Kilduff and De Bakey 1942). The following classification has been found by the author to be extremely useful in practice, and is followed throughout this thesis as a basis for study.

**CLASSIFICATION OF TRANSFUSION REACTIONS**

1. Pyrexial Reactions
2. Transmission of Disease
   (a) From Donor to Recipient
   (b) Extraneous Contamination
3. Mechanical Reactions
   (a) Circulatory Overloading
   (b) Air Embolism
   (c) Pulmonary Embolism
4. Haemolytic Reactions
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CHAPTER II

FEBRILE REACTIONS.

Introduction

This type of reaction is characterised by chills and fever which appear during or a few minutes to a few hours after transfusion, and persist for not more than a few hours. According to Riddell (1939) this is the commonest complication of blood transfusion, although in its simplest form this reaction produces no discomfort in the patient, but a simple rise in temperature to 100°F. (37.2°C.). In its most severe form there is a rigor followed by a rise of temperature to the region of 104°F. (40°C.).

The frequency of febrile reactions varies considerably from author to author. Certain clinicians have declared that they "have never seen a reaction", (Riddell 1939). The explanation of such an opinion is probably the fact that the patient is not visited or closely observed after the transfusion. Again some observers have not regarded manifestations less severe than a genuine rigor as a reaction. On the other hand, Rondouet and Smithies (1925) state that practically all transfusions if carefully observed are followed by some rise of temperature, though this is likely to be symptomless.

Indeed one of the outstanding impressions from a review of the literature on febrile transfusion reactions is the extreme variability in the incidence of such reactions. Beck (1934) presented a statistical analysis of the incidence of reactions following transfusion, and found that the reported average incidence was from 10 to 30 per cent. At the
Mayo Clinic during the three year period 1937 to 1939 inclusive, 9823 transfusions were performed, and in this series "untoward reactions" of various grades occurred in 921, an incidence of 9.4 per cent (Lundy et al. 1940). In a series of 3077 transfusions of banked blood reported by Hoxworth and Skinner (1941) there was an incidence of approximately 6 per cent. Kilduffe and De Bakey (1942) stated that in their experience febrile reactions have occurred in about 1 per cent of transfusions. De Gowin and Hardin (1940) reported that in a series of 2423 transfusions of fresh and preserved blood the incidence of pyrogenic reactions was 2.9 per cent, and from the same service using the same methods came a report by De Gowin (1945) of a series of 5,386 transfusions in which the incidence of chills and fever was 1.8 per cent.

The incidence of reactions will depend upon the observer's definition of a pyrexial reaction. Whereas some consider a febrile reaction to be any rise in temperature following a transfusion, others require in their definition the presence of an actual chill or rigor. For statistical purposes it is essential to define exactly what is meant by the term Pyrexial Reaction. For this purpose the classification suggested by Riddell (1939) is very convenient, and it is this classification which is adopted in the present studies.

**Pyrexial Reactions**

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<th>Grade</th>
<th>Description</th>
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<tr>
<td>Grade I</td>
<td>Associated with a rise of temperature to 100°F. (37.8°C.), but no other objective features.</td>
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<tr>
<td>Grade II</td>
<td>Associated with a similar or greater rise of temperature, and subjectively with feeling cold and shivery, but without having an actual rigor.</td>
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Grade III: Associated with a definite rigor, the so-called post-transfusion chill of some writers.

Another important factor which will influence reports on the incidence of transfusion reactions is the care with which the observations are made. As Riddell (1939) remarks it is very easy to deceive oneself about the frequency of reactions. The observation that "a reaction has never been seen" may be the result of not visiting the patient soon after the transfusion. Riddell (1939) has emphasised the danger of relying upon impressions. In his own experience he had formed the impression that his pyrexial reaction rate had fallen to under 5 per cent, but on analysis found that 11 out of his 100 cases transfused had rigors.

The cause of febrile reactions was improperly understood until Seibert (1923) demonstrated that certain non-pathogenic bacteria, commonly occurring in river water, can multiply in distilled water and produce substances which cause fever when injected parenterally into animals. These substances were named pyrogens. They are carbohydrates which are not destroyed by the heat employed to autoclave rubber tubing and other apparatus for parenteral injection. The term pyrogen is restricted to bacterial products. Occasionally other substances cause febrile reactions when introduced into the circulation. The finely divided sulphur formerly used in curing rubber tubing is such an agent, but tubing made expressly for parenteral injection apparatus no longer contains this material. The dried denatured plasma proteins in improperly cleaned tubing and apparatus produce violent chills and fever when injected intravenously.
The appreciation of the importance of avoiding the injection of pyrogenic material has resulted in a reduction in the incidence of pyrexial reactions. Lewisohn and Rosenthal (1933) stated that it was possible to decrease the incidence of these febrile reactions following transfusion from 12 per cent to 1 per cent by instituting a trained personnel for the correct preparation of transfusion paraphernalia.

The present studies on pyrexial Reactions were undertaken from three different angles in an attempt to obtain co-ordinated and comprehensive information on the incidence and practical causes of these reactions in modern transfusion practice. The following studies were undertaken.

**STUDY A** Controlled observations on 380 transfusions and infusions given to healthy volunteers, with particular reference to the effect of

(i) Duration of Blood Storage
(ii) Temperature of the Infusion Fluid
(iii) Rate of Administration

on pyrexial reactions, and the elucidation of the cause of pyrexial reactions.

**STUDY B** Observations on 2,483 transfusions of blood, and 1,328 infusions of plasma in hospital practice with particular reference to

(i) The incidence of reactions using the various transfusion fluids.
(ii) The severity of these reactions.

**STUDY C** Investigations into the cause of pyrexial transfusion reactions in hospital practice, and the result of eliminating the causal factor.

The methods of investigation, and the results of each of these Studies will be given in turn, before discussing the significance of the observations as a whole.
During the training of several hundred transfusion orderlies of the R.A.M.C., it was possible for the author to make some simple, but controlled observations on Transfusion Reactions (Wallace and Richards 1943). Many of these orderlies volunteered to have blood withdrawn, and subsequently to have their own blood reinfused. The volunteers were each venedected to the extent of 440 ml. of blood collected into 100 ml. 2 per cent Disodium Hydrogen Citrate, and after an interval varying from one hour to seven days, their own citrated blood was reinfused. By using the volunteer's own blood there was no danger of blood group incompatibility complicating the observations, and the choice of healthy subjects eliminated the side effect of disease on the incidence of reactions. In this way 324 collections and reinfusions of blood were performed, and in addition 56 infusions of isotonic glucose-saline, each of 540 ml., were given. The febrile reactions observed are described.

Febrile Reactions: All apparatus and fluids were prepared under the author's personal supervision. Oral temperatures were recorded by leaving a thermometer in situ for at least three minutes, and records were taken before and at hourly intervals for four hours after infusions.

Out of a total of 324 infusions of blood, a rise of temperature was observed on nine occasions, an incidence of 2.8 per cent. Using the classification of Riddell (1939), described in the introduction to these studies, seven of the reactions were Grade II (2.2 per cent), and two were Grade III (0.6 per cent). It was possible to state the probable cause of these nine reactions in all but one case.
Investigation of the first reaction showed that a new orderly had inadvertently taken an administration set from the non-sterile section awaiting sterilisation. The reaction was Grade II, and that it was not more severe was probably explained by the fact that the set had been recently assembled from carefully washed components, and by the fact that the blood was fresh and therefore still retained some bacteriostatic properties.

The second febrile reaction occurred in a volunteer reinfused with his own blood which had been collected into a sample of sodium citrate free from moulds, but containing numerous small particles. These particles were probably derived from the asbestos filter used in the preparation of the fluid. It was the general practice to discard flasks in which the anticoagulant contained this particulate matter, but on this occasion the volunteer agreed to the use of this particular flask. A Grade III reaction ensued, but an interesting feature of this case was that the onset of the rigor was delayed until some two hours after the end of the transfusion.

For the third observed febrile reaction no explanation could be found, but the remaining six reactions had an interesting and significant explanation. It was customary to use administration sets which had been recently sterilised, but on one occasion it was decided to use sets which had been sterilised eighteen months previously. Although the "cellophane" wrapping had lost its "crispness", these sets were still sealed. Out of eight infusions six subjects developed a febrile reaction, five of these being
Grade II, and the sixth a Grade III reaction.

No febrile reactions have followed the 56 infusions of isotonic glucose-saline, but it is of interest to note one false reaction. In this particular case it was found that the subject had a temperature of 102°F. (38.9°C.) four hours after the infusion. He was, however, found to be suffering from acute follicular tonsillitis.

An attempt was made in the course of this study to observe the effect of certain variations in transfusion technique on the incidence of reactions. The variable factors introduced were:-

(i) Duration of Blood Storage
(ii) Temperature of the Infusion Fluid
(iii) Rate of Administration

The following observations were made:-

Duration of Blood Storage - In most cases the reinfusion was started within one hour of collection, but in 11 cases the blood was stored for two days and in 20 cases for seven days before reinfusion. The blood was cleanly and properly collected, and immediately, accurately, and continuously refrigerated between 2 and 6°C. In none of these 31 cases did a febrile reaction follow the infusion.

Temperature of the Infusion Fluid - In 36 infusions of glucose-saline the fluid was given at room temperature, while in the other 20 the fluid was warmed in a bucket of water at approximately 4°C. As previously noted, febrile reactions did not occur in any of these saline infusions. The infusions of blood were performed at room temperature, the refrigerated blood being warmed to that
temperature. In three cases blood was taken straight from the refrigerator and immediately reinfused (as was the practice in several field transfusion units on active service) without any reaction apart from venous spasm in one case.

Rate of Administration - The saline infusions were given at a standard rate of approximately 40 ml. a minute and no reactions were observed. With blood the usual rate of administration was 20 ml. a minute, but in 24 cases a rapid infusion was given at rates varying from 80 to 110 ml. a minute. The following observations were made during these 24 rapid infusions:

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<thead>
<tr>
<th>Nature of Reaction</th>
<th>No. of Cases in which Observed</th>
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<tr>
<td>Rise in temperature</td>
<td>0</td>
</tr>
<tr>
<td>Flushing</td>
<td>9</td>
</tr>
<tr>
<td>Venous filling</td>
<td>9</td>
</tr>
<tr>
<td>Fullness in head</td>
<td>7</td>
</tr>
<tr>
<td>Shivering and feeling cold</td>
<td>3</td>
</tr>
<tr>
<td>Feeling cold without shivering</td>
<td>3</td>
</tr>
<tr>
<td>Tingling of lips</td>
<td>14</td>
</tr>
</tbody>
</table>

These reactions were transient, and in each case passed off a few minutes after ending the infusion.

STUDY B

Most of the large hospitals in Great Britain maintain a Blood Bank which is stocked by the Regional Transfusion Centre. This blood is withdrawn from the donor by the Mobile Units of the Transfusion Service using apparatus prepared at the Regional Transfusion Centre. In most cases the blood is administered with sets prepared by the Regional Transfusion Centre.

In addition to the issue of Whole Blood to hospitals the Regional Transfusion Centre may prepare and issue Concentrated Human Red Blood Corpuscles, Liquid Human Plasma and Dried Human Plasma (British Pharmaceutical Codex, 1949).
An attempt has been made in the present study to ascertain the incidence of pyrexial reactions following the administration of these various transfusion fluids in hospital practice, and to assess the severity of such reactions.


The fluid used for the preservation of blood was a modification of the Disodium-Citrate-Dextrose Solution recommended by Loutit and Mollison (1943), and contained -

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disodium Hydrogen Citrate</td>
<td>2 gm.</td>
</tr>
<tr>
<td>Anhydrous Dextrose</td>
<td>3 gm.</td>
</tr>
<tr>
<td>Water</td>
<td>100 ml.</td>
</tr>
</tbody>
</table>

This solution was autoclaved at 121°C. (250°F.) for 30 minutes. To 100 ml. of this fluid is added 440 ml. of blood.

The blood was stored between 2°C. and 6°C. (35.6°F. and 42.8°F.) for periods varying from 24 hours to 20 days before administration.

The following observations were made -

(a) A series of 1541 recipients of either whole blood or concentrated cells was observed particularly from the point of view of pyrexial reactions developing. This observation was made during the winter of 1947-48.

The patients studied were chosen at random from different hospitals in the Western Region of Scotland, and there was no selection of patients according to the clinical indications for transfusion. The classification of Riddell (1939) was used throughout.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Actual Number of Reactions</th>
<th>Percentage Incidence of Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td>II</td>
<td>18</td>
<td>1.2</td>
</tr>
<tr>
<td>III</td>
<td>7</td>
<td>0.5</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>1.8</td>
</tr>
</tbody>
</table>
(b) Similar observations were made on 242 recipients
of whole blood or concentrated cells during the very warm
summer of 1949, with the following results.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Actual Number of Reactions</th>
<th>Percentage Incidence of Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>0.6</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>0.6</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>1.1</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>2.3</td>
</tr>
</tbody>
</table>

There is no significant difference between the
total incidence of pyrexial reactions in the two series, when
the figures are examined statistically.

\[ n = 1, \quad \chi^2 = 0.73, \quad P \text{ is larger than 0.30} \]
i.e. more than 30 times in 100 tests would we reach so
large a difference by chance.

Combining the figures observed in the two
series (a) and (b) gives a total incidence of Pyrexial
Reactions of 49 among 2,483 patients studied, i.e. approximately
2.0 per cent.

(c) The series of 942 patients observed in (b) above
was subdivided into reactions among the recipients of whole
blood and the recipients of concentrated red blood corpuscles.

<table>
<thead>
<tr>
<th></th>
<th>Number of Patients</th>
<th>Number of Pyrexial Reactions</th>
<th>Percentage Incidence of Pyrexial Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Blood</td>
<td>302</td>
<td>19</td>
<td>2.4</td>
</tr>
<tr>
<td>Concentrated Corpuscles</td>
<td>140</td>
<td>3</td>
<td>2.1</td>
</tr>
</tbody>
</table>

There is no significant difference in the incidence
of Pyrexial Reactions among the recipients of whole blood and
of concentrated corpuscles.

\[ n = 1, \quad \chi^2 = 0.05, \quad P \text{ is larger than 0.80}. \]
Liquid Human Plasma and Dried Human Plasma.

The plasma used in this study was separated aseptically from preserved blood which had been collected from the donor more than 21 days previously, or from fresh blood used for the preparation of concentrated corpuscles. The method of processing was that described by Haizels (1944) using Kaolin, and plasma drying was performed in a unit similar to the Medical Research Council Unit (Greaves, 1946).

A series of 1,328 recipients of human plasma (including 101 recipients of Dried Plasma and 1,227 of Liquid Plasma) was observed closely for the onset of Pyrexial Reactions. These patients were chosen at random in a similar manner to that indicated for recipients of blood (paragraph (a) vide supra).

The result of these observations was:

<table>
<thead>
<tr>
<th></th>
<th>Number of Patients</th>
<th>Number of Pyrexial Reactions</th>
<th>Percentage Incidence of Pyrexial Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid Plasma</td>
<td>1227</td>
<td>13</td>
<td>1.1</td>
</tr>
<tr>
<td>Dried Plasma</td>
<td>101</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>Total</td>
<td>1328</td>
<td>14</td>
<td>1.05</td>
</tr>
</tbody>
</table>

Of the 14 reactions, 10 were Grade III; the remaining 4 were Grade II.

There is no significant difference in the incidence of Pyrexial Reactions among the recipients of Liquid Plasma and of Dried Plasma.

The incidence of Pyrexial Reactions among the recipients of Blood was compared statistically with similar reactions among recipients of Plasma. Thus -
Number of Individuals
Without Reactions With Reactions

| Transfused | Blood | 2434 | 49 |
| Plasma     | 1314  | 14  |

\[ n = 1, \quad \chi^2 = 3.92. \]

\( P \) is slightly less than 0.05, i.e. the incidence of Pyrexial Reactions among the recipients of Plasma in the series observed was just significantly lower than the incidence of Pyrexial Reactions among the recipients of Blood.

**The Severity of Pyrexial Reactions.**

In the present study there were 27 Grade III reactions among the 63 Pyrexial Reactions observed with the various transfusion fluids. However, in no case did the pyrexial reaction appear to have any serious consequences.

In those cases where the patient died within 48 hours of the transfusion there was abundant evidence of gross pathology, and it can be truly said that the patient died in spite of transfusion.

Although no serious consequences followed the pyrexial reactions in the present study, the author has been consulted on several occasions regarding the sudden death of a patient following a febrile transfusion reaction. In six of the cases investigated in this way by the author, it can be stated that in all probability a Pyrexial Transfusion Reaction was directly responsible for the death of the patient.

There are certain features of these six cases which are of interest. The indications for transfusion or infusion were as follows -

1. Severe dehydration following Hyperthermic treatment (Wallace and Bushby, 1944). The occurrence of pyrexial
reactions following glucose-saline infusions in such cases are discussed in Study O (vide infra). There were two fatalities among these cases.

(ii) Moderate anaemia in two cases of gastric carcinoma, and in one case of cirrhosis of liver.

(iii) Hypoproteinaemia in one case of Pyloric Stenosis.

All six patients were seriously ill at the time of the transfusion, but in no case was it thought that death was imminent. In one case the rigor developed after the administration of approximately 100 ml., but in the other five cases between 200 and 300 ml. had been given before the onset of the reaction. In each case there was a violent rigor and sharp rise in temperature. There was marked prostration and hypotension, but no evidence of urticaria or other allergic manifestations. Death occurred within six hours of the onset of the reaction.

There was no bacterial contamination of the fluid (vide Chapter 3) administered to any of these cases, nor was there any evidence of a haemolytic transfusion reaction in the cases receiving blood (vide Chapter 5).

Post-mortem examination excluded circulatory overloading (vide Chapter 4) as the cause of death. The salient features found at autopsy in these cases were as follows -

(a) The two patients who had undergone Hypertherm treatment showed acute hepatic necrosis. The danger of liver failure in patients subjected to this form of therapy was stressed by the author in the "Hazards of Hypertherm Treatment" (Wallace and Bushby 1944).
(b) The two patients with gastric carcinoma had metastases in the liver.

(c) The patient suspected of having cirrhosis of liver did in fact have this disease, and the suspected pyloric stenosis was in fact a case of cirrhosis of liver.

It should be noted therefore that all six patients had a pathological condition affecting the liver.

STUDY C

From time to time in the transfusion practice of a hospital there may occur a high incidence of pyrexial reactions, the cause of which should be actively sought and removed. This study records the author's observations on the investigation of three incidents of this nature in different hospitals.

Observation I

The treatment of gonococcal infections which had become resistant to chemotherapy with sulphonamide drugs constituted a serious problem in the armed forces during the early years of World War II. King (1942) found that Hypertherm treatment combined with chemotherapy gave excellent results in these resistant cases. The author had the opportunity of observing patients undergoing this treatment, and reported on the "Hazards of Hypertherm Treatment" (Wallace and Bushby 1944).

When Hypertherm treatment was first introduced many patients suffered from dehydration as a result of excessive loss of water and salt. In these cases it was customary to give an infusion of glucose-saline for up to 48 hours following the hypertherm treatment. By the time these post-hypertherm
infusions were administered, the patient's temperature had returned to normal, so that it was possible to recognise pyrexial reactions.

Of 130 patients observed, a pyrexial reaction followed the post-hypertherm infusion in 71 cases, i.e. an incidence of 54.6 per cent. The 71 reactions were classified as 35 of Grade II, and 36 of Grade III (Riddell 1939). This high incidence of pyrexial reactions demanded investigation, particularly as two fatalities occurred (vide supra Study B).

On making investigations it was found that the distilled water used to prepare the intravenous solutions was not fresh. Water was distilled in the hospital dispensary, where it remained for 3 or 4 days before the infusion fluids were prepared and sterilised. Following this discovery the fluids were prepared under the author's supervision, using only freshly distilled water to prepare the solutions.

Thereafter, in a series of 61 patients receiving post-hypertherm infusions there were no cases in which pyrexial reactions developed.

The difference in the incidence of pyrexial reactions in these two series is highly significant - \( n = 1, \chi^2 = 49.9 \) and \( P \) is very much less than 0.01.

**Observation II**

From the records of a busy general hospital it was noted that there had been 7 pyrexial reactions among 50 patients given a transfusion of blood. This incidence of 14 per cent was undesirably high, and the routine transfusion practice of the hospital was carefully scrutinised.

The probable source of the trouble seemed to
lie in the cleaning of the administration sets used for the blood transfusions. This cleaning was performed by the overworked staff of the operating theatre, and consisted of the dismantling of the used set, soaking the parts in cold water for half-an-hour, and then reassembling prior to sterilisation.

This routine practice was then altered in that all transfusions were administered with sets prepared under the author's direction, paying strict attention to the following points. New rubber tubing was boiled for one hour in 1 per cent sodium hydroxide, then rinsed with hot tap water under pressure, pulled through with a small brush, and boiled for 15 minutes in tap water. The tubing was then drained and used immediately for the assembly of sets which were sterilised without delay. Used rubber tubing was soaked overnight in cold water, and then treated in the same way as new rubber, except that the boiling in alkali was omitted.

Following this change in routine the first 100 patients transfused with blood were observed for the development of febrile reactions. There was one Grade II reaction among these patients.

There is, therefore, a highly significant reduction in the incidence of pyrexial reactions following the adoption of this careful cleaning technique for administration sets as shown by statistical analysis. \( n = 1, \chi^2 = 8.63 \). \( P \) is less than 0.01.

Observation III

The records of a general and maternity hospital showed that 10 out of 26 patients transfused with blood had developed a pyrexial reaction.
On more detailed investigation it was found that all these reactions occurred in patients in the medical or surgical wards, and not in the obstetric unit. The distribution of cases and reactions was as follows -

<table>
<thead>
<tr>
<th>Patients Transfused</th>
<th>Pyrexial Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical and Surgical Wards</td>
<td>14</td>
</tr>
<tr>
<td>Maternity Wards</td>
<td>12</td>
</tr>
</tbody>
</table>

There was a most important difference in the routine practice of the two sections of the hospital. The administration sets used by the medical and surgical wards were prepared by the operating theatre staff, whereas the maternity section used sets prepared by the Regional Transfusion Centre in a manner similar to that described in Observation II.

Accordingly, all departments in the hospital proceeded to use sets prepared by the Regional Transfusion Centre with the following results -

<table>
<thead>
<tr>
<th>Patients Transfused</th>
<th>Pyrexial Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical and Surgical Wards</td>
<td>13</td>
</tr>
<tr>
<td>Maternity Wards</td>
<td>13</td>
</tr>
</tbody>
</table>

This is a significant reduction in pyrexial reactions in medical and surgical cases ($n = 1$, $X^2 = 7.04$, $P$ is less than 0.01). However, the occurrence of 3 pyrexial reactions among 18 recipients was undesirably high.

All departments of the hospital obtained blood from the Regional Transfusion Centre, but there were two refrigerators in the hospital used for the preservation of blood. One of these cabinets was used by the maternity unit, and although no thermometer was normally used in this cabinet, it was found on investigation that the cabinet temperature was the optimum for blood preservation, namely 20°C to 60°C (35.6°F to 42.8°F).
On the other hand it was found that the refrigerator used by the medical and surgical wards, which again carried no thermometer, had a cabinet temperature between 80° and 120° (46.4°F. and 53.6°F.).

The thermostatic control of this latter cabinet was adjusted to give the optimum temperature for blood preservation. Thereafter the incidence of pyrexial reactions was

<table>
<thead>
<tr>
<th>Patients Transfused</th>
<th>Pyrexial Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical and Surgical Wards</td>
<td>27</td>
</tr>
<tr>
<td>Maternity Wards</td>
<td>20</td>
</tr>
</tbody>
</table>

Although there is an actual reduction in the incidence of pyrexial reactions in the Medical and Surgical Wards following the adjustment of the cabinet temperature, this reduction is not significant (n = 1, \( \chi^2 = 2.44 \), \( \chi^2 P \) is greater than 0.10).

**Discussion**

The Incidence of Reactions

In Study A healthy individuals were given a reinfusion of their own blood. The effect of disease and of minor blood group incompatibility on the development of pyrexial reactions was thus eliminated. From this study it appeared that pyrexial reactions might almost be eliminated by strict attention to such matters as the preparation of preservative solutions, the care of withdrawal and administration equipment, and the conditions of blood storage. While there were no reactions among 56 recipients of glucose-saline, nine pyrexial reactions followed the reinfusion of blood to 324 recipients, i.e., an incidence of 2.8 per cent. However, even this low
figure is artificially high, because in the majority of these reactions the anticoagulant solution or the administration sets were considered unsatisfactory for normal use, but were in fact utilised to ascertain whether a pyrexial reaction ensued. Indeed eight of the nine reactions were the direct result of using faulty equipment; in the remaining case the reaction was unexplained.

This initial study was performed in a self-contained transfusion unit in which rigid control of all aspects of technique was possible. Even under these conditions it appeared that a minor departure from strict supervision might result in the occurrence of reactions among the recipients. That it is possible in the transfusion practice of hospitals supplied from a modern Regional Transfusion Centre to maintain close supervision of transfusion arrangements, and thereby reduce the incidence of pyrexial reactions to a very low level, is demonstrated by the observations in Study B. Here the incidence of febrile reactions from the transfusion of blood was approximately 2 per cent. Beck (1934) reported an incidence of pyrexial reactions from 10 to 30 per cent with blood. Lundy et al. (1940) stated 9.4 per cent, and Hoxworth and Skinner (1941) found the incidence to be 6 per cent. The author's observation is very similar to the incidence of 1.8 per cent with blood reported by De Gowin (1945). Indeed it appears that in recent years a better understanding of the causes of pyrexial reactions, and a stricter supervision of transfusion technique have resulted in a progressive reduction in the incidence of these reactions.

The incidence of pyrexial reactions with concentrated red blood corpuscles in the present study is not
significantly different from that of whole blood. This observation confirms the opinion of Macquaild and Mollison (1940) and of Williams and Davie (1941), although the latter workers had a considerably higher reaction rate than the author with whole blood and concentrated red corpuscles. These workers had a gross reaction rate of 19.5 per cent in 77 transfusions, compared with the author's observed rate of 2.1 per cent in 140 infusions of concentrated red corpuscles. Williams and Davie (1941) attributed their high reaction rate to the fact that enemy action had destroyed the apparatus necessary for the production of distilled water. While the pyrexial reaction rate with concentrated red corpuscles is no higher than the rate with whole blood, there is a greater danger of bacterial contamination with concentrated corpuscles than with whole blood (Chapter 3).

The author's present observation that the pyrexial reaction rate with plasma is of the order of 1 per cent confirms the opinion of Alsever (1949) that the reaction rate with plasma infusions can be brought as low as 1 per cent. Miller and Tisdall (1945) reported a febrile reaction rate of 2 per cent in a series of 10,000 liquid plasma transfusions conducted during World War II in hospitals of the United States Army. In that study the administration sets were prepared in the Army hospitals, and the plasma processed in an Army centre. It was felt that the frequent turnover of technical personnel in the hospitals and in the centre was largely responsible for the high reaction rate of 2 per cent. Weinstein (1942) reported a reaction rate of 1 per cent in 1,500 transfusions of plasma, and Lozner and Nowhouser (1944) recorded 1.1 per cent reactions in a series of 1,751 transfusions of liquid plasma.
The Causes of Pyrexial Reactions

The present studies clearly demonstrated some important practical causes of febrile transfusion reactions. In the preliminary study causal factors were impure anticoagulant solution, unsterile apparatus and imperfect administration sets.

Ever since the introduction of sodium citrate as an anticoagulant a controversy has existed with regard to the toxicity of the compound when injected in transfusion. Beck (1934) found in an analysis of 17,000 transfusions that the average incidence of reactions following citrate transfusions was approximately 30 per cent in contrast to an incidence of 11 per cent with unmodified blood. On the other hand Lewisohn (1915, 1923, 1924, 1937) contends that the number of reactions following the citrate method is no greater than that following the use of unmodified blood. Kilduffe and De Bakey (1942) review the evidence, and consider that the greatest number of febrile reactions previously attributed to the use of citrate are caused by lack of scrupulous cleansing of apparatus. De Gowin (1949) also states that most reactions ascribed to the citrate radical were observed before the discovery and elimination of pyrogens from fluids and apparatus. The same author claims that with new methods of cleaning and preparing equipment for transfusion, the prejudice against citrated blood has largely disappeared. The present studies confirm that sodium citrate is not a toxic compound when injected intravenously in the quantity required to prevent blood coagulation. But at the same time these studies demonstrate
that anticoagulant solutions may be a source of pyrogenic material. In Study A one febrile reaction resulted from the use of anticoagulant solution containing particulate matter visible to the naked eye. All anticoagulant solutions should be inspected before use to ensure that the liquid is clear. Again, in Study C, it is shown that pyrexial reactions may result from the use of stale distilled water in the preparation of intravenous fluids. It is therefore important to use freshly distilled water for the preparation of all fluids, including the anticoagulant. The chemicals used for intravenous solutions should be of a high grade purity to eliminate another source of pyrogens. This latter point is emphasised by De Gowin (1949) who states that disodium hydrogen citrate is not readily available in the United States in a form free from pyrogens. The author has not encountered this difficulty with acid citrate in this country. The present observations show that febrile reactions following the administration of blood collected into acid citrate are infrequent. The author has throughout used chemicals of a high grade of purity, and has made use of activated charcoal and cotton-asbestos pads to remove pyrogens from the solutions of chemicals.

In the preliminary study one febrile reaction was the result of using an unsterile administration set. This is a danger which must be avoided by adequate precautions. In a busy transfusion department where space is limited it is particularly important to prevent confusion between sterile and unsterile equipment. The particular incident mentioned in Study A was brought about in the first place by entrusting to an inexperienced member of staff a task which was simple.
yet responsible. This error would probably have been detected if the sterile administration sets had borne a visible mark to show that sterilisation had been performed. At that time the sets were wrapped in calico bags before being sterilised, and remained in these bags after sterilisation. Following this incident the sets were wrapped in self-sealing cellophane, and a set was never used unless the cellophane was completely sealed.

It was shown in the preliminary study that it is dangerous to use administration sets which may have been sterilised at a much earlier date. In the actual incident there was eighteen months between sterilisation and use. During that period the sets were sealed in a cellophane wrapping in a closed tin. The observation is important in that many hospital blood banks are stocked with administration sets in excess of their immediate requirements, with the result that there may be a long interval before the set is used.

