DEFECTIVE GAS TRANSPORT FUNCTION OF STORED RED BLOOD CELLS. WITH OBSERVATIONS ON THE OXYGEN DISSOCIATION CURVE IN ANAEMIA.

by

Arthur Colville Kennedy

M.B., Ch.B., M.R.C.P.E., F.R.F.P.S.G.

Thesis presented to the University of Glasgow for the Degree of Doctor of Medicine.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

PREFACE

The experimental work contained in this thesis was performed in the University Department of Medicine, The Royal Infirmary, Glasgow and grateful acknowledgement is made to Professor L.J. Davis for his ever-helpful advice and for the generous facilities granted.

The author also wishes to express his appreciation to Dr. D.J. Valtis of the University of Salonika who, as a visiting research worker, shared in some aspects of the project and without whose aid it would have been physically impossible to have performed all of the many blood gas estimations within the time limits imposed by the experimental techniques.

Doctor J. Wallace of the West of Scotland Blood Transfusion Service gave ideal cooperation in the transfusion studies by providing citrated blood which had been stored for specified periods. Doctor A.G. Baikie of the University Department of Medicine, The Royal Infirmary, Glasgow gave assistance in the spectrophotometric observations.

CONTENTS

CHAPTER 1

INTRODUCTORY

Blood transfusion is now universally accepted by physician, surgeon and obstetrician alike as an extremely valuable and, indeed, often life-saving, therapeutic measure. The demand for blood is so great and so constant that a National Blood Transfusion Service, holding large stocks of stored blood, voluntarily donated, is now an integral part of the medical services of the country.

The search for ideal storage conditions, begun in America some forty years ago by Rous and Turner (1916) and in this country by Robertson (1918) became intensified, for obvious reasons, during the 1939 - 1945 war. With the introduction of the now familiar acid-citrate/dextrose mixture by Loutit and his associates in 1943 there was a substantial measure of agreement that a suitable preservative solution for red cells had been obtained; the mixture is still in general use.

Mollison (1954) has recently defined the criteria of successful red cell preservation....."One satisfactory feature/

feature of studying the preservation of red cells is that the objective can be precisely defined; the red cells should undergo no haemolysis during storage and when transfused should survive in the recipient's circulation just as well as freshly drawn red cells." Because of the labour involved in determining the survival time of red cells many workers have sought to appraise red cell storage conditions in a simpler fashion, from the physical, chemical and morphological changes occurring in vitro. Among these are spontaneous haemolysis (De Gowin et al 1940), alteration in osmotic fragility (Bagdassarow 1937), dimensional changes of the erythrocyte (Drew et al 1939), and changes in the distribution of phosphorus (Aylward et al 1940, Maizels 1943) or potassium and sodium (Jeanneney and Servantie 1938). Several studies (Maizels 1941, Mollison and Young 1941 and 1942, Loutit et al 1943) have demonstrated that these in vitro changes give little or no indication of the viability of stored red cells yet Rapoport (1947) still contends that they may be a "useful preliminary procedure."

When we consider that the major purpose of transfusion/

transfusion of whole blood, as compared with the transfusion of plasma or plasma products, is to provide the recipient with red cells which can perform their normal function of transporting oxygen to, and removing carbon dioxide from, the tissues it would seem reasonable to suggest that, in addition to studying the viability of the stored red cell, it would be desirable to determine whether storage affects, adversely or otherwise, the vital physiological function of gas transport. It is indeed surprising that this question should have received scant attention particularly in view of the very considerable volume of research expended on the subject of blood transfusion and storage conditions. The only observations in this field appear to be those of Belk et al (1939) in America, Scarborough and Thomson (1940) in Edinburgh, and Denstedt et al (1941) in Canada,who reported that the oxygen capacity of blood is unimpaired by storage. It is fallacious to assume, however, (Rapoport 1947) that the oxygen capacity provides an indication of the rate of reaction between haemoglobin and oxygen on which depends the release of oxygen to the tissues.

This/

This functional activity of the red cell may be satisfactorily investigated by studying the oxygen and carbon dioxide dissociation curves and their inter-relationship. Such an investigation has been attempted and in this thesis the author presents observations on

a) the oxygen and carbon dioxide dissociation curves of red blood cells stored in the widely used acid-citrate/dextrose mixture.

b) the effect of transfusion of stored citrated red cells on the oxygen dissociation curve of the recipient.

c) the causes and possible method of prevention of the abnormalities detected.

In the course of the investigations it was found necessary to determine the influence of anaemia per se on the position of the oxygen dissociation curve before the effects of transfusion of stored citrated blood could be properly assessed. The findings, together with a review of the previous work in this field, are presented in Chapters 8 - 10.

CHAPTER 2

THE DEVELOPMENT OF BLOOD TRANSFUSION

In any scientific study it is not only fitting, but indeed salutary, to pause and consider the findings, problems and aspirations of those who performed the initial critical experiments, successful and unsuccessful, and to trace through their discoveries the steps by which our present day knowledge has evolved. Only thus may we arrive at a true assessment of the worth, or otherwise, of current research studies.

From ancient times blood has been associated in the minds of men with life and it is set forth in the Old Testament (Leviticus xvii, 11) that "the life of the flesh is in the blood". For many centuries it was customary to ascribe to disorders of this vital fluid the great majority of the ailments to which the human frame is subject. It is not surprising, therefore, that man should have conceived the idea of giving the blood of the healthy to the ill, the strong to the weak, the sane to the insane, and the young to the old. In her History of Medicine (1947), Mettler records/

records that no less a mediaeval authority than Galen advised draughts of the blood of the goat as a remedy for renal stone and dropsy, of domestic fowls for cerebral haemorrhage, of the lamb for epilepsy, and (an exotic touch) of the land crocodile as a specific for acuteness of vision. Even in the present century (Frazer 1922) the primitive tribes of central Australia commonly give human blood to the sick and aged in the hope of strengthening them; their belief in the potency of this form of treatment is so great that in some instances the blood will not be taken internally but will merely be sprinkled on the body of the infirm person.

There could clearly be no move from these primitive beliefs towards the idea of transferring blood from the arteries or veins of one individual into the veins of another before the early part of the 17th century when the brilliant work of William Harvey gave to the world an understanding of the circulation of the blood. Later in the century Richard Lower, a distinguished Oxford anatomist and physiologist, performed further experimental work on the circulation of the blood and was the first in this country to publish observations (1666)/

(1666) on the transfusion of animal blood to animals and later animal blood to humans. Meanwhile similar work was being undertaken on the Continent by Jean Denys (1667) and there is some dispute as to which of the two was first in the field. It is probable that several other enthusiasts followed the lead given by the work of Harvey and the experiments of Lower and Denys and since animal blood is incompatible with human blood it is likely that there were many untoward, if not fatal, sequelae; those patients who received a "successful" transfusion were probably fortunate in that the volume of animal blood transfused was mercifully small.

James Blundell, a London medical man who served simultaneously on the staffs of Guy's and St.Bartholomew's Hospital, had the distinction of being the first to transfuse a patient with human blood. For this transfusion, performed in 1818 and described in 1824, Blundell used a syringe and cannula and gave rather less than a pint of blood derived from several donors; the procedure, although technically successful, was performed on a dying man but Blundell a few years later (1829) carried/

carried out the first of a small number of transfusions with apparent benefit to the recipient remarking that "It is worthy of notice that the patient expresses herself very strongly on the benefits resulting from the injection of blood; her observations are equivalent to this - that she felt as if life were infused into her body". Throughout the remainder of the 19th century several obstetricians and surgeons sought to improve upon the techniques employed by Blundell and numerous ingenious devices were constructed to enable the collection and transfusion of blood to be carried out before clotting occurred. A few abortive experiments were also made with defibrinated blood while Hicks (1869) attempted to prevent clotting by the addition of an anticoagulant; unfortunately, sodium phosphate, which is toxic, was used but, in Hick's own words, the method was brought forward "rather as a hint, and as an earnest of better things". In the latter part of the 19th century a serious, but limited, attempt was made by a French Army surgeon by the name of Roussel to counteract the effects of severe blood loss from war injuries by the transfusion of human blood and his experiences/

experiences with seven cases of "wounds on the field of battle" in the Franco-Prussian conflict of 1870 - 1871 are set forth in his publication of 1877 together with records of a larger number of transfusions performed on civilians. Less than a century later blood and plasma transfusions to war casualties were commonplace and to be numbered in their thousands. Thus in the operations following the invasion of Normandy in 1944 it is on record (Chambers's Encyclopedia 1950) that the British forces alone used in four months 101,284 pints of blood and blood substitutes.

Up until the beginning of the 20th century there was no knowledge of the different blood groups of man. It is therefore likely that reactions due to the transfusion of incompatible blood were common and may well have aroused antagonism to the use of blood transfusion as a therapeutic measure. The discovery by Landsteiner and Jansky at the beginning of the century of the four basic blood groups was an immense step forward for although many finer subdivisions in blood grouping have since been made it was possible from that time to provide what was, in a broad sense, compatible/

compatible blood for transfusion.

The real difficulty that remained before blood transfusion could become the relatively simple procedure of today was to find a suitable method of preventing clotting of the donor blood. Until this could be done it was necessary to use a direct transfusion technique in which an artery (usually the radial) of the donor was connected, either directly or by a thin silver or rubber tube, with a vein of the patient. In addition to the very considerable time and skill required for its successful execution the direct technique had the disadvantage that it was impossible to measure the amount of blood transfused. The problem developed a real urgency during the 1914-18 war when many thousands of young men died from acute blood loss. For a time wax-coated vessels (e.g. the Kimpton-Browne tube) were used but they had many shortcomings. Observations on the use of sodium citrate as an anticoagulant for blood transfusion purposes were actually published at the beginning of the war (Hustin 1914, Agote 1915, Lewishon 1915) but it was not until three years later that the method was used,/

used, and even then only on a small scale, for the treatment of war casualties (Robertson 1918). Had scientific progress been more rapid it is probable that many thousands of lives might have been saved.

In the years following the war there was a steady growth in the demand for blood for transfusion and in this country the need was met, in a typically British fashion, by a considerable body of men and women who voluntarily formed panels of donors at first in London and later in the other cities; the voluntary system of donation has happily persisted.

For many years after the 1914-1918 war only blood freshly drawn into a citrate anticoagulant was used for transfusion purposes although Rous and Turner in 1916 and Robertson in 1918 had conceived the idea of employing blood withdrawn at leisure and stored until required and had made preliminary observations, noting in particular the value of adding glucose to the anticoagulant solution. The fresh blood system with the donor being called as required was able to meet the growing, but still relatively small, demands of the interwar years but with the threat of many thousands of war casualties attention was, of necessity, focussed once/

once more on the question of storage. The Spaniards were the first to feel the urgent stimulus of war and the techniques devised by them for the collection and storage of blood during the Civil War of 1937 - 1939, and subsequently described in the medical press of this country by Duran-Jorda (1939), provided valuable information for those responsible in Britain for the required expansion of the blood transfusion service in the critical years of 1939 - 1945. At the outbreak of the war a trisodium-citrate/dextrose anticoagulant medium was employed and remained in use until the work of Loutit et al in 1943 showed conclusively that the acid disodium citrate salt was superior to the trisodium salt. An acid-citrate/dextrose anticoagulant solution, such as the one studied in this thesis, has been in general use since that time. Blood could now be stored at a low temperature for periods of two or three weeks, or in emergencies rather longer, and large stocks of blood could thus be maintained in blood banks available for instant use. It has been calculated that some two million donations of blood were made in this country during the war years and it is certain that the transfusion of blood or blood derivatives played/

played a very large part in the recovery of thousands of casualties.

With the introduction of the acid-citrate/dextrose anticoagulant medium and the demonstration that the viability of red blood cells was not significantly affected by a two or three week period of storage therin, most authorities accepted that a suitable preservative solution for red cells had been obtained. Further research tended to be directed towards other problems of which perhaps the most important were the serological complexities related to the Rhesus factor, the control of haemolytic disease of the newborn by exchange transfusion, the preservation and transfer of leucocytes, platelets and the other complex factors concerned in blood coagulation, the development of blood derivatives and substitutes, and the storage of red cells at very low temperatures for prolonged periods. In addition, the survival time of transfused red cells was used as a means of investigating various blood disorders, in particular the haemolytic anaemias.

In this thesis attention is redirected to the question of preservative solutions for stored red cells. The observations presented refer to the presence, causation,/

causation, and prevention of a shortcoming of the present-day storage conditions which has not been previously commented upon, namely a deleterious effect on the gas transport function of the red cells. The findings clearly indicate that in the generally used acid-citrate/dextrose medium we have not yet the ideal preservative solution for red cells. Moreover, they suggest that any appraisal of storage conditions is not complete without a study of the ability of the stored red cell to transport oxygen.

CHAPTER 3

NORMAL OXYGEN AND CARBON DIOXIDE TRANSPORT

Before considering the effect of storage in a citrate anticoagulant on the gas transport function of red blood cells it is desirable that a brief account should be given of the normal processes of oxygen and carbon dioxide transport and, in particular, of the factors, physiological and pathological, known to influence this essential biological activity.

The Transport of Oxygen

The manner in which oxygen is taken up from air in the lungs, transported in the blood in combination with haemoglobin, and then released to the tissues has been a fascinating study for many workers among whom the most eminent are perhaps Bohr, Hufner, Barcroft, Henderson, Adair, Bock, and Van Slyke. The pioneer work of these investigators has given us our present day understanding of this physiological process which is essential to life itself.

When the iron-containing pigment haemoglobin is exposed to oxygen a chemical reaction occurs with the formation/

formation of oxyhaemoglobin. This reaction is reversible and at low oxygen partial pressures dissociation occurs with the formation of reduced haemoglobin.

$$Hb + O_2 \rightleftharpoons HbO_2$$

reduced haemoglobin — oxyhaemoglobin

The capacity of the blood for absorbing oxygen depends upon the amount of haemoglobin present. If normal arterial blood is saturated with oxygen, 100 volumes of blood combine with 20 volumes of oxygen; of these 19.6 volumes are in chemical combination with haemoglobin, 1 gram of haemoglobin combining with 1.34 ml. of oxygen. The remaining 0.4 volumes are in physical solution in the plasma; although this amount is small it is of great importance since the plasma oxygen tension determines the amount of oxygen held in combination with haemoglobin.

It is essential to oxygen transport that haemoglobin circulates in the body highly concentrated within the red cells (30 grams of haemoglobin per 100 ml. of blood). The membrane of the red cell permits gaseous exchange and the biconcave shape of the cell presents a larger surface area than would be present if the cell were spherical. More important, if haemoglobin were not enclosed/

enclosed within the red cell but were present as a solution in the plasma it would, within a few days, become transformed to methaemoglobin and thus be inactive as regards gas transport.

The oxygen dissociation curve

The amount of haemoglobin present as oxyhaemoglobin and the amount as reduced haemoglobin depends on the partial pressure of oxygen and the relationship can be shown as a curve which is known as the oxygen dissociation curve of haemoglobin. Such a curve shows the points of equilibrium in the equation $Hb + O_2 \rightleftharpoons HbO_2$ at different oxygen partial pressures. The oxygen dissociation curve is obtained by exposing samples of haemoglobin to known oxygen partial pressures in closed containers (tonometers) and rotating these tonometers at body temperature until equilibration is present. The haemoglobin samples are then analysed, the proportion of oxyhaemoglobin to reduced haemoglobin determined and the results plotted as in Figure 2.

The oxygen dissociation curve for a dialysed haemoglobin solution was found by Barcroft to be a rectangular hyperbola while that of whole blood, or laked/

laked blood, is S shaped (Figure 2). The S shape of the curve is of great advantage to the organism. Thus at any partial pressure of oxygen above about 70 mm. Hg haemoglobin is practically wholly saturated with oxygen while at the lower partial pressures of oxygen which are present in the tissues dissociation rapidly occurs and the oxygen is released. The greater the need for oxygen in the tissues the lower will the partial pressure of oxygen be and the greater the release of oxygen. Under resting conditions only about thirty per cent of the oxygen carried by the haemoglobin is released to the tissues but in severe exercise the proportion may rise to seventy, eighty or even ninety per cent. In contrast, a solution of haemoglobin would not give up any significant amount of the carried oxygen until the oxygen partial pressure in the tissues was extremely low.

While the advantage of the S shape of the curve can be readily appreciated the mechanism responsible for it is more complex. Following the work of Adair (1925a, 1925b), Pauling (1935) and Coryell et al (1939) there is now a large measure of agreement that the S shape/

shape of the oxygen dissociation curve is related to the manner in which the iron in the molecule of haemoglobin combines with oxygen under physiological conditions, namely a high concentration of haemoglobin within red blood cells, a temperature of 37°C, and a carbon dioxide tension of 40 mm. Hg. or more. The haemoglobin molecule, containing 4 haeme groups and 4 ferrous atoms of iron, is believed to react with oxygen in four stages each of which increases the speed of the following.

$$Hb_4(Fe)_4 + O_2 \rightleftharpoons Hb_4(Fe)_3(FeO_2) + O_2 \rightleftharpoons Hb_4(Fe)_2(FeO_2)_2 + O_2$$
$$\rightleftharpoons Hb_4(Fe)(FeO_2)_3 + O_2 \rightleftharpoons Hb_4(FeO_2)_4$$

In performing oxygen dissociation curves in the laboratory the blood samples must be shaken for half an hour with the appropriate atmospheres before equilibration can be considered complete. In the body however, haemoglobin combines with oxygen in the lungs and releases oxygen to the tissues at great speed as a result of complex enzymatic activity. Experiments suggest that the time required is less than one hundredth of a second. In spite of the marked difference in the speed of the reaction between haemoglobin and oxygen in vitro/

vitro and in vivo, it is known from the work of Riley et al (1946) that in vitro studies do give an accurate representation of what happens in vivo with the important proviso that the processes occur very much more rapidly in vivo owing to enzymatic catalytic processes. The oxygen dissociation curve gives a static representation of the dynamic equilibrium between haemoglobin and oxygen and it is important to realise that small alterations in the in vitro oxygen dissociation curve signify very considerable alterations in the velocity of the reaction (Barcroft 1914).