In addition to demonstrating that stale distilled water is a source of pyrogens, the observations in Study C prove the need for scrupulous care in cleaning the component parts of administration sets. Lewisohn and Rosenthal (1933) found that it was possible to reduce the incidence of pyrexial transfusion reactions from 12 per cent to 1 per cent by the correct preparation of equipment.

De Gowin and Nardin (1940) found that there was no significant difference between the incidence of febrile reactions from fresh and from properly preserved blood, and stated that the observation tended to exclude the possibility that pyrogens were formed by bacterial growth after the blood was collected. On the other hand Cappell (1919) is of
the opinion that most febrile reactions from blood are the result of bacterial contamination, the growth of the contaminant being checked by refrigeration thereby preventing a more serious reaction. An attempt has been made in the present studies to obtain information on this aspect of febrile reactions. In Study C it was shown that correction of blood storage temperature from 10°C. \((50°F.)\) to the optimum 4°C. \((39.2°F.)\) caused a reduction in the incidence of pyrexial reactions from blood, but the decrease was not statistically significant. During warm weather there may be a greater chance of bacterial growth as blood may be exposed to the atmospheric temperature for a period after collection prior to refrigeration, and again in transit from the Regional Transfusion Centre to a hospital Blood Bank. In Study B one series of observations was made in winter, and another in summer. The actual incidence of pyrexial reactions from blood was higher in summer than in winter, but the difference was not statistically significant. In the same study it was observed that the incidence of febrile reactions from plasma was just significantly lower than the incidence from blood. This difference might be attributed to the removal of bacterial contaminants from the original blood by the filtration of plasma through sterilising pads. There are however other possible explanations of this difference. The asbestos sterilising pads not only remove bacteria from the liquid which is being filtered, but adsorb pyrogens (De Gowin 1949). Again the Kaolin which is used to remove the clotting elements in the processing of plasma also adsorbs pyrogens (Maizels 1944). The present studies
therefore, although suggestive, produce no significant evidence that pyrogens are formed by bacterial growth in preserved blood.

The Prognosis.

The danger of a febrile reaction in its severest form is that a rigor may bring about myocardial failure and a fatal pulmonary oedema (Riddell 1939). On the other hand,

Kilduff and De Bakey (1942) observe that febrile reactions are fortunately of a mild character. Whitby (1942) agrees with this benign nature of pyrexial reactions in general, but states that in a patient already in a precarious condition the development of a febrile reaction may prove fatal.

De Gowin (1949) observes that a pyrexial reaction is not usually dangerous except in patients who are extremely ill from other disease.

As a result of the observations reported herein the author is in agreement with the opinions expressed by Whitby (1942) and De Gowin (1949), but the author has not confirmed the opinion of Riddell (1939) that pulmonary oedema is the cause of death following a pyrexial reaction. There was no evidence of pulmonary oedema in the six fatalities described in these studies. It is interesting, and perhaps significant, that all six patients showed gross pathology of the liver. The liver has an important part to play in rendering toxic substances less dangerous (Hicks 1936), and it may well be in the liver that pyrogens are rendered harmless. Pyrexia produces an increase in metabolic rate, and this imposes a greater load on the liver (Wright 1940). Again Wallace and Bushby (1944) have emphasised that prolonged elevation of body temperature impairs liver function.
It is the author's opinion that in the six fatal cases described there was impairment of liver function before transfusion, and that the febrile transfusion reaction aggravated the liver failure and caused death.

While serious consequences from pyrexial reactions are rare, the fact that such reactions may occasionally be dangerous, emphasises the need of avoiding pyrexial reactions by scrupulous attention to the points discussed under the Causes of Pyrexial Reactions.

Rate of Administration.

Riddell (1939) states that the occurrence of febrile reactions can be reduced to a minimum by certain precautions of which one of the most important is introducing blood at drip rate - 40 drops a minute. Maycock and Whitby (1941) state that too fast a rate can cause a rigor, which ceases instantly if the rate is slowed. The latter workers recommend slowing the rate of administration when a pyrexial reaction develops during transfusion. In study A no rigors occurred in the cases of rapid infusions, but some of the subjects felt cold and shivery. This shivering passed off almost immediately after the end of the infusion, and it should be noted that no rise of temperature was associated with the shivering. In the author's opinion these shivering attacks and rigors associated with rapid infusions are not true febrile reactions. From clinical experience of true febrile reactions the author has noted that slowing the rate of administration does not abolish the shivering of a true febrile reaction. Transfusions accompanied by chills only, and not fever, have been reported by other workers (De Gowin and Hardin 1940, De Gowin 1945).
The exact nature of these non-febrile shivering attacks sometimes associated with a rapid transfusion is not clear. It is of interest to note in Study A that the three cases of shivering and the three cases of feeling cold during the rapid infusion did not show any signs of vaso-dilatation such as fullness in the head, flushing or distended peripheral veins; indeed in three of these six cases the subject became pale. Conversely, none of the subjects showing signs of vaso-dilatation during the rapid infusions developed a feeling of cold or shivering. It is suggested that the sudden introduction of a large volume of fluid at room temperature into the circulation produces, in some subjects, a compensatory vaso-constriction, with a resultant feeling of cold. In a previous observation made by the author (Sharpey-Schafer and Wallace, 1942a, 1942b) on rapid infusions in subjects who had been venesected and then infused with serum, there were no rigors or shivering attacks even in those who showed no signs of vaso-dilatation, although the volumes of fluid infused were much larger than in the present studies. In the previous series, however, the infused fluid was warmed to body temperature, whereas in the present study the blood was given at room temperature.

Temperature of the Infusion Fluid.

If the above explanation of these shivering attacks and rigors associated with rapid transfusions is correct it raises the practical problem of the advisability of warming transfusion fluids. The observations in Study A indicate that transfusion fluids can be administered safely and without pyrexial reactions at room temperature or even
at the temperature of the blood bank. Occasionally a feeling of cold or actual shivering will ensue if fluid is transfused rapidly at room temperature, but as discussed above these shivering attacks should not be regarded as pyrexial reactions.

It is theoretically sound to warm all transfusion fluids to body temperature, but in the author's experience of busy resuscitation wards during World War II it was usually impossible in practice. In those cases of oligaeemic shock which developed a shivering attack or a rigor during a rapid infusion of fluid at room temperature, it has been the author's practice to continue the rapid infusion if the level of the blood pressure demanded a rapid infusion. Those suffering from oligaeemic shock who developed these shivering attacks improved dramatically with the onset of the shivering as if there had been a stimulation of the vasomotor centre. It must be emphasised that before continuing the rapid infusion in these cases it is essential to exclude circulatory overloading (Chapter 4), and a haemolytic reaction (Chapter 5), as the cause of the shivering attack.

The decision as to whether transfusion fluids should be warmed to body temperature or not will be discussed in more detail in Chapter 5, as there is a danger in overheating blood and causing a serious haemolytic reaction. As far as pyrexial reactions are concerned, the present studies indicate that administration of transfusion fluids at room temperature does not increase the incidence of pyrexial reactions.

**Duration of Blood Storage.**

De Gowin and Hardin (1940) found no significant difference between the incidence of febrile reactions from fresh
and from properly preserved blood, the average incidence of pyrogenic reactions being 2.9 per cent. In the carefully controlled observations of Study A it was shown that blood which was properly stored could be administered seven days after withdrawal without pyrexial reactions developing. In Study B blood was preserved for varying periods up to 21 days, and in a large series the pyrexial reaction rate was only 2.0 per cent. In the author's opinion pyrexial reactions are not increased by blood storage up to 21 days provided the method of preservation is satisfactory, i.e., blood should be cleanly and properly collected, immediately, accurately, and continuously refrigerated between 2° and 6°. (35.6° and 42.8°F.) in an acid citrate-dextrose mixture.

Pseudo-pyrexial Reactions.

Pyrogenic reactions must be differentiated from febrile manifestations of the primary disease. The importance of this point was well illustrated by one false reaction in Study A in which the subject had a temperature of 102°F. (38.9°C.) four hours after an infusion of glucose-saline. This subject appeared well at the time of the infusion, but when examined later complained of a sore throat, and was found to be suffering from acute follicular tonsillitis.

CONCLUSIONS

1. In a self-contained transfusion unit pyrexial reactions can be almost eliminated, and in a modern Regional Transfusion Service the incidence of pyrexial reactions from whole blood and concentrated corpuscles can be brought as low as 2 per cent, and from plasma as low as 1 per cent. These results can only be achieved provided there is
acrupulous attention to all aspects of transfusion technique. Even a minor departure from a strict routine may result in a sharp rise in the pyrexial reaction rate.

2. The most important practical causes of pyrexial reactions are the use of stale distilled water and impure chemicals for the preparation of solutions, and the use of administration sets which have been imperfectly cleaned, improperly sterilised or been kept for too long a period after sterilisation. Sodium citrate is not a toxic compound to the human subject in the quantity used in blood transfusion work. Minimal bacterial contamination may be a source of pyrogens, and careful attention should be paid to the conditions of blood storage.

3. The vast majority of pyrexial reactions are harmless to the patient, but on rare occasions such a reaction may prove fatal. It is suggested that pyrexial reactions are particularly serious in patients suffering from advanced liver disease.

4. Rapid administration of transfusion fluids does not induce true pyrexial reactions, but will on occasions cause a shivering attack without elevation of temperature. It is suggested that these occasional shiverings are the result of the rapid administration of a large volume of fluid below body temperature.

5. The administration of transfusion fluids at room temperature or even at the temperature of the blood bank does not induce true pyrexial reactions.

6. Blood stored for periods up to 21 days in an acid citrate dextrose preservative can be administered with a pyrexial reaction rate no greater than fresh blood.
7. True pyrexial reactions must be differentiated from febrile manifestations of the primary disease.

SUMMARY

The following studies on pyrexial Reactions were undertaken -

Study A

The collection of 440 ml. of blood, and the reinfusion of this citrated blood were performed on 324 individuals. An infusion of 540 ml. of glucose-saline was given to 56 individuals.

Febrile reactions occurred in nine of the 324 infusions of blood, but in none of the infusions of glucose-saline. The causes of these reactions are elucidated.

The effect of the duration of preservation, the temperature, and the rate of administration of the infused fluid on reactions are described.

Study B

Observations were made on 2,483 recipients of whole blood or concentrated corpuscles, and on 1,328 recipients of liquid or dried plasma. All recipients were patients in hospitals receiving transfusion fluids from the Regional Transfusion Centre.

From blood the pyrexial reaction rate was 2 per cent, and there was no significant difference between the rate with whole blood and concentrated corpuscles.

From plasma the incidence of pyrexial reactions was 1 per cent, and there was no significant difference between the rate with liquid and dried plasma.

The severity of reactions was assessed, and six fatalities are described.
Three incidents involving a high rate of pyrexial reactions in hospital practice were investigated.

Observation I showed that stale distilled water was the source of pyrogens.

Observation II demonstrated that imperfect cleaning of administration sets was the cause of the high rate.

Observation III revealed faulty cleaning of administration sets, and probably wrong storage conditions for blood, as the cause of the high incidence.

The incidence of reactions, the causes of pyrexial reactions, the prognosis, rate of administration, temperature of the infusion fluid, duration of blood storage and pseudo-pyrexial reactions are discussed.

REFERENCES (CHAPTER II)


--- (1923). Ibid., 80, 247.


CHAPTER III

TRANSMISSION OF DISEASE.

Introduction

The problem of transmitting disease by blood transfusion is twofold. In the first place, there is the possibility of transmitting a disease from which the donor is suffering to the recipient, and in the second place there is the danger of contaminating by faulty handling the blood which has been collected from a healthy donor. These two aspects will be considered separately.

Transmission of Disease from Donor to Recipient

In theory many infections may be transmitted by blood transfusion. The practice of accepting as blood donors only those persons who appear healthy, and who give a history of good health, eliminates the risk of transmitting most infections. There are a few conditions in which the infecting agent may exist in the blood stream without producing clinical manifestations at the time the donor presents himself. The literature (Kilduffe and De Bakey 1942) on the transmission of disease by transfusion records rare accidents such as the transmission of influenza, relapsing fever, measles and smallpox, but in practice the three main diseases which must be considered are, Syphilis, Malaria and Homologous Serum Jaundice.

SYPHILIS

The first recorded case of syphilis transmitted by blood transfusion was probably that of Dade, reported by Fordyce (1915). Then Bernheim (1917) and Sydenstricker et al. (1917) each reported a case.
Probably as a result of the increased use of blood transfusion this complication has been reported more frequently. Rein et al. (1938) collected sixty-eight cases from the literature and knew of nineteen unreported cases. Kilduffe and De Bakey (1942) report over 100 cases in the literature, and emphasise that there have been many unreported cases, since the occurrence of such a complication is not conducive to publication. The majority of the reported cases have come from the United States of America. It seemed appropriate to try and assess the potential danger as it exists in this country at the present time.

**STUDY**

In this observation the serological reactions of 50,000 consecutive blood donors were studied. Because of the large number of tests to be performed it was necessary to find a simple yet reliable test for serological evidence of syphilis. The test which was selected was the Presumptive Kahn Test using sensitized antigen (Kahn 1940). The test may give an occasional false positive result. To avoid the award of a true positive assessment to such pseudo-positive observations, confirmatory tests were performed on all sera giving a positive result on the screening test. The confirmatory tests consisted of the Wassermann Reaction (Mackie and McCartney 1945), and the Standard Kahn Test (Kahn 1940). During the period of the observations it was possible to perform the same tests on 10,000 women attending Ante-Natal Clinics in the same area.

The results were as follows:
<table>
<thead>
<tr>
<th>Total Observed</th>
<th>Positive Serological Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Donors</td>
<td>50,000</td>
</tr>
<tr>
<td>Number of Ante-natal Cases</td>
<td>10,000</td>
</tr>
</tbody>
</table>

\[ n = 1, \chi^2 = 45.15, \text{ } P \text{ is less than } 0.01, \text{ i.e., } \text{ the incidence of positive serological reactions among Donors was significantly less than the incidence among ante-natal patients.} \]

The blood of the donors found to be sero-positive was discarded, and not transfused. During the period of the observation, approximately 15,000 patients were transfused with blood, and no cases of syphilis were known to occur in the recipients.

It is important to record that during the same period two donors with clinical syphilis, but with negative serological reactions were recognised. Each of these donors gave a history of good health at the time of donation, and both had given blood previously. At all donations the Presumptive Kahn test was negative. In the case of one donor, eight days after donation, and in the case of the other donor, twelve days after donation, a message was received to say that the donor was suffering from primary syphilis, and had volunteered the information that a blood donation had recently been given. The blood in each case had been preserved in Acid Citrate Dextrose at 4°C (39.2°F), and at the end of nine days in one case, and thirteen days in the other, there were no spirochaetes demonstrable in the blood of which films were treated with 1 in 10 dilution of Giemsa stain for twenty-four hours (Van Rooyen 1937).

DISCUSSION

Two important points emerge from these observations
(i) the low incidence of positive serological reactions among blood donors in this country, and (ii) the potential danger of the sero-negative donor.

Keller and Leathers (1939) found that out of 14,246 blood donors examined 9.5 per cent were sero-positive, while Hoxworth and Skinner (1941) found a 7.5 per cent incidence of positive serological reactions among 3,487 donors. These workers were concerned with the professional blood donor, whereas in this country at the present time blood donation is on a voluntary basis. Riddell (1939) states that almost all the recorded cases of transmission of syphilis have occurred when using paid donors or untested relatives or friends. This is one of the strongest criticisms against the professional system — namely, that a less reliable member of the community is attracted to service when there is a prospect of gain.

The incidence of sero-positive reactions among donors in the present series is significantly lower than the incidence in women attending ante-natal clinics in the same area, and lower than among thousands of war-time donors with an incidence of positive serological reactions less than 0.5 per cent (Whitby 1942). The civilian donor in the post-war voluntary transfusion service in Great Britain has a strong sense of altruism. His code of morals is such that he rarely contracts syphilis, and as illustrated in the present observations, is so alive to the danger of transmitting the disease as to volunteer the information about recent blood donation should syphilis be contracted.

The danger of transmitting syphilis by transfusion is, therefore, very much less in a voluntary donor.
system such as exists in this country, than in the professional system which is practised in many areas of the United States of America and the Continent of Europe. At the same time the danger exists even in this country. In the Western Region of Scotland 25,000 donations each year are required to maintain supplies of blood and blood products to hospitals. On the basis of one donation every six months, this involves 12,500 blood donors with an expected incidence of 10 sero-positive reactions based on the observed figures. On a population basis this means that an average of 200 sero-positive reactions will be found among voluntary donors during the course of one year in the Blood Transfusion Services in the United Kingdom. It is important, therefore, to ensure that a serological test is performed on every donor at each donation.

An analysis of the reported cases of the transmission of syphilis by transfusion reveals that the condition was transferred usually in the early phases of the disease in the donor. In some instances the transmission occurred before the serological reaction of the donor had become positive (Kilduffe and De Bakery 1942). In eighteen of forty-one cases of transfusion syphilis reviewed by Eichenlaub and Stolar (1939) a blood Wassermann test would not have aided in detecting syphilis in the donor. The present observations have brought to light two blood donors whose serological reactions were negative, but clinically were suffering from syphilis a few days after blood donation. These cases emphasise the limitations of even routine serological tests in detecting the presence of syphilis.

In these two cases in the present series,
Spirochaetes were not demonstrable in the preserved blood 9 and 13 days respectively after the donation. It may be that the search for these organisms was not sufficiently intense. On the other hand, the problem of the prophylaxis of syphilis is apparently somewhat simpler when stored blood is used for transfusion. Turner and Diseker (1941) found that "under conditions obtaining in blood-banks, Treponema pallidum undergoes progressive deterioration in citrated whole blood during the storage period. There is a corresponding reduction in the risk of transmitting syphilis by transfusion, and it is probable that blood stored for four days or longer can no longer transmit this disease". In this connection, the author has estimated that out of all blood transfused in the Western Region of Scotland, only 5 per cent is less than four days old when transfused. This fact, together with the very low incidence of syphilis among voluntary blood donors, makes the danger of transmitting syphilis by transfusion remote.

**SUMMARY**

1. There was an observed incidence of 39 positive serological reactions among 50,000 blood donors, compared with the significantly higher incidence of 34 positive reactions among 10,000 patients attending ante-natal clinics.

2. The danger of the sero-negative donor in the early stages of clinical syphilis is illustrated and emphasised.

3. The risk of transmitting syphilis by transfusion in a voluntary donor service such as exists in this country is discussed.

**MALARIA**

A large number of instances of the accidental
transmission of malaria by blood transfusion have been recorded (Riddell 1939). One of the earliest cases is that recorded by Woolsey (1911) who reported the development of clinical manifestations of malaria in a 59-year old male with pernicious anaemia twenty-four hours following transfusion by artery-to-vein anastomosis from a malarial donor. Since then, several cases have been reported. Wright (1938) reviewed the literature from which he collected 23 cases, and reported six additional cases. Of these 29 cases, the only English example was that of Thomas et al. (1936). Then McCulloch (1937), Gurian (1941), Marks (1941), Zusman and Silver (1938), and Gardner (1938) have described cases, all of which were in America; in England cases have been reported by Nabarro and Edward (1939), Rogers (1947) and Roden (1947).

Riddell (1939) states that in the reported instances of the transmission of malaria, most of the donors were unaware of ever having suffered from the disease. In the case reported by Nobécourt (1932), the donor, who was unaware of having had malaria, and who had left the endemic area, infected a recipient years later. McCulloch (1937) reported a case of transmission of quartan malaria from father to daughter by blood transfusion in Canada, where the quartan type of malaria is almost unknown. The father had left Rumania 25 years before, and did not know that he had ever had malaria. In the case described by Rogers (1947) the donor had never had clinical malaria, and had lived five months in a malarious area seven years before.

Moreover in cases of clinical malaria the infection may have been latent for years, and yet still be
transmissible. In the case reported by Jankelson (1931), the quartan malaria was transmitted to a 6-week-old infant from the father who had had malaria 37 years previously in Italy. Similarly, Naveiro (1934) reported a case in which the donor had been symptomless for 37 years. In other reported cases the donor had been free of symptoms for 25 years (McCluskie 1939), and for 18 years (Gardner and Dexter 1938).

Furthermore, De Goviro (1949) points out that malarial parasites may be present in such small numbers that the most assiduous search of blood films will not reveal their presence.

The clinical manifestations of malaria transmitted by blood transfusion usually appear from one week to one month following the transfusion, although in the reported cases the incubation period has varied from one to sixty days. In the majority of cases the condition was suspected shortly after the onset of chills and fever, and most of them responded satisfactorily to appropriate therapy. However, in the case reported by Nabarro and Edward (1939) the infant died of a malignant infection.

From these observations it appears that there is a real danger of accidentally transmitting malaria by blood transfusion. According to Kilduffe and De Bakey (1942) malaria is, next to syphilis, the most important disease transmitted by transfusion. Whitby (1942) states that malaria is a hazard difficult to avoid, especially in malarial endemic centres. The danger is associated with blood transfusion only, as Whitby (1942) stated, and Lozner and
Newhouser (1943) demonstrated, that the risk of transmission of malaria by plasma is practically non-existent. The risk of transmitting the disease by whole blood raises the problem of the prophylaxis of transfusion malaria.

Ideally, no-one should be used as a donor who has ever resided in a malarial area, and certainly no-one who has ever had an attack (Thomas et al. 1936). When these ideals are impossible, some protection is afforded by cinchonisation of the potential donor or of the recipient for some days beforehand (Thoroughgood 1940). It was suggested by Hutton and Shute (1939) that civilian donors who had lived in the tropics or subtropics should not be used if other blood donors were available. Now that so many ex-Service men and women are potential donors, there may be a tendency for this advice to be disregarded (Rogers 1947).

No great study has yet been made of the survival of malarial parasites under conditions of blood storage (Whitby 1942). Keeping the donor's blood at low temperature for days or even weeks will not destroy Plasmodia if they are present (Hutton and Shute 1939). This statement is contrary to the observations of Ackermann and Filator (1934) and of Antsohelowitch (1937) who demonstrated gradual degeneration of the parasite in stored blood. The former investigators found that transmission of malaria did not occur when blood was stored in the refrigerator for five days or longer, while the latter worker stated that persons could be infected up to the eighth day.

While the safest procedure is to reject all donors who give a history of having had malaria or of residing
In a malarious area, such rejection in the United Kingdom at the present time would seriously reduce the number of available blood donors. In Glasgow and West of Scotland, the author has found that 1 volunteer out of every 10 on the average, would have to be rejected on these grounds. De Gowin (1949) states that since World War II, when so many soldiers were exposed to malarial infections or had the disease, some authors have accepted as donors those persons who have been free from symptoms for two years without the influence of malarial suppressants, but is of the opinion that further experience is needed to determine whether this measure is an adequate safeguard.

The author during the past three and one half years has adopted a definite policy regarding the malarial donor in the Regional Transfusion Service under his direction. Each donor is asked specifically whether he has suffered from malaria, and whether he has lived in a malarious area. Any donor answering one or both questions in the affirmative will be accepted as a donor provided he satisfies other tests of fitness, but if the last clinical attack of malaria or the last period of residence was within the previous three years, that donor's blood will be used for processing into plasma, and not as whole blood. If the interval since the last attack or period of residence is more than three years, the donor's blood may be used as whole blood.

During the period that this policy regarding malarial donors has been pursued by the author, approximately 2,000 patients have received blood from donors who, more than three years previously, had either suffered from malaria.
or had lived in a malarious area. There has been no known case of malaria among the recipients.

The only reaction concerned with malaria that the author has observed was ironically enough in a donor who had suffered from malaria while serving in the Army overseas. At the time of his blood donation he appeared very fit, and had been free of symptoms of malaria for 13 months. Accordingly, 440 ml. of blood was withdrawn to be used for processing into plasma. A few hours after this donation the donor developed clinical malaria. The blood which had been donated was converted to plasma. The removal of a pint of blood apparently stimulates the marrow, a common latent focus, so that the donor may suffer a malarial attack after years of freedom (Whitby 1942).

**SUMMARY**

1. The literature on the accidental transmission of malaria by blood transfusion is reviewed.
2. The danger of transmitting malaria in this way is aggravated by the fact that individuals who have resided in a malarious area without developing clinical malaria may nevertheless transmit the disease. Such potentially dangerous donors cannot be recognised by assiduous search of blood films for parasites.
3. The influence of refrigeration on the survival of malarial parasites in preserved blood is in doubt.
4. The author's policy in relation to the acceptance of donors who have suffered from malaria or lived in a malarious area is described. There have been no known instances of malaria developing among 2,000 recipients of blood from potentially dangerous donors.
The danger to the donor of blood withdrawal from a donor who has suffered from malaria is illustrated.

**Homologous Serum Jaundice**

The development of jaundice in patients who had received subcutaneous injections of convalescent measles serum was reported by MacNalty (1938). Of 109 recipients, 41 developed jaundice. Thereafter, similar reports appeared, and in 1943 a memorandum was published (Lancet, 1943) by Medical Officers of the Ministry of Health reviewing the literature on Homologous Serum Jaundice. This memorandum reported that recipients of human plasma or serum transfusions had developed jaundice. The importance of this complication of jaundice following the injection of pooled human serum or plasma was illustrated by Sawyer et al. (1944) who reported 28,585 cases among United States troops, following inoculation against yellow fever with vaccine suspended in human serum.

Considerable interest in the development of Homologous Serum Jaundice following transfusion of whole blood, plasma or serum was stimulated by these reports. Beeson (1943) reported four cases in which jaundice resulted from small transfusions of whole blood within four months, and three cases after the use of plasma. Steiner (1944) recorded three cases following whole blood alone, and two following plasma. Rappaport (1945) found 33 cases of jaundice after transfusion, including two patients who received only whole blood. Loutit and Mannsell (1945) found no case of frank homologous serum jaundice in a follow-up of a selected series of 213 blood transfusions. Similarly, Spurling et al. (1946) reported no case of homologous serum jaundice among 391 patients.
transfused with whole blood only, but found 77 cases among 1054 recipients of serum or plasma, i.e. an incidence of 7.3 per cent. In the follow-up of 4,430 patients who had received transfusion therapy (Lehane et al. 1949) it was found that 134 (3 per cent) of the recipients had developed jaundice. The same workers observed that the incidence rates of homologous serum jaundice for small-pool plasma (1.3 per cent), and for whole blood (0.8 per cent) are not significantly different, but these rates differ very considerably from that of large-pool plasma (11.9 per cent).

The studies of Neefe et al. (1945) suggest that Homologous Serum Jaundice is caused by a filterable virus which is distinct from that which produces the epidemic type of infectious hepatitis. These workers gave successive inoculations of infective material from patients with both diseases to groups of human volunteers, and demonstrated that an attack of one disease did not protect against the other, although the first attack protected against subsequent inoculations with the same infective agent. Furthermore, the incubation period of epidemic hepatitis is said to be fifteen to thirty-four days, whereas that of homologous serum jaundice is fifty-six to 154 days (Havens 1947). Both diseases may be transmitted by the serum from infected persons. The infective agent of epidemic hepatitis has been demonstrated in the faeces of patients with the disease, but a transmissible virus has not been encountered in the excreta of those with homologous serum jaundice.

Of the 77 cases of homologous serum jaundice investigated by Spurling et al. (1946), only one was serious,
the patient being in a comatose condition for several days. No deaths occurred among their proved cases of post-transfusion jaundice. On the other hand, Lehane et al. (1949) observed that seven out of 131 cases of post-transfusion jaundice terminated fatally, although the majority of the patients were not severely ill.

The following study records the author’s experience of this important complication of transfusion therapy.

**STUDY**

The author's first experience of post-transfusion jaundice came in 1941 following the administration of pooled human serum to volunteers in studies on circulatory overloading (Sharpey-Schafer and Wallace 1942a, 1942b). In these and other studies more than 50 individuals received serum infusions, and at least three cases of post-transfusion jaundice were known to have occurred. In one of these cases the incubation period was 117 days, and in the other two cases approximately 90 days. The illness was only moderately severe. As to other cases of jaundice in recipients of the same batch, it was impossible to obtain the information.

During four and one-half years in the Army Blood Transfusion Service the author is not aware of having seen a frank case of homologous serum jaundice, but patients were usually seen for a short interval after transfusion, and not at the time when serum jaundice might have developed.

The author was surprised to learn that in Glasgow and the West of Scotland, prior to 1946, no cases of homologous serum jaundice had been reported to the Regional Transfusion Centre, and it was not until 1947 that the first case was reported.
Case I. A married woman, aged 24 years, received 1 pint of blood, and 2 pints of plasma on account of circulatory collapse associated with an incomplete abortion. Seventy-nine days after the transfusion the patient complained of nausea and vomiting. She was admitted to hospital suffering from jaundice which cleared completely within two weeks. The case was regarded as a mild attack of homologous serum jaundice.

Investigations - The donor of the whole blood transfused in this case was interviewed, and gave a clean bill of health before and after the donation. There had never been any illness suggestive of hepatitis.

The laboratory records showed that the two bottles of plasma administered had come from different batches of processed plasma. There was the possibility of 94 donors having contributed an iatrogenic agent in one batch, and 73 donors in the second batch.

Each donor contributing to these two batches was sent a personal letter explaining the reasons for which information about personal health was sought. Apart from general questions, the donor was asked specific questions about Colds, Rheumatism, Bilious attacks, Skin Rashes and Jaundice.

Replies were received from 86 out of 94 donors in the first batch, and from 65 out of 73 donors in the second batch. In no case was there any suggestion of an illness resembling hepatitis. Indeed it is interesting to learn of the terms in which these 151 donors described their general health -

<table>
<thead>
<tr>
<th>Health Level</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfect</td>
<td>8</td>
</tr>
<tr>
<td>Excellent</td>
<td>12</td>
</tr>
<tr>
<td>Very Good</td>
<td>43</td>
</tr>
<tr>
<td>Good</td>
<td>80</td>
</tr>
<tr>
<td>Fine</td>
<td>3</td>
</tr>
<tr>
<td>Normal</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>151</strong></td>
</tr>
</tbody>
</table>
The majority of these donors were giving blood regularly every six months. There was no apparent deterioration in health as a result of blood donations.

From these two batches of plasma 76 bottles had been issued to hospitals. Although the author's records showed exactly to which hospital each individual bottle had been sent, it was only possible to establish with certainty the exact fate of 25 of these bottles. The hospital case sheets were incomplete in many cases, and no plasma batch number was shown. These 25 bottles had been transfused to 13 patients including the present patient, and of these patients three had died. The remaining nine patients had had no illness resembling hepatitis in the six months following the transfusion. The three patients who died had succumbed from the illness for which the plasma infusion was administered.

The patient in this case had had no known contact with a case of infectious hepatitis, and in view of the development of jaundice 79 days after transfusion with blood and plasma, was considered to be suffering from Homologous Serum Jaundice. There was, however, no evidence of any of the donors or other recipients having suffered from jaundice.

Case 2. A married woman, aged 35 years, received a transfusion of one pint of blood and two pints of plasma for a retained placenta and post-partum haemorrhage. Then 98 days later she was admitted to hospital suffering from jaundice. She remained in hospital for three weeks with a moderately severe hepatitis. There had been no recent contact with a case of infectious hepatitis, and in view of
the history of previous transfusion this was regarded as a case of homologous serum jaundice.

Investigations - The donor of the blood given to this patient was found to be in perfect health. She was the wife of a physician who was convinced that his wife had never had an illness resembling hepatitis.

The two bottles of plasma administered were from the same batch of processed plasma, and from the first cordial within the batch. The author used throughout the Kaolin method for processing plasma (Haizels 1944). Any icterogenic agent responsible for the patient's jaundice should have been present in the first cordial to which 16 donors contributed.

These 16 donors were followed-up as in Case 1, and 15 out of 16 replied. The health of all these donors was good, the only complaint being an occasional head cold. There had been no illnesses resembling hepatitis.

At first sight it might appear that an icterogenic agent present in the first cordial would only appear in the first few bottles of filtered plasma. However, the same closed system of apparatus is used for up to ten cordials of plasma, and it is possible that an icterogenic agent in the first cordial may be present in any of the individual flasks of plasma derived from the whole batch. Accordingly, all the bottles in this batch were followed up.

The batch contained 89 flasks of plasma which had been issued to hospitals. Of these, 31 were traced to the recipients, the hospital records being incomplete in the remaining cases. The 31 flasks traceable had been administered to 22 patients including the present case.
Six of these patients died soon after transfusion. Of the remainder 13 were traced, and none of these had an illness resembling homologous serum jaundice within six months of transfusion.

As in Case 1, the patient appeared to be suffering from homologous serum jaundice, but there was no evidence of jaundice among the donors or other recipients of plasma from the same batch.

Subsequent to these reports and investigations, the author decided to ascertain the incidence of homologous serum jaundice following the transfusion of whole blood and plasma to patients in hospitals in Glasgow and the West of Scotland.

Method of Follow-up.

The survey was carried out on the following lines - (1) Hospitals report to the Regional Transfusion Centre the names and addresses of patients receiving transfusions, the condition for which the transfusion is given and other relevant details. (2) After a period of six months from the date of transfusion had elapsed, a letter was sent by the author to the patient's home address. This letter took the form of a general inquiry regarding the patient's health since discharge from hospital. The patient was asked to answer specific questions about Colds, Rheumatism, Skin Rashes, Bilious attacks and Jaundice in addition to the questions about general health.

If a reply indicated that jaundice or an illness suggestive of hepatitis had developed, the particular patient was visited to obtain more information, particularly
as to the occurrence of jaundice in other members of the patient's household. In addition, whenever possible the patient's medical practitioner was consulted as to the nature of the illness.

A diagnosis of homologous serum jaundice was made, provided the following conditions were fulfilled: (1) the development of jaundice from one to six months after transfusion; (2) absence of history of contact with cases of infectious hepatitis; and (3) absence of other pathological causes of jaundice.

The result of the survey is shown in the accompanying table.

<table>
<thead>
<tr>
<th></th>
<th>Blood Alone</th>
<th>Plasma Alone</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of patients</td>
<td>150</td>
<td>450</td>
<td>600</td>
</tr>
<tr>
<td>followed-up</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients traced</td>
<td>133</td>
<td>369</td>
<td>502</td>
</tr>
<tr>
<td>Number of deaths within 6</td>
<td>14</td>
<td>45</td>
<td>59</td>
</tr>
<tr>
<td>months of transfusion (not</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with jaundice)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of surviving patients</td>
<td>119</td>
<td>324</td>
<td>443</td>
</tr>
<tr>
<td>upon whom incidence of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>jaundice is based</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number who developed jaundice</td>
<td>1</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>attributed to transfusion</td>
<td>(0.8%)</td>
<td>(3.7%)</td>
<td>(2.9%)</td>
</tr>
<tr>
<td>within 6 months of transfusion</td>
<td></td>
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The patients to whom letters were sent were chosen at random.

Although the incidence of jaundice with plasma (3.7 per cent) appears considerably higher than that with blood (0.8 per cent), the difference is not significant.
in the present survey.

\[ n = 1, \quad \chi^2 = 1.6. \quad P \text{ is slightly greater than } 0.2. \]

Other observations made during the survey were:

**Incubation Period** - varied from 31 days to 126 days.

**Nature of Illness** - All cases were mildly or moderately ill.

The period in bed varied from three days to three weeks.

There were no fatal cases in the present survey.

**Age Incidence** - varied from 8 years to 65 years.

The donor of the whole blood which caused post-transfusion jaundice was found to be perfectly healthy, and had never had an illness resembling hepatitis.

The plasma pools used for the preparation of plasma were derived from 120 to 200 donors. The twelve cases of jaundice were drawn from eleven different batches.

Since this Survey was made, the author has received reports from time to time of what appear to be true cases of homologous serum jaundice. All these cases have been mild attacks apart from one case which ended fatally.

This was a youth, aged 19 years, who had been given 2 pints of plasma when the operation of thoracoplasty was performed. He made a good recovery from the operation, and was allowed to go home, where he continued to make satisfactory progress. Then 91 days after receiving plasma he became ill with what his own medical practitioner called catarrhal jaundice.

This condition was treated at home for seven days by which time the patient was comatose. He was then admitted to hospital, where he died on the 9th day of the illness. The main findings at autopsy were acute hepatic necrosis, and multiple haemorrhages. There was no history of contact with a case of infectious hepatitis.
It would appear that this was a fatal case of homologous serum jaundice. The patient in the next bed also had a thoracoplasty performed, and received two bottles of plasma from the same batch. This latter patient remained well however, and showed no sign of jaundice in the six months following transfusion. Thirteen other recipients of the same batch who were traced, had had no illness resembling homologous serum jaundice.

DISCUSSION
The present study confirms that jaundice may follow the transfusion of blood or plasma. The incidence following plasma in this survey was 12 cases of jaundice among 324 patients transfused (3.7 per cent) compared with 1 case of jaundice among 119 recipients of blood (0.8 per cent). Although this difference in the present survey is not statistically significant, the trend is such as to conform with the observations of Spurling et al. (1946) and Lehane et al. (1949), that the incidence of jaundice is higher following large pool plasma than following blood.

The incidence of jaundice following plasma was reported as 7.3 per cent (Spurling et al. 1946), and as 11.9 per cent (Lehane et al. 1949), but in both these reports the plasma pools were larger than in the present study, being derived from 120 to 800 donors compared with from 120 to 200 donors in this study. Lehane et al. (1949) have shown that by using small-pool plasma in which each pool is derived from not more than 10 donors, the incidence of jaundice may be reduced to 1.3 per cent. The incidence of jaundice following plasma in the present study was 3.7 per cent which
is intermediate between the incidence for large pool plasma and for small pool plasma reported by these other workers. As the size of the plasma pool was also intermediate between the sizes used by the other workers, this observation tends to confirm the view of Lehane et al. (1949) that the incidence of post-transfusion jaundice can be reduced by using small pool plasma. The pooling of many plasmas and sera to make therapeutic or prophylactic sera is ill advised, as oneicterogenic plasma or serum may contaminate the whole pool. In addition to this danger of large pool plasma, it has been suggested (Andrews 1944) that dilution of a virus may increase its virulence.

Of the 77 cases of jaundice investigated by Spurling et al. (1946), only one was serious, and there were no deaths. On the other hand, most of the 134 cases of post-transfusion jaundice reported by Lehane et al. (1949) were not serious, but seven terminated fatally. The present observations emphasise that although most cases of post-transfusion jaundice are not seriously ill, the disease may end fatally.

The long incubation period, from 81 to 126 days, and the age incidence from 8 to 65 years observed in the present study are in agreement with the findings of Lehane et al. (1949).

Apart from the thirteen cases of homologous serum jaundice discovered by the survey in the present study, only nine other cases in Glasgow and the West of Scotland have been reported to the Regional Transfusion Centre during the course of two years. During the same period a conservative estimate of the number of patients transfused with plasma would be 5,000. Assuming that 80 per cent of these patients survive for 6 months after transfusion, and that the recorded incidence
of 3.7 per cent for plasma jaundice in true, then one would expect to have had 148 cases of homologous serum jaundice from plasma alone. The fact that the observed incidence of post-transfusion jaundice in this study agrees with the observations of other workers, suggests that the disease is occurring in the West of Scotland, but is not being recognised as Homologous Serum Jaundice. Many of the cases appear to be mild, and may well be regarded by the patient's practitioner as a bilious attack, or if more severe, as "catarrhal jaundice" or infectious hepatitis.

Prophylaxis - In the past the only prophylactic measure has been to reject those donors who have had jaundice within six months of the time they present themselves for the donation of blood. This precaution has not proved efficacious, as attested by the fact that many cases of the disease have been reported in recipients of plasma or whole blood from donors who were screened in this manner. It is recognised that some persons harbour the icterogenic virus in the blood without exhibiting clinical manifestations of disease (De Gowin 1949). The present observations confirm that the donors contributing to a plasma pool which was icterogenic were apparently healthy.

The incidence of post-transfusion jaundice following plasma can be reduced to the same order as the incidence following whole blood by using plasma pools derived from not more than ten donors (Lehane et al. 1949). The same workers observe, however, that fatalities may occur even from the use of whole blood and small pool plasma.

Since the virus may apparently be inactivated by ultra-violet light, Wolf et al. (1947) gave irradiated plasma
to 21 volunteers without untoward reaction, but they offered no evidence to show that the virus was actually inactivated. MacCallum (1946) failed to inactivate the virus with the doses of ultra-violet light recommended by Oliphant and Hollaender (1946), but Blanchard et al. (1948) have now shown that a practical and effective method of irradiating icterogenic serum and plasma can be evolved. The Ministry of Health plans to establish a plant for the irradiation of plasma (Maycock 1949). Irradiation cannot of course be applied to whole blood.

**SUMMARY**

Only nine cases of homologous serum jaundice have been reported to the Regional Transfusion Centre in the West of Scotland during two years. One of these cases terminated fatally.

In a survey of 443 patients who had received transfusion therapy, 13 (2.9 per cent) cases of jaundice were discovered. The incidence of jaundice following pooled plasma was 3.7 per cent, and following whole blood 0.3 per cent. None of these cases were fatal.

It is suggested that many cases of homologous serum jaundice are missed, because the disease is mild, or in more severe cases it is regarded as infectious hepatitis.

The incubation period varied from 31 to 126 days, and the age incidence from 8 to 65 years in the survey reported.

The importance of using small plasma pools to reduce the incidence of jaundice is discussed, but complete safety will only be achieved by destroying the icterogenic virus by some agent such as ultra-violet irradiation.

**ALLERGIC REACTIONS**
Urticaria and occasionally angioneurotic oedema may occur after the transfusion of blood or blood derivatives. In some cases there is evidence of natural hypersensitivity either in the recipient or in the donor.

Goodall (1938) reported the case of a woman who was receiving a transfusion when she suddenly developed urticarial symptoms. The transfusion was stopped, and after injection of adrenaline, was resumed. When questioned upon her reactions to food the patient stated that the only ingredient which she knew she could not take was gin. It always produced urticaria, and she had not taken it for 15 years. When the donor was asked how much gin he had had that morning he confessed (in some alarm) to having had one drink.

Redstrom (1934) recorded the case of a donor who had urticaria, at the time that his blood was withdrawn, from eating strawberries: the recipient developed urticaria immediately following the transfusion.

Similar cases of allergic reactions in which there was evidence of hypersensitivity in the donor or recipient have been reported by Ramirez (1919), Holder and Diefenbach (1932), Stewart and Bates (1938), and De Gowin and Hardin (1940).

STUDY F

As reported in Study B, Chapter II, observations were made on 942 recipients of whole blood or concentrated red corpuscles during the very warm summer of 1949. In addition to observing these cases for the development of pyrexial
reactions, an attempt was made to note the development of urticaria or other allergic manifestations.

Results - An allergic reaction was observed in eight patients among the 942 transfused, i.e. an incidence of 0.85 per cent.

In each case urticaria developed during or soon after the transfusion. There was a generalised urticaria in one case, but in the other cases it was localised usually to the arms. Apart from severe discomfort from pruritus these reactions were not serious. There was no evidence of the more serious allergic manifestations such as angioneurotic oedema or bronchial spasm.

No special treatment was given in five of these cases, and the urticarial eruption disappeared within four hours. In two cases a hypodermic injection of 7 minims of 1 in 1000 adrenaline hydrochloride was given, and the urticaria disappeared within half-an-hour. The eighth case was interesting in that an anti-histamine drug was used. The patient who suffered from haemophilia developed an urticarial eruption on the face and arms during the administration of the first pint of blood. As it was desired to give three pints of blood, the patient was given 0.15 Gm. 'Anthisan' (May and Baker). The remainder of the blood was transfused without extension of the eruption to other areas, and without the development of other allergic manifestations.

Questioning of the recipients and donors failed to reveal any history of known hypersensitivity.

DISCUSSION

Urticarial reactions follow about one per cent
of transfusions (Wiener et al. 1941, Whitby 1942). In a series of 2423 transfusions (De Gowin and Hardin 1940) the incidence of urticaria was 1.1 per cent. Again in another series of 5386 transfusions (De Gowin 1945) this complication occurred in 0.8 per cent of the cases. Hardin (1949) reports that the occurrence is so constant that he employed the reported incidence of urticaria as an index of the accuracy with which transfusion reactions were reported from various military units in the European Theatre of Operations. If the incidence of urticarial reactions was reported as much less than one per cent, it could be assumed that the recipients were not being closely observed.

The incidence of urticarial reactions in the present study, 0.85 per cent in 942 cases, is in agreement with the reports of these other workers. The present observations also support the contention of Riddell (1939) and De Gowin (1949) that, although this complication is classified as allergic on account of its clinical manifestations, it is not possible in most cases to prove allergy. It is probably unwise to accept as donors any persons giving a history of allergic manifestations.

The suggestion (De Gowin 1949) that adrenaline will cause the eruption to subside is confirmed, and the possibility of continuing the transfusion in cases in which this complication arises by the use of anti-histamine drugs is illustrated.

The allergic reactions described in the present study were not serious, but De Gowin (1949) observes that although the majority of these reactions are not serious, such a
reaction may be very serious. The same worker emphasises the
danger of generalised angioneurotic oedema, and describes a case
in which this complication precipitated the death of a patient
already seriously ill.

**SUMMARY**

Eight cases of urticaria were observed among
942 recipients transfused. The reactions were not serious.

No history of hypersensitivity was elicited
on questioning the donors and recipients.

The value of adrenaline or anti-histamine
drugs in relieving symptoms and in enabling the transfusion
to be continued is discussed.

**CONTAMINATION OF TRANSFUSION FLUIDS**

**Introduction**

Stored blood, and unfiltered plasma or serum,
carry the risk of bacterial contamination sufficient to induce
at least a pyrexial reaction, and at most, fortunately rarely,
a virulent and fatal septicaemia (Whitby 1942). The same
worker states that in his experience the contamination-rate of
blood is fairly high, and if there are also the manipulations
necessary to separate plasma or serum the incidence is increased.

Refrigeration at 2 to 6°C. (35.6 to 42.8°F.) is some protection
in that it inhibits rapid growth, but many bacteria survive
and even multiply at this temperature, and certainly if the
refrigerated blood be allowed to warm up (Whitby 1942).

Whole blood cannot be sterilised, and any chance
bacterial contaminants are prevented from multiplying only if the
temperature of the blood is kept constantly below 6°C. (42°F.)
(Tovey 1950). Bacterial growth may convert a life-saving
fluid into a highly toxic one.

The danger of bacterial contamination of blood is mentioned by De Gowin (1949) who agrees with Whitby (1942) that serious complications from contaminated blood are fortunately rare.

STUDY G

This study records the author's observations on the occurrence and dangers of bacterial contamination of blood and plasma used for transfusion purposes.

Incident I - This episode occurred during an experimental attempt to produce an immune anti-P agglutinin in the human subject. Four volunteers including the author, all of whom were P negative, each received an intravenous injection of 1.0 ml. Group O, P Positive blood. There was no reaction to these injections. Then one week later each volunteer received an intravenous injection of 0.5 ml. of the same blood which had been stored in the interval in acid citrate dextrose between 2 and 6°C. (35.6 and 42.8°F.). On inspection of this stored blood before injection there was no evidence of haemolysis.

Following this second injection each volunteer had a reaction which varied very much in severity. The individual least affected developed a headache three hours after the injection and suffered from "sore bones and muscles" for 24 hours. This individual never felt cold or shivery, and did not think that his temperature was elevated. In the second subject nothing unusual was noticed until six hours after the injection when shiverings were noticed, and there was a complaint of generalised aches for 24 hours. This individual described his symptoms as being like "a dose of 'flu", and although he
did not take his temperature he felt that it was above normal.
The third individual complained of nausea a few hours after the
injection, and vomited on several occasions during the next 24
hours. This subject's temperature was elevated to 102°F.
(38.9° C.).

The fourth victim was the author, and in this
case the reaction was more severe. About six hours after the
injection the subject began to feel cold and shivery. Body
temperature was then 100°F. (37.8° C.). Shortly thereafter the
patient had a violent rigor, and the temperature rose to
103.4°F. (39.7° C.). There was severe frontal headache, pains
in the large joints, nausea and extreme restlessness. After a
night with very little sleep the subject got out of bed, and
regained consciousness lying on the bedroom floor. This was
the first time that the subject had ever fainted. The fever
and the constitutional symptoms persisted for 48 hours.

Investigations - At first the possibility of these reactions
being caused by immunisation to the P antigen was considered,
although it seemed improbable that sensitisation would have
occurred after only one injection of a weak antigen. In
fact there was no evidence of anti-P agglutinins in the serum
of the four subjects, either 72 hours after the second injection
or 14 days later.

A more likely explanation of these reactions
in all four subjects was bacterial contamination of the
injected blood. The remainder of the blood which had been
preserved in acid citrate dextrose at 2 to 6°C. (35.6 to 42.8°F.)
was examined 72 hours after the second injection. On
centrifuging a sample it was found that marked haemolysis

77.
 existed. Direct microscopic observation of a slide preparation of the blood showed a moderate number of organisms. These organisms were in the form of Gram negative rods of the coliform type. Cultures on Agar showed that these organisms multiplied rapidly at 37°C. (98.7°F.), and at room temperature, but only multiplied slowly at 4°C. (39.2°F.).

Enquiries were made as to the storage of the blood used for the injections. At the time of the first injections the container had been exposed to the air for a few seconds when it was opened to withdraw blood. There was some doubt as to how soon it had been returned to the refrigerator. In all probability the container remained in a laboratory for 2 hours before being refrigerated again. Although the contaminating organism multiplied in the refrigerator, its growth was so slow that it was more probable that the blood had been allowed to warm up at one period, thereby causing marked bacterial contamination.

Incident II - A patient suffering from Aplastic Anaemia had received multiple transfusions of homologous group blood (Group 0, D positive). The therapeutic response to transfusion had been satisfactory, and no reactions had been associated with these transfusions.

On the occasion of the incident compatible concentrated red corpuscles were supplied by the Regional Transfusion Centre, and were delivered at the Porter's Lodge of the hospital concerned as was the custom at the time. Unfortunately, the porter did not inform the medical officer of the arrival of the blood until 24 hours later, and then did not disclose the fact that the blood had been in the
lodge for that length of time. The blood was then placed in a refrigerator at 4°C. (39.2°F.) where it remained for another three days before being transfused.

The first bottle (approximately 250 ml. of concentrated red corpuscles) was transfused without reaction, but soon after the commencement of the second bottle the patient had a rigor and became very collapsed. There was vomiting and diarrhoea. The marked prostration persisted, and the patient died within six hours of the onset of the reaction.

Inspection of the blood in the bottle which caused the reaction revealed a purple colour characteristically associated with bacterial contamination. A slide preparation of the blood showed numerous organisms when observed directly with a microscope. These organisms were in the form of Gram negative rods of the coliform type. Cultures on Agar showed that these organisms multiplied rapidly at 37°C. (98.6°F.) and at room temperature, but did not multiply at 4°C. (39.2°F.).

Incident III - A male patient aged 56 years was being transfused on account of a refractory anaemia. After approximately 150 ml. of concentrated red corpuscles had been administered the patient developed vomiting and diarrhoea. The faeces were fluid and greenish in colour. The temperature rose to 102°F. (38.9°C.), and the patient became rapidly comatose. Death followed within six hours of the onset of the reaction.

Investigations - The concentrated red corpuscles had been supplied as compatible blood from the Regional Transfusion Centre. The blood grouping tests were repeated as follows -
Patient Group O, Rh negative (cDe, cDe)
Donor Blood Group O, Rh positive (CDe, cDe)

Compatibility tests in which the patient's serum was tested against the donor's cells suspended in saline and AB serum were performed at 0°C. (39.2°F.), room temperature, and at 37°C. (98.7°F). All these tests were perfectly satisfactory.

The concentrated corpuscles remaining in the bottle had a purple colour suggesting bacterial contamination, and a slide preparation of the blood showed numerous coliform organisms when examined microscopically. These organisms multiplied rapidly at 37°C. (98.7°F.) and at room temperature when cultured on Agar, but did not multiply at 4°C. (39.2°F.).

The history of the blood was that the bottle had been taken from a refrigerator at the Regional Transfusion Centre, and the supernatant plasma withdrawn by a closed system. The bottle was packed in a cardboard container, and sent by rail to the hospital concerned. The journey from the time of leaving the Regional Transfusion Centre until arrival at the hospital blood-bank occupied four hours, and this in the middle of summer. The blood was not transfused the same day, but was stored overnight in the hospital blood-bank.

**DISCUSSION**

The reactions described in this study all resulted from bacterial contamination of blood. Experience has shown that, even with highly skilled blood collection, from 1 to 5 per cent of bottles may contain an odd contaminant derived from the skin or the air (Resuscitation, 1944). The author
has made a practice of culturing random bottles of blood collected by Mobile Teams of the Regional Transfusion Centre, and has shown that the contamination rate can be reduced to less than one per cent provided that the blood is cleanly and properly collected, and then refrigerated without delay. Even with a careful technique for blood collection, bacterial contamination of blood will occasionally occur, and every bottle of blood must be regarded as being potentially contaminated. The importance of refrigeration as a protection against bacterial growth is emphasised (Whitby 1942), and according to Resuscitation (1944) the only safeguard is immediate, continuous and accurate refrigeration. Once blood has been removed from a refrigerator it must be used without delay; it must never be allowed to warm up, and then be restored to the refrigerator for another occasion (Whitby 1942).

The two fatal reactions in hospital patients described in the present study, emphasise the danger of not observing continuous refrigeration of blood. In one case the blood had remained at room temperature for 24 hours, and in the other case the blood had been exposed to atmospheric temperature at the height of summer for four hours.

Another important feature of these two fatal reactions was the fact that concentrated red corpuscles was the fluid administered. The manipulations required in packing the cells increase the chances of bacterial contamination, even when a strict technique is used. Whitby (1942) has shown that the manipulations necessary to separate plasma or serum increase the incidence of bacterial contamination in blood. In addition to the increased risk of contamination there is an important
practical hazard in the use of concentrated corpuscles. Grossly contaminated blood is characterised in most cases by the complete diffusion of pigment throughout the plasma, but the removal of this supernatant plasma in packing cells eliminates this visual aid, and makes recognition of bacterial contamination difficult. Alsever (1949) states that concentrated red cell suspensions prepared from blood collected into acid citrate dextrose solution can be preserved in a refrigerator for as long as whole blood in the same preservative solution. The author disagrees profoundly with this statement, and believes that the dangers of manipulation and loss of visual aid are so great that concentrated red corpuscles should be administered as soon as possible after preparation, and certainly within 24 hours of packing the cells.

These two fatal cases illustrate two important practical problems in the administration of a Regional Blood Transfusion Service. The first of these is an arrangement whereby blood which is delivered at a hospital from the Regional Transfusion Centre is immediately placed in the hospital blood-bank. Whitby (1942) has emphasised that care of a blood-bank is a great responsibility, and the fate of such a precious, yet dangerous, fluid as blood on arrival at a hospital should not be entrusted to an irresponsible individual. The second of these administrative problems is a means of refrigerating blood which has been despatched from the Regional Transfusion Centre to a hospital. Blood which has been removed from a refrigerator takes time to warm up to room or atmospheric temperature, but if the journey from the Regional Transfusion Centre to the hospital blood-bank is likely to occupy two hours or more, the blood should be carried in cooled insulated boxes.
Half-hearted gestures with lumps of ice are potentially dangerous. Indeed, it is inadvisable to put loose ice in contact with bottles of blood, in that corpuscles nearest the side of the bottle tend to become fragile and later, haemolysed.

The actual contaminant in the two fatal reactions was a Gram negative rod of the coliform type, and it was clearly demonstrated that the growth of the organism in the two cases was inhibited at the optimum storage temperature for blood, i.e., 4°C. (39.2°F.). In the author's experience the organism most frequently encountered in contaminated blood is a coliform type of organism. Fortunately, in most cases this type of organism is held in check at refrigerator temperature, although occasionally, as in Incident I, the organism may multiply slowly at 4°C. (39.2°F.). Most bacterial contaminants cause visible haemolysis, but unfortunately this is not always the case. The contaminated blood used in Incident I only showed definite haemolysis after shaking up the blood and then centrifuging.

The reactions described in Incident I show that there is a considerable individual variation in the severity of the reaction following a sub-lethal injection of contaminated blood. The volume of blood, and presumably the bacterial content, injected was the same in each case. The actual volume of blood injected was only 0.5 ml., and the reaction in one case was severe. This observation suggests that even a small volume of stored blood which is bacterially contaminated may prove fatal, especially in a patient who is already ill. The four subjects observed in this study were healthy volunteers. Another interesting feature of the reactions described under Incident I was the delay between the injection and the onset of
symptoms. Although the injection was intravenous, the latent period varied from three to six hours. On the other hand when larger volumes of contaminated blood are injected as in the actual transfusion of patients, the onset of symptoms is much earlier. Symptoms may appear with as little as 50 ml transfused (Resuscitation 1944).

The symptoms which appear following the transfusion of contaminated blood include respiratory distress, cerebral symptoms, rigor, vomiting, diarrhoea and loss of sphincter control (Resuscitation 1944). The two fatal cases described had marked constitutional symptoms affecting the gastro-intestinal tract in addition to profound prostration. Death occurred in both cases approximately six hours after the onset of the reaction.

Like stored blood, unfiltered plasma or serum carry the risk of bacterial contamination. Indeed with the manipulations necessary to separate plasma or serum the risk is greater. Unfiltered plasma is a dangerous fluid; its natural turbidity obscures any turbidity due to bacterial contamination—the simplest macroscopic test of fitness for use (Whitby 1942). Most Transfusion Centres in this country abandoned the use of unfiltered plasma because of this danger in the early part of World War II, but for many years the Aberdeen Blood Transfusion Service has used unfiltered liquid plasma without complications (Cruickshank 1950). The success of the Aberdeen unit is the result of extremely careful bacteriological control, which would not be possible in the larger Transfusion Centres. This problem is however raising its head again in this country. Most Regional Transfusion Centres are using dried plasma which has been prepared from unfiltered liquid plasma. There is a definite
danger of bacterial growth during transportation from the Regional Transfusion Centre to the central Drying Plant. Sterility tests must be performed at the drying unit to ensure the safety of the final product. The author feels that the risk of transfusing contaminated plasma can be reduced to a minimum by using filtered liquid plasma (Haisels 1944), and it is this product which is issued by the Glasgow and West of Scotland Transfusion Service. Each flask of liquid plasma, after filtration through sterilising pads, is incubated for 21 days at 22°C. (71.6°F.) which is the optimum temperature for detecting a bacterial contaminant in plasma (Gruickshank 1950). Only plasma which is absolutely clear at the end of this period is issued to hospitals.

The importance of not tampering with a flask of liquid plasma until immediately before use is well illustrated by a recent experience of the author's. A particular hospital was supplied with standard flasks containing only 200 ml. filtered liquid plasma. This was to be used for the treatment of gastro-enteritis in children, and to save time the hospital decided to add Hartmann's solution to the plasma already in the flask. The Hartmann's solution was sterile, and a supposedly aseptic technique was used for the transfer. After storing the mixture for a few days at room temperature it was noted that a turbidity had developed in the fluid which was then returned to The Regional Transfusion Centre. The author found that the mixture was contaminated with a Gram negative organism of the coliform type. On investigation it was discovered that the technique used for transfer had not been correct.

The author is not aware of any reaction having
occurred in the Region under his direction from bacterial contamination of plasma.

**SUMMARY**

1. Three incidents are described to illustrate the danger to the recipient of contaminated blood. Two patients died as a result of the transfusion of grossly contaminated blood.

2. The importance of continuous refrigeration of blood is discussed.

3. The increased risk with concentrated red corpuscles is illustrated.

4. The care of a hospital blood-bank should be entrusted to a responsible person.

5. The risk of bacterial contamination of plasma is discussed.

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CHAPTER IV

MECHANICAL REACTIONS

Introduction

The Mechanical Reactions which may complicate transfusion are -

(i) Circulatory Overloading
(ii) Air Embolism
(iii) Pulmonary Embolism

Of these complications, the most important in practice is Circulatory Overloading, and special attention has been devoted to this hazard in this thesis. The author's experiences with the complications of Air Embolism and Pulmonary Embolism are also discussed.

Circulatory Overloading

According to Riddell (1939) circulatory failure is the commonest cause of a fatality following blood transfusion. It may be due directly to overloading of the circulation, or it may develop secondarily to a rigor.

In an individual whose blood volume is not reduced, the production of circulatory failure following the intravenous infusion of fluid depends on three main factors; the volume of fluid introduced, the rate of its introduction, and the mechanical efficiency of the cardiac muscle at the time.