Factors Influencing the Position of the Oxygen Dissociation Curve

An appreciation of the factors which influence the position of the oxygen dissociation curve is essential to an understanding of oxygen transport. Some of these factors are operative under physiological conditions (e.g. variations in carbon dioxide pressure) while others come into play only in pathological states (e.g. carbon monoxide poisoning). Alteration in the position of the oxygen dissociation curve is of great importance for a shift to the right indicates a greater release/

release of oxygen to the tissues while a shift to the left signifies an increased affinity of haemoglobin for oxygen resulting in a decreased oxygen release to the tissues.

One of the most important factors influencing the position of the oxygen dissociation curve is the reaction of the blood. Bohr in the early years of this century was the first to describe the effect of different pressures of carbon dioxide on the curve and Barcroft (1928) showed that the effect is due to plasma pH alteration and can be produced by the addition of acid. Increase of the hydrogen ion concentration of the plasma produces a shift to the right of the oxygen dissociation curve while a decrease has the reverse effect. The oxygen dissociation curve at a carbon dioxide tension of 40 mm. Hg. represents the oxygenation of haemoglobin at the normal plasma pH of 7.4. The effect of pH change on the position of the oxygen dissociation curve is of value to the organism for the carbon dioxide and lactic acid produced by tissue activity thereby facilitate the release of oxygen to the tissues. The pH of the red cell, which is lower than that of the plasma,/

plasma, is also of great importance in determining the position of the oxygen dissociation curve: a fall in the cell pH displaces the curve to the right while an increase displaces it to the left.

A rise in temperature produces a shift to the right of the curve and a fall a shift to the left. In poikilothermic animals, whose body temperature varies with that of the environment, this is of great importance for as the body temperature falls the supply of oxygen to the tissues is decreased. In man there is normally no significant alteration in the internal body temperature but the effect of temperature change is seen in marked pyrexia, where the release of oxygen to the tissues may be increased.

The effect of altitude on the position of the oxygen dissociation curve has been the subject of many studies. Barcroft (1925) showed that at altitudes of approximately 14,000 feet the oxygen dissociation curve is shifted to the left indicating an increased affinity of haemoglobin for oxygen. This was confirmed by Keys et al (1936) who, in addition, found that at still higher altitudes the curve was displaced to the right. Aste-Salazar/

Aste-Salazar and Hurtado (1944) also found a slight rightward shift of the oxygen dissociation curve at high altitudes (approximately 15,000 feet) and concluded that it indicated a compensatory adjustment to the low oxygen pressure of the atmosphere.

Carboxyhaemoglobin, sulphaemoglobin, and methaemoglobin are all derivatives of haemoglobin which are inactive as regards oxygen carrying power but in addition their presence influences the position of the oxygen dissociation curve. Douglas et al (1912) and Stadie and Martin (1925) showed that the presence of carboxyhaemoglobin produced a shift to the left of the oxygen dissociation curve and it is this shift which is responsible for the disproportionately severe anoxaemic symptoms which are a feature of carbon monoxide poisoning. The position was graphically described by Haldane (1922) in the following passage....." miners may be doing their ordinary work though their haemoglobin percentage is reduced to half or less by ankylostomiasis.....whereas a person whose blood is half saturated with carbon monoxide is practically helpless." A similar shift to the left of the oxygen dissociation/

dissociation curve is produced by methaemoglobin (Darling and Roughton 1942) and by sulphaemoglobin (Barcroft et al 1926).

The oxygen dissociation curve of foetal haemoglobin is significantly shifted to the left compared with that of adult haemoglobin (Eastman et al 1933) and it is believed that this is due to a difference in the globin fraction (Rimington 1951) and not to pH or carbon dioxide differences. As a result of this increased affinity of foetal haemoglobin for oxygen at low oxygen partial pressures the blood of the foetus is adequately oxygenated despite the relatively low oxygen partial pressures that prevail in the maternal sinuses.

In many types of anaemia the oxygen dissociation curve is shifted to the right and it is believed that this is a compensatory phenomenon to increase tissue oxygenation which would otherwise be reduced as a result of the lowered oxygen capacity of the blood. The previous work in this field is reviewed in Chapters 8 - 10 where the author's original observations on the oxygen dissociation curve in anaemia are presented.

Carbon Dioxide Transport/

Carbon Dioxide Transport

The transport of carbon dioxide by the blood is of equal importance to that of oxygen and is intimately related to it. The normal adult produces, at rest, approximately 200 ml. of carbon dioxide per minute and the amount is greatly increased by exercise. But for an efficient transport and excretion mechanism there would inevitably be a significant alteration in the pH of the blood.

The carbon dioxide produced as the result of tissue activity is transported:

a) partly in physical solution in the plasma and red cells

b) partly as bicarbonate

c) partly as carbaminohaemoglobin.

Physically dissolved carbon dioxide

A small proportion (about 5 per cent) of the carbon dioxide is carried physically dissolved in the plasma and red cells; the quantity is proportional to the partial pressure of the gas and to its solubility coefficient. The dissolved gas is present both as carbon/

carbon dioxide and carbonic acid but in calculations the dissolved gas is usually considered to be present as carbonic acid - the one is a constant fraction of the other at a given temperature.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

Although the amount of carbonic acid present is very small it is of the greatest importance since it determines the amount of carbon dioxide present as bicarbonate. The relationship between the amount of carbonic acid and of bicarbonate is kept fairly constant; the relationship is expressed in the Henderson - Hasselbalch equation

$$pH = pK_1 + \log \frac{BHCO_3}{H_2CO_3}$$

pK_1 is a composite constant with the value of 6.1 in normal plasma; with the normal plasma pH of 7.4 the equation becomes

$$7.4 = 6.1 + \log \frac{BHCO_3}{H_2CO_3}$$

thus $$\frac{BHCO_3}{H_2CO_3} = \frac{20}{1}$$

Carriage as bicarbonate/

Carriage as bicarbonate

The greatest part of the carbon dioxide is carried as bicarbonate. As carbon dioxide diffuses from the tissues into the blood carbonic acid is formed. This reaction takes place much more rapidly in the red cells because they contain an enzyme, carbonic acid anhydrase, which catalyses the reaction (Roughton 1935). Practically all the carbonic acid so formed within the red cells is transformed to bicarbonate; the base used is largely derived from haemoglobin. The other proteins of the blood play a less important part.

$$H_2CO_3 + KHb \rightleftharpoons HHb + KHCO_3$$

Haemoglobin provides the greatest quantity of base because:

a) haemoglobin forms the bulk of the blood proteins

b) the haemoglobin molecule is polyvalent and holds several base groups

c) oxyhaemoglobin (isoelectric pH about 6.6) is a stronger acid than haemoglobin (isoelectric pH about 6.8) so that at the red cell pH of about 7.25 oxyhaemoglobin binds more base than does haemoglobin. Thus in the tissues as oxyhaemoglobin/

oxyhaemoglobin loses its oxygen it becomes more able to provide base for combination with carbonic acid.

The chloride - bicarbonate shift

Although most of the bicarbonate is formed in the red cells by virtue of their content of carbonic anhydrase, most of it is actually transported in the plasma. When carbon dioxide enters the blood from the tissues the increase of bicarbonate within the cells increases the ratio HCO_3^- cell to HCO_3^- plasma. According to the Donnan principle the balance between HCO_3^- ions, Cl^- ions, and H^+ ions should be as below

$$\frac{(HCO_3^-)\ \text{cells}}{(HCO_3^-)\ \text{plasma}} = \frac{(Cl^-)\ \text{cells}}{(Cl^-)\ \text{plasma}} = \frac{(H^+)\ \text{plasma}}{(H^+)\ \text{cells}}$$

To restore this balance HCO_3^- ions diffuse from the cells into the plasma and Cl^- ions from the plasma to the cells. In the lungs the reverse occurs. In the cells the HCO_3^- ions are combined with K^+ ions derived from haemoglobin and in the plasma with Na^+ ions derived from the plasma NaCl; the Cl^- ions which enter the cells combine with K^+ ions to form KCl. In addition to the ionic exchange there is a movement of water into the cells with the uptake of carbon dioxide and out of the cell when it is released.

Transport as carbaminohaemoglobin

It is known from the work of Roughton and Ferguson (Roughton 1935, Ferguson and Roughton 1935) that an amino group in the haemoglobin molecule is able to react reversibly with carbon dioxide to form carbaminohaemoglobin

$$HbNH_2 + CO_2 \rightleftharpoons Hb.NHCOOH$$

The reaction takes place directly and rapidly between the free gas and haemoglobin and the enzyme carbonic anhydrase is not involved. Of great importance is the fact that reduced haemoglobin is able to combine with approximately three times as much carbon dioxide as oxyhaemoglobin. Thus the reaction above moves to the right in the tissues and to the left in the lungs. It is believed that the carbaminohaemoglobin mechanism accounts for about twenty five per cent of the carbon dioxide released in the lungs.

Carbon dioxide capacity of reduced and oxygenated blood

Although the influence of carbon dioxide on the position of the oxygen dissociation curve was known from the work of Bohr in the early years of the century the influence/

influence of the oxygenation of haemoglobin on the carbon dioxide curve dissociation curve was not appreciated until the work of Christiansen et al in 1914 showed that reduced whole blood was able to carry more carbon dioxide than oxygenated blood.

The beneficial effect of the increased carbon dioxide capacity of reduced blood is obvious; in the tissues reduction of haemoglobin enables it to take up more carbon dioxide while in the lungs oxygenation of haemoglobin permits release of the carbon dioxide. There are two factors responsible for the greater carbon dioxide combining power of reduced blood

a) reduced haemoglobin being a weaker acid than haemoglobin requires less base so that more is available for bicarbonate formation

b) reduced haemoglobin is able to combine with more carbon dioxide to form carbaminohaemoglobin than haemoglobin.

Integration of carbon dioxide and oxygen transport

The venous blood which passes from the right auricle to the lungs has a higher partial pressure of carbon dioxide and a lower partial pressure of oxygen than/

than the alveolar air. As a result carbon dioxide diffuses from the blood into the alveoli to be exhaled and oxygen diffuses from the alveoli into the blood to form oxyhaemoglobin. The carbon dioxide pressure in the plasma and cells falls and more carbon dioxide is released from combination as carbonic acid, bicarbonate, and carbaminohaemoglobin and in turn is expired. In contrast the oxygen partial pressure rises in the plasma and cells and the conversion of the weaker acid haemoglobin into the stronger acid oxyhaemoglobin further facilitates the release of carbon dioxide.

In the blood of the capillaries the above reactions are reversed. The oxygen partial pressure of the blood is higher than that of the tissues and the carbon dioxide pressure is lower. Carbon dioxide diffuses from the tissues into the blood, the plasma carbon dioxide partial pressure rises, and the various reactions which result in the formation of carbonic acid, bicarbonate, and carbaminohaemoglobin are set in motion. At the same time oxygen passes from the blood to the tissues, the oxygen partial pressure in the plasma falls and oxyhaemoglobin dissociates into oxygen and haemoglobin; the base liberated facilitates the/

the formation of bicarbonate and of carbaminohaemoglobin.

It is thus seen that both in the lungs and in the tissues the oxygen and carbon dioxide changes are intimately related and mutually beneficial.

CHAPTER 4

MATERIAL AND METHODS

Preparation of Blood Samples for In-Vitro Studies

Samples of stored blood were obtained from blood supplied by the West of Scotland Blood Transfusion Service for routine transfusions, or from blood obtained from healthy volunteers and stored in universal containers for the purposes of this investigation. The anticoagulant medium was composed of disodium citrate 2 grams, dextrose 3 grams, and water to 100 ml.; this anticoagulant medium is commonly known as the A.C.D. medium. The medium was used in a proportion of 100 ml. to 440 ml. of blood, and the temperature of storage of the citrated blood was 4°C. The pH of this anticoagulant-blood mixture is approximately 7.1 initially, 6.8 at the end of 7 days of storage, and 6.6 after 20 days (Loutit et al 1943).

For fresh blood studies the anticoagulant medium, of pH 7.4, was 4 per cent sodium fluoride in an 0.2 per cent heparin solution, in a proportion of 1 drop to 3 ml. of blood (Riley et al 1946). Blood stored with/

with this anticoagulant at 4°C undergoes no plasma pH alteration up to 20 days. For studies of fresh blood at low plasma pH ranges this heparin-fluoride anticoagulant was acidified with a normal solution of lactic acid in saline solution. 0.1 ml. of the lactic acid solution added to 10 ml. of heparinized blood gave a final plasma pH of approximately 6.8

A few observations were also made on blood samples stored in a trisodium - citrate / dextrose anticoagulant, and on laked blood buffered solutions. The laked blood solutions were prepared by the methods described by Brooks (1935) and by Darling and Roughton (1942).

The plasma pH of the blood samples was partially restored by substituting the acid plasma with fresh plasma obtained from the same patient or, when this was not possible, from an individual of the same blood group. To bring the plasma pH still further to normal in some cases the red cells were washed with fresh plasma prior to the substitution. In these manoeuvres care was taken to avoid exposure to atmospheric air.

Oxygen and Carbon Dioxide Dissociation Curves/

Oxygen and Carbon Dioxide Dissociation Curves

The tonometers, of 300 ml. capacity, were of the type described by Rappaport (1949, pages 62 - 63) as these allow easy removal, by slight negative pressure, of the blood samples direct into an Ostwald-Van Slyke pipette or into a syringe in which dead space has been eliminated with oil. The tonometers were air tight, being capable of maintaining an absolute vacuum for at least 4 hours.

The tonometers were filled at the desired gas tensions using the apparatus described by Austin et al (1922) with a mercury manometer in place of gas burettes as suggested by Van Slyke et al (1923). The oxygen, carbon dioxide, and nitrogen, supplied by the British Oxygen Company, were highly purified as, in the case of oxygen and carbon dioxide, for medical use. The tonometers were filled with oxygen, carbon dioxide and nitrogen at room temperatures; the partial pressures required to produce the desired partial pressures at 37°C were calculated from the following equation, which is a slight modification of that given by Peters and Van Slyke (1932)

$$Pt = P37 \frac{273 + t}{(273 + 37) \times c}$$

where

Pt = gas tension at room temperature read from the manometer (without correction) in mm. of Hg.

P37 = desired tension at 37°C in mm. of Hg.

t = room temperature in degrees Centigrade.

c = mercury correction factor which depends on the diameter of the mercury reservoir and manometer.

The slight correction for the effect of the blood sample, both as regards its volume and the gaseous exchanges occurring during equilibration, was obtained from a modification of the equation given by Austin et al (1922)

$$Pi = P37 + 7.8\,\Delta\,\frac{Vbl}{Vtn - Vbl}$$

when absolute temperature of tonometer during saturation is 37°C

where

Pi = initial tension of specified gas in tonometer at beginning of saturation in mm. of Hg.

P37 = final tension of specified gas in tonometer at end of saturation in mm. of Hg.

Vbl = volume of blood in tonometer in ml.

Vtn = total volume content of tonometer in ml.

$\triangle$ = increase in total (free and combined) millimolecular concentration of the specified gas in the blood caused by changing the blood from its original state to that at the end of saturation.

The corrections were always small as might be expected from the small volume of blood relative to the volume of the tonometer.

The volume of blood added to each tonometer was 3 ml. The blood samples were equilibrated at the gas tensions of the tonometer by mechanical rotation in a water bath at 37°C. The rotation was produced by directing a jet of water from the mixing pump of the bath on to tin-plate vanes fitted as a collar around the tonometer. During the rotation the blood sample was spread in a thin film over the surface of the tonometer.

Four points were determined for the majority of the oxygen dissociation curves (at partial pressures of oxygen of 20, 40, and 100 mm. Hg and atmospheric air) while in a few cases additional determinations were made at 60 and 80 mm. Hg. The partial pressure of carbon/

carbon dioxide in the tonometers was 40 mm. Hg. The oxygen capacity of the blood samples was obtained by rotation in tonometers containing atmospheric air at 37°C. This temperature, in preference to room temperature, was used for the reasons given by Roughton et al (1944), and, perhaps more important, to avoid differences in the amount of oxygen in solution and in vapour pressure. Two points were determined on each carbon dioxide dissociation curve from oxygenated blood and one point on each carbon dioxide dissociation curve from reduced blood.

The blood gas analyses were performed in the Van Slyke manometric apparatus (Peters and Van Slyke 1932) using 1 ml. blood samples and the lactic acid, ferricyanide, urea reagent (King et al 1948, Rappaport 1949). The use of this reagent enables oxygen, carbon dioxide and carbon monoxide estimations to be made from a single sample of blood and, in addition, the instrument is easily cleaned after its use (King et al 1948).

Correction of the position of the oxygen dissociation curve to the standard plasma pH (pH_s) of 7.4/

7.4 was effected by using the equation

$$\frac{\Delta \log pO_2}{\Delta\ pH_s} = 0.048$$ (Keys et al 1936, Aste-Salazar and Hurtado 1944)

The value 0.048 was confirmed for fresh blood of plasma pH down to 7.0, but at lower plasma pH levels the value was found to be 0.046.

Correction of the position of the oxygen dissociation curve to the standard cell pH (pH_c) was effected by using the equation given by Dill in the paper of Keys et al (1936)

$$\frac{\Delta \log pO2}{\Delta\ pH_c} = 0.058$$

or by using the procedure outlined by Dill in the same paper using Figure 1 which is the graphic representation of the above two equations.