A large volume of blood introduced slowly may give rise to no symptoms, whereas a small volume introduced rapidly may produce heart failure. The mechanical efficiency of the heart may be reduced in chronic anaemia, and in such a case a transfusion of normal volume given at normal speed may so increase the venous return that the balance between income and output is disturbed, and the myocardium fails.
Plummer (1936) reported four deaths due to circulatory failure. One of these was an example of straightforward overloading of the circulation in a patient with mitral stenosis, but the other three cases only developed signs of heart failure following a rigor. All these patients had been anaemic for a long time. Acute pulmonary oedema was the most striking feature at necropsy in these cases. Two similar cases were reported by De Gouin (1938), in which there was no reaction of any kind to the introduction of blood, but which collapsed and died following a rigor.

While Riddell (1939) maintains that circulatory overloading of this nature is the commonest cause of a fatality following blood transfusion, he produces very scanty evidence to support his contention. On the other hand, Kilduffe and De Bakey (1942) state that this complication has never been observed by them, and on the basis of a statistical analysis consider that this complication occurs relatively rarely. This analysis is made on a collected series of 43,284 transfusions reported by 18 different observers. In the whole series there were 45 deaths, but only 6 of these fatalities (13.3 per cent) were due to cardiac failure.

In view of the wide divergence of opinion on the frequency of this type of reaction in practical transfusion work, it seemed important to study the haemodynamics of the circulation in the human subject. The following studies have been undertaken:

**STUDY II** Blood Changes following Controlled Haemorrhage in Man.

**STUDY I** Retention of Injected Serum in the Circulation in Man.
STUDY J Circulatory Overloading following Rapid Intravenous Injections in Man.

STUDY K The Circulating Blood Volume in Patients suffering from Anaemia.

STUDY L Cases of Circulatory Overloading in Hospital Transfusion Practice.

The methods used and the results obtained in each study will be reported consecutively, and the significance of the observations discussed.

STUDY H

The object of this work has been to investigate the course of dilution following controlled haemorrhage in man (Wallace and Sharpey-Schafer 1941). Haemoglobin percentages, plasma proteins, blood-pressure and pulse changes have been followed, as well as blood volume, plasma-chloride and blood urea in a few cases.

METHODS

Volunteers were venesected and 23 observations were made. The subjects were convalescent hospital patients, and each one had a thorough clinical examination. All were found to have normal circulatory systems apart from three cases (Nos. 6, 7 and 9) who had hypertension. The clinical diagnoses of those with normal circulatory systems were neuroses (nine cases), chronic nervous diseases (eight cases), chronic bronchitis (two cases), convalescent lobar pneumonia (two cases), uncomplicated gastric ulcer, orthostatic albuminuria, undescended testicles and headaches (each one case). Two subjects (Nos. 2 and 7) were found to have low initial haemoglobin; both had been previously bled and regeneration of haemoglobin was not complete. Nos. 7 and 9, and 13 and 24 are the same subjects venesected on
on different occasions.

Venesection was performed in the usual manner through a wide-bore needle, the subject resting on a couch with the trunk at an angle of 30°. Care was taken to keep the subject warm and at a constant temperature. An initial resting period was allowed for blood-pressure, pulse and haemoglobin estimations to reach an equilibrium.

Haemoglobin was estimated by taking 0.02 ml. blood samples from a freely flowing ear puncture and estimating the percentage by a photoelectric method (Hill and Pincock 1941) accurate to 0.4% (standard error).

Plasma-proteins, plasma-chlorides and blood-urea were estimated by the methods of King, Haslewood and Delory (1937).

Blood-volumes were estimated in three subjects by a modified carbon-monoxide method. This was in principle similar to that described by Hartmann (1937), except for the use of red and infrared light instead of green and yellow light for measuring carboxyhaemoglobin.

**RESULTS**

The main data are shown in the table. (see p. 94)

**Blood-pressure** - Sixteen subjects showed a fall in blood-pressure, but in eight of these the pressure had returned to its previous level within an hour; three cases (Nos. 12, 26 and 28) showing this temporary reduction in blood-pressure developed a simultaneous bradycardia of 60 beats per minute or less; in eight subjects the fall in blood-pressure was prolonged up to as much as 24 hours, a group which included two hypertensive subjects.
**RESULTS OF CONTROLLED HEMORRHAGE IN MAN**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>Blood withdrawn (comb.)</th>
<th>Blood pressure (mm. Hg)</th>
<th>Pulse-rate (per min.)</th>
<th>Haemoglobin (%)</th>
<th>Plasma-proteins (g. per 100 c.c.m.)</th>
<th>Blood-volume (litres)</th>
<th>Plasma-chlorides (mgs. per 100 c.c.m.)</th>
<th>Blood-area (mg. per 100 c.c.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>M</td>
<td>M1150 12</td>
<td>105/70 70/58 PR+</td>
<td>19 hr.</td>
<td>8 hr.</td>
<td>8 hr.</td>
<td>8 hr.</td>
<td>8 hr.</td>
<td>8 hr.</td>
</tr>
<tr>
<td>2</td>
<td>44</td>
<td>M</td>
<td>M1150 15</td>
<td>115/70 105/58 PR+</td>
<td>20 min.</td>
<td>10 min.</td>
<td>10 min.</td>
<td>10 min.</td>
<td>10 min.</td>
<td>10 min.</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>M</td>
<td>M1150 10</td>
<td>115/80 105/80 PR+</td>
<td>15 min.</td>
<td>15 min.</td>
<td>15 min.</td>
<td>15 min.</td>
<td>15 min.</td>
<td>15 min.</td>
</tr>
<tr>
<td>4</td>
<td>49</td>
<td>F</td>
<td>M1150 8</td>
<td>120/80 120/90 PR+</td>
<td>20 min.</td>
<td>20 min.</td>
<td>20 min.</td>
<td>20 min.</td>
<td>20 min.</td>
<td>20 min.</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>F</td>
<td>M1150 11</td>
<td>140/70 45/1 PR+</td>
<td>8 hr.</td>
<td>8 hr.</td>
<td>8 hr.</td>
<td>8 hr.</td>
<td>8 hr.</td>
<td>8 hr.</td>
</tr>
<tr>
<td>6</td>
<td>54</td>
<td>M</td>
<td>M1150 16</td>
<td>200/100 80/40 PR+</td>
<td>24 hr.</td>
<td>24 hr.</td>
<td>24 hr.</td>
<td>24 hr.</td>
<td>24 hr.</td>
<td>24 hr.</td>
</tr>
<tr>
<td>7</td>
<td>42</td>
<td>M</td>
<td>M1150 12</td>
<td>200/100 44/1 PR+</td>
<td>24 hr.</td>
<td>24 hr.</td>
<td>24 hr.</td>
<td>24 hr.</td>
<td>24 hr.</td>
<td>24 hr.</td>
</tr>
<tr>
<td>8</td>
<td>40</td>
<td>M</td>
<td>M1150 18</td>
<td>90/70 90/70 PR+</td>
<td>6 hr.</td>
<td>6 hr.</td>
<td>6 hr.</td>
<td>6 hr.</td>
<td>6 hr.</td>
<td>6 hr.</td>
</tr>
<tr>
<td>9</td>
<td>42</td>
<td>M</td>
<td>M1150 12</td>
<td>120/120 172/18 PR+</td>
<td>6 hr.</td>
<td>6 hr.</td>
<td>6 hr.</td>
<td>6 hr.</td>
<td>6 hr.</td>
<td>6 hr.</td>
</tr>
<tr>
<td>10</td>
<td>56</td>
<td>M</td>
<td>M1150 10</td>
<td>120/80 115/89 PR+</td>
<td>6 hr.</td>
<td>6 hr.</td>
<td>6 hr.</td>
<td>6 hr.</td>
<td>6 hr.</td>
<td>6 hr.</td>
</tr>
</tbody>
</table>

**PR +** = Exaggerated response of blood-pressure to postural changes.  
**Vs.** = Venesection.  
**Max.** = Maximum.
The fall in pressure usually occurred with dramatic suddenness at the end of venesection, which was stopped at the first sign of a fall. Other symptoms appeared at the same time. The face became pale, the skin cold and clammy, and the respiratory rate frequently increased. Yawning was also a prominent feature. The patient complained of faintness, dizziness and nausea. There was frequently a desire to defecate and urinate, and one subject actually passed urine. Unconsciousness often supervened, with depression of respiration, cyanosis, twitching of the limbs, rotation of the eyes, disappearance of the heart sounds and absence of pulsation in the large arteries. Bradycardia occurred with the first fall in blood-pressure and persisted. Loss of consciousness was usually brief, but case 6 was unconscious for five minutes. Case 28 had a fall of blood-pressure, bradycardia and syncope after the removal of 500 c.cm. blood; he was then allowed to recover, and half an hour later a further 600 c.cm. was withdrawn without a significant change in blood-pressure or a recurrence of other symptoms.

In nine subjects the effect of posture on systolic blood-pressure and pulse was investigated after venesection. All showed a striking and immediate fall in systolic blood-pressure on sitting sharply upright on the couch. The extent of this fall was in every case greater than 40 mm. Hg and always occurred within 10 seconds of sitting upright. If the sitting position was maintained, in most cases the systolic blood-pressure rose within 30 seconds to its previous resting level or to a figure slightly lower than that resting level, but in a few cases syncope terminated the observation.
Fig. 1 illustrates this exaggerated response of systolic blood-pressure to alteration in posture compared with the normal response in the same individual after he had restored his original blood-volume.

The heart-rate, which was followed by continuous electrocardiographic tracings, showed a tachycardia with an increase of more than 30 beats per minute, except in two cases where a bradycardia developed. On lying back there was in every case a rapid return of the blood-pressure and heart-rate to normal.
The nine subjects who showed this response are indicated in the table by \( \text{hr} \). This exaggerated response to posture could be repeated at will, and some subjects still showed the response 5 or 6 hours after venesection.

**Pulse-rate** - Six subjects (Nos. 1, 5, 6, 12, 26, 28) showed bradycardia, always associated with a fall of blood-pressure; another five (Nos. 2, 3, 8, 9, 15) showed tachycardia with a rise of heart-rate of more than 20 beats a minute; the remainder had little or no change in heart-rate.

**Haemoglobin percentage and blood-volume** - Initial dilution as judged by the haemoglobin values was small. Eight subjects (Nos. 1, 3, 5, 6, 8, 19, 26, 28) showed a fall of Hb. of 5% or more and the rest less than 5%. The majority of the cases showed a maximum dilution which corresponded closely with the theoretical dilution calculated on the assumption that the blood-volume was five litres. Three subjects (Nos. 6, 21, 22), however, showed a maximum dilution which was considerably greater than the calculated figure, and in two of these subjects actual blood-volume estimations confirmed that there had been an overdilution, and that the blood-volume had increased over the initial value. In cases 19, 21 and 22 the theoretical dilution is calculated from the observed blood-volume. Case 13 seemed to show failure to dilute to the theoretical figure, though followed for a considerable period. A week later the same subject (24) was again venesected, and on this occasion showed a normal dilution. The time taken to reach a maximum dilution showed great variation, from 3 to 90 hours; no reason has yet been observed why some subjects take a long and others a short time to reach maximum dilution.
Plasma-protein, plasma-chloride and blood-urea - There was but little change in plasma-proteins while dilution was taking place. Cases 9 and 21 showed a fall, case 21 being one of those with over-dilution, while case 8, who had a rapid dilution, showed a rise in plasma-proteins. There was no significant change in plasma-chlorides after venesection. Blood-urea changes were also negligible except in cases 6, 7 and 9, who had hypertension with some renal involvement. The duration of the azotaemia in case 9 is illustrated in Fig. 2.

![Graph](image)

**Fig. 2**—Azotaemia following bleeding in subject 9 with malignant hypertension. Venesection of 1100 c.cm. was carried out at zero time.

**DISCUSSION**

The mechanism of dilution and regeneration of
of plasma-proteins and haemoglobin after experimental haemorrhage in animals has been reviewed by Whipple (1936). In man, Stead (1940) removed 760-1220 c.cm. of blood from six subjects. Five showed a sudden fall of blood-pressure and bradycardia. There was an immediate small dilution with protein-poor fluid, and dilution continued up to 72 hours without further changes in plasma-proteins. Our results show that the rate of dilution varies greatly in different subjects, and that therefore the haemoglobin percentage estimated at an arbitrary time after haemorrhage from an injury may give no indication of the extent of the haemorrhage. The rate of dilution may also vary in the same subject, as illustrated by cases 13 and 24, the same subject being vasectomised on two separate occasions at an interval of 5 weeks. There was no obvious change in conditions between the two observations, and regeneration of blood had taken place.

The dilution of haemoglobin is not paralleled by a dilution in plasma-proteins. In only one subject, case 21, was the small initial dilution associated with the passage of a protein-poor fluid from the tissues into the blood. Further fall of haemoglobin continued without dilution of plasma-proteins, suggesting that there are available proteins in the tissues which pass into the blood-stream and maintain its colloidal content.

The mechanism of this mobilisation of proteins is unknown, although there is evidence that the liver is an important source (Whipple 1936) and the cells of the reticuloendothelial system may play a part (Cutting and Gutter 1935). Over-dilution may occur occasionally, a phenomenon that might be of clinical importance when considering the transfusion of patients with low haemoglobin levels after haemorrhage, and indicating the
advantage of actual blood-volume estimations in such states.

The fall of blood-pressure in certain subjects has a practical application to "shocked" casualties and the bleeding of donors. Emotional causes seem unlikely in our subjects, since the fall occurred only at the end of a large venesection, and a sudden drop of blood-volume appears to be the initiating mechanism. The prolonged fall in a few cases is in accord with well-known clinical observations after haemorrhage. The exaggerated postural response may be part of the same mechanism, for it is known to happen in other conditions in which there is reduced venous return, such as long standing in the erect posture (Turner 1927) and untreated Addison's disease (Berry, Horton and Maclean 1940). Although an exaggerated postural response can occur in conditions in which there is no reduction of blood-volume, yet its presence in casualties suffering from haemorrhage may be one further indication that diminished blood-volume is present. Thus the postural response may be of value clinically to demonstrate reduction of blood-volume after suspected haemorrhage. The pulse-rate seems to be of little value after haemorrhage, since it may be slow, fast or show no change, an observation that agrees with the findings in air-raid casualties that have bled extensively.

The insignificant alteration in plasma-chloride confirms the observations made by Black (1940) after experimental haemorrhage in dogs. The same worker observed a slight rise in blood-urea, but this is not confirmed except in those cases in which there is pre-existing renal damage.

SUMMARY

Twenty-seven convalescent patients with normal...
circulatory systems were bled up to 1150 c.cm. and blood-pressure, pulse-rate, haemoglobin percentage, plasma-protein, plasma-chloride, blood-urea, and, in a few subjects, blood-volume were followed.

Sixteen showed a fall of blood-pressure with syncope; six of these had bradycardia. In half, the fall of blood-pressure was temporary; in the rest it was prolonged up to 24 hours. The response to posture (fall of blood-pressure on sitting up) was exaggerated in all of nine subjects investigated.

The heart-rate may be slowed, increased or unchanged after haemorrhage.

The time taken to reach maximum haemoglobin dilution varied greatly, from 3 to 90 hours average (32 hours). Three subjects showed over-dilution.

Plasma-protein concentration showed little or no change while dilution was taking place. Plasma-chloride showed no change, nor did the blood-urea except in cases with known renal impairment.

STUDY I

Introduction

The extensive use of solutions of human plasma proteins in therapeutics suggested an investigation into the behaviour of such solutions in the circulation. The fate of these solutions in the circulation has an important bearing on possible circulatory overloading.

Freeman and Wallace (1938), using the dye T-182, calculated the plasma volume in two dogs before and for three hours after the injection of concentrated serum. It was found
that the serum was retained in the circulation for this period, while saline and glucose solutions were not. Twenty-four hours later, however, there was no difference in plasma-volume whether serum or saline had been injected. Robertson (1938) found that both serum and saline disappeared rapidly from the circulation of normal anaesthetised cats, and of cats after an acute haemorrhage. In this species haemorrhage is followed by rapid dilution (Robertson 1935), while in man the process of dilution is slower (Ebert et al. 1941; Wallace and Sharpey-Schafer 1941). Florey and Jennings (1941) also found rapid disappearance of serum from the circulation of cats, and that there was no change in plasma proteins after injection of concentrated serum. Marriott and Kekwick (1940) state that the plasma volume remains constant after whole-blood transfusion to man, provided the transfusion is slow, suggesting that the plasma portion of the transfusion is eliminated from the circulation. In studies on the dilution of haemoglobin after injecting saline and serum (Hill et al. 1940) it was found that in subjects with stable blood volumes, dilution after saline was not maintained, while in four subjects after serum it was maintained. Figures for haemoglobin after serum injection published by Hayward (1942) also show dilution over one, two or more days in several cases.

The term "stable blood-volume" is used here to mean that the circulating volume has not been acutely reduced or increased by experimental procedures prior to the observations. In view of individual variations it is preferred at the present stage to the term "normal blood-volume", since the precise definition of normality in this connection is difficult.
The present study (Sharpey-Schafer and Wallace 1942) describes the effect on haemoglobin concentration of injecting serum into subjects with (a) stable blood-volumes, (b) blood volumes acutely reduced by venesection.

**METHODS**

The observations were made on convalescent subjects without circulatory disease.

The use of serial haemoglobin determinations to follow the retention of injected protein solutions in the blood-stream is open to possible criticism. The method of determination and of sampling, proper mixing, stores of red corpuscles, and effects of posture and temperature have all to be considered. It is also necessary to assume that injected serum has no considerable specific effect on the recipient's corpuscles. Since comparative readings only are required, no absolute haemoglobin standard, related to iron content or oxygen-carrying power, is necessary. There appears to be no evidence in man that stores of red corpuscles may be swept into the circulation and give false readings (Ebert and Stead 1941). Hahn and others (1942), from experiments on dogs, state that 20 per cent of the total plasma volume is contained in peripheral, cell-free, sluggishly moving plasma films, or in small vessels in which no red cells are present. It may therefore that haemoglobin determinations only reflect changes in the "rapidly circulating blood-volume".

Experiments were performed recumbent, with the trunk at an angle of 30°. Time was allowed at the beginning for the haemoglobin percentage to adjust itself to any small changes in posture. The subject was kept at a warm uniform
temperature. The standard hospital diet had been taken for some days previously, and no attempt was made to control water intake. All observations were started two hours after the midday meal. Haemoglobin was estimated by a photo-electric method (Hill and Pincock 1941), 0.02 ml. samples of blood being obtained from a freely flowing ear puncture. Plasma proteins were estimated by the method of King, Haslewood and Delory (1937). Protein solutions used were as follows.

(1) Dried serum, each bottle containing dried solids from 200 c.cm. human serum diluted to 200 c.cm. with distilled water, referred to in the text as serum; the estimated protein content of these solutions lay between 6 per cent and 8 per cent. (2) Similar bottles of dried serum diluted to 50 c.cm. referred to as concentrated serum; the protein concentration was between 25.6 and 33.4 per cent. (3) Serum-saline (Clegg and Dible 1940) was used in five experiments.

Recording of Results - The time scale in the graphs of haemoglobin percentages is arbitrary, since initial changes are rapid and thereafter slower; the same time scale has been used throughout. The control haemoglobin value in each experiment has been designated 0, and changes therefrom minus or plus percentages of haemoglobin. The actual haemoglobin value of each graph is given in the legend. In experiments performed after venesection, zero represents the haemoglobin percentage directly after venesection.

RESULTS

Stable blood-volume; serum injected - Haemoglobin percentages are shown in Fig. 1. In many subjects, initial dilution was considerable, but in none was it sustained, and over minutes or
hours the concentration of haemoglobin tended to return to the initial level.

![Fig. 1](image)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex and age (yr.)</th>
<th>Weight (lb.)</th>
<th>Initial Hb. (%)</th>
<th>Serum injected (c.cm.)</th>
<th>Duration of injection (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M. 21</td>
<td>123</td>
<td>88</td>
<td>1000</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>M. 27</td>
<td>135</td>
<td>100</td>
<td>1000</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>M. 57</td>
<td>165</td>
<td>110</td>
<td>1000</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>M. 30</td>
<td>145</td>
<td>85</td>
<td>1700</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>M. 42</td>
<td>151</td>
<td>103.2</td>
<td>800</td>
<td>27</td>
</tr>
<tr>
<td>6</td>
<td>M. 59</td>
<td>150</td>
<td>95</td>
<td>2100</td>
<td>16</td>
</tr>
<tr>
<td>7</td>
<td>M. 17</td>
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<td>102.3</td>
<td>1600</td>
<td>14</td>
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<tr>
<td>8</td>
<td>M. 42</td>
<td>126</td>
<td>64.5</td>
<td>1620</td>
<td>20</td>
</tr>
<tr>
<td>9</td>
<td>M. 49</td>
<td>**</td>
<td>100.2</td>
<td>1250</td>
<td>20</td>
</tr>
<tr>
<td>10</td>
<td>M. 55</td>
<td>161</td>
<td>100.2</td>
<td>1350</td>
<td>22</td>
</tr>
<tr>
<td>11</td>
<td>M. 50</td>
<td>155</td>
<td>104</td>
<td>2000</td>
<td>24</td>
</tr>
<tr>
<td>12</td>
<td>M. 31</td>
<td>150</td>
<td>68.5</td>
<td>1200</td>
<td>13</td>
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<td>M. 18</td>
<td>120</td>
<td>105.3</td>
<td>1200</td>
<td>18</td>
</tr>
</tbody>
</table>

When the initial dilution was great, there was evidence of acute overloading of the circulation; the venous pressure
became elevated, the vital capacity diminished, the X-ray contour of the heart enlarged, and there were changes in blood-pressure and pulse rate. These measurements will be described in detail elsewhere (Study J). In subjects 1, 2, 3, 4 and 5 initial dilution was small or nonexistent, and there was little or no evidence of overloading of the circulation. No special feature has been found to distinguish these subjects from the others. The experiments were not performed in abnormal weather conditions, when some change in water balance might be expected.

Transient generalised weal formation was seen in the skin on four occasions (subjects 2, 5, 9, 10). It appeared towards the end of, or a few minutes after, injection. This phenomenon has been noticed in dogs after the injection of serum (Freeman and Wallace 1938).
Stable blood-volume; concentrated serum injected. - Results (Fig. 2) are similar to those found with serum; thus in some experiments there is initial dilution and loss of diluting fluid over the next twenty-four hours; in others little or no initial dilution occurs. Initial dilution in some experiments is greater than would be produced theoretically by the addition to the circulation of the amount of "fluid" injected.

Concentrated serum solutions are of course also hypertonic salt solutions, and it is to be expected that they will behave in the well-known manner of such solutions, so that by drawing water into the circulation the resulting volume of diluting "fluid" becomes much greater than the original volume injected.

Subjects 16, 17, 18 and 19 showed a progressive fall of haemoglobin.
to levels lower than the initial fall. Plasma proteins showed no change directly after injection.

![Fig. 3](image-url)

Fig. 3

INJECTION OF SERUM AFTER VENESECTION

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex and Age (yr.)</th>
<th>Height (in.)</th>
<th>Initial Ht. (%)</th>
<th>Amount of Venesection (c.cm.)</th>
<th>Duration of Venesection (min.)</th>
<th>Serum (c.cm.)</th>
<th>Duration of serum injection (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>M 52</td>
<td>129</td>
<td>88</td>
<td>500</td>
<td>14</td>
<td>1200</td>
<td>20</td>
</tr>
<tr>
<td>24</td>
<td>M 57</td>
<td>165</td>
<td>106, 3</td>
<td>720</td>
<td>8</td>
<td>700</td>
<td>5</td>
</tr>
<tr>
<td>25</td>
<td>M 59</td>
<td>151</td>
<td>84</td>
<td>1050</td>
<td>19</td>
<td>2100</td>
<td>25</td>
</tr>
<tr>
<td>26</td>
<td>M 59</td>
<td>163</td>
<td>83</td>
<td>1080</td>
<td>16</td>
<td>300x</td>
<td>7</td>
</tr>
<tr>
<td>27</td>
<td>F 53</td>
<td>107</td>
<td>95</td>
<td>900</td>
<td>7</td>
<td>1300</td>
<td>23</td>
</tr>
<tr>
<td>28</td>
<td>M 50</td>
<td>115</td>
<td>95</td>
<td>1100</td>
<td>12</td>
<td>2100</td>
<td>25</td>
</tr>
</tbody>
</table>

x Concentrated serum
Reduced blood-volume: serum injected - Results (Fig. 3) indicate that serum injection given immediately after a large venesection produced immediate dilution which was maintained. Observations 6 and 25, 11 and 28, 3 and 24, 16 and 26 were made on the same subjects; the amount and rate of injection were the same when the blood-volume was stable and when it was reduced. There was no evidence of overloading of the circulation when these doses of serum were given after venesection. The venous pressure, which on bleeding showed either a slight fall or no change, did not rise above the control level on injecting serum; three subjects who showed a fall of blood-pressure and fainting after venesection were rapidly restored by the serum injection.

Reduced blood-volume: saline injected - In comparison with serum injection after venesection, similar quantities of 0.85% saline produced some initial dilution (Fig. 4), but thereafter the rising haemoglobin concentration indicates a loss from the circulation, and it was not until twenty-four hours or more that the normal process of dilution after haemorrhage again produced a fall in haemoglobin percentage. Plasma proteins fell immediately after saline injection, as has been shown by Gilligan, Altshule and Volk (1938).
The results of this work show two main features: the degree of retention in the circulation of injected serum depended on whether the blood-volume was stable or reduced; no significant change in plasma proteins was detected after injection of concentrated serum, although in some subjects there was little or no change in haemoglobin percentage.

It must be pointed out that in these experiments the reduction of blood-volume is acute, and there is no implication that the same results will be obtained in states where the blood-volume is chronically reduced and perhaps stabilised at a lower level. The retention of serum after a large venesection agrees with the results obtained in casualties suffering from acute blood loss (Hill et al. 1941). The possibility that the immediate sustained fall in haemoglobin represents a natural process of dilution after venesection may be rejected, since in 28 similar subjects no such immediate fall has been seen after simple venesection of the same amount of blood (Wallace and Sharpey-Schafer 1941), an observation
confirmed by Ebert, Stead and Gibson (1941). A comparison, too, with the results of saline injection after venesection shows that it is the injected serum which is responsible for sustained dilution.

Less is known of the effects to be expected from acute increase in the blood-volume, such as may occur when a large injection of serum is given rapidly to a normal person. In clinical medicine a blood-volume greater than normal is found only in exceptional circumstances such as polycythaemia, overdosage with desoxycorticosterone acetate (Ferrebee et al. 1939), and possibly congestive heart-failure (Wollheim 1929), arteriovenous shunts (Holman 1940) and over-dilution following haemorrhage (Ebert, Stead and Gibson, 1941, Wallace and Sharpey-Schafer 1941). In all these, the increase is relatively slow. It is not surprising, in acute experiments, that the physiological mechanisms of a normal man are directed against any lengthy endurance of a raised blood-volume with such sequels as high venous pressure, diminished vital capacity, enlarged heart, &c. Though there is a considerable individual variation in time, it appears that the blood-volume tends to return to the original lower level by a loss of excess fluid from the circulation. Our results in man, therefore, confirm experiments in the dog and cat, though in the cat elimination of injected serum appears to be very rapid (Florey and Jennings 1941).

Experiments in which there is no fall of haemoglobin and no change in plasma proteins after the injection of large quantities of concentrated serum allow of two explanations, provided it is accepted that haemoglobin figures
are not altered by processes such as the sweeping into the
circulation of large stores of corpuscles; either large
quantities of protein leave the blood-stream in a few minutes,
or else a natural flow of proteins into the blood-stream is
suddenly stopped. The latter seems improbable since in some
experiments it would mean a natural flow of some 80 g. of
protein in ten minutes, and observations on haemodilution and
plasma-protein levels after haemorrhage do not suggest that the
human body is capable of such a rate of production (Wallace and
Sharpey-Schafer 1941). The constant level shown by the plasma
proteins in these experiments, and in dilution following
haemorrhage, is further evidence in favour of the view put
forward by Madden and Whipple (1940) that plasma protein "is
part of a balanced system of body proteins. A steady state or
ebb and flow exists between it and a portion of the cell and
tissue body protein".

The occurrence of transient weal formation in a
few experiments suggests that loss of fluid may occur generally
in the tissues. Further work, however, is necessary on the
 eventual fate of "lost" protein. It does not appear in the
urine, and a few preliminary observations indicate that there
is no increased nitrogen excretion in the days following the
injection of large amounts. Daft, Robscheit-Robbins and
Whipple (1938), in similar experiments on dogs, showed that
large doses of protein could be injected for many days before
"protein intoxication" and increase in urinary nitrogen
appeared. A total dose of 700 g. or more of protein would be
required in man, if the response was similar to the dog.
The degree of retention of injected serum has been followed by serial haemoglobin determinations in subjects with stable and acutely reduced blood-volumes.

When the blood-volume is stable, serum tends to leave the circulation, and, though in many subjects, it is retained longer than an equal quantity of saline, in a few it is lost with great rapidity.

Concentrated serum behaves in a similar manner, and since no change in plasma proteins was detected there is evidence in some experiments that protein left the circulation rapidly.

When the blood-volume is acutely reduced by a large venesection, serum injected immediately is retained in the circulation. Saline is not retained under the same circumstances.

It is suggested that mechanisms in man dealing with injected protein solutions are directed towards attaining the previous stable blood-volume.

**STUDY J**

In this study observations have been made on the complication of circulatory overloading following rapid intravenous injections (Sharpey-Schafer and Wallace 1942b).