Correction of the position of the oxygen dissociation curve for the presence of carbon monoxide (Riley et al 1946) was required only where storage was for more than thirty days; blood samples stored for less than thirty days do not contain significant amounts of carbon monoxide. The correction is derived from Haldane's second law which states that in blood exposed to/

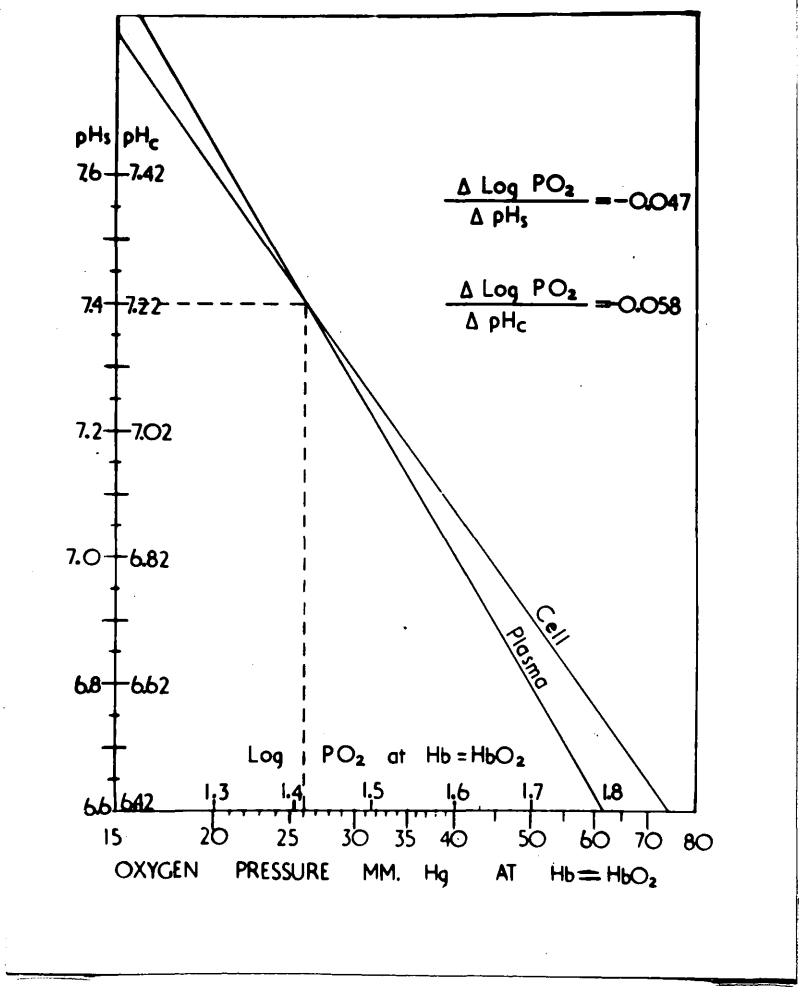

Figure 1 The relationship between the position of the oxygen dissociation curve at Hb = HbO_2 and plasma pH and cell pH (modified from Dill).

to oxygen at a partial pressure pO_2 and to carbon monoxide at a partial pressure pCO, the total haemoglobin saturation is the same as if no carbon monoxide were present and pO_2 equalled pO_2 MpCO (total effective gas tension). MpCO was calculated from the Haldane equation

$$\frac{\text{MpCO}}{pO_2} = \frac{\text{CO content}}{O_2 \text{ content}}$$

where M is the relative affinity constant of haemoglobin for carbon monoxide compared to oxygen; in man it is approximately 210.

The abscissa then becomes pO_2 MpCO and the ordinate

$$100 \times \frac{(HbO_2) + (COHb)}{(HbO_2) + (COHb) + (Hb)}$$

Correction of the position of the oxygen dissociation curve for temperature was not necessary as the water bath temperature was controlled at 37°C ± 0.01°

Determination of Plasma pH and Cell pH

The plasma pH of true plasma, obtained from blood equilibrated at 37°C with arterial gas tensions, was determined/

determined both with a glass electrode standardised with buffers of pH 6.5 and 7.4 and by the Henderson-Hasselbalch equation

$$pH_s = pK' + \log \frac{(BHCO_3)_s}{(H_2CO_3)_s}$$

where

pH_s = plasma pH

pK' = 6.11 (Dill et al 1937a, 1940)

$(H_2CO_3)_s$ = free CO_2 of the serum = 0.696 (pCO_2) volume per cent in accordance with the solubility coefficients of Van Slyke at 37°C and one atmosphere (Van Slyke et al 1928)

$(BHCO_3)_s$ = combined CO_2 of the serum = the total CO_2 content of plasma (Total CO_2)s - $(H_2CO_3)_s$

The solubility coefficient of carbon dioxide in serum at 37°C given by Van Slyke et al has been used for the calculation of free carbonic acid in the above equation despite the fact that the plasma in the present studies contained citrate. Any error so produced in the in vitro studies is small because the solubility coefficient/

coefficient of carbon dioxide in a citrate solution is lower than that of carbon dioxide in water (Van Slyke et al 1928) and the value is very near that for the plasma. Indeed the calculated values for plasma pH, obtained at 37°C, agreed closely (± 0.02) with those obtained with a glass electrode at room temperature, after the temperature difference has been allowed for. To avoid small errors resulting from variations in room temperature and the loss of carbon dioxide to the atmosphere during the glass electrode determinations the calculated plasma pH values are used.

The total carbon dioxide of the plasma (total $CO_2)_s$ was determined directly from plasma obtained from blood saturated with oxygen at a carbon dioxide partial pressure of 40 mm. Hg. The total carbon dioxide of oxygenated blood (total $CO_2)_o$, and of reduced blood (total $CO_2)_r$, was also determined directly.

The pH of the cells of the stored and fresh blood samples was also calculated from the Henderson-Hasselbalch equation

$$pH_c = pK'_c + \log \frac{(BHCO_3)_b}{(H_2CO_3)_b}$$

where

pH_c = the cell pH

$(BHCO_3)_b$ = T40 (the total CO_2 of oxygenated blood at 40 mm. Hg. partial pressure of CO_2) - $(H_2CO_3)_b$

and $(H_2CO_3)_b$ = H_2CO_3 content of blood calculated from the formula $(H_2CO_3) = apCO_2$ where a is the solubility coefficient of carbon dioxide in blood (from data by Dill in the paper of Keys et al 1936 and Dill et al 1940)

pK'_c was obtained from the alignment chart and procedure given in the paper of Keys et al modified in such a manner for the pK'_c to be 5.98 for normal blood (Dill et al 1937a, 1937b) giving a cell pH of 7.20 - 7.25 The other values for pK'_c derived from this alignment chart were changed proportionately.

The cell pH of the recipient before and after transfusion was determined by the same methods, after the blood samples, which were from anaemic individuals, had been concentrated by removal of plasma under oil, to bring the packed cell volume into the normal range. This manipulation permits more accurate comparative study/

study for if the methods are applied directly to anaemic blood where the cell phase is small experimental errors may be considerable. It is shown in Chapter 9 that concentration does not alter the position of the oxygen dissociation curve.

Spectrophotometry

A 'Unicam S.P. 600' spectrophotometer was used for the spectrophotometric examination of the blood samples. For methaemoglobin and sulphaemoglobin the methods of Evelyn and Malloy (1938) were employed and for carboxyhaemoglobin and other abnormal pigments the methods described by Heilmeyer (1943).

Transfusion Studies

The blood used in the transfusion studies was prepared in the standard manner by the West of Scotland Blood Transfusion Service. The period of storage was from half an hour to twenty days. For the plasma transfusion, plasma freshly separated from citrated blood stored for twenty days was used.

The observations were made on ten patients, all severely/

severely anaemic, who received fourteen transfusions. The blood or plasma was administered at a rate of a pint in one or two hours. Oxygen dissociation curves and determinations of blood and plasma carbon dioxide were done in all the cases immediately before and immediately after the transfusion. In some of the cases additional observations were carried out from half an hour to twenty days later. The time-consuming nature of these investigations prevented more frequent estimations. The data for the gas studies were obtained from venous blood, taken under the standard conditions described for gas analysis, using the heparin-fluoride anticoagulant.

CHAPTER 5

IN-VITRO STUDIES WITH FRESH BLOOD AND STORED BLOOD

Oxygen and carbon dioxide dissociation curves, together with relevant determinations of plasma and cell pH, were made on over 100 samples of blood and the detailed results are presented in Tables 1 - 11 and Figures 2 - 9. The position of the oxygen dissociation curve, to which most reference will be made, is indicated in the Tables and in Figures 6 and 7 by the partial pressure of oxygen at which 50 per cent of the haemoglobin is oxygenated (PO_2 for Hb = HbO_2).

Fresh Blood (Table 1)

Before investigating the gas transport in stored blood it was clearly desirable to establish the accuracy of the methods employed. Estimations of the oxygen dissociation curve, the carbon dioxide dissociation curve, the plasma pH (pHs), and the cell pH (pHc) were made on 6 normal blood samples collected in the heparin-fluoride anticoagulant medium. The values obtained (Table 1, Figure 2) agree closely with those given by previous workers (Dill 1928, Keys et al 1936, Aste-Salazar and Hurtado 1944).

The precise significance of the column headings in Table 1 and subsequent Tables is as under:

HbO_2 = oxyhaemoglobin capacity.

CO = carbon monoxide content.

$(CO_2)_r$ = total carbon dioxide of reduced blood at CO_2 partial pressure of 40 mm. Hg.

$(CO_2)_o$ = total carbon dioxide of oxygenated blood at CO_2 partial pressure of 40 mm. Hg.

$\frac{\Delta\ CO_2(r-o)}{HbO_2}$ = relation between the difference $(CO_2)_r - (CO_2)_o$ and oxyhaemoglobin.

$(CO_2)_s$ = total carbon dioxide of true plasma from oxygenated blood at CO_2 partial pressure of 40 mm. Hg.

$\Delta\ CO_2(s-o)$ = difference between $(CO_2)_s$ and $(CO_2)_o$

PO_2 for Hb = HbO_2= partial pressure of oxygen at which oxyhaemoglobin saturation is 50% at the plasma and cell pH of the recipient, at standard plasma pH, and at standard cell pH.

Plasma pH determined in true plasma from blood saturated with oxygen when PCO_2 = 40 mm. Hg.

TABLE 1

In vitro results with fresh blood samples.

SAMPLE NO.	HbO_2 vol.%	CO vol.%	$(CO_2)_r$ vol.%	$(CO_2)_o$ vol.%	$\frac{\Delta CO_2 (r-o)}{HbO_2}$ vol.%	$(CO_2)_s$ vol.%	ΔCO_2 (s-o) vol.%	BLOOD pH_s	BLOOD pH_c	PO_2 for $Hb = HbO_2$ at BLOOD pH_s and pH_c	at pH_s 7.4	at pH_c 7.22
1	21	0.5	59	52	0.34	62.5	10.5	7.43	7.26	25	26	26
2	18.5	0.7	54.5	48	0.38	56.5	8.5	7.39	7.22	27	27	27
3	20	0.5	48.5	42	0.33	50	8	7.33	7.17	28	25.5	26
4	20	0.25	55.5	48	0.38	58.5	10.5	7.40	7.23	26	26	26
5	18	0.5	54	47	0.39	56.5	9.5	7.38	7.21	26	25.5	26
6	19	0.5	57.5	50	0.39	61	11	7.42	7.24	26	26.5	25.5
Mean	19.5	0.5	54.5	48	0.38	57.5	9.5	7.39	7.21	26.5	26	26

Effects of Dilution, Haemolysis, and pH change (Table 2)

Since blood stored for transfusion purposes is diluted (440 ml. of blood to 100 ml. of A.C.D. medium), is kept at a pH considerably lower than the normal pH of blood, and moreover sometimes undergoes slight haemolysis, it was an essential preliminary in this study to determine the effect of these changes per se on blood gas transport.

Fresh blood samples were slightly haemolysed and diluted with plasma to the degree present in stored blood and it is apparent from Table 2 and Figures 6 and 7 that there was no effect on the position of the oxygen dissociation curve or on the quotient $\frac{\Delta CO_2 (r-o)}{HbO_2}$ which indicated the amount of carbon dioxide released from the blood for each volume per cent of oxygen saturation of haemoglobin (Table 2 and Figure 8). That dilution with plasma has no effect on the oxygen dissociation curve of blood was first shown by Richards and Strauss (1927) in their investigations on the oxygen dissociation curve in anaemia. The difference between the total carbon dioxide of plasma and of oxygenated whole blood, on which depends the difference between the plasma pH and cell pH, is altered proportionately/

proportionately to the change in the oxyhaemoglobin capacity. The position of the oxygen dissociation curve corrected to the standard plasma pH of 7.4 coincides with that corrected to the standard cell pH of 7.22 ($Hb=HbO_2$ at 26 mm. Hg partial pressure of oxygen).

In fresh acidified heparinised blood the position of the oxygen dissociation curve and the quotient $\frac{\Delta CO_2\ (r-o)}{HbO_2}$ are altered in keeping with the low plasma pH. The difference between the plasma pH and the cell pH is decreased but proportionately to the pH fall. After the pH has been partially restored with fresh plasma there is no abnormality.

TABLE 2

In vitro results with fresh blood samples after dilution, slight haemolysis, and acidification.

SAMPLE NO.	HbO_2 vol.%	CO vol.%	$(CO_2)_r$ vol.%	$(CO_2)_o$ vol.%	$\frac{\Delta CO_2 (r-o)}{HbO_2}$ vol.%	$(CO_2)_s$ vol.%	ΔCO_2 (s-o) vol.%	BLOOD pH_s	BLOOD pH_c	PO_2 for Hb = HbO_2 at BLOOD pH_s and pH_c	at pH_s 7.4	at pH_c 7.22
Effects of dilution and haemolysis												
1	15	0.5	58	53	0.34	61	8	7.42	7.23	25.5	26	25.5
2	14	0.75	53	47.5	0.39	55	7.5	7.38	7.16	25.5	25	25
3	15	0.25	61.5	55	0.43	62	7	7.43	7.25	25	26	26
Mean	14.7	0.5	57	51.5	0.39	59	7	7.41	7.22	25.3	25.7	25.5
Acidified fresh blood												
1	19	0.7	17	14.5	0.13	16.2	1.7	6.8	6.72	50	26	26.5
2	18	0.3	27.5	24	0.2	28	4	7.07	6.94	37	26	26
3	16	0.3	21.5	19	0.15	21	2	6.93	6.83	41	25	25.5
4	20	0.5	35.5	30.5	0.25	37	6.5	7.20	7.06	32	26.5	26.5
5	17	0.3	29	25	0.24	30	5	7.10	6.96	36	26	26
6	18	0.5	25	22.5	0.14	26	3.5	7.03	6.90	39	26	26
Partial restoration of pH with fresh plasma												
1	18	0	38	44	0.34	52	8	7.35	7.18	27.5	26	25.5
2	19	0.2	30	35.5	0.29	41	5.5	7.25	7.10	30	26	26
3	16	0.2	32	37	0.31	43	6	7.28	7.12	30	26.5	26

Blood Stored in A.C.D. Medium

The results are presented in Tables 3 - 7 according to the duration of storage which extended from ½ an hour to 2 months and in Figures 2 - 8. The majority of the studies are from the period of storage up to 20 days, which is the clinically important period.

The oxygen dissociation curve shows a shift to the left which is progressive with storage, and the quotient $\frac{\Delta CO_2(r-o)}{HbO_2}$ is decreased. The changes are very slight after 1 day (Table 3), pronounced after 7 days (Table 4) and even more pronounced, although not to the degree expected from the further fall in plasma pH (from 6.8 to 6.6), after 20 days (Table 5). After 2 months storage (Table 6) the shift of the oxygen dissociation curve to the left is still greater, but after correction for the presence of carboxyhaemoglobin the position of the curve does not differ significantly from that of citrated blood stored for 20 days. The progressive shift of the oxygen dissociation curve is clearly seen in Figure 4.

There is also a progressive alteration in the relationship/

relationship between the plasma and the cell pH. After 24 hours storage the difference between the total carbon dioxide of plasma and of oxygenated blood is decreased out of proportion to the fall of plasma pH and the difference between plasma and cell pH is reduced. After 7 days storage the total carbon dioxide of plasma is actually less than that of oxygenated blood and the relationship between the plasma and cell pH is reversed the cell pH being less acid than the plasma pH. This change is also present in the samples stored for 20 days.

Calculated correction of the position of the oxygen dissociation curve to the standard plasma and cell pH makes more apparent the shift to the left of the curve (Figures 2 and 3). It is also evident that the oxygen dissociation curve corrected to the standard cell pH lies less far to the left than that corrected to the standard plasma pH; it would appear that correction to standard cell pH diminishes, although it does not abolish, the abnormality in the position of the oxygen dissociation curve.

The effect of partial restoration of the plasma pH with/

with fresh plasma was observed in the stored citrated blood samples and it is seen (Tables 3 - 5) that although the abnormalities described above remain, they are lessened by this manoevre. Contact with fresh plasma apparently has some effect on the restoration of the normal relationship between the total carbon dioxide of plasma and whole blood, and therefore on the plasma to cell pH relationship.

The addition of citrated plasma derived from blood stored in the A.C.D. medium for 20 days to fresh red cells does not produce the abnormalities in the position of the oxygen dissociation curve, in the relationship of the total carbon dioxide of plasma and whole blood, and of the plasma to cell pH relationship, seen in the blood samples stored in the A.C.D. medium (Table 5 and Figure 4).

TABLE 3

In vitro results with blood samples stored in A.C.D. medium for ½ to 24 hours.

SAMPLE NO.	STORAGE TIME hours	HbO_2 vol.%	CO vol.%	$(CO_2)_r$ vol.%	$(CO_2)_o$ vol.%	$\frac{\Delta CO_2 (r-o)}{HbO_2}$ vol.%	$(CO_2)_s$ vol.%	ΔCO_2 (s-o) vol.%	BLOOD pH_s	BLOOD pH_c	PO_2 for Hb = HbO_2 at BLOOD pH_s and pH_c	at pH_s 7.4	at pH_c 7.22
1	½	16	0.3	29	26	0.2	29	3	7.09	6.98	35	25.5	26
2	1	17	0.5	27	24	0.18	26	2	7.03	6.94	38	25	27
3	16	15.5	0.3	25.5	23	0.17	25	2	7.03	6.93	39	25.5	27
4	24	14.5	0.7	27	25	0.15	26	1	7.03	6.97	37	25.5	26.5
5	4	16.5	0.5	27.5	25	0.16	26	1	7.07	7	37	26	27.5
6	20	17	0.7	23	21	0.14	22	1	6.94	6.88	40	25.5	26.5
7	22	15	0.5	31	28	0.2	29	1	7.09	7.01	35	25.5	27
8	20	17	0.7	25	22.5	0.15	23.5	1	6.97	6.90	39	25	26
Partial restoration of pH with fresh plasma													
9		16	0.1	39.5	35	0.29	40	5	7.24	7.10	30	25	26.5
10		15	0.1	41	38	0.2	41	3	7.25	7.14	31	26	28
11		16	0.2	37.5	34	0.22	37	3	7.20	7.09	30	24	26

TABLE 4

In vitro results with blood samples stored in A.C.D. medium for 7 days.

SAMPLE NO.	HbO_2 vol.%	CO vol.%	$(CO_2)_r$ vol.%	$(CO_2)_o$ vol.%	$\frac{\Delta CO_2 (r-o)}{HbO_2}$ vol.%	$(CO_2)_s$ vol.%	ΔCO_2 (s-o) vol.%	BLOOD pH_s	BLOOD pH_c	PO_2 for Hb = HbO_2 at BLOOD pH_s and pH_c	at pH_s 7·4	at pH_c 7·22
1	15.5	0.5	18	17	0.06	15	-2	6.76	6.78	40	19.5	22.5
2	16	0.7	20.5	19.3	0.07	17	-2.3	6.81	6.85	38	20	23.5
3	15	0.5	18	17	0.06	15.5	-1.5	6.78	6.78	39	20	22
4	17	0.2	19	17.8	0.07	16	-1.2	6.78	6.80	38	19	22
5	16	0.5	21	20.5	0.03	18.3	-2.2	6.84	6.88	35	20	23.5
6	18	0.5	23	21	0.11	18.8	-2.2	6.85	6.90	36	20	24
7	14	0.2	16	15.5	0.04	13	-2.5	6.68	6.74	42	19.5	23
Partial restoration of pH with fresh plasma												
8	15	0.5	30	28.5	0.1	30	1.5	7.1	7	29	21	22.5
9	16	0.7	41	39	0.13	41	2	7.25	7.14	25	21.5	22.5
10	15	0.5	32	30	0.13	31.2	1.2	7.12	7.02	28	20.5	22
11	17	0.7	46	43	0.18	45.5	2.5	7.29	7.17	24	21.5	22.5

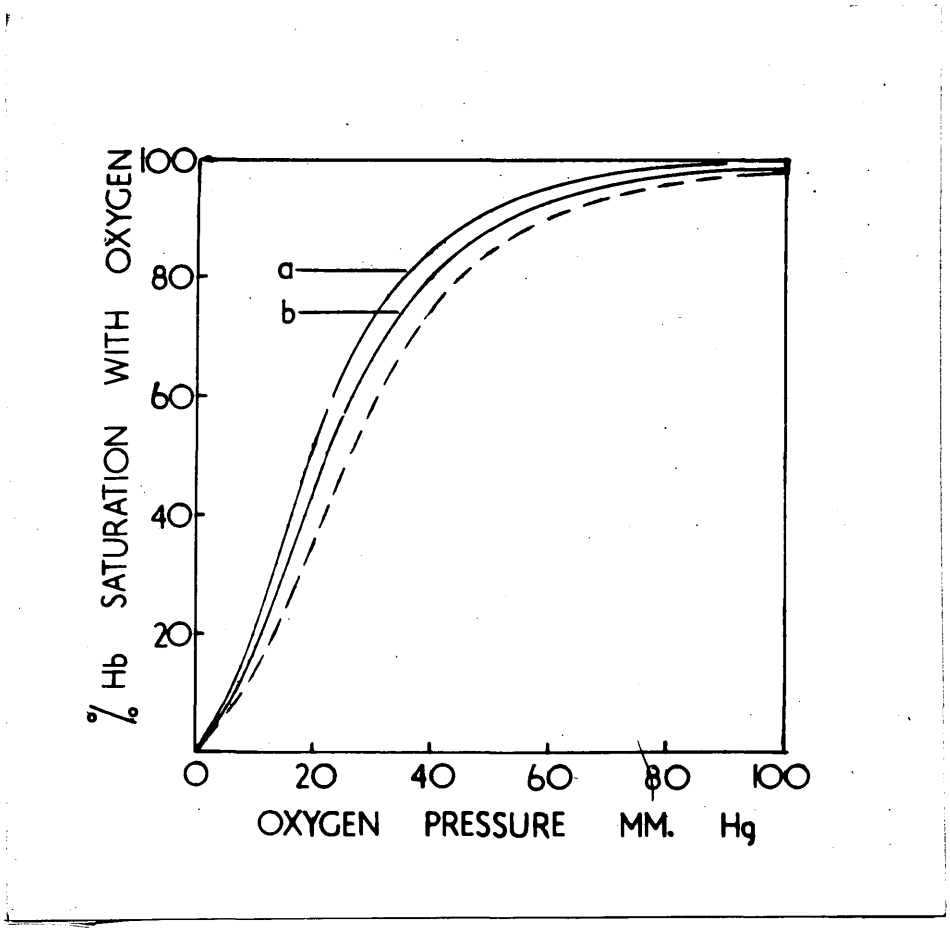

Figure 2 Oxygen dissociation curves from blood stored for 7 days in A.C.D. medium.

Curve a - At plasma pH 7.4

Curve b - At cell pH 7.22

Interrupted line - Fresh normal blood.

TABLE 5

In vitro results with blood samples stored in A.C.D. medium for 20 days.

SAMPLE NO.	HbO_2 vol.%	CO vol.%	$(CO_2)_r$ vol.%	$(CO_2)_o$ vol.%	$\frac{\Delta CO_2 (r-o)}{HbO_2}$ vol.%	$(CO_2)_s$ vol.%	ΔCO_2 (s-o) vol.%	BLOOD pH_s	BLOOD pH_c	PO_2 for Hb = HbO_2 at BLOOD pH_s and pH_c	at pH_s 7·4	at pH_c 7·22
1	15.5	1	16	15	0.06	12.5	-2.5	6.65	6.73	40	18.3	21
2	16	0.7	15.5	14	0.1	12	-2	6.63	6.69	41	18.5	21.5
3	14.5	0.7	16	15	0.07	12.5	-2.5	6.64	6.73	41	18.3	21.5
4	17	0.5	14	13	0.06	11	-2	6.58	6.65	42.5	18	20
5	16.5	1	16	14.5	0.09	12.5	-2	6.65	6.71	41	19	21
6	15	0.7	15	14	0.07	12.5	-2.5	6.65	6.69	40	18.5	21
Partial restoration of pH with fresh plasma												
7	15	0.5	29.5	28	0.1	28.7	0.7	7.08	7	28	20	21
8	12.5	0.7	32	30	0.14	30.5	0.5	7.11	7.02	26	19	20.5
9	16	1	40	38	0.13	40	2	7.24	7.11	23	19.5	20.5
10	15	0.7	42.5	40	0.16	41.5	1.5	7.26	7.14	22	19.5	20.5
Plasma from 20-day stored citrated blood + fresh red cells												
11	17	0.3	24	21.5	0.15	23	1.5	6.97	6.87	41	26	26
12	18	0.5	23	20	0.17	21	1	6.93	6.82	42	25.5	25
Partial restoration of pH with fresh plasma												
13	16	0.3	39	35	0.25	40	5	7.24	7.10	30	25.5	26

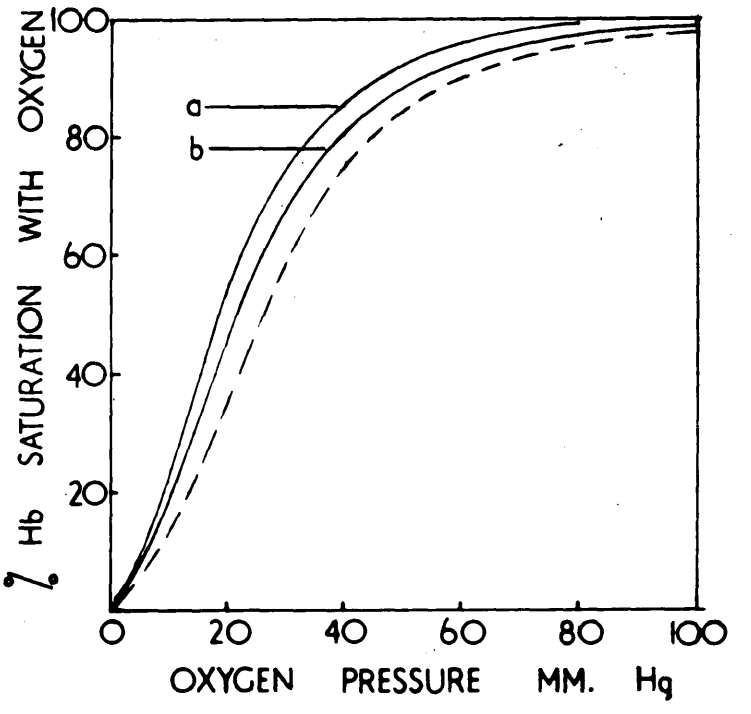

Figure 3 Oxygen dissociation curves from blood stored for 20 days in A.C.D. medium.

Curve a - At plasma pH 7.4

Curve b - At cell pH 7.22

Interrupted line - Fresh normal blood.

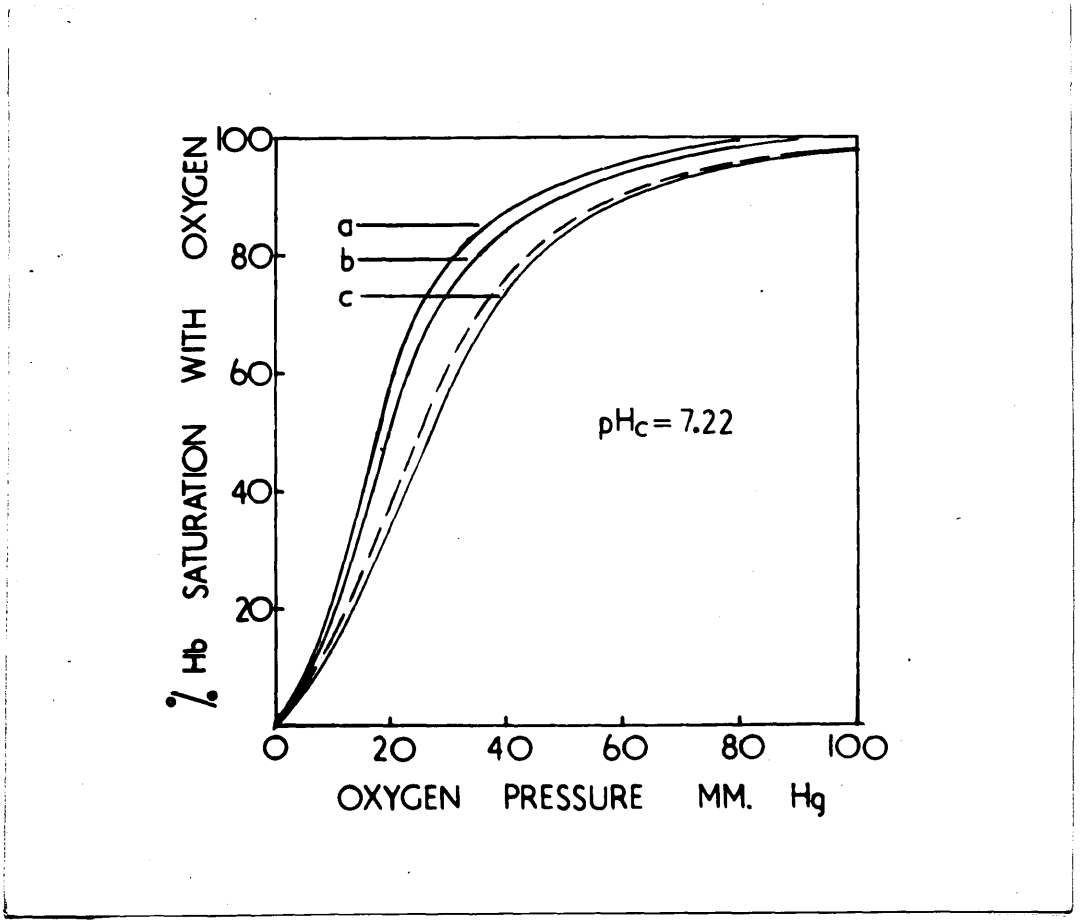

Figure 4 Oxygen dissociation curves at cell pH 7.22

Interrupted line - Fresh normal blood.

Curve a - Blood stored for 20 days in A.C.D. medium.

Curve b - Blood stored for 7 days in A.C.D. medium.

Curve c - Blood stored for 1 day in A.C.D. medium and fresh cells plus stored citrated plasma.

TABLE 6

In vitro results with blood samples stored in A.C.D. medium for 2 months.

SAMPLE NO.	HbO_2 vol.%	CO vol.%	$(CO_2)_T$ vol.%	$(CO_2)_O$ vol.%	$\frac{\Delta CO_2 (T-O)}{HbO_2}$ vol.%	$(CO_2)_S$ vol.%	ΔCO_2 (S-O) vol.%	BLOOD pH_S	BLOOD pH_c	PO_2 for Hb = HbO_2 at BLOOD pH_S and pH_c	at pH_S 7·4	at pH_c 7·22
1	14	2*	11.6	11	0.04	9.5	-1.5	6.5	6.57	41	15	18.5
										47*	17.5*	20.5*
2	15.5	2	13	12	0.06	9	-3	6.47	6.59	40	15.5	18
										46*	18*	20.5*
3	14.5	2.5	12	11	0.07	8.5	-2.5	6.42	6.57	40	16	18.5
										48*	17.5*	21*

* After correction for carbon monoxide.

The Oxygen, Carbon Dioxide and Carbon Monoxide Content of Stored Citrated Blood.

Table 7 below shows that the carbon dioxide and carbon monoxide content of stored citrated blood becomes progressively increased with storage. This is accompanied by a fall in the oxygen content and oxyhaemoglobin capacity and a rise in the calculated partial pressure of carbon dioxide from 20 to 65 mm. Hg.

TABLE 7

SAMPLE NO.	STORAGE TIME days	HbO_2 vol.%	CO vol.%	O_2 (as HbO_2) vol.%	CO_2 vol.%	Plasma pH	Calculated pressure of CO_2 (approx.) mm. Hg
1	0	17	0.5	9	40	7.1	20
2	7	17.5	0.5	8	42	6.8	35
3	20	17	1	6	45	6.6	55
4	60	16	2	4	46	6.5	65

Using the formula 5 from Table 4 of Austin et al (1922) and 0.15 as solubility coefficient for CO_2 at that temperature.

Blood Stored in other Anticoagulants

A relatively small number of observations were made, for comparative purposes, on blood samples stored for 7 days in anticoagulant media other than the A.C.D. medium and the results are presented in Table 8 and Figures 5 - 8. These studies show that

a) blood samples stored for 7 days either with trisodium citrate or with an acidified heparin solution show basically the same abnormalities as blood samples stored for the same period in the A.C.D. medium but in lesser degree.

b) storage of blood with a heparin-fluoride anticoagulant, which does not lower the pH, does not effect the position of the oxygen dissociation curve or the relationship between the total carbon dioxide of plasma and whole blood and therefore the plasma to cell pH relationship.

Studies beyond the 7 day period were impossible in heparinised samples because of gross haemolysis.

TABLE 8

In vitro results with blood samples stored in various anticoagulants for 7 days.