**Introduction**

Authorities differ widely in advising the amount of serum or blood to be injected and the rate at which it should be given. Many stress the dangers of overloading the circulation. In 1894 Bayliss and Starling showed that rapid injection of crystalloid solutions or blood caused a rise of
venous pressure, observations which have been amply confirmed by others in the experimental animal (Bainbridge 1915; Wiggers 1921). Meek and Eyster (1922) showed in dogs that the immediate effect of an intravenous injection was a rise of venous pressure associated with an increase in the diastolic size of the heart; these effects were only transitory, and there was evidence that the peripheral capillaries and venules were acting as reservoirs. There have been fewer observations in man. Eyster and Middleton (1924) investigated 4 normal subjects. In the case showing the most pronounced change 600 c.cm. of blood was given at the rate of 43 c.cm. a minute; this produced a rise in venous pressure of 2 c.m. H2O but no increase in the size of the heart. Gaughey (1935) found no rise of venous pressure in normal subjects receiving 1,500 c.cm. of saline at the rate of 50 c.cm. a minute, while a rise of up to 6 cm. H2O was produced in some patients with heart disease. No attempt was made to measure the degree of retention of injected saline in the circulation. Altschule and Gilligan (1938) gave from 500 to 1,500 c.cm. of crystalloid solutions to 35 subjects at rates of 6 to 71 c.cm. a minute. The venous pressure was raised more than 4 cm. H2O in only 6 subjects, pulse and blood-pressure changes were small, the cardiac output was increased, but there was no change in vital capacity. This paper reports the results of rapid intravenous injection of saline, serum, and, in a few cases, blood on the venous pressure and certain other cardiovascular measurements.

**METHODS**

The observations were made on convalescent subjects without cardiovascular disease. The conditions of study have been given elsewhere (Sharpey-Schafer and Wallace 1942a). (see Study I)
The haemoglobin was estimated on 0.02 ml. freely flowing ear samples by a photo-electric method (Hill and Pincock 1941). Venous pressures were estimated in the antecubital vein by a continuous citrate method (Wood, 1940), and changes were checked by direct observation of the neck veins (Lewis 1937). Up to 45 minutes may have to be allowed for the venous pressure to reach a steady level. Vital capacity was measured on a Benedict-Roth spirometer. Radiographs of the heart were taken in the sitting posture, with the tube at 6 ft. The exposure was 1 second to obtain the diastolic heart size. Differences in inclination or respiratory excursion are indicated in the text. Usually two control and several post-injection films were taken. The right pectoral-F or the right pectoral-R leads (Wood and Selzer 1939) were employed in the electrocardiographic studies. The following solutions were used: (1) 0.9% saline; (2) normal strength reconstituted lyophil serum; (3) 4-times-concentrated reconstituted lyophil serum; (4) citrated blood.

**RESULTS**

The results are shown in Table 1. When the rate of injection was between 50 and 150 c.cm. a minute the venous pressure began to rise after about 1,000 c.cm. of saline or serum had been given, and reached a maximum toward the end of injection (Fig.1). If the rate of injection was very rapid, as little as 500 c.cm. raised the venous pressure. The subject shown in Fig. 2 received the first 500 c.cm. of serum at a rate of 250 c.cm. a minute. All subjects given saline or serum showed a rapid fall of venous pressure once the injection had been stopped. Saline often left the circulation quickly, as
<table>
<thead>
<tr>
<th>Case</th>
<th>Age and Sex</th>
<th>Amount Injected (c.cm.)</th>
<th>Rate of Injection (c.cm./min)</th>
<th>Initial Hb %</th>
<th>Immed. Hb % Fall</th>
<th>V.P. Rise (cm. H₂O)</th>
<th>Pulse Before</th>
<th>Vital Capacity</th>
<th>E.G.</th>
<th>Symptoms</th>
</tr>
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<tbody>
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<td>0</td>
<td>44 M</td>
<td>1,620</td>
<td>80</td>
<td>64·5</td>
<td>15·5</td>
<td>11·0</td>
<td>3·8</td>
<td>3·4</td>
<td></td>
<td></td>
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<td>1a</td>
<td>17 M</td>
<td>1,600</td>
<td>114</td>
<td>102·3</td>
<td>23·5</td>
<td>10·5</td>
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<td>9·0</td>
<td>105</td>
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<td>2a</td>
<td>52 M</td>
<td>2,000</td>
<td>82</td>
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<tr>
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<td>700</td>
<td>154</td>
<td>95</td>
<td>18·6</td>
<td>8·5</td>
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<td>18·3</td>
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<td>1,400</td>
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<td>62·8</td>
<td>5·9</td>
<td>4·0</td>
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<td>75</td>
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<tr>
<td>7a</td>
<td>61 M</td>
<td>1,500*</td>
<td>60</td>
<td>54</td>
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<td>75</td>
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<tr>
<td>8a</td>
<td>40 M</td>
<td>1,000*</td>
<td>71</td>
<td>54</td>
<td>3·0</td>
<td>3·0</td>
<td>4·0</td>
<td>75</td>
<td>105</td>
<td>Slight constriction in chest. Headache</td>
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</tbody>
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**Concentrated Serum**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age and Sex</th>
<th>Amount Injected (c.cm.)</th>
<th>Rate of Injection (c.cm./min)</th>
<th>Initial Hb %</th>
<th>Immed. Hb % Fall</th>
<th>V.P. Rise (cm. H₂O)</th>
<th>Pulse Before</th>
<th>Vital Capacity</th>
<th>E.G.</th>
<th>Symptoms</th>
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<td>9a</td>
<td>59 M</td>
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<td>30</td>
<td>84·2</td>
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<td>10·0</td>
<td>10·0</td>
<td>10·0</td>
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</tr>
<tr>
<td>10b</td>
<td>21 M</td>
<td>300</td>
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<td>95</td>
<td>19·9</td>
<td>6·0</td>
<td>6·0</td>
<td>6·0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11b</td>
<td>17 M</td>
<td>300</td>
<td>21</td>
<td>93·2</td>
<td>11·6</td>
<td>4·0</td>
<td>4·0</td>
<td>4·0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12b</td>
<td>48 M</td>
<td>300</td>
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<td>66·6</td>
<td>9·6</td>
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</tr>
<tr>
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<td>0·5</td>
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</table>

* Blood.
indicated by a rising haemoglobin percentage (Fig. 1.) The venous pressure still fell in a matter of minutes, however, if the haemoglobin remained at the lower post-injection level (Fig. 2.).

Fig. 1.—Case 1. Venous pressure and Hb% change after saline. Zero represents control level of venous pressure or Hb%.

One subject given blood showed a rise of venous pressure that persisted for 50 minutes. When the blood volume was first
reduce by a large venesection, injection of serum or saline in similar quantities and at similar rates produced little or no change in venous pressure (Table II and Fig. 3).

Table II.—Injection after Venesection

<table>
<thead>
<tr>
<th>Case</th>
<th>Age and Sex</th>
<th>Initial Hb%</th>
<th>Amount of Venesection (c.cm.)</th>
<th>Rate of Venesection (c.cm./min.)</th>
<th>Hb% Change directly after Venesection</th>
<th>Amount Injected (c.cm.)</th>
<th>Rate of Injection (c.cm./min.)</th>
<th>Immed. Hb% Fall</th>
<th>V.P. Change (cm. H$_2$O)</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>31 M</td>
<td>108</td>
<td>900</td>
<td>90</td>
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<td>1,650</td>
<td>138</td>
<td>15.5</td>
<td>0</td>
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<tr>
<td>3</td>
<td>37 M</td>
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<td>740</td>
<td>105</td>
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<td>140</td>
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<td>+2.5</td>
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<tr>
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<td>57 M</td>
<td>108</td>
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<td>1,500</td>
<td>88</td>
<td>9.0</td>
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<table>
<thead>
<tr>
<th>Case</th>
<th>Age and Sex</th>
<th>Initial Hb%</th>
<th>Amount of Venesection (c.cm.)</th>
<th>Rate of Venesection (c.cm./min.)</th>
<th>Hb% Change directly after Venesection</th>
<th>Amount Injected (c.cm.)</th>
<th>Rate of Injection (c.cm./min.)</th>
<th>Immed. Hb% Fall</th>
<th>V.P. Change (cm. H$_2$O)</th>
<th>Symptoms</th>
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</thead>
<tbody>
<tr>
<td>2b</td>
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<td>104.7</td>
<td>1,100</td>
<td>100</td>
<td>-3.0</td>
<td>2,000</td>
<td>77</td>
<td>33.5</td>
<td>0</td>
<td>Fall of B.P. Syncope</td>
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<tr>
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<td>59 M</td>
<td>84</td>
<td>1,080</td>
<td>81</td>
<td>-4.0</td>
<td>2,100</td>
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</tr>
<tr>
<td>4c</td>
<td>17 M</td>
<td>108.4</td>
<td>1,100</td>
<td>36</td>
<td>-5.8</td>
<td>1,600</td>
<td>80</td>
<td>20.6</td>
<td>0</td>
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</tr>
<tr>
<td>5e</td>
<td>54 M</td>
<td>106.3</td>
<td>740</td>
<td>146</td>
<td>-1.0</td>
<td>700</td>
<td>140</td>
<td>10.8</td>
<td>0</td>
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</tr>
<tr>
<td>5f</td>
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<td>900</td>
<td>112</td>
<td>-4.0</td>
<td>1,500</td>
<td>80</td>
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<td>Fall of B.P. Syncope</td>
</tr>
<tr>
<td>9b</td>
<td>59 M</td>
<td>83</td>
<td>1,080</td>
<td>71</td>
<td>0</td>
<td>300*</td>
<td>43</td>
<td>17.3</td>
<td>0</td>
<td></td>
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</tbody>
</table>

* Concentrated serum.

Fig. 3.—Case 12a and 12b: interval of 10 days between observations.

The subject of Fig. 3 showed a small fall of venous pressure after venesection, associated with a fall of blood pressure and syncope. In this and other subjects with syncope after
Large venesections (Wallace and Sharpey-Schafer 1941) a small fall of venous pressure (1 to 2 cm.) preceded the fall of blood pressure, and there was a further fall of venous pressure with collapse of the circulation. If there is no fall of blood pressure after venesection, there is, however, an abnormal fall on sitting upright.

Weiss, Wilkins, and Haynes (1937) showed that the fall of blood pressure and syncope in the upright posture after sodium nitrite is preceded by a fall in venous pressure. Injection of serum immediately after venesection restored venous pressure, blood pressure, or postural response to normal. When giving intravenous injections at rates of 100 c.cm. or more a minute considerable pressure must be maintained in the bottle of fluid; measurements of venous pressure in the opposite antecubital vein might therefore merely reflect this transmitted pressure. That this is not so, and that a rise of venous pressure is due to an acutely increased blood volume, is shown by the following considerations: (1) When the blood volume has not been previously reduced a rise of venous pressure does not occur until about 1,000 c.cm. has been given, at rates of about 100 c.cm. a minute.

(2) There is a statistically significant correlation between the rise of venous pressure and the fall of haemoglobin (Fig. 4).

(3) There is little or no rise of venous pressure when saline or serum is injected immediately after blood-volume reduction by venesection. (4) If, however, enough time elapses after venesection for the haemoglobin to fall considerably, transfusion of similar quantities then causes a rise of venous pressure.

Vital Capacity - The vital capacity, measured within 2 minutes of the end of injection, showed an average fall of 1470 c.cm. in 6 of 7 subjects (Table I). McMichael and McGibbon (1939) have
shown that there is a fall in the lung volume and vital capacity of normal subjects on assuming the horizontal posture;

![Graph showing relation between rise of venous pressure and fall in haemoglobin. Coefficient of correlation (r) = 0.84.](image)

they ascribed the fall to the presence of more blood in the lungs. Venesection, on the other hand, increases the vital capacity (Glaser and McMichael 1940). That the fall of vital capacity on overloading the circulation is due to increased blood in the lungs is borne out by the X-ray appearances. None of these subjects showed clinical evidence of pulmonary oedema, and the respiratory rate and tidal air showed no change.

**Radiograph of Heart** - Films were taken before and immediately after injection. The results are shown in Table III. The area of the cardiac shadow was measured by a planimeter, and the right, left, and oblique cardiac and transverse chest diameters were also measured. In 5 of the 8 subjects examined the average increase in the heart area was 15.3%. Table I shows
<table>
<thead>
<tr>
<th>Case</th>
<th>Heart Area (sq. cm.)</th>
<th>Right Diam. (cm.)</th>
<th>Left Diam. (cm.)</th>
<th>Oblique Diam. (cm.)</th>
<th>Transverse Chest (cm.)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
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<tr>
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<td>5b</td>
<td>150</td>
<td>158</td>
<td>4.0</td>
<td>4.7</td>
<td>10.6</td>
</tr>
</tbody>
</table>

Diaphragm 1 cm. higher in films after slight inclination of body forward in films after Diaphragm 1.2 cm. higher in films after

that there was a considerable rise of venous pressure and fall of haemoglobin in each of these 5 subjects. Although the latter changes were present in Case 2, there was but little cardiac enlargement. So far as could be judged from postero-anterior films the enlargement appeared to involve all chambers. In addition, the following changes were present after injection: the superior vena cava shadow became prominent in those subjects in whom there was a considerable rise of venous pressure, the pulmonary arteries and vascular markings in the lungs increased in size and density, and the whole lung fields were relatively less translucent (Figs. 5 and 6).

**Electrocardiogram** - The right pectoral-R arm or right pectoral-L leg electrocardiogram is the chest lead that gives one of the earliest indications of acute right heart stress, as from packed pulmonary embolism (Wood 1941). Changes in the T-wave are in the direction of inversion when chest leads are recorded in the manner agreed by the Cardiac Society of Great Britain.
and Ireland and the American Heart Association (British Medical
Journal, 1938, 1, 187). Only 4 of 12 subjects showed
electrocardiographic changes, the T-wave becoming flattened or
inverted. Gilligan and Altschule found increased amplitude of the P-wave in lead II in a few of their cases. There were no P-wave changes in leads II, III, or V in this series. The X-ray findings suggest that enlargement of the heart shadow may involve all chambers in some subjects. Electrocardiographic changes in the direction of preponderance of ventricle would therefore not be expected, since it is well known that cardiac enlargement can take place without them. However, it has not proved possible so far to observe the correlation, if any, between electrocardiographic changes and X-ray appearances after acute overloading of the circulation.

Heart Rate - The heart rate showed no change or slowing in 9 subjects, while 6 showed an increase.

Symptoms - Eight subjects had headache; 4 complained of a feeling of constriction in the chest during injection; in 1 it was severe, but not severe enough to necessitate termination of the injection. It passed off rapidly as soon as intravenous fluid was stopped. The most striking sign was vasodilation; it was present in all subjects with any degree of retention of saline or serum in the circulation. The face became redder in tint, and the peripheral veins became prominent and distended.

SUMMARY

Up to 2,000 c.cm. of saline, serum, and blood was injected into subjects without cardiovascular disease at rates of from 54 to 168 c.cm. a minute. The venous pressure was raised up to 11 cm. H₂O when there was considerable retention of injected fluid in the circulation, as indicated by the fall in haemoglobin. Radiographs showed an increase in the diastolic size of the heart, enlargement of the pulmonary arteries, and
prominence of the vascular markings in the lung fields. Vital capacity was diminished, but there was no evidence of pulmonary oedema. In spite of the rise of venous pressure many subjects had no increase in heart rate. Of 12 subjects 4 showed electrocardiographic changes indicating slight right heart stress. Symptoms were absent or unimportant. There was a rapid fall of venous pressure to normal on ceasing injection, except in one subject given blood. There is evidence that the peripheral and pulmonary capillaries and veins dilate to accommodate the increased blood volume.

When the blood volume was first reduced by a large venesection, saline or serum injected in similar amounts and at similar rates caused little or no rise of venous pressure.

STUDY K

This study is concerned with the circulating blood-volume in patients suffering from Anaemia.

The commonest indication for blood transfusion is anaemia either from blood loss or from other causes. It is of practical importance to determine what changes, if any, occur in the circulating blood volume in the severely anaemic patient.

The observations of Gibson et al. (1939) suggested that the total blood volume was below normal in chronic anaemias, irrespective of the aetiology. It is important however, to distinguish between anaemia due to blood loss and anaemia from other causes. A reduction in blood-volume follows blood loss (Robertson and Bock, 1919). The physiological restoration of blood-volume after acute haemorrhage in man may be slow (Schert et al. 1941, Wallace and Sharpey-Schafer 1941). It is difficult
therefore, in patients suffering from haemorrhage, to ascertain whether reduction in blood volume is part of a state of chronic anaemia or is due to recent haemorrhage. In diseases such as Addisonian anaemia any reduction in blood volume must result from physiological disturbances other than haemorrhage.

Methods for the clinical study of blood volume require careful selection. In the past, two methods have been available, the carbon monoxide method and the dye injection method. Plasma volume measurement by various dyes is technically difficult and open to sources of error. The dye must leave the blood stream slowly, and it must not be absorbed by the red cells. The carbon monoxide method may be dangerous in anaemic patients.

Red blood cells are natural inhabitants of the circulation, and may require to be administered to anaemic patients. The concentrated corpuscle-haemoglobin (CC-Hb) method (Hill 1941) for the estimation of blood volume in anaemic patients is useful. This study reports the results obtained by this method in ten patients suffering from anaemia.

**Concentrated Corpuscle-Haemoglobin (CC-Hb) Method**

Compatible donor blood of the same group as that of the recipient is selected. The concentrated corpuscles are prepared by siphoning off the supernatant plasma from bottles of whole blood which have been centrifuged in a bottle-centrifuge or have been allowed to settle by standing. The infusions used in the present study varied in volume from 400 ml. to 650 ml., and in haemoglobin content from 99 to 141 per cent Haldane. The corpuscles were injected rapidly, the haemoglobin of the recipient being measured before and after transfusion. The initial blood-volume ($x$) was calculated from the formula
\[ x = V \frac{(Hb_y - Hb_2)}{Hb_2 - Hb_1} \]

where \( V \) = volume of blood injected in ml., \( Hb_y \) = haemoglobin percentage of injected blood, \( Hb_1 \) = haemoglobin percentage of recipient's blood before transfusion, and \( Hb_2 \) = haemoglobin percentage of recipient's blood after transfusion.

The above formula is based on the assumption that (1) mixing is complete and sampling accurate, (2) injected corpuscles are not lost from the circulation, (3) there are no plasma shifts.

The last is the most serious source of possible error for if \( y \) ml. of plasma are displaced out of the recipient's circulation before \( Hb_2 \) is estimated, then \( x \cdot Hb_1 + V \cdot Hb_y = (x + V - y) \cdot Hb_2 \) and thus

\[ x = \frac{V(Hb_y - Hb_2)}{Hb_2 - Hb_1} + \frac{y \cdot Hb_2}{Hb_2 - Hb_1} \]

Hill (1941) assumed that \( y \) was zero, but if \( y \) is 100 to 200 ml. (a loss that would be impossible to detect) then the error in Hill's formula increases as the ratio \( \frac{Hb_2}{Hb_1} \) increases. For example

\[ \frac{Hb_2}{Hb_1} = 5 \]

if \( y = 200 \) ml. and \( \frac{Hb_2}{Hb_1} = 5 \) then Hill's formula gives an initial blood volume which is low by 1000 ml. When \( Hb_2 \) is small and \( Hb_2 - Hb_1 \) is large, the possible error is decreased. The method is therefore least liable to this error in severely anaemic patients. If on the other hand fluid is added to the circulation during transfusion, Hill's formula gives a result which is too high.

The studies were made at various times of the day with the patient supine. Haemoglobin was estimated on a freely flowing ear sample of blood by a photo-electric method (Hill and Pincock 1941). Duplicate samples were taken before and 3 - 5 minutes after transfusion.

**RESULTS**

The results obtained by the CO - Hb method are shown in Table I.
### TABLE I - Results with the CO - Hb Method

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex and Age (yrs.)</th>
<th>Weight (kg.)</th>
<th>Blood Pressure</th>
<th>Initial Hemoglobin</th>
<th>Hb % of Trans. Fused blood</th>
<th>Initial Blood Volume (litres)</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M, 68</td>
<td>60</td>
<td>120/50</td>
<td>25</td>
<td>45</td>
<td>130</td>
<td>2.04</td>
</tr>
<tr>
<td>2</td>
<td>F, 51</td>
<td>53</td>
<td>130/65</td>
<td>32</td>
<td>48</td>
<td>144</td>
<td>2.61</td>
</tr>
<tr>
<td>3</td>
<td>F, 65</td>
<td>53</td>
<td>140/75</td>
<td>50</td>
<td>54</td>
<td>132</td>
<td>3.39</td>
</tr>
<tr>
<td>4</td>
<td>F, 45</td>
<td>54</td>
<td>120/60</td>
<td>29</td>
<td>53</td>
<td>121</td>
<td>1.79</td>
</tr>
<tr>
<td>5</td>
<td>M, 38</td>
<td>62</td>
<td>110/75</td>
<td>32</td>
<td>55</td>
<td>140</td>
<td>2.07</td>
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<td>6</td>
<td>F, 69</td>
<td>54</td>
<td>125/60</td>
<td>30</td>
<td>45</td>
<td>136</td>
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</tr>
<tr>
<td>7</td>
<td>F, 34</td>
<td>67</td>
<td>105/65</td>
<td>33</td>
<td>49</td>
<td>136</td>
<td>2.72</td>
</tr>
<tr>
<td>8</td>
<td>F, 40</td>
<td>55</td>
<td>125/70</td>
<td>43</td>
<td>56</td>
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<tr>
<td>10</td>
<td>F, 48</td>
<td>62</td>
<td>130/60</td>
<td>50</td>
<td>52</td>
<td>130</td>
<td>3.26</td>
</tr>
</tbody>
</table>

Cases 1 to 4 and Case 10 may be classified as chronic anaemia not due to haemorrhage. The systolic blood pressure in these cases was within normal limits at the time of the blood-volume estimation, although all show an increased pulse pressure. The venous pressure was raised above normal in cases 1 and 4. In Cases 5 to 9 there had been previous haemorrhage, but the blood pressure was well maintained except in case 7.
in which haemorrhage was probably recent and acute.

**DISCUSSION**

The primary object of this work was the estimation of the circulating blood volume in patients suffering from anaemia, and then to observe the relationship of any blood volume changes to circulatory dynamics.

The finding of a marked reduction of circulating blood volume in post-haemorrhagic anaemia (Cases 5 to 9) has an obvious explanation. Gibson et al. (1939) also found a reduction of the circulating blood volume in chronic anaemia, but the reduction in cases 1, 2 and 4 of the present series was unexpectedly great. However these cases had a more severe grade of anaemia than those of Gibson et al. (1939), and since it appears from Gibson's findings that the more severe the anaemia the lower the blood volume, results round 2.0 litres in a few cases by the CC - Hb method are to be expected. These findings are in agreement with those of McNicholas et al. (1943) using the CC - Hb method.

It was of interest to find a normal systolic blood pressure and a low diastolic pressure in association with gross reduction of circulating blood volume. In acute blood loss a reduction in blood volume to 2 litres is associated with profound circulatory collapse. It is apparent therefore that chronic reduction of blood-volume is associated with certain physiological adjustments which ensure the maintenance of a normal or an increased cardiac output.

The venous pressure was raised above normal in cases 1 and 4, which showed a marked reduction of blood volume. As these cases presumably had a normal or increased cardiac output, it is probable that the high filling pressure of the heart is
maintained by an increase in venous tone. If this is indeed a fact, then transfusion in such cases is potentially dangerous. The addition of fluid to the circulation will raise the filling pressure even more, and may lead to a falling cardiac output and pulmonary oedema.

**SUMMARY**

The circulating blood volume was estimated in five cases of chronic anaemia and in five cases of post-haemorrhagic anaemia by the concentrated-corneal haemoglobin method.

All cases showed a diminished blood-volume, with two cases as low as 2 litres. In spite of this marked reduction, the systolic pressure was maintained. This indicates the association of physiological adjustments to maintain cardiac output.

The potential danger of transfusion in such cases is suggested.

**STUDY I**

The preceding studies in this chapter have clearly demonstrated that circulatory overloading is a potential danger in transfusion therapy, particularly in cases in which the volume administered is large and the rate is rapid. The danger appears to be greatest in the severely anaemic patient, for in these cases there is a reduced blood volume with a compensatory increase in venous pressure in order to maintain the cardiac output.

In the present study an attempt has been made to assess the frequency and the severity of circulatory overloading as a complication of blood transfusion in hospital practice. This report is based on the author's experience in a Regional Transfusion Service during the past three and one half years.
The cases described have been observed by the author, and particular attention has been given to the clinical condition for which transfusion was given, and to the volume and rate of transfusion. During the period in which these observations were made, approximately 27,000 patients received blood, and the ten cases described are the only cases in which circulatory overloading was thought to have occurred.

Case 1 - This patient was a male aged 35 years, suffering from ulcerative colitis with gross anaemia, the haemoglobin being 5 g. per cent.

The patient was transfused with blood of the homologous Group O, Rhesus positive (D positive). After the administration of approximately 200 ml. of this blood in one hour, the patient was very flushed and complained of fullness in the head and a feeling of warmth. There was also tightness in the chest. The patient became rapidly dyspnoeic, and there was marked cyanosis. The temperature was 98.4°F., pulse 90 and the respiratory rate 20 per minute. As there was also fullness of the neck veins, it was considered that the patient was suffering from circulatory overloading, and the transfusion was discontinued. Thereafter the patient's condition rapidly improved.

The donor blood was investigated and found to be perfectly normal in appearance and sterile. The donor blood was rechecked as Group O, Rhesus positive (D positive), and the recipient was also Group O, Rhesus positive (D positive). Compatibility tests were perfectly satisfactory. In all probability this was a case of circulatory overloading resulting from the rapid infusion of blood to a patient with a severe anaemia.
This patient was a married woman aged 36 years who had previously borne six healthy children. She was pregnant for the seventh time, and it was discovered at the ante-natal clinic that she had a marked anaemia for which hospital treatment was advised, but the patient refused to go into hospital. The baby was born at home, the delivery being spontaneous, and the third stage was completed five minutes after the birth of the child; the duration of labour was 3½ hours.

The patient was so weak immediately after delivery that her own medical practitioner admitted her to hospital.

On admission she was very weak, and had a marked pallor with slight cyanosis of the lips. There was pitting oedema of the ankles; the liver and spleen were not enlarged, and there were no palpable superficial glands. No abnormality of the respiratory system was detected on physical examination. The heart showed no enlargement, but there was a soft systolic murmur present at the apex, probably of the functional variety; the pulse was rapid and of poor quality; the blood pressure was 140/80.

The haemoglobin was 3.6 g. per cent, and the red cell count 1.2 million per c.mm. The blood film was characteristic of a hypochromic anaemia. In view of the marked anaemia it was decided to transfuse the patient immediately.

A transfusion of blood of the homologous Group A, Rhesus positive (D positive) was commenced, but after the administration of 250 ml. in 1½ hours, the patient had a severe rigor and complained of tightness in the chest. There was marked dyspnoea, cyanosis, and engorgement of the neck veins. The transfusion was stopped, but the patient continued to have
acute respiratory distress, and died a few hours later. Permission for a post-mortem examination was refused. The donor blood was re-examined and found to be normal in appearance. There was no bacterial contamination, and the blood was checked as Group A, Rhesus positive (D positive). On clinical grounds this appeared to be a case of circulatory overloading due to the rapid infusion of a patient with a severe anemia. The death of the patient was the immediate result of circulatory overloading.

Case 3 - This patient was a male aged 70 years, who had been under treatment for some years on account of pernicious anemia. On admission to hospital the blood findings were as follows: Haemoglobin 7 g. per cent, red cell count 1.5 m. per c.mm., P.C.V. 23 volumes per cent, M.C.V. 153 cu. μ, M.C.H.C. 30 per cent. The blood film showed anisocytosis, poikilocytosis, and macrocytosis.

It was decided to transfuse the patient with blood of the homologous Group O, Rhesus positive (D positive). Two bottles of compatible blood were provided by the Regional Transfusion Service. After the transfusion of approximately 500 ml. in two hours, the patient developed severe dyspnoea with cyanosis and engorgement of the neck veins. Physical examination of the chest revealed numerous rhonchi and rales. The transfusion was immediately discontinued, and the patient's respiratory embarrassment subsided.

The donor blood was rechecked as Group O, Rhesus positive (D positive) and found to be perfectly compatible with the patient's serum. The clinical impression was that this reaction was due to overloading of the circulation.

Case 4 - This patient was a male aged 69 years. He had
recently had a coronary thrombosis, and from time to time suffered severe attacks of angina, mostly on effort, but occasionally at rest. As the patient was severely anaemic it was considered that raising the haemoglobin level with small blood transfusions might relieve the symptoms of angina. He was, accordingly, given a transfusion of the homologous Group O, Rhesus positive (D positive). After infusion of 200 ml. of this blood in one hour, the patient had a severe attack of angina. The transfusion was discontinued, and the attack passed off. The clinical impression was that the additional load on the heart, resulting from the infusion of blood, precipitated the anginal attack.

**Case 5** — This patient, a male aged 32 years, was admitted to hospital with a severe anaemia, the blood picture being as follows: Haemoglobin 5.6 g. per cent, red cell count 1.2 m. per c.mm., white blood count 1,200 per c.mm., E.C.V. 18 volumes per cent, M.C.V. 150 cu. µ M.C.H.C. 31.1 per cent. The blood film was characteristic of a macrocytic anaemia. The patient was transfused with blood of the homologous Group A, Rhesus positive (D positive). After the administration of 100 ml. in 30 minutes the patient became extremely dyspnoeic with marked cyanosis and engorgement of the neck veins. There were numerous rhonchi and rales on physical examination of the chest. The transfusion was discontinued. Respiratory embarrassment was not relieved, and the patient died three hours later.

A post-mortem examination showed that the patient had been suffering from pernicious anaemia. There was an early tuberculous infection of the lungs. The lungs also showed considerable oedema. It was considered that the immediate cause
of death was circulatory overloading following blood transfusion.

Case 6 - This patient was a married woman, aged 39 years, who had recently been delivered of her third child. She was admitted to hospital soon after delivery on account of severe anaemia which was considered to be a megaloblastic anaemia of pregnancy. Her blood picture on admission was haemoglobin 3.6 g. per cent; red cell count 0.9 m. per c.mm., white cell count 3,400 per c.mm. In addition to the anaemia the patient had a mitral stenosis.

Two bottles of compatible blood Group O, Rhesus positive (D positive) were sent from the Regional Transfusion Service. After the administration of approximately 500 ml. in two hours the patient had a severe rigor, and complained of tightness in the chest. There was cyanosis and respiratory embarrassment. The transfusion was discontinued, and the patient improved rapidly.

The patient's blood and the donor blood were rechecked and found to be perfectly compatible. There was no evidence of bacterial contamination, and it was considered that the clinical reaction had been caused by circulatory overloading of a heart already impaired functionally by anaemia and mitral stenosis.