SAMPLE NO.	HbO_2 vol.%	CO vol.%	$(CO_2)_T$ vol.%	$(CO_2)_O$ vol.%	$\frac{\Delta CO_2 (r-o)}{HbO_2}$ vol.%	$(CO_2)_S$ vol.%	ΔCO_2 (s-o) vol.%	BLOOD pH_s	BLOOD pH_c	PO_2 for Hb = HbO_2 at BLOOD pH_s and pH_c	at pH_s 7.4	at pH_c 7.22
BLOOD STORED IN HEPARIN + LACTIC ACID												
1	18	0.7	27	25	0.11	25.5	0.5	7.01	6.96	36	23.5	25
2	19	1	22	20.5	0.09	20.5	0	6.90	6.87	40	23	25
3	20	0.7	32.5	30	0.12	30.7	0.7	7.10	7.04	33	23.5	26
Partial restoration of pH with fresh plasma												
4	18	0.7	44	40.5	0.2	46.5	6	7.3	7.15	26.5	25	24
5	19	1	33	30	0.16	33	3	7.15	7.02	31	24	25
BLOOD STORED IN TRISODIUM CITRATE												
6	17	0.5	41	38	0.17	41	3	7.25	7.14	27	23	24
7	16	0.5	37.5	35	0.16	38	3	7.21	7.10	27.5	22.5	23.5
BLOOD STORED IN HEPARIN + SODIUM FLUORIDE												
8	20	0.7	57	50	0.35	58.5	8.5	7.41	7.25	25	25.5	26
9	21	1	52	45.5	0.32	54.5	9	7.38	7.20	26	25.5	25.5
10	19.5	0.7	52	45.5	0.33	53.5	8	7.37	7.20	26	25	25.5

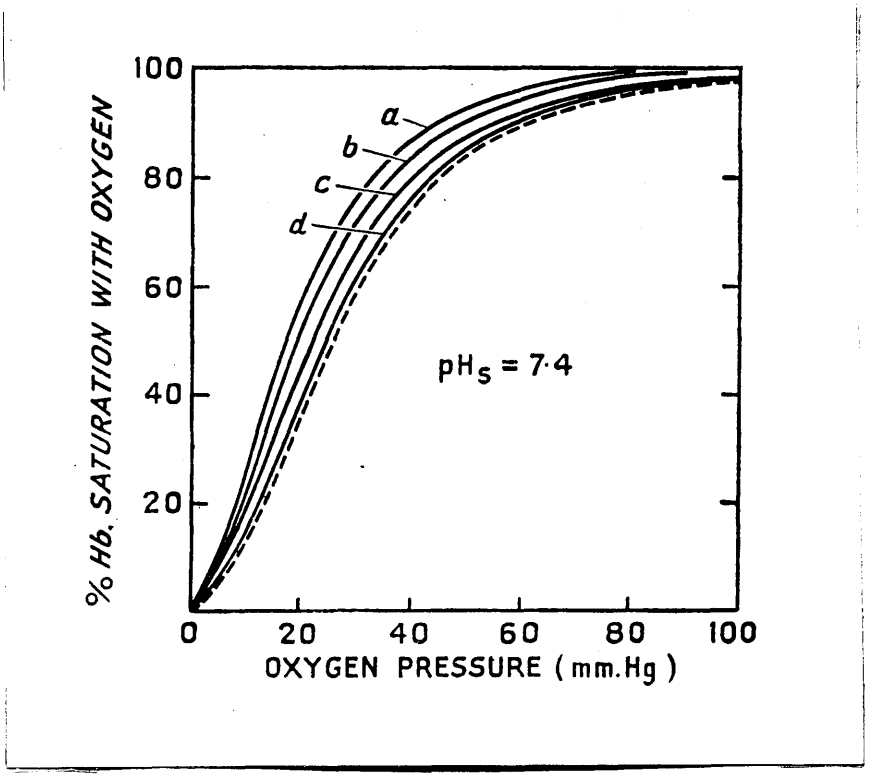

Figure 5 Oxygen dissociation curves at plasma pH 7.4

Curve a - Blood stored in A.C.D. for 20 days.

Curve b - Blood stored in A.C.D. for 7 days.

Curve c - Blood stored in trisodium citrate or in acidified heparin for 7 days.

Curve d - Blood stored in A.C.D. for 1 day.

Interrupted line - Fresh normal blood.

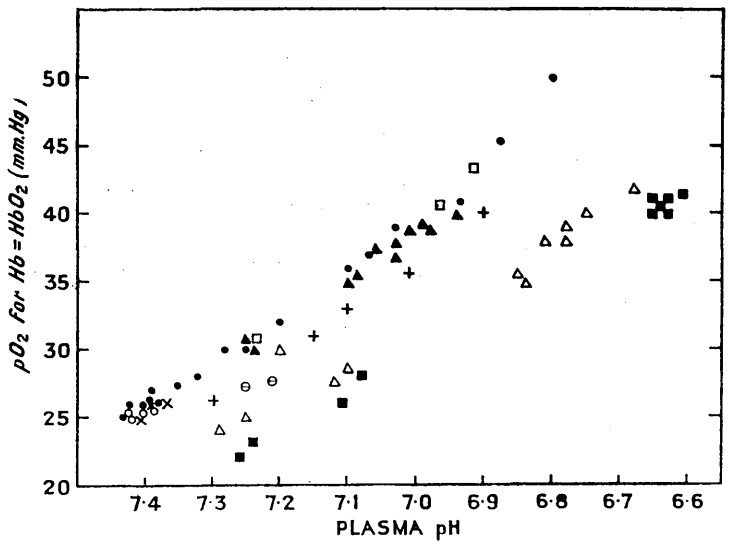

Figure 6 Relation between position of oxygen dissociation curve and plasma pH of fresh blood and blood stored in various anticoagulants.

- ● Fresh normal blood.
- o Fresh blood haemolysed and diluted.
- ▲ Stored in A.C.D. ½ - 24 hours.
- △ Stored in A.C.D. 7 days.
- \+ Stored in acid heparin 7 days.
- ⊖ Stored in trisodium citrate 7 days.
- x Stored in heparin fluoride 7 days.
- ■ Stored in A.C.D. 20 days.
- □ Plasma from stored blood plus fresh red cells.

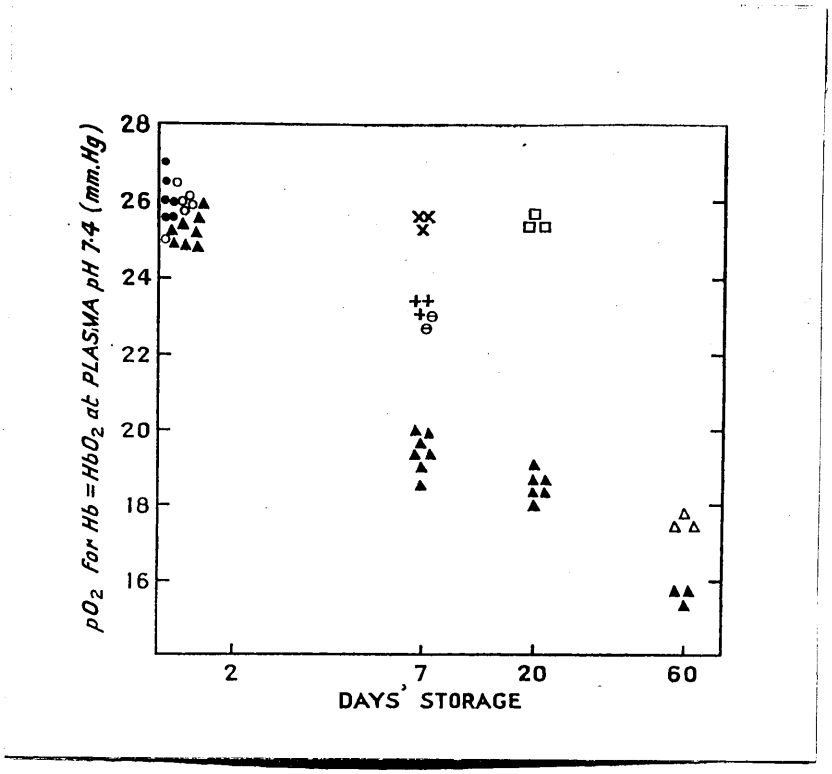

Figure 7 Position of oxygen dissociation curve at plasma pH 7.4 of fresh blood, acidified fresh blood, and blood stored in various anticoagulants for different periods of time. Note change of time scale.

- ● Fresh normal blood.
- o Acidified fresh blood.
- ▲ Stored in A.C.D.
- Δ Stored in A.C.D. after CO correction.
- \+ Stored in acid heparin.
- ⊖ Stored in trisodium citrate.
- × Stored in heparin fluoride.
- □ Plasma from stored blood plus fresh red cells.

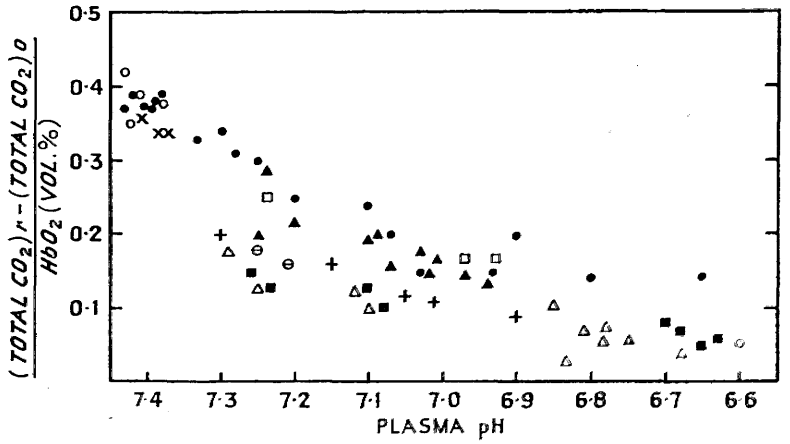

Figure 8 Relation between $\frac{\Delta CO_2\ (r-o)}{HbO_2}$ and plasma pH of fresh blood and of blood stored in various anticoagulants.

- ● Fresh normal blood.
- o Fresh blood haemolysed and diluted.
- ▲ Stored in A.C.D. ½ - 24 hours.
- Δ Stored in A.C.D. 7 days.
- \+ Stored in acid heparin 7 days.
- ⊖ Stored in trisodium citrate 7 days.
- × Stored in heparin fluoride 7 days.
- ■ Stored in A.C.D. 20 days.
- □ Plasma from stored blood plus fresh red cells.

Laked Blood Solutions

Table 9 gives the results of the oxygen studies on buffered solutions of laked blood prepared from fresh blood and from blood stored in the A.C.D. medium for 1 day, 20 days, and 2 months.

The oxygen dissociation curve from fresh blood is the same in position and form as that from citrated blood stored for 1 and for 20 days; that from citrated blood stored for 2 months lies slightly to the left of the normal position but this is due to the presence of carboxyhaemoglobin.

TABLE 9

PO_2 mm. Hg.	Fresh Blood	Stored 1 day	Stored 20 days	Stored 2 months HbO_2 vol.%	
	HbO_2 vol.%	HbO_2 vol.%	HbO_2 vol.%	-	Corrected for CO
15	29	28	28	30	28
20	48	47	47	50	46
30	77	75	76	78	75
40	85	84	84	87	85
21	50	50	50	53	50

Effect of Addition of Electrolytes to Stored Citrated Blood

As it seemed possible that the alterations observed in stored citrated blood were related to a disturbance of the normal electrolyte balance between cells and plasma due to the citrate content of the anticoagulant medium it was decided to observe the effects of adding electrolytes to stored blood samples. The results, which are presented in Table 11 and Figure 9 were most instructive.

After the addition of a molecular solution of disodium phosphate sufficient to raise the plasma pH of a 20-day stored citrated blood sample to 7.3 or 7.4 the oxygen dissociation curve was found to lie less to the left of normal than the curve of the untreated citrated blood sample corrected by calculation to the same plasma pH. This result suggested the use of electrolyte solutions of the same pH as the stored blood sample. The addition, in a proportion of 10 per cent of molecular phosphate buffer (disodium phosphate plus monosodium phosphate) of the same pH (6.6) as the plasma pH of the stored blood sample also had the effect of partially correcting the displacement of the oxygen dissociation curve although in this case there was no alteration in the plasma pH./

plasma pH. Even more striking results were obtained with a molecular solution of sodium chloride which added to a 20-day stored citrated blood samples in a proportion of 10 per cent completely corrected the displacement of the oxygen dissociation curve without producing a change in the plasma pH of the samples.

The difference between the total carbon dioxide of plasma and of oxygenated blood and the difference between the plasma pH and cell pH are considerably smaller after the addition of phosphate buffer. The addition of sodium chloride has a more marked effect and restores these relationships to normal. In 20-day stored citrated blood, after rotation in a tonometer for 1 hour at 37°C and arterial gas tensions, the normal relationship between cell and plasma chloride anion is reversed (Table 10). The addition of sodium chloride restores to normal this relationship. A further noticeable effect of the addition of sodium chloride to citrated blood is the diminution of spontaneous haemolysis in blood samples stored over prolonged periods.

TABLE 10

Chloride content* in mg.% of whole citrated blood and true plasma before and after addition of sodium chloride.

WHOLE BLOOD	TRUE PLASMA
Before sodium chloride	
328	294
325	289
After sodium chloride	
780	877
791	877

*Chloride estimations kindly performed by Dr. J.C. Eaton, Biochemist to the Royal Infirmary, Glasgow.

TABLE II

TABLE 11

Effect of addition of different electrolytes in vitro.

SAMPLE NO.	DESCRIPTION OF SAMPLE	PACKED CELL VOLUME	HbO_2 vol. %	$(CO_2)_o$ vol. %	$(CO_2)_s$ vol. %	$\Delta CO_2(s-o)$ vol. %	BLOOD pH_s	BLOOD pH_c	PO_2 for Hb = HbO_2		
									at BLOOD pH_s and pH_c	at pH_s 7·4	at pH_c 7·22
1	Blood stored for 20 days	41	15.5	15	12.5	-2.5	6.65	6.73	40	18.3	21
	Restoration of pH - fresh plasma	-	15	40	41.5	1.5	7.26	7.14	22	19.5	20.5
	Plus M/Disodium phos. to pH 7.3	-	13	38	40.5	2.5	7.25	7.12	25	21	22
	Plus M/Disodium phos. to pH 7.4	-	12.5	54	58	4	7.4	7.26	22	22	-
	Plus M/Phosphate buffer of pH 6.6	32	14	13	12.5	-0.5	6.65	6.65	47	21	22
	Plus molecular NaCl	33	14	12	12.5	0.5	6.65	6.62	55	25	25
2	Blood stored for 20 days	46	17	13	11	-2	6.58	6.65	42.5	18	20
	Plus M/Phosphate buffer of pH 6.6	36	15	12	12	0	6.63	6.62	49	21.5	22.5
	Plus molecular NaCl	34	15	11	12	1	6.63	6.58	57	25	25.5
3	Blood stored for 20 days	40	14.5	15	12.5	-2.5	6.64	6.73	41	18	20
	Plus M/Phosphate buffer of pH 6.6	31	13	14	13	-1	6.65	6.65	49	22	23
	Plus molecular NaCl	32	13	11.5	12	0.5	6.63	6.60	57	25	25

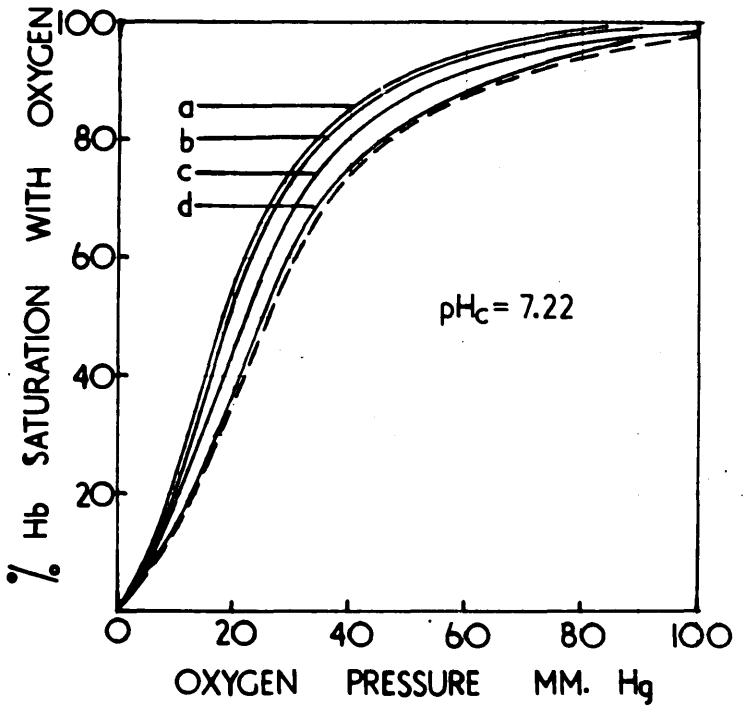

Figure 9 Oxygen dissociation curves at cell pH 7.22

Curve a - Blood stored for 20 days in A.C.D. medium.

Curve b - Blood stored for 20 days in A.C.D. medium after plasma replacement.

Curve c - Blood stored in A.C.D. medium for 20 days after addition of phosphate buffer.

Curve d - Blood stored for 20 days in A.C.D. medium after addition of NaCl

Interrupted line - Fresh normal blood.

SUMMARY OF IN-VITRO STUDIES

In blood samples stored in an acid-citrate/dextrose medium at 4°C, studies of oxygen and carbon dioxide dissociation curves reveal the following changes

1. a substantial shift to the left of the oxygen dissociation curve.
2. a reduction in the amount of carbon dioxide released for each volume per cent of haemoglobin saturation with oxygen.
3. a reduction in the difference between the plasma and cell pH with eventual reversal of the normal relationship so that the cell pH becomes less acid than the plasma pH.

The changes are slight after one day of storage, considerable after seven days and slightly more marked after twenty days.

Addition to citrated blood samples of a molecular solution of disodium phosphate or a molecular phosphate buffer partially corrects the changes; they are wholly corrected by the addition of sodium chloride in a proportion of 10 per cent.

CHAPTER 6

TRANSFUSION STUDIES

The subjects on whom the following observations were made were suffering from severe anaemia and pre-transfusion investigations showed that the oxygen dissociation curves of many of them were shifted to the right. Many of these pre-transfusion studies are presented in greater detail in Chapter 9 along with data derived from severely anaemic individuals who were not transfused. It may be noted here that the major part of the displacement of the oxygen dissociation curve in anaemia appears to be due to an alteration in the cell pH and that after correction to standard cell pH the abnormality of position largely, but not completely, disappears.

The results of the transfusion studies are presented in Tables 12 - 15 and in Figures 10 - 14.

Oxygen Dissociation Curve of the Recipient

It will be seen that the transfusion of 1 pint of blood thirty minutes after its withdrawal into the A.C.D. medium (Table 12, Case 1) did not produce any significant/

significant change in the position of the recipient's oxygen dissociation curve immediately after the transfusion, whereas the transfusion of 2 pints of blood twenty hours after withdrawal into the A.C.D. medium (Case 2) produced a slight shift to the left of the recipient's oxygen dissociation curve at the standard plasma pH (Figure 10).