Case 7 - This patient was a woman aged 43 years who had a severe anaemia following menorrhagia. It was decided to transfuse her with one pint of blood of the homologous Group A, Rhesus positive (D positive), sent from the Regional Transfusion Service.

After the administration of 200 ml. in one hour, the patient complained of tightness in the chest. There was dyspnoea and cyanosis. The transfusion was immediately stopped.
and the patient rapidly recovered.

The patient's blood and the donor blood were rechecked and both were found to be Group A, Rhesus positive (D positive). There was no evidence of any blood group incompatibility, nor was there any evidence of bacterial contamination. It was considered that this was a case of circulatory overloading.

Case 8 - This patient was a married woman aged 40 years who was suffering from severe anaemia. One bottle of compatible blood of the homologous Group B, Rhesus negative (D negative) was sent from the Regional Transfusion Centre.

After the administration of 250 ml. in one hour the patient had a severe rigor with respiratory embarrassment and engorgement of the neck veins. Transfusion was discontinued, and the patient rapidly improved.

The groups of the donor and the patient's blood were rechecked and found to be Group B, Rhesus negative (D negative). There was no evidence of incompatibility, and no evidence of bacterial contamination. It was considered that this was a case of circulatory overloading.

Case 9 - This patient was a multiparous woman aged 37 years. She was admitted to hospital soon after delivery suffering from severe anaemia. A transfusion of the homologous Group O, Rhesus positive (D positive) was immediately given, and after the administration of 400 ml. in 1½ hours the patient had a severe rigor. There was marked respiratory embarrassment and engorgement of the neck veins. The transfusion was immediately stopped, but the patient did not improve, and died within a few hours. A post-mortem examination was not performed.
On clinical examination it was considered that the patient died from circulatory overloading. The donor blood was rechecked as Group 0, Rhesus positive (D positive), and there was no evidence of bacterial contamination.

Case 10 - The patient was a married woman, aged 51 years, suffering from nephrosis. She was given approximately 200 ml. of double strength plasma on account of hypoproteinaemia. After the administration of this volume in 30 minutes the patient had a rigor and complained of a choking sensation in her throat. Her pulse became very rapid and her respiratory rate 40 per minute. The transfusion was stopped, and she was given 0.5 ml. adrenalin hydrochloride subcutaneously. Her condition was poor for 24 hours following transfusion. Thereafter she improved. On clinical observation it was considered that this was a case of circulatory overloading following transfusion.

DISCUSSION

The salient features of these ten cases are summarised in the accompanying Table.

"Synopsis of Cases of Circulatory Overloading"
"Synonyms of Cases of Circulatory Overloading"

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex and Age (yrs)</th>
<th>Diagnosis</th>
<th>Volume Transfused</th>
<th>Time taken Transfusion</th>
<th>Clinical Manifestations</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M.35</td>
<td>Ulcerative Colitis; Anaemia</td>
<td>200</td>
<td>1</td>
<td>Fullness in head; Drapemos; Tightness in chest; cyanosis; venous engorgement.</td>
<td>Recovery</td>
</tr>
<tr>
<td>2</td>
<td>F.36</td>
<td>Anaemia of Pregnancy</td>
<td>250</td>
<td>1½</td>
<td>Rigor, tightness in chest; Drapemos; cyanosis; venous engorgement.</td>
<td>Death</td>
</tr>
<tr>
<td>3</td>
<td>M.70</td>
<td>Pernicious Anaemia</td>
<td>500</td>
<td>2</td>
<td>Dyspnoea; cyanosis; venous engorgement; Khonchi and rales.</td>
<td>Recovery</td>
</tr>
<tr>
<td>4</td>
<td>M.69</td>
<td>Angina; Anaemia</td>
<td>200</td>
<td>1</td>
<td>Severe angina.</td>
<td>Recovery</td>
</tr>
<tr>
<td>5</td>
<td>M.32</td>
<td>Pernicious Anaemia</td>
<td>100</td>
<td>½</td>
<td>Dyspnoea; Cyanosis; Venous engorgement; Khonchi and rales.</td>
<td>Death</td>
</tr>
<tr>
<td>6</td>
<td>F.39</td>
<td>Anaemia of Pregnancy; Mitral Stenosis</td>
<td>500</td>
<td>2</td>
<td>Rigor; Tightness in chest; cyanosis.</td>
<td>Recovery</td>
</tr>
<tr>
<td>7</td>
<td>F.43</td>
<td>Menorr: Ilangia; Anaemia</td>
<td>200</td>
<td>1</td>
<td>Tightness in chest; Dyspnoea; cyanosis.</td>
<td>Recovery</td>
</tr>
<tr>
<td>8</td>
<td>F.40</td>
<td>Anaemia</td>
<td>250</td>
<td>1</td>
<td>Rigor; venous engorgement.</td>
<td>Recovery</td>
</tr>
<tr>
<td>9</td>
<td>F.37</td>
<td>Anaemia of Pregnancy</td>
<td>400</td>
<td>1½</td>
<td>Rigor; venous engorgement.</td>
<td>Death</td>
</tr>
<tr>
<td>10</td>
<td>F.51</td>
<td>Nephrosis</td>
<td>200</td>
<td>½</td>
<td>Rigor; Choking.</td>
<td>Recovery</td>
</tr>
</tbody>
</table>

x Case 10 was given 200 ml. double strength Plasma.

Kilduff and De Bakoey (1942) observed no cases of circulatory overloading in 5000 transfusions, but in reviewing the statistics of other workers found six fatalities from cardiac

137.
failure among 43,000 recipients. In the present series there were three fatalities among 17,000 recipients. It appears therefore that fatal circulatory overloading is a real hazard of transfusion therapy, although such fatalities are infrequent.

Whithy (1942) quotes six fatalities from circulatory overloading in anaemic patients with an enfeebled cardiac muscle. In the present study the three fatal cases all had a severe anaemia which was associated with pregnancy in two cases, and with pulmonary tuberculosis in the third. It is of interest that these three fatal cases were all under 40 years of age. All ten cases in the present series had an anaemia which was severe, except in case 10 which was slight. In only two cases (4 and 6) was a heart lesion known to be present before transfusion.

The volume of fluid administered to the ten patients in this study varied from 100 ml. to 500 ml. This relatively small volume would not have embarrassed a normal myocardium (Study J, Sharpey-Schafer and Wallace, 1942b), suggesting that in all the present cases there was an enfeebled cardiac muscle. It might appear surprising that a small volume of 100 ml. should result in a fatal pulmonary oedema (Case 5). However the author is of the opinion that once initiated pulmonary oedema may become irreversible. This was illustrated by an unpublished case which the author observed. The patient who was suffering from advanced mitral stenosis was given 500 ml. physiological saline intravenously in 15 minutes. As a result the patient developed a fatal pulmonary oedema. The patient's haemoglobin was determined immediately before the infusion, and again at the end of the infusion. A haemodilution occurred, but subsequent haemoglobin estimations showed that this was transient, for not
only did the haemoglobin return to the pre-transfusion level, but haemoconcentration occurred. This haemoconcentration was progressive as the pulmonary oedema became more severe.

The rate of administration in this study varied from 170 ml. to 270 ml. per hour (except in Case 10 in which the rate was equivalent to 800 ml. per hour). This relatively slow rate would not have embarrassed a normal myocardium (Study J, Sharpey-Schafer and Wallace, 1942b). However these rates are considerably faster than those recommended by Marriott and Kelwick (1940) in order to avoid circulatory overloading when transfusing patients with chronic anaemia. The latter workers concluded that the rate should never exceed 140 ml. per hour in a ten stone individual, and when the initial haemoglobin is below 25 per cent the rate should be reduced to 70 ml. per hour. Marriott and Kelwick (1940) consider that transfusion in anaemia is rendered safe by observing their principles. Whitby (1942) supports this view.

According to Marriott and Kelwick (1940) careful watch should be kept for manifestations of cardiac failure (dyspnoea, cough, basal rales), and the transfusion must be suspended if they appear. The author has found that observation of the filling pressure of the neck veins is the most useful clinical guide (Sharpey-Schafer and Wallace, 1942b), and Sharpey-Schafer (1945) recommends that this should be done with the patient in the propped-up position.

An interesting clinical manifestation in the present study was that five of the ten patients developed a rigor, and two of the three fatal cases had a rigor. Riddell (1939) states that circulatory failure as a complication of transfusion may be due directly to overloading of the
The occurrence of a non-febrile rigor or shivering attack was discussed previously (Chapter II, p.40). The observations in Study A showed that these non-febrile shivering attacks were associated with rapid infusions only, and that the shivering passed off almost immediately after the end of the infusion. It was noted by Maycock and Whitby (1941) that too fast a rate can cause a rigor, which ceases instantly if the rate is slowed. The exact nature of these non-febrile shivering attacks or rigors is not clear, but evidence was produced (Chapter II, p.41) suggesting that the sudden introduction of a large volume of fluid at room temperature into the circulation causes a compensatory vaso-constriction, with a resultant feeling of cold. Whatever the correct explanation, the author is of the opinion that there is a non-febrile type of rigor, and that such a reaction is associated with overloading of the circulation either transient or persistent. Riddell (1939) is probably correct in stating that a rigor
imposes an increased burden upon the heart, but the author believes that all cases of circulatory failure following transfusion result directly from overloading of the circulation.

Much light has been thrown on this problem of circulatory overloading from studies, using cardiac catheterisation (McMichael and Sharpey-Schafer, 1944), on patients suffering from haemorrhage or chronic anaemia (Sharpey-Schafer 1945; Howarth and Sharpey-Schafer 1947). These workers demonstrated that in severe anaemia the right auricular pressure is normal or increased, and the cardiac output is increased. The blood-volume may be reduced to low levels, but the high filling pressure of the heart is maintained by an increase in venous tone. The systolic blood-pressure is within normal limits, but the pulse pressure is increased. Howarth and Sharpey-Schafer (1947) observed that in some cases of haemorrhage the heart behaves similarly to the heart in severe anaemia. Raising the filling pressure in these cases of haemorrhage or severe anaemia by transfusion may lead to a falling cardiac output and pulmonary oedema. Small transfusions with concentrated corpuscles given very slowly are needed if transfusions have to be given at all.

**SUMMARY**

The clinical features of ten cases of circulatory overloading are described. These ten cases occurred among 17,000 recipients. Three of the ten cases proved fatal.

The three fatal cases were suffering from severe anaemia. The danger of overloading a heart already functionally impaired by anaemia or an organic lesion is discussed, and principles regarding the rate of transfusion in such cases stated.

When transfusing patients with anaemia a careful
watch should be kept for manifestations of cardiac failure. In particular, the patient should be observed in the propped-up position for the filling pressure of the neck veins.

The significance of a rigor as a manifestation of circulatory overloading is discussed.

Changes in haemodynamics including a raised right auricular pressure are sometimes seen after haemorrhage as well as in severe anaemia. Small volume transfusions of concentrated corpuscles given very slowly are safest in cases with raised venous pressure.

AIR EMBOLISM

This complication of transfusion results from faulty technique. Air is forced through the transfusion apparatus with a Higginson's syringe (Simpson 1942; Bolton et al. 1945; Doyle and Frosdren 1949). Tovey (1950) describes how the presence of fibrin clot converted the blood-filter inside the bottle into a cylinder, which permitted an inrush of air through the blood-line, although the blood bottle was little more than half empty. The same author considers that this danger is so great that those who are ignorant of the potential dangers should be forbidden to apply positive pressure. If a rapid transfusion is desired, Drummond (1949) considers that it is better to use a wide bore needle rather than force the fluid through a narrow gauge needle with the aid of positive pressure, and the attendant risk of air embolism.

The author has never observed this complication in the administration of a transfusion fluid, but emphasised the potential danger (Wallace and Richards 1948), particularly from the emptying of the transfusion bottle when using the
British Army overseas pattern set with its one-way air inlet, which permitted the build-up of a considerable positive pressure inside the bottle. Although the author has not observed this complication in administering fluids, one case has been observed in collecting blood from a donor.

This donor was a healthy young man aged 26 years who had previously donated blood without complications. On this particular occasion the venepuncture was cleanly performed, and there was a free flow of blood. After approximately 100 ml. of blood had been withdrawn the Orderly noticed that the flow of blood stopped, and he tried to adjust the position of the needle in the vein, but without success in restarting the flow of blood. The medical officer was then called, and immediately it was observed by palpation along the vein above the site of puncture that bubbles of air were entering the vein. The needle was withdrawn from the vein immediately, and there was a spurt of blood and air from the needle. The patient was pale, and complained of pain in the praecordium. The pulse rate was 80 per minute and of good volume. The blood pressure was 115/75.

It was noticed on inspection that the airway of the withdrawal set was filled with blood, the cotton wool filter being saturated. Apparently the blood which entered the bottle was impinging on the outlet needle of the airway, and some blood was sucked back into the airway by negative pressure. This blood caused the cotton-wool in the airway to swell and block the airway.

The donor complained of severe praecordial pain while resting, but the pulse and blood pressure were maintained. There was no respiratory embarrassment. As the pain persisted
it was decided to admit the donor to hospital for observation. Morphia was administered, and this relieved the pain. The following day the donor felt well, and an electrocardiogram showed no abnormality. The donor was discharged from hospital, and remained well.

This case illustrates one of the dangers of blood collection. Blocking of the airway results in a positive air pressure developing in the bottle. The entry of air from the bottle to the vein can be prevented by maintaining a pressure of 50 mm. Hg. in the cuff on the donor's arm. Apart from a blocked airway, this complication may result from the use of a Higginson's syringe which blows air into the bottle instead of sucking it out.

**PULMONARY EMBOLISM**

It is theoretically possible for Pulmonary Embolism to complicate transfusion. The clot may be derived from the lining of the vein or from the fluid which is being transfused. Small fibrin clots are present in many bottles of preserved blood, and occasionally in processed liquid plasma. It is essential to have a filter on the administration set to hold back any clots which may be present in the fluid, and prevent them entering the recipient's circulation.

The author has seen one case in which pulmonary embolism may have played some part in the death of a patient following transfusion. The patient was a male aged 46 years who was suffering from anaemia following melena. It was thought that the patient had a duodenal ulcer. The author was consulted on transfusion therapy, and set-up a transfusion of 500 ml. whole blood which it was intended should take four
hours to administer. A telephone message was received two hours later stating that the patient had received 250 ml., and was feeling cold and shivery. The author advised the resident medical officer to stop the transfusion. This suggestion was carried out, but the apparatus was left with the needle still in the vein. Some five hours later the resident medical officer decided to restart the transfusion. On opening the screw regulator no blood flowed, so positive pressure was applied with a Higginson's syringe, and after vigorous application the blood suddenly started to flow. Almost at once the patient gave a sighing respiration and died. Unfortunately, permission for a post-mortem examination was not given.

The sudden death of this patient coincided with the attempt to restart the transfusion, and it seemed reasonable to conclude that the two events were related. The sudden application of pressure may have displaced a loose clot from the vein at the site of the puncture, or there may have been a sudden rush of air into the vein. Whether pulmonary embolism or air embolism was responsible for the fatality or not, the incident illustrates the danger of trying to restart in the same vein a transfusion which has been stopped for a considerable period. If a transfusion has ceased to flow for a length of time sufficient to permit clotting, then it is advisable to restart the transfusion only if another site is chosen.

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CHAPTER V

HEMOLYTIC REACTIONS.

Introduction

Prior to the epochal discoveries by Landsteiner (1900, 1901) of isoagglutinating and isoagglutinable substances in human blood, the successful performance of blood transfusion had been hindered by the grave manifestations of agglutination and haemolysis from the mixture of incompatible bloods. In spite of subsequent improvement and development of blood grouping and matching, incompatible reactions have occurred with sufficient frequency to warrant serious consideration.

Statistics of the past from various sources have indicated a prominent rate of incompatible (haemolytic) transfusion reactions. Kilduffe and De Bakey (1942) compiled the results of 43,284 blood transfusions by 13 different groups of workers. There were 1.8 haemolytic reactions in every 1,000 transfusions, and of these 1.04 per 1,000 (57.7 per cent) were fatal. Approximately three-quarters of the above reports appeared prior to the discovery of the Rhesus blood group antigens by Landsteiner and Wiener (1940). The routine use of Rhesus typing in transfusions no doubt would have lowered the quoted haemolytic reaction rate. Yet, it may well be that iso-immunisation by various ODE-ode (Rh-Hr) sub-group antigens and other less understood blood antigens in addition to regrettable clerical-technical errors will continue to make this an important problem. Certainly the present upsurge in the use of whole blood transfusions lends additional emphasis to this question.

Clinical Manifestations - The clinical features developing in a patient receiving incompatible blood are usually quite characteristic and rather dramatic (Kilduffe and De Bakey 1942).
The original description of Denis (1667) remains a classic (Sievers 1938). The patient was given a second transfusion of calf's blood in an attempt to cure his insanity, and "As soon as the blood entered into his veins, he felt the same heat all along his arm and in his armpits which he had done before; His Pulse was forthwith raised, and while after we observed a great Sweat sprinkled all over his face. His pulse at this moment was very much altered; and he complained of a great Pain and Illness in his Stomach and that he should be presently choaked, unless we would let him go -- -- By and by he was laid in his bed, and after he had for two hours sustained much violence, vomiting up divers liquors which had disturbed his Stomach, he fell into a profound Sleep about ten a clock, and slept all that night without intermission till eight a clock the next day -- -- When he awakened he seemed wonderfully composed and in his right mind, expressing the Pain and universal weariness that he felt in all his members. He pitt a large glass full of such black Urine, that you would have said it has been mixed with soot -- -- --

Clinical Course of Haemolytic Transfusion Cases - The clinical course of a patient experiencing a haemolytic reaction may be divided into three phases as presented in Table I (Muirhead et al. 1948).

**TABLE I - Clinical Course, Haemolytic Reaction Case**
**TABLE I - Clinical Course, Haemolytic Reaction Case**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>&quot;Reaction Shock&quot; (1st day)</td>
</tr>
<tr>
<td>II.</td>
<td>Renal Insufficiency (1 - 12 days)</td>
</tr>
<tr>
<td>III.</td>
<td>Salt Losing Diuresis (8th - 16th day)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Haemolysis and Hypotension</th>
<th>Tubular damage</th>
<th>Tubular recovery or regeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Apprehension, backache, etc.</td>
<td>2. Azotaemia, hypertension.</td>
<td>2. Severe dehydration if water and salt not supplied.</td>
</tr>
<tr>
<td>4. Hypotension, mental confusion.</td>
<td>4. Depressed serum Na, Cl, CO₂ combining power, Calcium.</td>
<td></td>
</tr>
<tr>
<td>5. Chill, fever.</td>
<td>5. Rising titres of agglutinins.</td>
<td></td>
</tr>
<tr>
<td>6. Haemoglobinæmia, haemoglobinuria.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The first phase may be termed the stage of "reaction shock". The onset of the reaction is usually sudden, and the patient displays the following manifestations: apprehension, generalised tingling sensations of the skin, tightness in the chest, severe backache, dyspnoea, cyanosis and mental confusion. Shivering or rigor with rise in temperature and hypotension are common. The duration of the hypotension varies, and may be influenced by other coexisting factors (blood loss, anaesthetic agent, anaemia, operation, etc.). Prolonged hypotension may in itself be conducive to factors that cause renal damage (Moon 1942, Corcoran and Page, 1943). A haemorrhagic tendency is not unusual soon after the reaction.
The patient may receive 40 to 500 ml. of the incompatible blood before the above manifestations become severe. The factors that determine the necessary volume of infused blood for the evident reaction to appear have not been fully elucidated. According to the observations of Hill et al. (1947), the violence and degree of haemolysis may be related to the number of antibody-antigen combinations (multiple R.B.C. antigens, and/or antigens determined by single or double gene doses). This concept indicates that homozygous red cells and red cells with multiple antigens for which antibodies are available react more promptly and more violently. The antibody titres may actually be diminished during this phase due to their adsorption by the specific antigen or antigens.

In an average sized individual it requires some 40 to 60 ml. of haemolysed blood to liberate a sufficient quantity of haemoglobin into the circulating plasma in order to give rise to the appearance of haemoglobin in the urine. The studies of Yuile (1942) have placed the renal threshold in animals for free haemoglobin in the vicinity of 30 to 150 mg. per 100 ml. plasma.

The second phase is the phase of renal insufficiency. By far the commonest cause of death has been uraemia. Oliguria is marked, but usually is not static, and each day a slightly greater volume of urine is excreted. Associated with the oliguria there is a marked decrease in the excretion of solid matter by the kidneys. Not only is the solid output scanty, but the concentration of various ingredients is substantially lowered.

The urine contains pathological ingredients
during the first few days. Free haemoglobin gives the first few specimens a dark red or reddish brown colour. Haemoglobin-aemia and its attendant haemoglobinuria usually disappear within 24 to 36 hours. Proteinuria is definite, however, even after the haemoglobinuria subsides. The presence of casts containing the breakdown products of haemoglobin is an important diagnostic feature. Intact red blood cells are commonly present in the urine, and white blood cells, frequently in clumps, are present during the oliguric period. The urine specific gravity is depressed (1.005 to 1.010), and remains low for a varying period after recovery.

A mounting azotaemia and slight to moderate hypertension occur in conjunction with the above changes. The serum potassium concentration is elevated probably on account of the breakdown of red cells rich in potassium. Other changes commonly include a decrease in the concentration of serum sodium, plasma chlorides, and the carbon dioxide combining power of the plasma. The serum calcium concentration is likewise frequently depressed.

During the stage of renal insufficiency the antibody titre of the patient’s serum may become elevated, very specific evidence of the occurrence of an incompatible blood transfusion.

The renal insufficiency phase lasts 8 to 12 days, and culminates either in death from uraemia or in recovery following a copious diuresis (third or salt losing diuresis phase). The ability of the kidneys to concentrate waste products improves, and the solid output increases. This increase in both urinary volume and solid output strongly
suggests a gradual recovery of damaged renal elements.

During the early phases of the diuresis not only is the 24-hour urine urea output markedly elevated, but at the same time the 24-hour urine salt output (mainly NaCl) may be greatly increased. Some 20 to 40 grams of salt may be lost daily by this mechanism. This finding suggests that the kidneys have recovered sufficiently to allow for urine volume production, but that the recovery is as yet insufficiently advanced to allow for the conservation of water and salts. Such failure to conserve water and salts lasts a few days only. Muirhead et al. (1943) have observed that the kidneys at this stage will discard body water and salts to the extent of causing pronounced dehydration or even death. Within certain limits, such diuresis occurs whether or not water from sources outside the body is furnished. This feature simulates the cases designated by Thorn et al. (1944) as "salt-losing nephritis".

Renal lesions - Rarely a patient may die at the onset of the reaction, but in the main it is renal complications which are the cause of deleterious sequelae. The factors which are necessary for the production of renal damage are not fully understood, but probably several are involved. Haemolysis alone and the intravascular liberation of products of damaged red cells are not sufficient for the development of renal failure. Experimentally an animal's circulating haemoglobin may be rapidly lowered to 25 per cent of normal by the injection of anti-red cell serum without the development of evident renal insufficiency (Tigges et al. 1940, Daneshekk and Schwartz 1938). Clinically many haemoglobinanaemias with
Haemoglobinuria show no evidence of impaired renal function. Substantial quantities of haemoglobin solution have been infused intravenously into humans without subsequent renal insufficiency (Yuile 1942). The same worker has shed considerable light on this question by demonstrating experimentally the consistent development of renal insufficiency in animals following the intravenous injection of haemolysed red cells after the kidneys had been damaged either by the temporary constriction of renal vessels, or by the administration of a nephrotoxic substance such as sodium tartrate. It would appear that primary renal damage, as might occur clinically in prolonged hypotension, severe anaemia, or acute oligaemia, is a prerequisite which together with haemolysis gives rise to the more severe renal damage and renal insufficiency.

Ross (1945) has reviewed the subject and summarised the various factors involved in the renal damage by singling out the following: lowered blood pressure, vasoconstriction of renal vessels, reduced renal blood flow, lowered alkali reserve and the secretion of large amounts of haemoglobin derivatives in an acid urine.

The pathology of the kidneys has received much attention in these cases (Bordley 1931, De Gowin et al. 1936, Daniels et al. 1941). Such terms as "haemoglobinuric nephrosis" (Hallory 1947) and "lower nephron nephrosis" (Lucke 1946) have been applied to these lesions. The kidneys are usually enlarged, and bulging of the cut surface denotes an increased subcapsular tension. The cortex is pale; the medulla is dark and dusky, and presents a well striated
appearance. Microscopically the main damage is to be found in the lower segment of the nephron in which degeneration or necrosis of the tubular epithelium are evident. Foci of inflammation (accumulation of neutrophils, lymphocytes, eosinophils and macrophages) can be seen where necrosis occurs. Haeme casts may be prominent in the form of brownish-granular or eosinophilic-granular material in distal segments and collecting tubules. Interstitial oedema is usually evident. Peritubular hyperaemia is often marked in the medullary region. There is acute parenchymatous degeneration of the upper segments. Apart from some thickening of the glomerular membrane, the morphological appearance of the glomeruli is normal.

Regeneration of the distal segments becomes evident by the eighth to tenth day (Lucke 1945). The damaged epithelial cells slough into the lumen and are replaced by new cells. The young cells are flat at first but later gain more and more substance. The inflammatory foci become replaced by fibrous tissue.

S T U D Y M

INVESTIGATION OF HAEMOLYTIC REACTIONS

The investigation of transfusion reactions suspected of being haemolytic in nature is greatly facilitated if certain samples are available at the time of investigation. These are (1) a sample of the donor blood, and (2) a pre-transfusion sample of the recipient's blood. It should be an invariable rule after every transfusion to recap the bottle containing the remnant of the donor blood, and keep this in the refrigerator for at least 24 hours after the transfusion. It is also desirable to obtain a clotted blood specimen from
the recipient before transfusion, and to keep it in a refrigerator until 24 hours after the transfusion, when the patient has been found to have responded favourably to the transfusion. In cases in which a haemolytic reaction is suspected a large sterile venous sample of blood should be obtained, and also a clean sample of urine. Many of the tests set out below will be inapplicable if more than 24 hours have elapsed since the transfusion.

Precautions to be Observed in Obtaining the Venous Sample - A large (20 ml.) sterile syringe with a needle of not less than 20 S.W.G. should be used: the syringe and needle should be absolutely dry or else must be rinsed in sterile isotonic saline. Venepuncture must be clean. The needles must be removed from the syringe before transferring the blood to the sample tubes. The sample should be divided between two plain tubes of which one is sterile.

The Sample of Urine - It will be sufficient to obtain a clean sample of urine, unless there is any risk of contamination with blood, e.g., during the menstrual flow or after delivery, in which case a catheter specimen must be obtained.

Examination of the Samples - The following tests may have to be performed:

(a) Donor's Blood (from the Transfusion Bottle if possible)

   (i) A sample should first be withdrawn from the bottle with a sterile pipette and kept in a sterile tube in case bacteriological examination is required. (ii) A mixed sample should next be centrifuged to determine the amount of free haemoglobin in the supernatant plasma. (iii) The blood should be regrouped, testing both cells and plasma; the RhEus Blood group should be determined in addition to the ABO group.
(iv) The cells should be tested against the recipient's serum, using the pre- and post-transfusion specimen. (v) The titre of the agglutinins in the donor's plasma should be estimated if necessary.

(b) The Recipient's Blood (Post-transfusion Sample)

(i) The sample in the sterile tube should be submitted for bacteriological examination. (ii) The serum from the second clotted tube should be examined by naked eye for the presence of haemoglobin or excess bilirubin. The amount of bilirubin or the icterus index should be estimated, and Schum's test (Fairley 1940) for methaemalbumin should be applied. The pre-transfusion sample, when available, is a valuable control in these examinations. A small amount of serum should be reserved for serological tests.

(iii) Serological Tests - (a) A suspension of the recipient's blood should be prepared by adding one drop of blood to approximately 2 ml. of isotonic sodium chloride to make a 2 per cent suspension approximately. This suspension should be examined under the low power of the microscope for the presence of agglutinates. With experience it is possible to recognise the difference between weak diffuse clumping due to auto-agglutination and the presence of small scattered but well-formed agglutinates lying in a field of free cells, the latter appearance resulting from the presence of clumps of donor cells in the suspension. (b) The blood should be regrouped, testing both cells for agglutinogens and serum for agglutinins. The Rhesus blood group of the cells should be determined and, if necessary, the serum examined for the presence of anti-Rh agglutinins. (c) The agglutinins in the serum should be
titrated for comparison with the results of titrations carried out on samples obtained later, and on the pre-transfusion sample when available. (d) If necessary, differential agglutination tests should be carried out.

Note on Methods of Differential Agglutination - This permits the recognition, in a sample of blood taken from the recipient, of foreign red cells derived from a previous transfusion. The donor cells can be recognised either by being agglutinated by a test serum which does not agglutinate the recipient's cells, or by their failure to be agglutinated by a test serum which completely agglutinates the recipient's cells. Many possibilities of differentiation exist if anti-A, anti-B, the various anti-Rh sera, anti-M, anti-N and anti-P sera are used.

Application of the method of differential agglutination is essential for the solution of many transfusion problems (Wiener 1942, Dacie and Hollison 1943). Mourant (1943) recommends the use of the direct anti-human-globulin test (Coombs, Mourant and Race, 1946) in the examination of obscure cases of transfusion reaction. After a transfusion which is incompatible owing to the presence of anti-Rh in the recipient's plasma, a direct anti-human-globulin test of the recipient's blood may appear weakly positive owing to the sensitization of the donor's cells. (e) Sample of Recipient's Urine - The urine should be inspected by the naked eye and its colour noted; it should then be examined spectroscopically for oxyhaemoglobin and methaemoglobin. If the supernatant fluid contains no pigments a sample should be centrifuged, and the deposit examined for the presence of pigment casts. The specific gravity and the reaction of the urine should be determined, and tests for albumin applied.
Interpretation of Tests

All the tests described above will not have to be performed in every case of alleged haemolytic transfusion reaction. The most likely cause should be investigated first, and it is only necessary to perform other tests when the first ones are negative or inconclusive. For example, regrouping the blood of the donor and recipient may reveal a mistake in ABO grouping, and render other tests unnecessary. Again, if the recipient is a woman who has given birth to an infant suffering from haemolytic disease of the newborn, the probability that the reaction has been caused by previous immunization of the recipient to one of the Rhesus blood group factors should be considered first.

A blood sample taken from the recipient immediately after an incompatible transfusion may contain an appreciable number of donor cells, and this may possibly result in a mistaken diagnosis of the recipient's group. However, in the case of ABO groups such mistakes are not likely to be made, because the appearance of partial agglutination is so different from the complete agglutination of the A and B reactions. Mistakes are more likely to arise in cases in which the incompatibility lies in the Rhesus blood group. When a Rhesus negative individual already sensitized to the Rh. antigen is transfused with Rhesus positive blood the post-transfusion sample may contain appreciable numbers of Rhesus positive cells although their destruction is proceeding rapidly, and this may lead to the impression that the recipient is Rhesus positive (Diamond 1942).

It is important to use powerful anti-sera to enable this appearance of partial agglutination to be recognised with
certainty from that of complete agglutination.

The titre of the offending agglutinin in the recipient's serum may be very low after transfusion, so that the donor's cells may appear to be compatible with the recipient's serum. For this reason the pre-transfusion sample of serum from the recipient should be used whenever possible, or else a sample taken at least five days after transfusion.

In recently delivered women immune agglutinins may be found in the serum owing to stimulation by foetal agglutinogens during pregnancy. When the foetal red cells contain either the A or B agglutinogens, and these are lacking in the mother, the anti-A or anti-B agglutinins in the maternal serum are increased in titre after delivery not only in a proportion of cases (Jonsson 1936), but in the great majority, reaching a maximum titre 10 to 20 days after delivery (Mollison 1943). Care must therefore be exercised in interpreting the results of serial titrations of agglutinins in recently delivered women.

Agglutinins in the recipient's serum which are not active at 37°C (98.6°F.) are not capable of causing the destruction of transfused red cells (Mollison 1943).

When preserved blood has caused a haemolytic reaction enquiries should be made as to the temperature and duration of storage, and the method of warming the blood before transfusion. The answers to these questions may show that a physical factor and not a blood group incompatibility is responsible for the haemolysis.

Only when the above causes have been excluded should the possibility that the signs of haemolysis are due to
destruction of the recipient's own red cells by high-titre incompatible agglutinins in the donor's plasma be considered. In such a case the application of the method of differential agglutination is practically essential in establishing that the signs are not due to destruction of the donor cells, although the agglutinins in the donor's plasma must also be titrated.

Case Records - The following cases have been observed and investigated personally by the author. These cases illustrate some of the points described in the text and some of the methods of investigation. All the cases have occurred within the past three and one half years in hospitals served by a modern Regional Transfusion Centre. The factors which were primarily responsible for the haemolytic reactions are stressed as it is only by a realisation of the causal factors that such reactions will be avoided in the future.

ABO Incompatibility. Cases 1 to 9

Case 1 - Donor Group AB; Recipient Group O.

A specimen of clotted blood was received at the Regional Transfusion Centre with a request for compatible blood for a patient M.P. The specimen tube was labelled M.P. The blood group was found to be AB Rhesus positive (D positive), and 500 ml. concentrated red corpuscles of the homologous group which had been found compatible with the patient's serum were despatched to the hospital. No confirmatory compatibility test was performed in the hospital, and the blood was transfused immediately. As the Haemoglobin was only 45 per cent Waldae it was decided to transfuse slowly, and the transfusion occupied eight hours. During the
Transfusion the patient felt cold and shivered slightly, but there was no rigor or other complaint. Immediately after the transfusion the patient's haemoglobin had risen to 56 per cent Haldane. It was noted about 24 hours after the end of the transfusion that the patient was jaundiced. There was no haemoglobinuria.

The donor blood was rechecked and found to be Group AB, but a fresh specimen from the patient appeared to be Group O with a few cells which were agglutinated by anti-A and anti-B sera. The recipient's anti-A titre was 3 and anti-B titre 4, both values being significantly lower than the normal titres in the serum of a Group O person. Three days after the transfusion the jaundice had cleared, but the patient's haemoglobin was only 43 per cent Haldane.

It appeared that the specimen sent originally to the Regional Transfusion Centre must have belonged to another patient. Blood specimens had been taken from three other patients in the same ward at the same time. The blood group of one of these patients was later found to be Group AB.

Comments - This case emphasises two points - (i) the importance of clerical accuracy in labelling specimens and (ii) the importance of performing a compatibility test, even in cases in which allegedly compatible blood is provided by the Regional Transfusion Centre. Other interesting features are the almost complete absence of early clinical features of a haemolytic reaction, and the return of the haemoglobin to the pre-transfusion figure within three days. The lack of early symptoms may be accounted for by the slow rate of administration.
Case 2 - Donor Group A; Recipient Group B

Compatible Group B blood was sent from the Regional Transfusion Centre to a hospital for a patient Mr. S. This blood which was carefully and specifically labelled for Mr. S. was placed in the hospital blood-bank. It so happened that the same day the patient's wife donated blood at the hospital, and a laboratory technician placed this bottle of blood in the blood-bank with a label which simply stated "S, Ward B". No grouping tests had been performed on this blood. A recently appointed medical officer removed this latter bottle from the blood-bank assuming that it was the compatible blood for Mr. S. After approximately 100 ml. had been transfused the patient developed a rigor, and complained of pain in the back. The transfusion was immediately stopped, and the patient developed no other untoward symptoms or signs.

The blood which had been transfused was found to be Group A.

Comments - The case illustrates the danger of leaving improperly labelled bottles in the blood-bank. Again this case shows how important it is for the medical officer who actually performs the transfusion to ensure that the blood which is to be administered is in fact compatible.

Case 3 - Donor Group A; Recipient Group O

A request was received by the Regional Transfusion Centre for two bottles of fresh blood for a patient suffering from haemophilia. The patient was known to be Group O, Rhesus positive (D positive) and had been repeatedly transfused. A donor session was in progress at the time of the request, and it was suggested to the medical officer in charge of the case...
that two bottles of fresh Group O blood would be sent and that compatibility tests should be performed in the hospital. Blood from two donors known to belong to Group O was confirmed as Group O, and instructions were accordingly given to the appropriate technician to label and despatch the bottles of blood to the hospital.

The medical officer in charge of the patient felt doubtful about one of the compatibility tests, but was assured by a colleague that it was rouleaux and not agglutination which he was observing. The first bottle of blood was transfused without a reaction, but after only 20 ml. of the blood about which there had been some doubt had been administered the patient complained of pain in the back, and developed a violent rigor. The transfusion was immediately stopped.

The donor blood which caused the reaction was found to be Group A. Unfortunately, there had been duplication of a flask number at the Regional Transfusion Centre. One flask of the particular number was the Group O blood which should have been issued; the other flask of the same number was the Group A blood which was in fact issued. This error would normally have been detected, but in this case the routine checks were not performed as it was an issue of fresh blood.

The patient recovered rapidly from the reaction. A specimen of the recipient's blood taken 24 hours after the transfusion showed no evidence of Group A cells among the Group O cells. The anti-A titre of the recipient's serum was 64, and 14 days later was 128.
The importance of clerical accuracy is again illustrated. If there is any doubt about a compatibility test it is better not to transfuse the blood until the opinion of an expert blood group serologist has been obtained.

The early classical features of a haemolytic reaction appeared after the administration of only 20 ml. of incompatible blood, and this small volume appeared to have very little effect on the titre of the corresponding antibody in the recipient's serum.

Case 4 - Donor Group A; Recipient Group O.

Two bottles of Group O blood were issued from the Regional Transfusion Centre to a hospital for the transfusion of a Group O patient who had a moderate anaemia following melaena. The first bottle was successfully transfused, but after approximately 200 ml. of blood from the second bottle had been given the patient had a severe rigor and the temperature rose to 104°F. (40°C). There was no complaint of lumbar pain. Haemoglobinuria was detected in the first urine specimen after transfusion.

The first bottle of blood was confirmed as Group O, but the second bottle was found to be Group A. This latter bottle was one of ten bottles which had been treated in an unusual fashion at the Regional Transfusion Centre. The ten bottles of blood had been collected under a donor replacement scheme in a hospital, and sent to the Transfusion Centre for grouping. No pilot tubes were sent with these bottles, and a specimen was taken from each bottle under sterile conditions for grouping purposes. The particular bottle which caused the reaction had had attached a pilot tube of blood taken
from the bottle, but although the labels on bottle and pilot tube corresponded, the blood in the pilot tube was Group 0 and in the bottle Group A. As the blood in the flasks with serial numbers immediately adjacent to the offending bottle was Group 0, it appeared that a clerical-technical error had been made in transferring the specimens from the bottles to the pilot tubes.

The recipient was confirmed as Group 0, and there was no evidence of Group A cells in a specimen taken 48 hours after the transfusion. The blood in the pilot tube was compatible with the patient's serum, but the blood in the bottle which caused the reaction was incompatible. The anti-A titre in the recipient's serum was 8 in the immediate post-transfusion specimen, but had risen to 128 in saline and 512 in AB serum when re-examined 14 days after transfusion.

Comments - One of the dangers involved in the use of pilot tubes is well illustrated. Clerical accuracy is of vital importance at every stage in blood transfusion technique.

The case illustrates the depression of antibody titre which occurs immediately after an incompatible transfusion, and the rise which takes place later as the result of antigenic stimulation.

Case 5 - Donor Group AB; Recipient Group 0

The patient was suffering from anaemia associated with acute rheumatism, and had been grouped by the medical officer in charge of the case as Group AB. Accordingly one bottle of Group AB blood was ordered from the Regional Transfusion Centre, and a compatibility test was stated to be satisfactory.

After the administration of approximately 250 ml.
of this blood the patient complained of pain in the back, had a violent shivering attack and became collapsed. The transfusion was immediately stopped, and the patient's condition rapidly improved following the application of heat. The first post-transfusion specimen of urine contained haemoglobin, and on the day following the transfusion the patient was jaundiced. Recovery thereafter was uneventful.

The donor blood was confirmed as Group AB, but the patient instead of being Group AB was in fact Group O. There were a few clumps of AB cells surviving in the specimen withdrawn from the recipient 24 hours after the transfusion. This specimen of the recipient's serum had an anti-A titre of 8, and an anti-B titre of 4. Ten days later the respective titres were in saline anti-A 256 and anti-B 64, and in AB serum anti-A 1024 and anti-B 512.

The recipient's serum was examined for the presence of cold agglutinins and these were demonstrable at 4°C. (39.2°F.) to a titre of 4, but were not active at room temperature. The mistake in finding a Group O patient to be Group AB was not therefore accounted for by the presence of cold agglutinins active at room temperature, and the most likely explanation was the interpretation of rouleaux as agglutination by an inexperienced observer. The failure to detect incompatibility was also the result of inexperience as this test had been wrongly performed by incubating the donor's serum with the recipient's cells.

Comment - The causal factor in this case was entrusting grouping and compatibility tests to an inexperienced medical officer.
The case illustrates the depression of antibody titre immediately following the haemolytic reaction, and the subsequent boosting of titre.

Case 6 - Donor Group A; Recipient Group O

The patient was suffering from haematemesis, and was given 2 litres Group A blood within 12 hours. The patient had seemed to belong to Group A, and the compatibility tests were considered satisfactory. The only unusual clinical feature was that haemoglobinuria developed and persisted for 24 hours after the transfusion.

This incident was not reported to the Regional Transfusion Centre until four weeks later, when it was shown that the patient was in fact Group O, and the anti-A titre was 64,000 in saline and 512,000 in AB serum. The donors were confirmed as Group A.

The compatibility test which had been performed in hospital had again incorrectly consisted of incubating the donor's serum with the recipient's cells.

Comments - The causal factor again was lack of experience in blood group serology.

The change in antibody titre as a result of the transfusion of an incompatible blood group antigen is illustrated.

It is interesting that the transfusion of 2 litres of incompatible blood produced few signs or symptoms usually associated with a haemolytic reaction.

Case 7 - Donor Group A; Recipient Group O

This patient was suffering from anaemia as the result of menorrhagia. As the patient was thought to be Group A a transfusion of Group A blood was commenced. After
the administration of only 100 ml. the patient had a violent rigor with a rise of temperature to 104°F. (40°C.), and the transfusion was discontinued. No other untoward features were noted.

Both pre- and post-transfusion samples of blood from the recipient were available. It was shown that the patient was in fact Group O. The anti-A titre in the recipient's serum was 32 immediately before transfusion, 8 immediately after transfusion and 256 in a specimen taken ten days later.

The donor blood was confirmed as Group A.

No explanation of the original mistake in determining the patient's group was forthcoming apart from the inexperience of the medical officer concerned. The grouping sera which had been used were checked, and found to be satisfactory.

Comments - The causal factor again appeared to be lack of experience on the part of the medical officer concerned.

Characteristic changes in antibody titre were again observed.

Case 8 - Donor Group A; Recipient Group B

This patient was suffering from gastric carcinoma, and it was decided to transfuse before operation. The patient's group was determined as A, and accordingly one bottle of Group A blood was obtained from the Regional Transfusion Centre. After the administration of 100 ml. of this blood the patient complained of backache and the transfusion was stopped.
No compatibility test had been performed, but after the reaction the medical officer concerned regrouped the blood which had been issued from the Regional Transfusion Centre, and claimed that it was Group B and not Group A, hence the reaction.

The bottle of blood which caused the reaction was returned to the Regional Transfusion Centre, and both the blood in the bottle and in the pilot tube were found to be Group A as stated on the labels at the time of issue.

Enquiry revealed that the medical officer had confused the anti-A and anti-B serum, and that this was in fact the first blood grouping test which he had performed since qualification.

The recipient's blood was examined eight days after the transfusion, and found to be Group B with an anti-A titre of 2048 in saline, and 4096 in AB serum.

**Comments** - Inexperience on this occasion in the nomenclature of standard anti-A and anti-B sera was responsible for the error in grouping.

No compatibility test was attempted.

The boosting of antibody titre by the injection of incompatible blood is illustrated.

**Case 2** - Donor Group AB; Recipient Group B

This patient was suffering from Congenital Haemolytic Anaemia. The patient's group was found by the resident medical officer to be AB, and two bottles of Group AB blood appeared to be compatible.

After approximately 700 ml. of blood had been transfused in 6 hours the patient started to shiver, and the
temperature rose to 103°F. (39.4°C.). The transfusion was stopped immediately. For a period of 24 hours following the transfusion there was haemoglobinemia and haemoglobininuria. The patient showed no rise in haemoglobin as a result of the transfusion.

The donor blood was confirmed as Group AB.

The patient was however found to be Group B.

The anti-A titre of patient's serum before transfusion was 256.

The anti-A titre of patient's serum after transfusion was 4.

These titres were estimated at 37°C. as the patient's serum contained in addition to anti-A, a non-specific cold agglutinin with a titre of 16 at 4°C. and a titre of 4 at room temperature.

It appeared that the presence of cold agglutinins in the patient's serum caused auto-agglutination, and the patient's blood group was misinterpreted as Group AB.

The compatibility test was incorrectly performed in that the donor's serum was incubated with the patient's cells.

Comments - The presence of auto-agglutinins active at room temperature resulted in the false grouping of the patient. This error would normally have been detected by the compatibility test, but this was incorrectly performed.

The depression of antibody titre immediately following a haemolytic transfusion reaction is demonstrated.

Rhesus Blood Group Incompatibility - Cases 10 to 14

Donor Rh. positive; Recipient Rh. negative, sensitized to Rh. blood group antigens.
Case 10 - The patient was an unmarried woman aged 30 years who was suffering from aplastic anaemia. Her blood group was B, and she had received 500 ml. Group B blood on each of two occasions without apparent reaction. On a third occasion she was given another 500 ml. of apparently compatible blood, and the patient's temperature rose to 101°F. (38.3°C.) immediately after transfusion. A fourth transfusion of 500 ml. of apparently compatible Group B blood was administered, and at the end of the transfusion the patient developed a rigor with a rise in temperature to 104°F. (40°C.). There were no other untoward manifestations. Ten days later it was decided to transfuse again, and a sample of blood was sent to the Regional Transfusion Centre. The patient was found to be Group B, Rhesus negative (D negative), and the serum contained anti-D agglutinins of the albumin variety to a titre of 16. Group B, Rhesus negative blood was subsequently transfused without reaction.

The four previous donors were traced, and all were found to be Group B, Rhesus positive (D positive).

Comments - The patient had apparently been sensitized to the D antigen by repeated transfusions of D positive blood. The sensitization appeared to develop slowly, and the transfusion reactions were progressively more severe. The incompatibility was not detectable in the ordinary compatibility test using a saline suspension of red cells as the Rh. antibodies were of the albumin variety.

Case 11 - The patient was a woman of 25 years who had
previously given birth to two healthy children. She was pregnant for the third time, and was admitted to hospital in an emergency with ante-partum haemorrhage. Her Rhesus blood group was unknown, and she was transfused rapidly with Group O, Rhesus positive (D positive) blood. After the administration of approximately 800 ml. of this blood the patient had a violent rigor and became extremely collapsed. The transfusion of blood was discontinued, and a plasma infusion was substituted. In spite of this infusion the blood pressure remained low, and the patient died a few hours later.

The donor blood was rechecked and found to be Group O, Rhesus positive (D positive). A sample of blood taken from the patient immediately after the reaction was found to be Group O, Rhesus negative (D negative). No Rhesus antibodies were detected in the recipient's serum, but a direct anti-human-globulin test on the recipient's blood showed small clumps of agglutinated cells among many unagglutinated cells. The agglutinated cells were probably Rhesus positive (D positive) cells to which had become attached Rhesus (anti-D) agglutinins.

There was no history of previous transfusions.

Comment - This Rh. negative (D negative) patient had probably become sensitized to the D antigen as a result of pregnancy with a Rhesus positive (D positive) foetus. The rapid transfusion of Rhesus positive (D positive) blood caused a severe haemolytic reaction which together with the severe blood loss proved fatal.

No attempt was made to perform a compatibility test before transfusion.

Case 12 - The patient was an unmarried woman of 34.
years who had been pregnant on several occasions. She had given birth to two full term infants which were perfectly healthy, and in addition had had several abortions. At the time of one previous abortion she had been given a transfusion of Group O, Rhesus positive (D positive) blood without apparent reaction.

The present admission to hospital was on account of an abortion at the 16th week of pregnancy. On admission she was given 500 ml. of Group O, Rhesus positive (D positive) blood which appeared to be compatible. This transfusion was followed by a rigor, and a sharp rise in temperature. Eight days later a specimen of the patient's blood was sent to the Regional Transfusion Centre, where it was found that the patient was Group O, Rhesus negative (D negative) with Rhesus (anti-D) antibodies in the serum to a titre of 8. These antibodies were detectable in albumin, but not in saline. The patient was subsequently transfused with Rhesus negative (D negative) blood without reaction.

Comments - The patient had become sensitized to the D antigen as the result of a previous transfusion of Rhesus positive blood, and probably also as a result of repeated pregnancies.

The incompatibility was not detectable in the ordinary compatibility test with a saline suspension of cells as the Rh. antibodies were of the albumin variety.

Case 13 - The patient was a man of 43 years who gave a history of repeated haematemesis over the past twenty years. On two occasions it had been necessary to give a blood transfusion, but no information was available as to the blood group transfused or the occurrence of reactions.
On the present admission to hospital he was found to be Group B, and was transfused with Group B, Rhesus positive (D positive) blood which appeared to be compatible with the patient's serum. After the administration of 200 ml. of this blood the patient had a rigor and the temperature rose to 104°F. (40°C.). No other untoward symptoms or signs were observed.

On examining an immediate post-transfusion specimen of blood it was found that the patient was Group B, Rhesus negative (D negative) and that there was a small number of Rhesus positive (D positive) cells detectable with a potent anti-D serum. A direct anti-human-globulin test on the recipient's blood showed a few clumps of agglutinated cells, presumably Rhesus positive cells to which Rh. antibody had become attached. The recipient's serum contained Rh. (anti-D) antibodies with a saline titre of 4 and an albumin titre of 32.

The patient was subsequently transfused with Group B, Rhesus negative (D negative) blood without reaction.

Comments - The patient had become sensitized to the D antigen by the previous transfusion of blood.

The anti-D agglutinins were mainly of the albumin variety, but sufficient saline antibody was present to cause agglutination of Rhesus positive (D positive) cells. This agglutination was probably missed at the time of the compatibility test in hospital as the agglutination was much finer than is seen in ABO incompatibility.

Case 24 - This case was published in detail as an example of severe renal failure after administration of apparently
compatible blood (Buchan and Wallace 1949).

A corporal aged 24 received a severe facio-maxillary injury on Jan 1, 1945. At that time he was given 5 pints (2.84 litres) of Group O blood in the course of 36 hours. His Service records make no mention of any reaction to these transfusions.

On July 24, 1946, a bone-graft operation was performed by Mr. A.B. Wallace. During the operation the patient's condition deteriorated, but a satisfactory rise in blood pressure followed the transfusion of 2 pints (1.14 litres) of Group O blood which appeared compatible on direct matching with the patient's serum.

On the morning of July 25 his condition was poor, his pulse rapid and of small volume, and his blood pressure 88/50. His collapse was attributed at the time to post-operative shock. One pint (570 ml.) of plasma and 1 pint of blood were given, and within four hours his general condition had improved, the blood pressure being 106/68. This latter blood was Group O, and appeared compatible with the patient's serum.

On the 27th he complained of nausea and vomiting, and his urine was blood-red in colour. The microscopical report on the urine was: "Vast numbers of red blood cells and a few pus cells." The urinary output was 560 ml. in 24 hours.

By the morning of the 29th the urine was clear to the naked eye, but the microscopical report stated that a few red cells were present. The nausea and vomiting were more severe. Next day his general condition had
deteriorated. Investigation of the blood chemistry gave the following results: urea, 540 mg. per 100 ml.; chlorides, 491 mg. per 100 ml.; uric acid, 4.1 mg. per 100 ml.; plasma albumin, 3.6 g. per 100 ml.; plasma globulin, 1.9 g. per 100 ml.

Analysis of the urine showed: chlorides, 0.1 g. per 100 ml.; urea, 1.3 g. per 100 ml.; urinary output, 660 ml. in 24 hours.

Because of the increasing vomiting, a slow glucose-saline infusion was started on July 30.

The patient remained in a state of uraemia until Aug. 4, when a diuresis occurred, 5.25 litres being excreted in 24 hours. The specific gravity of the urine, which had remained constant at 1010 since the operation, now began to vary, thereby showing the return of the kidney's concentrating power. From this time the blood chemistry revealed a gradual return to normal. On Aug. 10 analyses showed: urea, 55 mg. per 100 ml.; uric acid, 4 mg. per 100 ml. The blood pressure on July 30 was 154/96 and on Aug. 3 it rose to 206/120.

Convalescence was slow. Renal function was investigated by the water concentration, the urea range, and urea-excretion tests in October, and all showed a normal degree of kidney function. Blood pressure was then 128/70.

Special Investigations - Not until six weeks after the transfusions had been given were these performed, so a complete investigation was not possible. The results were: patient's blood group, 0, Rh. negative (genotype rr); patient's serum contained anti-A agglutinin, titre 1/32, anti-B agglutinin, titre 1/16, and incomplete anti-D agglutinin, titre 1/8.

Compatibility Tests - The routine test of the patient's serum against twelve random Group O cells suspended in physiological
saline showed no agglutination. However, when the red-cell suspensions were prepared with AB serum instead of saline there was agglutination of the Rh. positive but not of the Rh. negative cells. Although no agglutination of the Rh. positive cells occurred in the saline suspension, these cells showed that they had been sensitized by a rhesus antibody when tested by the indirect anti-human globulin reaction (Coombs et al. 1946).

In the accompanying Table the total fluid intake, urinary output, daily urinary excretion of sodium chloride and urea, and the level of the blood urea are shown.

### TABLE SHOWING URINALYSIS

<table>
<thead>
<tr>
<th>Day after Transfusion</th>
<th>Total Fluid Intake (ml.)</th>
<th>Urinary Output (ml.)</th>
<th>Total Daily urinary Excretion Chloride (g.)</th>
<th>Urinary Excretion Urea (g.)</th>
<th>Blood Urea (mg. per 100 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2nd</td>
<td>-</td>
<td>150</td>
<td>-</td>
<td>-</td>
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<td>3rd</td>
<td>-</td>
<td>560</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4th</td>
<td>2490</td>
<td>660</td>
<td>0.6</td>
<td>8.1</td>
<td>340</td>
</tr>
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<td>2150</td>
<td>660</td>
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<td>8.6</td>
<td>340</td>
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<tr>
<td>7th</td>
<td>2580</td>
<td>1080</td>
<td>1.1</td>
<td>13.4</td>
<td>320</td>
</tr>
<tr>
<td>8th</td>
<td>2730</td>
<td>1380</td>
<td>3.5</td>
<td>18.4</td>
<td>340</td>
</tr>
<tr>
<td>9th</td>
<td>3090</td>
<td>1920</td>
<td>4.9</td>
<td>25.5</td>
<td>340</td>
</tr>
<tr>
<td>10th</td>
<td>4050</td>
<td>2970</td>
<td>3.9</td>
<td>36.6</td>
<td>340</td>
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<tr>
<td>11th</td>
<td>5100</td>
<td>3000</td>
<td>3.0</td>
<td>42.0</td>
<td>325</td>
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<tr>
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<td>4860</td>
<td>5190</td>
<td>2.1</td>
<td>72.0</td>
<td>230</td>
</tr>
<tr>
<td>14th</td>
<td>5010</td>
<td>4800</td>
<td>6.2</td>
<td>92.0</td>
<td>230</td>
</tr>
<tr>
<td>15th</td>
<td>4470</td>
<td>4740</td>
<td>5.2</td>
<td>74.2</td>
<td>150</td>
</tr>
<tr>
<td>16th</td>
<td>3840</td>
<td>3570</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>17th</td>
<td>3870</td>
<td>3430</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>18th</td>
<td>4080</td>
<td>3390</td>
<td>6.1</td>
<td>66.0</td>
<td>55</td>
</tr>
<tr>
<td>19th</td>
<td>2850</td>
<td>2790</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Before the onset of a copious diuresis on the twelfth day there had been a gradual daily increment in the 24-hour urinary output. For a few days after the onset of the diuresis the urinary output was greater than the total fluid intake.
Muirhead et al. (1948) found that with the onset of diuresis not only was the water excretion markedly raised but the daily urinary excretion of sodium chloride was greatly increased. These workers suggested that the renal tubules had recovered sufficiently to allow for urine volume production, but that the recovery was not yet advanced enough to allow for the conservation of water and salts. In the present case there was a marked increase in the daily excretion of urea, but the excretion of sodium chloride, while increased, remained within normal limits. It would seem that the kidney had recovered sufficiently to conserve sodium chloride. It has been emphasized (Muirhead et al. 1948) that with the onset of the diuresis the kidneys may discard water and salts to the extent of causing pronounced dehydration or even death. It is therefore essential in the treatment of this phase to replace the water and salt lost from the body.

**Physical Haemolysis - Cases 15 to 21**

**Case 15 - Freezing and Thawing of Blood.** (Wallace 1950).

This patient received a blood transfusion following the operative removal of a uterine fibroid. The patient's blood group was not known at the time of transfusion, but she had not been transfused previously and had never been pregnant. Accordingly, she was given Group O, Rhesus positive, blood from the hospital blood-bank. This blood had been withdrawn from the donor 10 days previously, and appeared to be compatible with the patient's serum. The blood was not warmed before transfusion.

Approximately 250 ml. of blood was given in two hours without immediate reaction, but the medical officer discontinued the transfusion because he "did not like the look
of the blood which showed no separation into cells and plasma and appeared to be darker in colour than normal.

On the day following the transfusion the patient developed jaundice, and passed 200 ml. blood stained urine. The urinary output on the third day following the transfusion was only 100 ml. and the blood urea was 220 mg. per cent. Thereafter, the patient developed complete anuria and died suddenly on the fourth day. Permission for a post-mortem examination was refused.

Investigations - A specimen of blood from the patient soon after the transfusion was found to be Group O, Rhesus Positive. There was no evidence of cells of other groups or of irregular antibodies. A direct anti-human-globulin test on the recipient's blood was entirely negative (Hollison et al. 1948).

Unfortunately the blood remaining in the bottle was washed out immediately after the transfusion. However, on inspecting the hospital blood-bank another bottle hard up against the ice coil was found to be completely haemolysed. This latter blood was the same age as the blood which had been transfused, and both bottles had been despatched from the Regional Transfusion Centre at the same time. The technician who examined the blood before despatch reported normal appearance of the blood. There was no delay in transport or in refrigerating the blood on arrival at the hospital. Examination of the haemolysed blood showed that it was sterile.

No thermometer was kept in the hospital blood-bank, but the engineer who inspected the refrigerator reported that on several occasions he had found that the thermostatic control had been adjusted to give a temperature lower than
It was also ascertained that the refrigerator was not reserved for blood but was used for the preservation of food by the nursing and domestic staff of the hospital.

It seemed a reasonable assumption that the blood transfused was haemolyzed as the result of freezing and thawing.

At a later date the blood donor was regrouped and the original grouping as 0, Rhesus positive, was confirmed.

Case 16 — Overheating Blood. (Wallace 1950)

A patient suffering from repeated haematemesis was transfused with three pints of Group 0 whole blood from the blood-bank. She had never been transfused previously and had had two normal pregnancies. Direct compatibility tests were satisfactory. Two hours after the transfusion it was observed that the patient had a low grade pyrexia 37.4°C. (99.4°F.) and haemoglobinuria. The post-transfusion serum was definitely icteric when compared with the pre-transfusion specimen. The haemoglobinuria persisted for 6 hours. Thereafter the patient made satisfactory progress.

Investigations — A post-transfusion specimen from the patient was Group 0, Rhesus Positive. There was no evidence of free unagglutinated cells when tested with a potent anti-D serum, and the direct anti-humaa-globulin test (Mollison et al. 1948) was negative.

The residue from each bottle of donor blood was found to be Group 0, Rhesus Positive, and the red cells were compatible with the post-transfusion specimen of the patient's serum. Unfortunately, the pre-transfusion specimens of the patient's serum was not available. In addition to examining the patient's serum against the donors' cells suspended in
albumin, it was tested against a variety of Group 0 cells without agglutination occurring. The indirect anti-human-globulin test on all these cells was negative.

On centrifuging blood from each of the three donor bottles it was found that there was marked haemolysis in one of the bottles. Cultures were taken from each bottle but no bacterial growth on agar occurred at 40°C. (39.2°F.) room temperature or at 37°C. (98.6°F.).