In all the patients who received 2 or 3 pints of blood which had been stored for seven days or more in the A.C.D. medium the recipient's oxygen dissociation curve (at plasma pH of 7.4) was substantially shifted to the left immediately after the transfusion. The shift was greater in those receiving 3 pints than in those receiving 2 pints. In certain patients further studies were made during the few hours following transfusion. In cases 3, 5, 7, 9, 9a and 10 oxygen dissociation curves were plotted from half an hour up to six hours after transfusion, when the shift to the left was still considerable (Figure 11). In cases 5, 6 and 9 the curve was still slightly abnormal at 24 hours. In case 10, who received 3 pints of blood stored for one or two weeks, the curve was still definitely abnormal/

abnormal at twenty-four hours after the transfusion (Figure 12). In case 9a a 3-pint transfusion was given four days after a 2-pint transfusion, when the oxygen dissociation curve was still slightly to the left; the shift of the curve after the second transfusion lasted for several days.

The degree of shift of the recipient's oxygen dissociation curve did not appear to increase with time of storage of the transfused blood beyond seven days.

Determinations of the cell pH of the recipient were made on five of the patients transfused with stored blood (cases 2, 3, 5, 6, 10). In the case of the patient who was given 2 pints of twenty-hour stored blood (case 2) the slight shift to the left of the oxygen dissociation curve apparent at standard plasma pH immediately after the transfusion was not present at standard cell pH suggesting that the shift of the curve is due to alteration in the cell pH of the recipient. In four of the patients who were given citrated blood of seven or more days storage (cases 3, 5, 6, 10) a large part of the substantial shift to the left of the oxygen dissociation curve at standard plasma pH was not present after correction of the pre- and post-transfusion/

transfusion curves to standard cell pH indicating that factors other than altered cell pH also play a part in the production of the shift of the curve to the left.

Carbon Dioxide and Plasma pH

In all the cases receiving stored citrated blood the difference between the carbon dioxide of the recipient's reduced and oxygenated blood ($\Delta CO_2(r-o)$) was altered but the relationship between the carbon dioxide difference and the oxyhaemoglobin capacity ($\frac{\Delta CO^2_2(r-o)}{HbO_2}$) remained unchanged.

The plasma pH of the recipient was in no case significantly affected by the transfusion of stored citrated blood.

TABLE 12

TABLE 12

Effect of transfusion of stored citrated blood

CASE NO.	SEX AGE	TIME OF ESTIMATION IN RELATION TO TRANSFUSION	VOLUME OF BLOOD (PINTS)	STORAGE TIME	PACKED CELL VOLUME	HbO_2	CO	$(CO_2)_r$	$(CO_2)_o$	$\frac{\Delta CO_2 (r-o)}{HbO_2}$ *	$(CO_2)_o$ con. *	$(CO_2)_s$	ΔCO_2 (s-con) +	BLOOD pH_s	BLOOD pH_c	PO_2 for Hb = HbO_2 at BLOOD pH_s and pH_c	PO_2 for Hb = HbO_2 at pH_s 7·4	PO_2 for Hb = HbO_2 at pH_c 7·22
						vol.%	vol./	vol.%	vol.%	vol.%	vol.%	vol.%	vol.%					
1	M 14 yrs.	Immediately before	-	-	14	7.5	0	60	57	0.40	-	63	-	7.45	-	29.5	31	-
		Immediately after	1	30 mins	20	9.5	0	62.5	59	0.37	-	65	-	7.46	-	28.5	30.5	-
2	M 73 yrs.	Immediately before	-	-	-	9	0.2	52	48.5	0.39	41	56	15	7.39	7.15	32	31.5	28.5
		Immediately after	2	20 hours	-	10.5	0.2	54	50	0.39	43	55	12	7.38	7.18	30.5	30	28.5
3	M 16 yrs.	Immediately before	-	-	20	9	0.2	51.5	48.5	0.33	43	53	10	7.37	7.17	28	27	26
		Immediately after	2	7 days	27	12	0.3	56	52	0.34	-	55.5	-	7.38	-	23	22.5	-
		25 minutes after	"	"	28	12.5	0.2	54	52	0.33	49	56	7	7.39	7.25	23	22.5	24
		24 hours after	"	"	28	12.5	-	51	47	0.33	43	53	10	7.36	7.18	27.5	26	26
4	M	Immediately before	-	-	14	6.5	0.2	53.5	51	0.39	-	58	-	7.41	-	32	29	-
		Immediately after	2	7 days	19	8.5	1.3	58.5	55	0.41	-	60	-	7.42	-	27	26	-

*$(CO_2)_o$ con. = total CO_2 of oxygenated blood after concentration.

+ΔCO_2 (s-con.) = difference between $(CO_2)_s$ and $(CO_2)_o$ con.

Other column headings as on page 48.

TABLE 12

TABLE 12 - Continued

CASE NO.	SEX AGE	TIME OF ESTIMATION IN RELATION TO TRANSFUSION	VOLUME OF BLOOD (PINTS)	STORAGE TIME	PACKED CELL VOLUME	HbO_2 vol.%	CO vol.%	$(CO_2)_r$ vol.%	$(CO_2)_o$ vol.%	$\frac{\Delta CO_2 (r-o)}{HbO_2}$ vol.%	$(CO_2)_o$ con. vol.%	$(CO_2)_s$ vol.%	ΔCO_2 (s-con.) vol.%	BLOOD pH_s	BLOOD pH_c	PO_2 for Hb=HbO_2 at BLOOD pH_s and pH_c	at pH_s 7.4	at pH_c 7.22
5	F 30 yrs.	Immediately before	-	-	10.5	6	-	52	50	0.37	41	57	16	7.4	7.14	32	32	28.5
		Immediately after	2	7 days	19	9.5	0.3	56.5	54	0.37	49	58	9	7.41	7.25	24	24	26.5
		6 hours after	"	"	20.5	10	0.3	56.5	52	0.35	46	56	10	7.38	7.20	28	28	27
		20 hours after	"	"	-	10	-	54	51	0.3	-	57	-	7.4	-	30.5	30.5	-
6	M 68 yrs.	Immediately before	-	-	21	8	0.5	54	51	0.37	42	57.5	15.5	7.4	7.16	29	29	27
		Immediately after	2	14 days	27	11	0.7	56	53	0.27	48	56	8	7.39	7.24	24	24	25
		24 hours after	"	"	-	11	0.5	56	52	0.36	46	59	13	7.41	7.20	27	27.5	27
		8 days after	"	"	-	14	-	58	53	0.36	-	61	-	7.42	-	29	30	-
7	M 28 yrs.	Immediately before	-	-	13	7	0	32.5	31	0.21	-	34	-	7.14	-	32	24	-
		30 minutes after	2	14 days	18	9.5	0.2	33	31	0.22	-	33	-	7.12	-	27	20	-
		16 hours after	"	"	-	9	0.2	31	29	0.23	-	32	-	7.1	-	33	24	-
8	F 41 yrs.	Immediately before	-	-	18	7	0	44.5	47	0.36	-	54	-	7.37	-	30	29	-
		Immediately after	2	18 days	25	10	0.5	51	47.5	0.35	-	53	-	7.36	-	24	23	-

TABLE 12

TABLE 12 - Continued

CASE NO.	SEX AGE	TIME OF ESTIMATION IN RELATION TO TRANSFUSION	VOLUME OF BLOOD (PINTS)	STORAGE TIME	PACKED CELL VOLUME	HbO_2 vol.%	CO vol.%	$(CO_2)_r$ vol.%	$(CO_2)_o$ vol.%	$\frac{\Delta CO_2(r-o)}{HbO_2}$ vol.%	$(CO_2)_o$ con. vol.%	$(CO_2)_s$ vol.%	ΔCO_2 (s-con) vol.%	BLOOD pH_s	BLOOD pH_c	PO_2 for Hb = HbO_2 at BLOOD pH_s and pH_c	PO_2 for Hb = HbO_2 at pH_s 7·4	PO_2 for Hb = HbO_2 at pH_c 7·22
9	M 70 yrs.	Immediately before	-	-	14.5	6.5	0.2	58.5	56	0.40	-	64	-	7.45	-	33	34	-
		Immediately after	2	16 days	18	8.5	0.7	60	57	0.35	-	62	-	7.43	-	26	26.5	-
		1 hour after	"	"	18.5	9	-	60	57	0.36	-	63	-	7.44	-	29	30	-
		24 hours after	"	"	-	-	-	-	52	-	-	59.5	-	7.41	-	31	31.5	-
		4 days after	"	"	23	10	-	54	50	0.4	-	58	-	7.4	-	32	32	-
9a	M 70 yrs.	Immediately before	-	-	23	10	0.2	54	50	0.4	-	58	-	7.42	-	32	32	-
		Immediately after	3	14 days	-	13	1	-	52.5	-	-	57	-	7.4	-	24	24	-
		1 hour after	"	"	30	13.5	-	59	54	0.38	-	58.5	-	7.41	-	25	25	-
		24 hours after	"	"	-	14	-	56	51	0.37	-	57	-	7.4	-	28	28	-
		3 days after	"	"	31	14.5	-	-	49	-	-	55.5	-	7.38	-	29.5	30	-
		8 days after	"	"	-	-	-	-	50	-	-	57	-	7.4	-	30	30	-
		20 days after	"	"	33	15	-	54	48	0.4	-	56	-	7.39	-	31	31	-
10	F 53 yrs.	Immediately before	-	-	18	7	0.2	55.5	53	0.36	40	62	22	7.43	7.12	33	34	28.5
		Immediately after	3	7-14 days	27	11	0.7	59	56	0.36	49	60	11	7.42	7.25	24	24.5	25
		4 hours after	"	"	27.5	11.5	0.5	59	55	0.36	47	60	13	7.41	7.22	26	26.5	26
		24 hours after	"	"	27	11	0.5	55	51	0.36	43	58	15	7.4	7.18	30	30	27.5

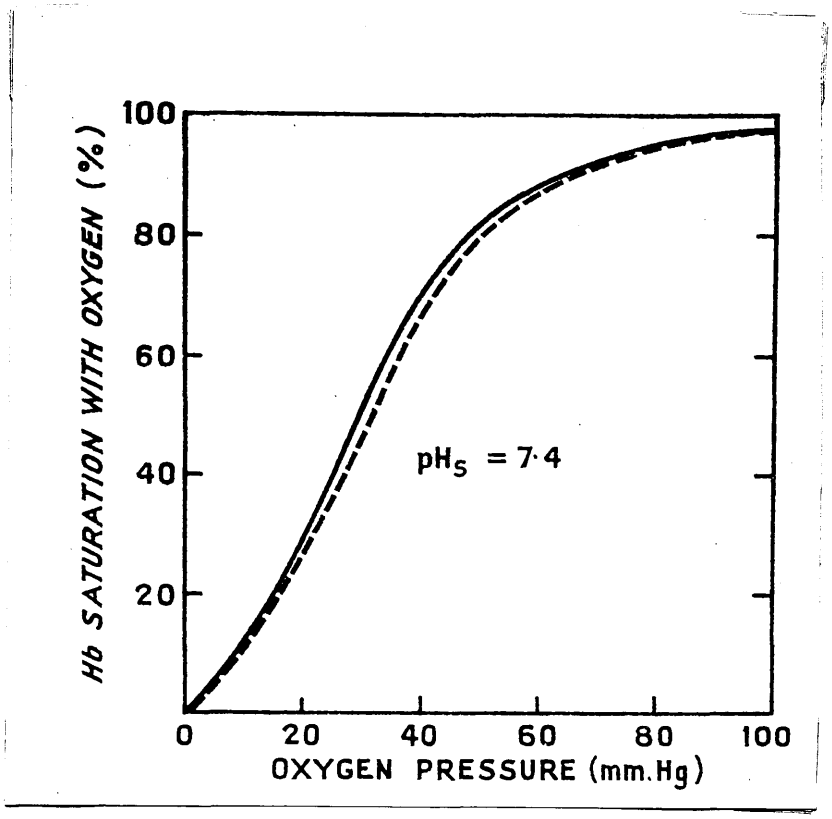

Figure 10 Position of plasma pH 7.4 of recipient's oxygen dissociation curve before (interrupted line), and after (continuous line) transfusion of 2 pints of 20-hour stored citrated blood.

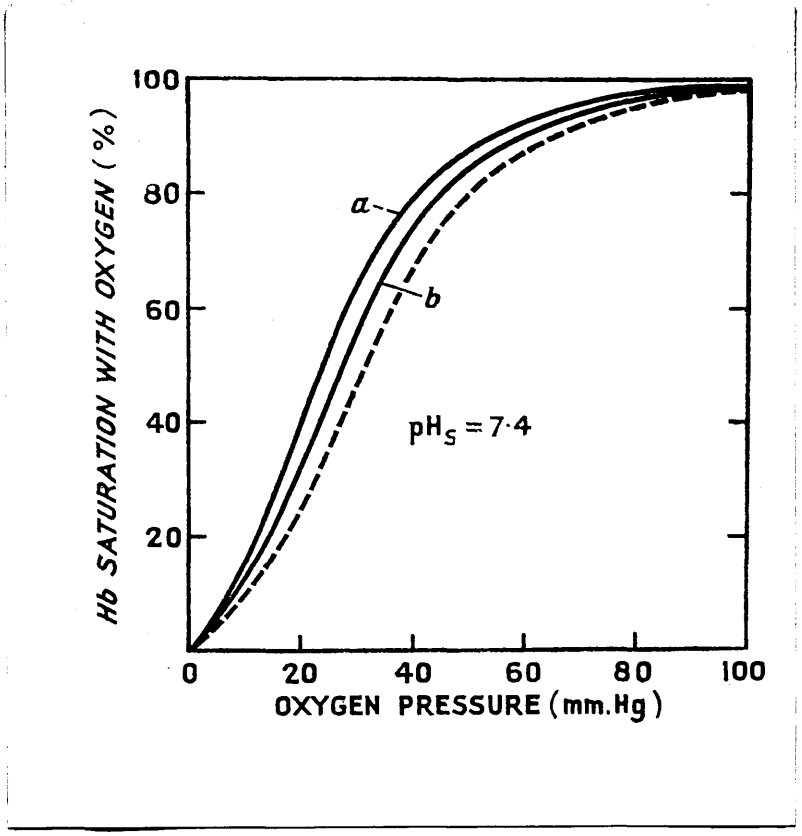

Figure 11 Position at plasma pH 7.4 of recipient's oxygen dissociation curve before (interrupted line), immediately after (a), and 6 hours after (b) transfusion of 2 pints of 7-day stored citrated blood.

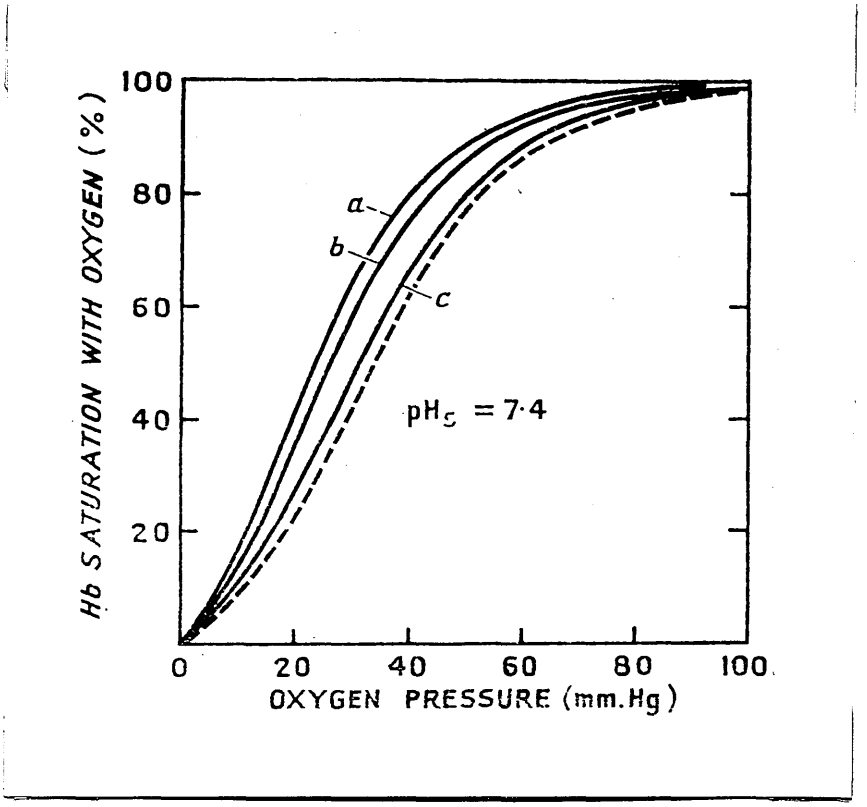

Figure 12 Position at plasma pH 7.4 of recipient's oxygen dissociation curve before (interrupted line), immediately after (a), 4 hours after (b), and 24 hours after (c) transfusion of 3 pints of 7-14 day stored citrated blood.

Plasma transfusion

In one individual (a male of 68 years) 600 ml. of plasma, derived from 3 pints of citrated blood stored for twenty days, were transfused (Table 13). This produced no alteration in the recipient's oxygen dissociation curve or plasma pH immediately after the transfusion.

TABLE 13

Transfusion of plasma derived from citrated blood stored for 20 days.

TIME OF ESTIMATION	PACKED CELL VOLUME	HbO_2 vol.%	CO vol.%	$(CO_2)_T$ vol.%	$(CO_2)_O$ vol.%	ΔCO_2 (T-O) vol.%	$\frac{\Delta CO_2 (T-O)}{HbO_2}$ vol.%	$(CO_2)_S$ vol.%	BLOOD pH_S	PO_2 for Hb = HbO_2	
										at BLOOD pH_S	at pH_S 7.4
Before	30	14	0.5	58	53	5	0.36	61	7.42	29	30
After	29	13.5	0.5	59	54	5	0.39	62	7.44	29	30.5

Carbon Monoxide and Abnormal Pigments

Gas analysis of the recipient's blood after transfusion of stored citrated blood did not reveal any significant amount of carbon monoxide (see Table 12, all cases). Spectrophotometry of the recipient's blood for methaemoglobin, carboxyhaemoglobin, or other abnormal pigments was also negative.