Investigation of the method of warming the blood before transfusion showed that the bottles had been taken from the refrigerator and placed in a cabinet in the ward kitchen. This cabinet was used to warm plates, etc. The haemolysed bottle had been the last to be transfused, and had probably been in the cabinet for the longest period. By placing specimens of fresh blood in the cabinet it was shown that marked haemolysis occurred within twenty minutes.

Cases 17 and 18 - Overheating Blood.

Case 17 was a patient suffering from aplastic anaemia. He was known to be Group 0, Rhesus positive (D positive), and had been transfused on many occasions with compatible blood sent from the Regional Transfusion Centre. There had never been any reactions from these transfusions.

On the occasion of this episode compatible blood had been sent to the hospital as usual. The patient developed a transient haemoglobinuria following this transfusion.

The concentrated corpuscles which caused the reaction were regrouped and confirmed as Group 0, Rhesus positive (D positive). These cells were perfectly compatible with the pre-transfusion specimen of the patient’s serum. The blood in
the bottle which caused the reaction was found to show marked haemolysis. The blood was examined for bacteria, and cultures on agar were set up, but there was no evidence of bacterial contamination.

On the day following the report of the reaction in Case 17, a similar report was received from the same ward in the same hospital about Case 18. Compatible concentrated red corpuscles of Group A, Rhesus positive (D positive) had been provided by the Regional Transfusion Centre for this patient who was suffering from anaemia associated with ulcerative colitis. After the administration of approximately 250 ml. of this blood the patient had a severerigor, and the transfusion was discontinued. This patient had a transient haemoglobinuria after transfusion.

The donor blood remaining in the bottle was examined, and there was gross haemolysis. It was noticed that there was a solidified layer of a brown colour resembling methaemoglobin at the bottom of the bottle. The appearance suggested that the blood had been overheated to produce haemolysis and partial coagulation.

It was then ascertained that the blood used in Cases 17 and 18 had in fact been warmed by a junior nurse in the ward. A careful watch was kept at the next transfusion to be given in the same ward, and the water provided by the nurse for warming the blood was near boiling point.

Case 19 - Overheating Blood

This patient was suffering from anaemia associated with menorrhagia. She was transfused with Group O, Rhesus negative (D negative) blood. After approximately 900 ml. had
been administered she developed a rigor, and her temperature rose sharply to 105°F. (40.6°C.). The transfusion was discontinued, and the patient improved rapidly, although there was haemoglobinuria for a few hours following the transfusion.

The patient was grouped and found to be Group O, Rhesus positive (D positive). The donor blood was Group O, Rhesus negative (D negative), and perfectly compatible with the patient's serum. The donor blood remaining in the bottle was markedly haemolysed, but there was no evidence of bacterial contamination. The age of the blood was only seven days, and storage conditions had been satisfactory.

Enquiries were made regarding the heating of the blood before transfusion, and it was ascertained that a member of the nursing staff had placed the bottle of blood in a bucket to which she had added water just off the boil. It appeared that overheating the blood had caused haemolysis.

Case 20 - Over-age Blood.

This patient was suffering from accidental haemorrhage for which she received 1,100 ml. of Group O, Rhesus positive (D positive) blood. Towards the end of this transfusion she became extremely collapsed and died within one hour.

A pre-transfusion-specimen of blood from the patient was found to be Group O, Rhesus positive (D positive). The donor blood was rechecked and found to be Group O, Rhesus positive (D positive). This blood was perfectly compatible with the patient's serum, but there was evidence of haemolysis in the donor blood remaining in the bottles. There was no bacterial contamination of the blood, the storage conditions
had been satisfactory, and the blood had not been warmed before transfusion. However the age of the blood administered was 28 days.

A post-mortem examination was performed, and the salient pathological feature was marked macroscopic haemorrhage into the adrenals. The kidneys appeared normal to the naked eye, but sections were not examined microscopically. While the patient was probably doomed on account of the adrenal haemorrhage it was felt that death had been precipitated by the administration of blood which was partly haemolysed as a result of prolonged storage.

Case 21 - Over-age Blood. (Wallace 1950)

The provisional diagnosis in this patient was pernicious anaemia. This diagnosis was based on the macrocytic peripheral blood picture, but the bone marrow was not examined. The anaemia did not respond to injections of liver extract, and as the haemoglobin was less than 4 gms. per cent it was decided to transfuse.

Approximately 1 litre blood of the homologous Group 0, Rhesus positive (D positive) was given in six hours. Towards the end of the transfusion the patient had a rigor and the temperature rose to 104°F. (40°C.). On the day following the transfusion it was noticed that the patient was jaundiced, but although the urinary output was scanty and albuminuria was present there was no haemoglobinuria. The high temperature persisted, and physical examination suggested a diagnosis of lobar pneumonia. The patient died two days later and permission for a post-mortem examination was not obtained.

As this reaction was not reported to the
Regional Transfusion Centre until after the death of the patient, it was not possible to make a thorough investigation. The salient feature which did emerge was that the blood transfused had been withdrawn 35 days before the transfusion, and had not been returned to the Regional Transfusion Centre on the expiry of 21 days from collection. Another important clinical observation was that no rise in haemoglobin followed the transfusion.

**DISCUSSION**

The salient features of the twenty-one cases observed are summarised in the accompanying Table II.
### TABLE II
Salient Features of 21 Cases of Haemolytic Reaction.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Cause of Reaction</th>
<th>Volume of Incompatible or Haemolysed Blood Trans: fused. (ml.)</th>
<th>Clinical Manifestations</th>
<th>Eventual Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ABO Incompatibility</td>
<td>500</td>
<td>Febrile reaction; Jaundice; No rise in haemoglobin.</td>
<td>Recovery</td>
</tr>
<tr>
<td>2</td>
<td>do.</td>
<td>100</td>
<td>Febrile reaction; Pain in back.</td>
<td>do.</td>
</tr>
<tr>
<td>3</td>
<td>do.</td>
<td>20</td>
<td>Febrile reaction; Pain in back.</td>
<td>do.</td>
</tr>
<tr>
<td>4</td>
<td>do.</td>
<td>200</td>
<td>Febrile reaction; Haemoglobinuria.</td>
<td>do.</td>
</tr>
<tr>
<td>5</td>
<td>do.</td>
<td>250</td>
<td>Febrile reaction; Pain in back; Collapse; Jaundice; Haemoglobinuria.</td>
<td>do.</td>
</tr>
<tr>
<td>6</td>
<td>do.</td>
<td>2000</td>
<td>Haemoglobinuria.</td>
<td>do.</td>
</tr>
<tr>
<td>7</td>
<td>do.</td>
<td>100</td>
<td>Febrile reaction.</td>
<td>do.</td>
</tr>
<tr>
<td>8</td>
<td>do.</td>
<td>100</td>
<td>Pain in back.</td>
<td>do.</td>
</tr>
<tr>
<td>9</td>
<td>do.</td>
<td>700</td>
<td>Febrile reaction; Haemoglobinuria. No rise in Haemoglobin.</td>
<td>do.</td>
</tr>
<tr>
<td>10</td>
<td>Rh. Incompatibility</td>
<td>500</td>
<td>Febrile reaction.</td>
<td>do.</td>
</tr>
<tr>
<td>11</td>
<td>do.</td>
<td>800</td>
<td>Febrile reaction; Collapse.</td>
<td>Death.</td>
</tr>
<tr>
<td>12</td>
<td>do.</td>
<td>500</td>
<td>Febrile reaction.</td>
<td>Recovery</td>
</tr>
<tr>
<td>13</td>
<td>do.</td>
<td>200</td>
<td>Febrile reaction.</td>
<td>do.</td>
</tr>
<tr>
<td>14</td>
<td>do.</td>
<td>1600</td>
<td>Collapse; Haemoglobinuria.</td>
<td>Severe Uraemia with eventual Recovery</td>
</tr>
</tbody>
</table>

183.
<table>
<thead>
<tr>
<th>Case No.</th>
<th>Cause of Reaction</th>
<th>Volume of Incompatible Blood Transfused (ml.)</th>
<th>Clinical Manifestations</th>
<th>Eventual Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Freezing</td>
<td>250</td>
<td>Haemoglobinuria</td>
<td>Death</td>
</tr>
<tr>
<td>16</td>
<td>Overheating</td>
<td>500</td>
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<td>Recovery</td>
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<tr>
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<td>do.</td>
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<td>do.</td>
</tr>
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<td>do.</td>
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</tr>
<tr>
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<td>Over-age Blood</td>
<td>1100</td>
<td>Collapse.</td>
<td>Death</td>
</tr>
<tr>
<td>21</td>
<td>do.</td>
<td>1100</td>
<td>Febrile reaction; Jaundice. No rise in haemoglobin</td>
<td>do.</td>
</tr>
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</table>

Clinical Manifestations.

The most frequent feature observed was the febrile reaction consisting of a shivering attack or rigor, and a sharp rise in temperature. The intense lumbar pain which is so characteristic of intravascular haemolysis (Whitby 1942), was observed in only four of the twenty-one cases. Indeed in the whole series only Case 5 presented all the classical symptoms and signs of the early phase of a haemolytic transfusion reaction. Hollison (1943) stated that reactions to incompatible transfusion seemed to be less severe than formerly, and it was emphasised by Drummond (1944) and by Buchan and Wallace (1949) that an incompatible transfusion might be given without early symptoms occurring. Whitby (1942) suggests that lumbar pain is helpful.
in differentiating intravascular haemolysis from the more violent non-specific febrile reactions, but this has not been the author's experience. Indeed it is the author's opinion that all patients who develop a febrile reaction from the transfusion of blood should be investigated for the possible occurrence of a haemolytic reaction. Four of the cases in the present series presented no clinical features other than a febrile reaction, and in each case investigations showed that there had been a haemolytic reaction.

Mollison (1943) suggested that haemolytic transfusion reactions had become less severe than formerly, because of the increasing tendency to administer blood at a drip rate compared with the more rapid rate previously adopted. In the present series most of the cases were transfused relatively slowly, and the volume of blood transfused was relatively small. Ten of the twenty-one cases received 250 ml. or less. Bordley (1931) stated that cases receiving larger volumes of incompatible blood tend to be more severely affected - a conclusion with which Muirhead et al. (1943) are in general agreement. In the severe reactions in the present series the volumes transfused were relatively large. Case 11 received 800 ml. rapidly on account of haemorrhage and shock; Case 14 was given 1600 ml. rapidly because of post-operative collapse. Case 20 was given 1100 ml. rapidly on account of accidental haemorrhage, and Case 21 received 1100 ml., admittedly slowly, for anaemia probably haemolytic in origin. While the author agrees in general with the views of these other workers that the more rapid the rate and the larger the volume of incompatible blood transfused, the more severe the haemolytic reaction, there are exceptions to this generalisation. Case 6 received 2000 ml.
of blood of the wrong group, and showed only a transient haemoglobinuria. The rate in this case was not rapid, as the transfusion occupied 12 hours. On the other hand Case 15 proved fatal although only 250 ml. of haemolysed blood was transfused in two hours.

In general, sensitization to the Rh. antigen which occurs as a result of repeated transfusions develops slowly, and early reactions are mild or inapparent (Mollison 1948). It is interesting to note in the present series of cases that lumbar pain, which according to Whitby (1942) is so characteristic of intravascular haemolysis, was observed in four of the nine cases of ABO incompatibility, but in none of the five cases of Rh. incompatibility. Of these five cases of Rh. incompatibility Cases 10, 12 and 13 are in accord with the observation of Mollison (1948) concerning the slow development and mild nature of early reactions. On the other hand the reaction in the other two cases of Rh. incompatibility was severe. In Case 11 the patient died, but the patient was already seriously ill from acute blood loss. Case 14 had a severe reaction with uraemia after virtually the second transfusion. The original transfusion of 5 pints of blood to this patient within 36 hours at the time of wounding can be regarded as a single antigenic stimulus. It is of comparative interest that Cappell (1948) has found that the first recorded manifestation of haemolytic disease of the newborn due to Rh. incompatibility is usually one of the severer forms, contrary to the widely held belief that the first one or two affected children in a family show only mild symptoms.

The danger of haemolytic reactions simulated by the transfusion of haemolysed blood is emphasised by Mollison (1948),
Drummond (1949), and Wallace (1950). The particular danger of allowing blood to freeze is emphasised by De Gowin (1949), and a typical haemolytic reaction with haemoglobinuria and jaundice following the transfusion of frozen blood is recorded by Riddell (1939). Two cases of haemolytic reactions from frozen blood are reported by Drummond (1949). One of these two cases ended fatally, but the patient was an infant with severe haemolytic disease of the newborn. Case 15 in the present series illustrates that the haemolytic reaction which follows the transfusion of frozen blood may end fatally. The reactions from overheating blood described in the present study were all mild, but Baker (1937) records a fatal case. Mollison (1943) describes a case in which the transfusion of seven week old blood caused haemoglobinaemia, but no haemoglobinuria or jaundice. Drummond (1949) reports a case in which oliguria followed the transfusion of approximately 1900 ml. of aged blood, and emphasises the danger of a fatal haemolytic reaction from the transfusion of a large volume of time-expired blood. Cases 20 and 21 in this study each received 1100 ml. of time-expired blood, and both patients died. One of these patients was suffering from severe accidental haemorrhage, and the other from profound anaemia complicated by pneumonia. It may well be that these patients would have died apart from a haemolytic reaction, but the occurrence of a reaction probably removed any slender chance of recovery.

The presence of vast numbers of red blood cells and a few pus cells in the urine in Case 14 seemed at first to be in favour of a diagnosis of nephritis rather than of a haemolytic reaction. However, the presence of intact red
cells in the urine is common, and white blood cells, often in clumps, are present during the oliguric period following an incompatible transfusion (Muirhead et al. 1943). Pigment granules, often about the size and shape of a red blood corpuscle (for which they are not infrequently mistaken), are seen in haemoglobinuria (Bywaters and Dible, 1943).

The Incidence and Mortality of Haemolytic Reactions

The twenty-one reactions reported in this communication occurred during a period in which approximately 17,000 patients were transfused with blood, i.e., an incidence of 1.2 haemolytic reactions in every 1000 transfusions, and of these 0.24 per 1000 (19 per cent) were fatal.

These figures compare favourably with the statistics of Kilduffe and De Bakey (1942) who compiled the results of 43,254 blood transfusions by 18 different groups of workers. There were 1.8 haemolytic reactions in every 1000 transfusions, and of these 1.04 per 1000 (57.7 per cent) were fatal.

Muirhead et al. (1943) reported their experience of 28,630 blood transfusions given in hospital practice. There were 17 known haemolytic reactions or an incidence of 0.6 reactions per 1000 transfusions. Of these reactions 3 (17.6 per cent) were fatal. These figures compare favourably with those reported by the author, but it should be remembered that the observations of Muirhead et al. (1943) were made in one hospital, whereas the present study is based on many different hospitals supplied by a Regional Transfusion Centre.

Prevention of Haemolytic Reactions

Although the incidence of haemolytic reactions
is not high, there is nevertheless a definite morbidity and mortality associated with this type of transfusion reaction. In the present study an attempt has been made to recognise and assess the factors which have been responsible for the reaction.

One point of fundamental importance in every aspect of blood transfusion work is clerical accuracy. This applies in particular to the correct labelling of specimens and of flasks of fluid. The reaction which occurred in Cases 1, 2, 3 and 4 was the direct result of clerical inaccuracy.

In spite of the undetected clerical errors in these four cases the reaction would have been avoided in three of these cases (1, 2 and 3) if only a compatibility test had been properly performed. Indeed the importance of a correctly performed compatibility test is well illustrated in the present study. In nine of the Cases (1, 2, 3, 5, 6, 7, 8, 9, 11) a compatibility test was either not performed or imperfectly performed.

While this study emphasises the value of the compatibility test it also exposes its limitations. The direct compatibility test in which the patient's serum is set up against saline suspensions of red cells will fail to detect the presence of "blocking" or albumin Rhesus antibodies. In Cases 10, 12 and 14 the irregular antibody which caused the haemolytic reaction was in the form of an albumin Rh. antibody. To detect such antibodies the red cells must be suspended in AB plasma or preferably in 20 per cent bovine albumin solution. Alternatively the compatibility test may be performed in the usual manner and then an indirect anti-human-globulin test performed on the washed cells. When facilities do not exist for the performance
of these more refined tests in such cases, it is possible to safeguard the patient by determining the patient's Rhesus blood group (D antigen) in all patients who have been previously transfused or have been pregnant. By testing with anti-D serum, and ensuring that D negative patients receive Rhesus negative blood (cde, cde) the danger to the patient is almost eliminated.

It is clear from the cases described in this study that lack of experience in blood group serology and in the care of the blood-bank is largely responsible for the occurrence of haemolytic transfusion reactions. Tovey (1950) emphasises that grouping and matching of blood should be undertaken by trained and experienced workers only, and that a clinician who is likely to have to perform these tests should first undergo instruction.

The cases in this study emphasise some important practical aspects in the care of blood and of a blood-bank. Case 15 resulted from the freezing and thawing of blood, and illustrated the following points -

(i) The importance of having a recording thermometer or a maximum and minimum thermometer in the refrigerator.

(ii) The importance of not putting bottles of blood hard up against the ice box in the refrigerator, and of not packing loose ice against bottles during transportation.

(iii) The danger of using a refrigerator for purposes other than blood storage, and of allowing all and sundry to have access to the cabinet (Drummond 1949).

Cases 16 to 19 emphasise the danger of overheating blood, particularly if the warming is entrusted to an
Wallace and Richards (1943) found that no untoward reactions occurred with blood transfused without warming. Whitby (1942) has pointed out that cold blood given to a patient with well developed cold agglutinins will induce a haemolytic crisis comparable to the disaster of a grossly incompatible transfusion. Wallace (1950) advises warming the blood to body heat before transfusion in such cases, although in general recommending that it is satisfactory and safe to allow blood which has been removed from the refrigerator to return slowly to room temperature only, before transfusion.

Cases 20 and 21 emphasise the importance of avoiding the use of time expired blood, particularly in anaemia of uncertain aetiology, and stress the danger of not returning blood to the Regional Transfusion Centre immediately after the expiry of 21 days from the date of blood withdrawal (Wallace 1950).

Treatment of Haemolytic Reactions

Many forms of therapy have been advocated in the treatment of haemolytic transfusion reactions - e.g., alkaline therapy, forced fluids, transfusion of compatible blood, splanchnic block, and decapsulation of the kidney (Kilduffe and De Bakey, 1942) - but, opinions differ on the value of these methods. Few observers are in a position to study a large controlled series of such cases, and this renders assessment of a particular form of therapy difficult. According to Buchanan and Wallace (1949) some patients recover spontaneously, while others die in spite of the therapeutic measures advocated.

Case 14 in the present study developed severe uraemia from which the patient recovered. This case illustrates that even severely damaged kidneys may recover completely.
without any heroic therapy. Indeed modern opinion regards former therapeutic measures as too heroic. Many patients with severe haemolytic reactions have died as a result of the administration of an excessive amount of fluid and alkali during the phase of urinary suppression (Muirhead et al. 1948).

Encouraging success has been attained by the careful control of water, salt and protein intake (Bull et al. 1949). These workers also emphasize the importance of correcting anaemia by transfusion of compatible blood.

**SUMMARY**

The incidence, clinical manifestations, and clinical course of haemolytic transfusion reactions are reviewed.

The methods of investigating haemolytic reactions are discussed.

The case records and the results of investigating haemolytic reactions in twenty-one cases are recorded. Most of these reactions were mild, but four patients died and another only recovered after severe renal failure.

In general the severity of the reaction is directly proportional to the volume of blood transfused and to the rate of transfusion, but there are exceptions to this generalization.

Reactions to Rh type blood group incompatibility tend to be mild in early transfusions, but severe renal failure at a second transfusion is described.

Haemolytic reactions have a definite, if small, morbidity and mortality. Preventive measures include clerical accuracy, properly performed compatibility tests, and the development of Rh type blood grouping. Experience in blood group
serology, and in the care of the blood-bank is essential among those who undertake transfusion therapy.

The broad principles of treatment are indicated.

REFERENCES (CHAPTER V)


Tovey, G.H. (1950). The Practitioner, 164, 171.


CHAPTER VI

GENERAL CONCLUSIONS

An attempt has been made in the reported studies to assess the nature, the incidence and the gravity of transfusion reactions which occur in modern blood transfusion practice.

The following classification of transfusion reactions has been adopted, and is recommended for use in clinical practice -

1. Pyrexial Reactions

2. Transmission of Disease
   (a) From Donor to Recipient
   (b) Extraneous Contamination of Transfusion Fluids.

3. Mechanical Reactions
   (a) Circulatory Overloading
   (b) Air Embolism
   (c) Pulmonary Embolism

4. Haemolytic Reactions

To these major reactions may be added a group of minor reactions which occur infrequently. A summary will be given of the author's experience of these minor reactions in addition to the general conclusions derived from the studies on the major reactions.

1. Pyrexial Reactions - This is the type of transfusion reaction which is most frequently observed.

In modern hospital practice in which transfusion fluids are supplied along with administration sets from the Regional Transfusion Centre, it should be possible to reduce the incidence of pyrexial reactions to 2 per cent following the administration of whole blood or concentrated corpuscles, and to one per cent or less following the administration of
This low incidence of reactions will only be maintained, provided there is scrupulous attention to all aspects of transfusion technique. Even a minor departure from a strict routine may result in a sharp rise in the pyrexial reaction rate.

In practice the most important causes of pyrexial reactions are stale distilled water, impure chemicals, and imperfectly prepared administration sets. Another source of pyrogens may be minimal bacterial contamination, so that blood should be immediately, accurately, and continuously refrigerated. Blood stored in this way in an acid citrate dextrose preservative for periods up to 21 days, gives a reaction rate no higher than that given by fresh blood.

Fortunately the vast majority of pyrexial reactions are harmless to the patient, but on rare occasions such a reaction may prove fatal. Pyrexial reactions appear to be particularly serious in patients suffering from advanced liver disease.

The temperature of the fluid transfused, and the rate of administration do not affect the incidence of pyrexial reactions, although occasionally the rapid administration of fluid may cause a shivering attack, not associated with elevation of body temperature.

It is important to differentiate true pyrexial reactions from febrile manifestations of the primary disease.

2. Transmission of Disease.

(a) From Donor to Recipient

Syphilis - There was an observed incidence of 39 positive serological reactions among 50,000 blood donors.
This was significantly lower than the incidence of 34 positive reactions among 10,000 patients attending ante-natal clinics in the same area.

Serological tests have limitations in that these tests will not detect the donor in the sero-negative phase of early syphilis.

However the incidence of syphilis among voluntary blood donors is so small, and the loss of infectivity of Treponema pallidum in preserved blood so rapid, that the danger of transmitting syphilis by transfusion under the present voluntary donor system is remote. It is advisable to perform serological tests on every donor at each donation.

Malaria - There is a danger of transmitting this disease by the use of donors who have suffered from malaria or have lived in a malarial area even many years previously. There is no test which will detect the potentially dangerous donor. It is necessary to depend upon the history given by the donor.

The influence of refrigeration on the survival of the malarial parasite in preserved blood is equivocal.

The policy of waiting three years since the last attack of malaria, or the last period of residence in a malarial country, before collecting blood for use as whole blood, appears to be satisfactory. No known instances of malaria developing among 2,000 recipients of blood from potentially dangerous donors have occurred under this policy.

The withdrawal of blood from a donor who has had malaria or has lived in a malarial area may precipitate an attack of malaria in the donor.
Homologous Serum Jaundice - The incidence of this condition following transfusion of blood was 0.8 per cent, whereas the incidence with pooled plasma (120 to 200 donors per pool) was 3.7 per cent. The size of the plasma pool appears to determine the incidence with plasma infusions, and by using small pools of eight donors or less it is possible to reduce the incidence to the same order as with blood. Complete safety in the use of plasma will only be achieved by destroying the icterogenic virus by some agent such as ultra-violet irradiation.

Most cases of homologous serum jaundice are only moderately ill, and some are not recognised, being regarded as bilious attacks. Occasionally a case may terminate fatally.

Allergic Reactions - Eight cases of urticaria were observed among 92 recipients of blood. It appears that if recipients are observed carefully, the incidence of this type of reaction is around one per cent.

In the majority of reactions of this nature there is no history of previous hypersensitivity on questioning the donor or recipient.

These reactions are not generally severe, and adrenaline and anti-histamine drugs are of value in relieving symptoms and allowing the transfusion to be continued.

(b) Extraneous Contamination of Transfusion Fluids.

Blood should be cleanly collected from the donor, but even with careful technique there is a potential danger of bacterial contamination. It is therefore important to ensure that blood is immediately, accurately, and continuously refrigerated, so that the growth of bacterial contaminants may be reduced to a minimum.
Failure to observe these precautions may have serious consequences, and two fatalities from the transfusion of contaminated blood have been reported in the present studies.

There is an increased risk of contamination in the use of concentrated red corpuscles, and it is recommended that these should be used as soon as possible after preparation.

The care of a hospital blood-bank is a great responsibility, and should be entrusted to an experienced person.

The danger of bacterial contamination with plasma is not as great as with blood, provided the plasma processing includes bacterial filtration. It is important not to open a flask of plasma to the atmosphere until it is required for use.

3. Mechanical Reactions

(a) Circulatory Overloading

The rate of haemodilution following haemorrhage in man is variable, but slow. The time taken to reach maximum haemoglobin dilution varied from 3 to 90 hours.

Overdilution may occur occasionally following acute haemorrhage. This phenomenon is of clinical importance when considering the transfusion of patients with low haemoglobin levels after acute haemorrhage.

When the blood volume is stable, injected serum tends to leave the circulation, and, though in many subjects it is retained longer than an equal quantity of saline, in a few it is lost with great rapidity. On the other hand, when the blood-volume is acutely reduced by a large venesection, serum injected immediately is retained in the circulation. Saline is not retained under the same circumstances.
The rapid injection of 2 litres of saline, serum and blood into subjects without cardiovascular disease results in a rise of venous pressure in cases in which there is considerable retention of injected fluid in the circulation. Radiographs in these cases show an increase in the diastolic size of the heart, enlargement of the pulmonary arteries, and prominence of the vascular markings in the lung fields. Vital capacity was diminished, but there was no evidence of pulmonary oedema. There is a rapid return to normal on ceasing the injection.

The circulatory blood-volume in severe anaemia is reduced. In spite of this the systolic pressure is maintained. The filling pressure of the heart is increased in order to maintain an adequate cardiac output. Transfusion is potentially dangerous in such cases as the addition of fluid to the circulation will raise the filling pressure even more, and may lead to a falling cardiac output and pulmonary oedema.

Circulatory overloading is a rare, but real hazard of transfusion therapy. Three fatal cases are reported in the present studies. The danger is greatest in severe anaemia, particularly if the heart is already functionally impaired by anaemia or by heart disease. Such patients must be transfused very slowly, preferably with concentrated red corpuscles, and the patient must be observed closely for signs of overloading. In particular, the patient should be observed in the propped-up position for the filling pressure of the neck veins.

(b) Air Embolism.

This is a rare, but dangerous complication of
transfusion therapy, which results from faulty technique.
In most cases it results from the over enthusiastic use of a
Higginson's syringe. This complication may also arise in blood
donors should the airway of the withdrawal set become blocked.

(c) Pulmonary Embolism.
This complication is more theoretical than real. It is important to ensure that there is a filter present in the administration set to retain any clots present in blood or plasma, and it is equally important not to attempt to restart at the same site, a transfusion which has stopped for a considerable period.

These reactions have a definite, if small, morbidity and mortality. Twenty-one cases among 17,000 patients transfused are recorded. Four of these cases died.
Renal failure is the cause of death in most fatal haemolytic reactions.

In general the severity of the reaction is directly proportional to the volume of blood transfused, and to the rate of transfusion, but there are exceptions to this generalisation.
Reactions to Rhesus blood group incompatibility tend to be mild in the first few transfusions, but may be severe at the second transfusion.
Preventive measures include clerical accuracy, properly performed compatibility tests, and the development of Rhesus blood grouping. Experience in blood group serology, and in the care of the blood-bank is essential among those who undertake transfusion therapy.
Venous Spasm - Occasionally there may be spasm of the vein at
the site of puncture so intense as to interfere with the
administration of the transfusion fluid. In many of these
cases the vein which was large, obviously visible, and easily
palpable suddenly became small and almost impalpable on the
insertion of the needle. It appears to be mechanical
stimulation of the vein which causes spasm. Another factor
which may cause venous spasm is the temperature of the
transfusion fluid. Blood transfused at refrigerator
temperature may cause venous spasm.

When venous spasm occurs the application of
local heat will relieve the spasm, and the transfusion may be
given without difficulty.

Haematoma - Extravasation of blood at the site of venepuncture is usually of little consequence, producing some
discolouration. Occasionally the haematoma may be so large
as to cause mechanical interference with the transfusion.

The most frequent cause of extravasation of
blood is imperfect entry of the needle into the vein.

Failure to release the tourniquet promptly may also result in
a haematoma.

Dermatitis from Adhesive Tape - Rarely a patient develops a
contact dermatitis from adhesive tape. In those known to be
sensitive some other type of bandage should be applied.

Abscess and Cellulitis - Venepuncture produces a portal for
possible entry of bacteria into the subcutaneous tissues.

Occasionally this may occur with the production of a localized
abscess or cellulitis of the arm. This complication should be
avoided by clean technique.

**Thrombophlebitis** - Needle puncture may permit the entry of bacteria into the vein with a resulting thrombophlebitis. This is usually a localised infection, but the author has seen a fatal staphylococcus aureus pyaemia result.

**Aseptic Thrombosis** - This complication is occasionally observed. At the site of venepuncture, and for a distance of two or three inches or more, the vein becomes hard and cord-like. Tenderness is not a special feature, and spontaneous resolution apparently occurs in a few weeks. Severely anaemic patients are more liable to develop this complication. This complication occurs even when the venepuncture has been cleanly and accurately performed, and cannot therefore be regarded as avoidable.

In conclusion it may be stated that transfusion reactions in general are relatively uncommon, but with the widespread use of transfusion in modern therapeutics these reactions are important. This importance is emphasised by the unfortunate fact that a transfusion reaction may prove fatal. The vast majority of these reactions are avoidable, and it is essential that blood transfusion therapy should only be undertaken by those who are fully aware of the hazards of blood transfusion.