The parenteral administration of ascorbic acid 1,000 mg. or of methylene blue 2 mg. per kg. of body weight immediately after the transfusion of 2 pints of fifteen-day stored blood did not prevent the shift of the oxygen dissociation curve to the left (Table 14).

TABLE 14

TABLE 14

Effect of administration of methylene blue and ascorbic acid.

CASE NO.	SEX AGE	TIME OF ESTIMATION IN RELATION TO TRANSFUSION	VOLUME OF BLOOD (PINTS)	STORAGE TIME DAYS	PACKED CELL VOLUME	HbO_2 vol.%	CO vol.%	$(CO_2)_r$ vol.%	$(CO_2)_o$ vol.%	ΔCO_2 (r-o) vol.%	$\frac{\Delta CO_2 (r-o)}{HbO_2}$ vol.%	$(CO_2)_s$ vol.%	BLOOD pH_s	PO_2 for Hb = HbO_2 at BLOOD pH_s	PO_2 for Hb = HbO_2 at pH_s 7.4
1	M 68 yrs	Immediately before	-	-	21	8	1	54	51	3	0.37	57.5	7.4	29	29
		Immediately after	2	14	27	11	1	56	53	3	0.27	56	7.39	24	24
		Administration of methylene blue													
		1 Hour after	-	-	-	11	1	57	53	4	0.36	58.5	7.41	24	24.5
2	M 28 yrs	Immediately before	-	-	13	7	0.5	32.5	31	1.5	0.21	34	7.14	32	24
		Immediately after	2	15	18	9.5	0.5	33	31	2	0.22	33	7.12	27	20
		Administration of ascorbic acid													
		1 Hour after	-	-	-	9	0.5	33	31	2	0.22	34	7.14	28	21

Effect of Addition of Sodium Chloride to Stored Blood

If stored citrated blood is modified immediately before transfusion by the addition of a molecular solution of sodium chloride in a proportion of 10 per cent, the marked shift to the left of the recipient's oxygen dissociation curve no longer occurs (Table 15). A small shift to the left is present which is very slightly augmented by correction of the slightly lowered plasma pH to the standard plasma pH. After correction to standard cell pH the pre- and post-transfusion oxygen dissociation curves almost coincide (Figure 13b), suggesting that sodium chloride not only affects the corpuscular pH but also the other factors concerned in the production of the shift to the left of the oxygen dissociation curve.

Effect of Administration of Ammonium Chloride to Recipient

The oral administration of 12 grams of ammonium chloride (2 gm. 4-hourly) produces a marked shift to the right of the oxygen dissociation curve, the shift being proportional to the fall in the recipient's plasma pH (Table 15, Case 3). After transfusion/

transfusion of 2 pints of stored citrated blood the oxygen dissociation curve is considerably to the left of the position it occupies after ammonium chloride but only slightly to the left of the normal position. Correction of the position of the curves to standard plasma pH or cell pH reveals that a marked shift has occurred this shift being masked by the ammonium chloride effect on the plasma pH (Figure 14).

TABLE 15

TABLE 15

Effect of addition of sodium chloride to stored citrated blood and the effect of administration of ammonium chloride to the patient.

CASE NO.	SEX AGE	TIME OF ESTIMATION IN RELATION TO TRANSFUSION	VOLUME OF BLOOD	STORAGE TIME	P. C. V.	HbO_2 vol.%	CO vol.%	$(CO_2)_o$ vol.%	$(CO_2)_o$ con. vol.%	$(CO_2)_s$ vol.%	ΔCO_2 (S-con.) vol.%	BLOOD pH_s	BLOOD pH_c	PO_2 for $Hb = HbO_2$ at BLOOD pH_s and pH_c	at pH_s 7.4	at pH_c 7.22
Effects of addition of NaCl in a proportion of 10% to transfused blood.																
1	F 56 yrs.	Immediately before	-	-	22	10	0.2	52	42	59	17	7.41	7.16	29	29	26
		Immediately after	2 pints	14 days	28	13.5	0.5	49	43	55	12	7.37	7.17	28	27	26
2	F 53 yrs.	Immediately before	-	-	16	7	0.5	55	43	59.5	16.5	7.42	7.17	30	31	28
		Immediately after	2 pints	15 days	21	10	0.7	50	43	57	14	7.39	7.17	29	29	27
Effects of administration of NH_4Cl to the patient																
3	M 16 yrs.	Before NH_4Cl	-	-	-	8.5	0	54	47.5	58.5	11	7.41	7.23	26	26	26
		Immediately before transfusion	-	-	19	8	-	42	41	46	5	7.30	7.15	30	27	27
		Immediately after	2 pints	14 days	16	11	0.5	46	44	50	6	7.34	7.20	25	23	23

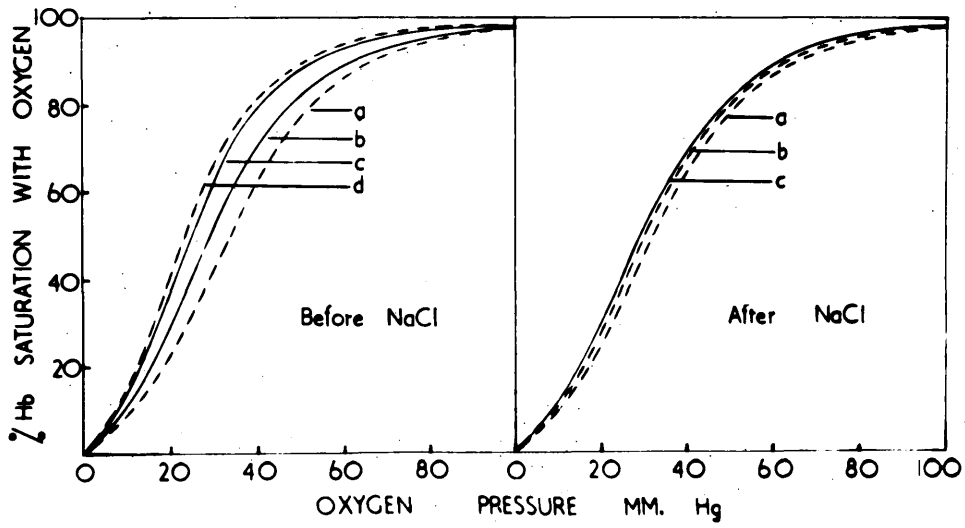

Fig. 13a Fig. 13b

Figure 13a The oxygen dissociation curve of the recipient of blood stored in A.C.D. medium.

Curve a - Before transfusion at plasma pH 7.4

Curve b - Before transfusion at cell pH 7.22

Curve c - Immediately after transfusion at cell pH 7.22

Curve d - Immediately after transfusion at plasma pH 7.4

Figure 13b The oxygen dissociation curve of the recipient of saline modified stored citrated blood.

Curve a - Before transfusion at plasma pH 7.4

Curve b - After transfusion at plasma pH 7.4

Curve c - Before and after transfusion at cell pH 7.22

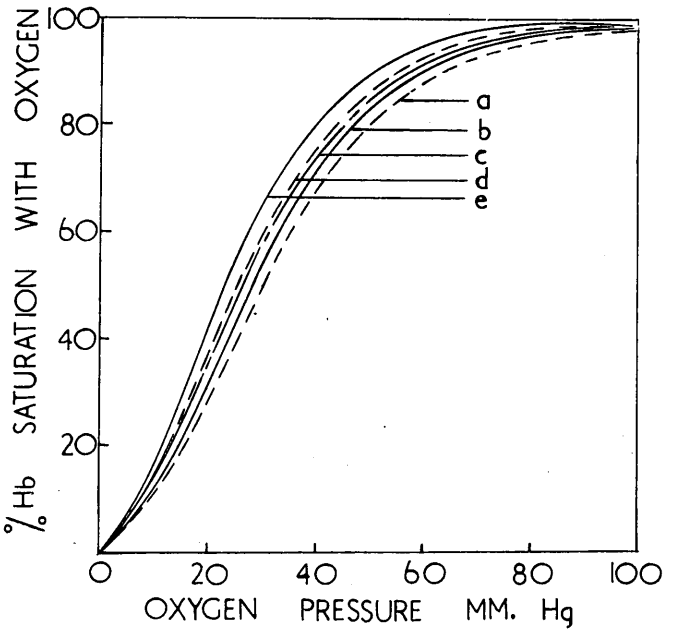

Figure 14 Effect of ammonium chloride on the oxygen dissociation curve.

Curve c - Before NH_4Cl

Curve a - After NH_4Cl at blood plasma pH and cell pH

Curve b - After NH_4Cl at standard plasma pH and cell pH

Curve d - After transfusion of stored citrated blood at plasma pH and cell pH.

SUMMARY OF TRANSFUSION STUDIES

The oxygen dissociation curve of patients after transfusion with citrated blood stored for seven days or more is substantially shifted to the left immediately after transfusion, and this effect lasts for several hours. The magnitude and duration of the shift are proportional to the volume and length of time of storage of the transfused blood. Correction of the position of the oxygen dissociation curve to standard cell pH largely corrects the leftward shift.

There is no alteration in the blood of the recipient of stored citrated blood as regards the amount of carbon dioxide released for each volume per cent of haemoglobin saturation with oxygen. The plasma pH of the recipient is not affected by the transfusion of stored citrated blood and there is no evidence of the presence of abnormal pigments.

The addition of sodium chloride to the stored citrated blood before transfusion, to give a final proportion of 0.4 to 0.5 per cent, corrects the leftward shift of the oxygen dissociation curve.

CHAPTER 7

SIGNIFICANCE, CAUSATION AND PREVENTION OF DEFICIENT GAS TRANSPORT FUNCTION OF STORED RED CELLS

Significance of the changes in oxygen transport

The in vitro studies reported in Chapter 5 show clearly that the oxygen dissociation curve of stored citrated red blood cells is shifted to the left of the normal position. The degree of the shift increases with time of storage up to seven days and is only slightly augmented by further storage. It is reasonable to suggest from these findings that red blood cells after storage in the universally used citrate anticoagulant might after transfusion be incapable, at least temporarily, of releasing as large a volume of oxygen to the tissues as normal red cells.

This surmise is borne out by the clinical studies detailed in Chapter 6. After transfusion of stored citrated blood the oxygen dissociation curve of the recipient is shifted substantially to the left and indeed the degree of the shift is actually greater than might be expected from the in vitro findings. The magnitude/

magnitude of the shift is proportional to the amount and to the age of the transfused blood. The shift is present immediately after the transfusion and, in the case of a transfusion of two or more pints of blood stored for seven days or more, remains for several hours. The curve may remain slightly abnormal for several days if several pints of stored blood are transfused.

The clinical significance of the alteration in the position of the oxygen dissociation curve of the recipient of stored citrated blood is well illustrated by the patient with a haemoglobin level of 35 per cent who received a transfusion of 3 pints of citrated blood stored for one or two weeks (Table 12 Case 10). Although the haemoglobin was increased to 55 per cent the shift of the oxygen dissociation curve to the left (Figure 12) means that after transfusion the patient's blood could not deliver to the tissues as much oxygen as it did before. Thus, given a normal oxygen partial pressure of 40 mm. Hg. in the tissues, this patient's blood before transfusion released to the tissues about 40 per cent of the oxygen carried (about 3 volumes of oxygen per cent). In contrast, for several hours after the transfusion/

transfusion of 3 pints of stored blood and given the same oxygen partial pressure in the tissues, the blood could release to the tissues only 20 per cent of the oxygen carried (about 2 volumes of oxygen per cent). The immediate post-transfusion hours are often extremely critical for the gravely ill patient and any adverse effect on oxygen transport in this vital period may be of serious import. It is recognised that the body has many compensatory mechanisms but in the critically ill patient it is conceivable that these may not operate rapidly enough or to a sufficient extent. It was several days before the oxygen dissociation curve returned completely to its pre-transfusion position, but the smaller percentage of oxygen released - e.g. after six hours - was more than compensated by the higher haemoglobin level.

Significance of the carbon dioxide alterations

The carbon dioxide alterations in stored citrated blood are of less importance than the oxygen changes described above and indeed it could be reasoned that they might be beneficial. For each volume per cent of oxygen saturation haemoglobin normally releases 0.35 - 0.4 volume/

0.35 - 0.4 volume of carbon dioxide at the standard plasma pH of 7.4 and 0.3 volume at a plasma pH of 7.3. The in vitro studies show that in stored citrated blood with a plasma pH of 7.3 the value is lowered to 0.2. This alteration suggests that the recipient of stored citrated blood will be able to release the normal volume of carbon dioxide in the lungs only if the tension of carbon dioxide in the tissues is increased. Such an increase will tend to lower the plasma pH and at the same time to stimulate the respiratory centre. Both these secondary effects would exert a beneficial influence on oxygen uptake in the lungs and release in the tissues.

The all-important clinical studies, however, show that the carbon dioxide abnormality observed in vitro, theoretically beneficial or otherwise, is not present after the transfusion of stored citrated blood.

Relation of anticoagulant media to gas transport changes

The changes observed in citrated blood stored for seven days are not present in blood stored for the same period in heparin at a pH of 7.4 or in acidified fresh blood; they are present, but in considerably lesser degree,/

degree, in blood stored for seven days in acid heparin or in trisodium citrate. These facts suggest that neither storage alone, acid reaction alone, nor citrate alone can be wholly responsible for the gas transport changes and that all three factors must be present together for the full development of the abnormalities.

Causation of the changes in gas transport

It would appear from the in vitro results and the transfusion studies that there is a close correlation between the deficient gas transport function of stored red cells and alteration of the cell to plasma pH relationship. Before this could be accepted as causal, however, it was necessary to consider several other possible mechanisms which might produce the leftward shift of the oxygen dissociation curve.

Changes in the plasma of stored citrated blood, resulting from red cell metabolism or from abnormal breakdown products of haemoglobin, may be readily excluded as the cause of the abnormality of the oxygen dissociation curve, for the leftward shift is not produced by the addition of plasma derived from stored citrated/

citrated blood to fresh red cells in vitro (Table 5) or by the transfusion of such plasma (Table 13).

An alkalosis would cause a shift of the oxygen dissociation curve to the left but the finding of Wurmser et al (1942) and Loutit et al (1943) that there is little or no alteration in the recipient's plasma pH after transfusion of stored citrated blood was confirmed (Table 12). Moreover, correction of the position of the recipient's oxygen dissociation curve to the standard plasma pH of 7.4 does not diminish the shift of the curve to the left.

Both carboxyhaemoglobin (Stadie and Martin 1925) and methaemoglobin (Darling and Roughton 1942) are known to shift the oxygen dissociation curve to the left. Appreciable amounts of carboxyhaemoglobin are present in blood stored in heparin at room temperature (Sjöstrand 1951b), and the optimum conditions for conversion of haemoglobin to methaemoglobin within the red cell are storage under low oxygen and high carbon dioxide pressure (Brooks 1935), conditions existing in stored citrated blood (Table 7). Sjöstrand (1951a) has also reported an increased alveolar concentration of carbon monoxide in the recipient after transfusion. In the/

the present investigation, however, no significant amount of carboxyhaemoglobin, methaemoglobin, or other abnormal pigments - for example sulphaemoglobin - was detected, either by gas analysis or by spectrophotometry, either in stored citrated blood or in the recipient's blood after transfusion. The absence of methaemoglobin is confirmed indirectly by the fact that the oxygen capacity of citrated blood stored seven days is slightly greater than that of fresh citrated blood (Table 7) possibly indicating the conversion of inactive pigment (Ammundsen 1941) to an active form, and by the failure of methylene blue and of ascorbic acid to prevent the shift of the curve to the left (Table 14).

A chemical change in haemoglobin structure or the presence of other chemical substances - e.g. glutathione (Litarczek et al 1931) - cannot be responsible for the changes in oxygen transport since no such changes are present in laked blood solutions prepared from stored citrated blood (Table 9).

Attention will now be directed to the part played by alteration in the cell to plasma pH relationship in the production of the leftward shift of the oxygen dissociation/

dissociation curve of stored citrated blood. Direct determinations of the pH of red cells stored in a citrate medium appear to have been performed previously only by Maizels (1943) who found that the cell pH became greater than the plasma pH. Thus, after one week of storage the cell pH was 7.13 and the plasma pH 7.07 and after four weeks the values were 6.97 and 6.82 respectively. Maizels gives his results with reservation in view of the inherent difficulties of the methods employed and, in addition, it may be noted that these pH studies, and indeed all the published electrolyte studies in stored blood, were performed on blood samples at room temperature and atmosphere - i.e. under conditions far removed from those obtaining in the body. The only exception to this is the work of Harris (1941) and Maizels (1949) on potassium levels in stored blood after incubation at 37°C. In the present investigation the blood samples were equilibrated with arterial gas tensions at body temperature before the cell pH was determined. The finding of Maizels was confirmed that the cell pH of stored citrated blood becomes greater than the plasma pH with storage (Chapter 5). The importance

of/

of this result is shown by the fact that in both the in vitro studies (Figures 2 and 3) and the transfusion studies (Table 12, Cases 2,3,5,6,10) if the position of the oxygen dissociation curve is corrected according to standard cell pH the abnormality in the position of the curve is largely corrected. Some explanation other than altered cell pH must be sought, however, for the remaining degree of displacement of the oxygen dissociation curve.

The position of the oxygen dissociation curve is dependent upon the red cell pH but it is also significantly influenced by the ionic and osmotic status of the red cell, which in turn are dependent on the electrolyte equilibria between cell and plasma and the condition of the red cell membrane. With regard to the osmotic status it is known that during storage swelling of the red cells occurs (Drew et al 1939, Crosbie and Scarborough 1941) and this increases the water content and decreases the haemoglobin concentration within the red cell; the Donnan ratio 'r' (as developed by Van Slyke) therefore increases resulting in a shift to the left of the oxygen dissociation curve. The leftward shift of the oxygen dissociation curve of stored/

stored citrated blood becomes less marked after the addition of molecular phosphate buffer (Table 11) and the packed cell volume returns to normal probably due to reversal of red cell swelling. The addition of the same volume of molecular sodium chloride produces an even more marked effect on the position of the oxygen dissociation curve although the phosphate buffer is more hypertonic and causes a greater reduction of packed cell volume. This suggests that, in addition to redistribution of water between cell and plasma, a redistribution of electrolytes has also taken place. Support for this theory is to be found in the fact that the total carbon dioxide of stored citrated blood is higher than the total carbon dioxide of the plasma of such blood (Chapter 5). The chloride ion freely penetrates the red cell membrane and, obeying Donnan's law, will move in accordance with the relationship between the total carbon dioxide of cell and plasma. The addition of sodium chloride restores the relationship of carbon dioxide and chloride between cell and plasma to normal (Table 10) and it is possible that this movement of chloride and carbon dioxide is accompanied by a/

by a similar redistribution of other ions the final effect being the restoration of the normal relationship between cell and plasma electrolytes, cell and plasma pH, and cell and plasma water content.

Prevention of the gas transport abnormalities

The in vitro experiments with phosphate buffers gave valuable information regarding the causation of the leftward shift of the oxygen dissociation curve of stored citrated blood but it is the effect of sodium chloride which is the more interesting and apparently valuable from the clinical viewpoint. The beneficial effect upon the recipient's post-transfusion oxygen dissociation curve of adding sodium chloride to the stored citrated blood prior to its administration is clearly demonstrated by Figure 13. The greater part of the sodium chloride effect is due to the osmotic and electrolytic redistribution discussed above. That the lowering of the plasma pH of the recipient plays only a small part can be judged from the very slight further shift in the position of the curve after correction to standard plasma and cell pH.

In/

In these studies the amount of sodium chloride added to the stored citrated blood produced a final concentration of approximately 0.45 per cent. Since this modification significantly improves the functional capacity of the stored red cell, as is shown by its effect on the oxygen dissociation curve, it is logical to expect that it might have a beneficial effect upon the survival of the transfused cells. An investigation to prove this was beyond the scope of the present study but it was observed incidentally that the addition of sodium chloride to stored citrated blood diminishes the degree of spontaneous haemolysis which occurs with long storage. It is also of interest to note that Gibson et al (1947) found a slightly hypertonic solution of sodium chloride to be the best diluent to add to packed red cells before transfusion.

CHAPTER 8

THE OXYGEN DISSOCIATION CURVE IN ANAEMIA

INTRODUCTION

It is a well-recognized clinical observation that the patient who has become anaemic gradually may experience relatively little in the way of symptoms from the low haemoglobin level he possesses. Thus it is not uncommon for an individual with a haemoglobin level well below 50 per cent, due, for example, to pernicious anaemia or chronic nutritional hypochromic anaemia, to carry on his normal activities. It is generally believed (Best and Taylor 1950) that the compensatory adjustment is in part due to increased utilization of oxygen by the tissues and in part to circulatory adaption. It has also been suggested, from studies of the gas transport of red cells in anaemia (Odaira 1923, Richards and Strauss 1927, Dill et al 1928, and Isac et al 1938), that it may in part be due to displacement of the oxygen dissociation curve to the right of normal whereby the amount of oxygen released to the tissues is increased; the

number/

number of patients observed in these investigations is small probably because of the difficulties and time-consuming nature of the methods. The fullest report is that of Richards and Strauss (1927) who indicated a shift to the right of the oxygen dissociation curve apparent, however, only at the abnormal plasma pH of 7.64 Only the reports of Dill and his co-workers (1928) and Isac et al (1938), demonstrate a shift to the right of the oxygen dissociation curve at the standard plasma pH of 7.4 There appear to be no observations on the oxygen dissociation curve at various stages after recovery from anaemia. Many of the patients in these early publications had received whole blood transfusions before the blood gas studies and, as has been shown in this thesis, this produces an alteration in the oxygen dissociation curve of the anaemic recipient. Various hypotheses, many of them contradictory, have been offered regarding the cause of the beneficial shift of the oxygen dissociation curve in anaemia but none of them affords a satisfactory explanation for the phenomenon.

For these reasons alone a further and more extensive/

extensive study of the oxygen dissociation curve in anaemia appeared warranted. For the author there was the additional reason that it was necessary to know the influence of anaemia on the oxygen dissociation curve before the effects of transfusion of stored citrated blood could be properly assessed.

The subjects chosen for study comprised eleven patients with megaloblastic anaemia in relapse and three in therapeutic remission, three patients with nutritional hypochromic anaemia, three patients with hypochromic anaemia secondary to chronic haemorrhage, two patients with aplastic anaemia, four patients with chronic nephritis, acute leukaemia, reticulosis, and scurvy respectively, and three patients with haemolytic anaemia. This gave a total of twenty-nine patients. Many of the observations were made before specific therapy was administered and subsequent curves were performed at different periods during and after recovery. None of the patients had received blood transfusions before the blood gas studies were performed, with the exception of one patient with aplastic anaemia of long standing (Table 17, Case 12).

The methods employed for the collection of blood/

blood samples, the equilibration in tonometers with the desired gas tensions, the gas analyses, and the pH determinations were as described in Chapter 4. The subjects were resting for at least one hour before the removal of the blood samples.

CHAPTER 9

THE OXYGEN DISSOCIATION CURVE IN ANAEMIA

RESULTS

The haematological data of the patients investigated and the results of the gas studies are presented in Tables 16 - 18 and in Figures 15 and 16. The position of the oxygen dissociation curve is indicated in Tables 16 and 17 by the partial pressure of oxygen at which 50 per cent of the haemoglobin is oxygenated.

Megaloblastic anaemia

In Table 16 are shown the individual data of eleven patients with megaloblastic anaemia in relapse and three in therapeutic remission; for comparison are shown the mean values obtained from six normal individuals (see Table 1 for individual results).

Of the patients in relapse the position of the oxygen dissociation curve at the plasma and cell pH of the subject was displaced to the right of normal in all but one (case 3). After correction to the standard plasma pH of 7.4 (Figure 15) the rightward shift/

shift was more pronounced except in cases 5 and 6; in these the curve was nearly normal at standard plasma pH. Serial observations (case 4 and cases 8 - 11) show that the displacement to the right of the oxygen dissociation curve did not alter during the earlier response to treatment, although by the time the red cell count had reached about the four million per cubic mm. level, between four and six weeks, some lessening of the displacement was apparent (Figure 16). In the two patients in whom the initial oxygen dissociation curves were normal at standard plasma pH (cases 5 and 6) a shift to the right developed during the early stages of treatment. The presence of reticulocytes did not appear to influence the position of the oxygen dissociation curve. The curve was normal in position in the three patients in therapeutic remission (cases 12 - 14).

The cell pH was determined in seven of the cases, of whom three were in relapse, two were during treatment, and two were in therapeutic remission. Of the cases in relapse, the difference between the cell and plasma pH was increased in those showing a shift of the oxygen dissociation curve to the right at standard/

standard plasma pH (cases 1 and 2), and normal in the case showing a normal oxygen dissociation curve at standard plasma pH (case 3). In the patients (cases 4 and 5) in whom the cell pH was determined when the red cell count had reached between three and four millions per cubic mm., at which time the oxygen dissociation curve was still slightly displaced to the right at standard plasma pH, the difference between the cell and plasma pH was normal. After correction to standard cell pH the position of the oxygen dissociation curve was unaltered. The cell pH was normal in the two patients in therapeutic remission (cases 12 and 13) both of whom showed a normal position of the oxygen dissociation curve.

TABLE 16

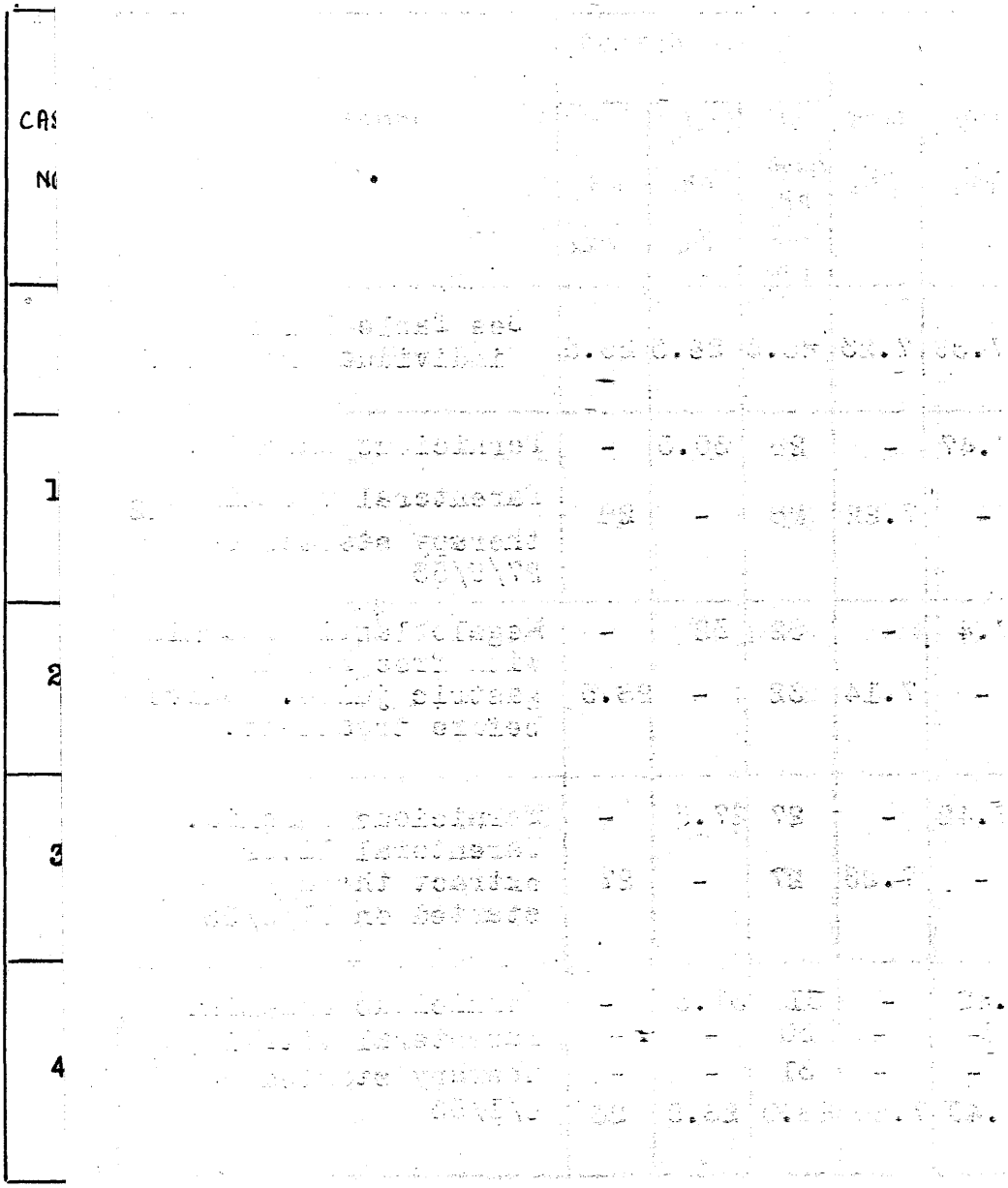

TABLE 16

Results in normal subjects and in megaloblastic anaemia

CASE NO.	SEX AGE (YRS.)	DATE OF ESTIMATION	RED CELL LEVEL mill./cu. mm.	HbO_2 vol.%	COLOUR INDEX	PACKED CELL VOLUME	$\frac{HbO_2}{P.C.V.}$	RETICS %	CO vol.%	$(CO_2)_s$ vol.%	$(CO_2)_o$ vol.%	ΔCO_2 (s-o) vol.%	BLOOD pH_s	BLOOD pH_c	Po_2 for $Hb = HbO_2$ at BLOOD pH_s and pH_c	Po_2 for $Hb = HbO_2$ at pH_s 7.4	Po_2 for $Hb = HbO_2$ at pH_c 7.22	COMMENTS
	Mean normal values from 6 subjects		-	19.5	-	-	-	-	0.5	57.5	48	9.5	7.39	7.23	26.5	26.5	26.5	See Table 1 for individual results.
1	M	26/9/53	2.2	9.5	1.1	23	0.41	1	0	65	57	8	7.47	-	28	30.5	-	Pernicious anaemia.
	79	After concentration		16	-	38	-	-	-	-	49	16	-	7.22	28	-	28	Parenteral vitamin B_{12} therapy started on 27/9/53
2	F	2/4/53	0.90	5.5	1.5	10.5	0.52	1	0	57	50	7	7.4	-	32	32	-	Megaloblastic anaemia with free HCl in gastric juice. Curve before treatment.
	30	After concentration	-	19	-	38	-	-	-	-	41	16	-	7.14	32	-	28.5	
3	F	9/5/53	2.05	12	1.4	24	0.5	3	0.2	59	53	6	7.42	-	27	27.5	-	Pernicious anaemia. Parenteral liver extract therapy started on 11/5/53
	70	After concentration	-	18	-	39	-	-	-	-	49	9.5	-	7.23	27	-	27	
4	M	13/1/53	1.39	7.5	1.3	22	0.34	14	0.2	59	-	-	7.42	-	31	31.5	-	Pernicious anaemia. Parenteral vitamin B_{12} therapy started on 6/1/53
		26/1/53	2.79	12	1.1	29	0.41	2.5	-	-	-	-	-	-	30	-	-	
		29/1/53	3.14	13	1.1	-	-	3	-	-	-	-	-	-	31	-	-	
	56	18/2/53	3.60	16.5	1.1	38	0.44	1	-	57	47	10	7.40	7.20	28.5	28.5	28	

TABLE 16

CASE NO.				
5				
6				
7				
8				

TABLE 16 - Continued

CASE NO.	SEX AGE (YRS.)	DATE OF ESTIMATION	RED CELL LEVEL mill./cu. mm.	HbO_2 vol.%	COLOUR INDEX	PACKED CELL VOLUME	$\frac{HbO_2}{P.C.V.}$	RETICS. %	CO vol.%	$(CO_2)_s$ vol.%	$(CO_2)_o$ vol.%	ΔCO_2 (s-o) vol.%	BLOOD pH_s	BLOOD pH_c	PO_2 for Hb=HbO_2 at BLOOD pH_s and pH_c	PO_2 for Hb=HbO_2 at pH_s 7·4	PO_2 for Hb=HbO_2 at pH_c 7·22	COMMENTS
5	M 36	23/2/53	1.51	8	1.3	18	0.44	2.5	0.5	51	47	4	7.35	-	28	26.5	-	Pernicious anaemia. Parenteral vitamin B_{12} started on 23/2/53
		28/2/53	1.85	10	1.4	25	0.40	31.5	-	-	-	-	-	-	26.5	-	-	
		17/3/53	3.38	14.5	1.1	40	0.36	2.5	1	62	50	12	7.44	7.24	26.5	28	28.5	
6	F 43	8/2/53	2.3	10	1.1	19.5	0.51	20	0.2	52	48	4	7.36	-	29	27.5	-	Pernicious anaemia. Parenteral vitamin B_{12} started on 2/2/53
		17/2/53	2.62	12	1.1	-	-	-	-	-	-	-	-	-	29	-	-	
		6/3/53	3.06	15.5	1.1	-	-	3.6	-	-	-	-	-	-	29	-	-	
		16/3/53	3.42	14.5	1.1	30	0.48	1	-	56	48.5	7.5	7.39	-	31	31	-	
		17/3/53	3.51	14.5	1.0	-	-	-	0.25	62	52	10	7.44	-	30	31.5	-	
7	F 58	3/3/53	1.77	8.5	1.2	19	0.44	2	0	62	56	6	7.44	-	30	31.5	-	Pernicious anaemia. Oral therapy with Hog's stomach and vitamin B_{12} started on 9/3/53
		16/3/53	1.47	8.5	1.5	18	0.47	-	-	58.5	53	4.5	7.41	-	29	29.5	-	
8	M 67	1/9/52	1.75	11.5	1.6	23	0.5	5.2	0.25	66	58.5	7.5	7.47	-	30	32	-	Pernicious anaemia. Oral therapy with Hog's stomach and vitamin B_{12} started on 2/9/53. Subsequent parenteral vitamin B_{12}
		8/9/52	2.6	14.5	1.4	31	0.47	12	-	-	-	-	-	-	30	-	-	
		7/11/52	4.49	20	1.1	43	0.47	-	-	-	-	-	-	-	27.5	-	-	
		5/2/53	5.02	20	1.0	46	0.44	-	0.5	57	45	12	7.40	-	27	27	-	

TABLE 16

TABLE 16 - Continued

CASE NO.	SEX AGE (YRS.)	DATE OF ESTIMATION	RED CELL LEVEL mill./cu. mm.	HbO_2 vol.%	COLOUR INDEX	PACKED CELL VOLUME	$\frac{HbO_2}{P.C.V.}$	RETICS. %	CO vol.%	$(CO_2)_s$ vol.%	$(CO_2)_o$ vol.%	ΔCO_2 (s-o) vol.%	BLOOD pH_s	BLOOD pH_c	PO_2 for Hb = HbO_2 at BLOOD pH_s and pH_c	PO_2 for Hb = HbO_2 at pH_s 7·4	PO_2 for Hb = HbO_2 at pH_c 7·22	COMMENTS
9	F	23/7/52	1.43	7.8	1.4	16	0.49	2.2	0.2	59	54	5	7.41	-	28.5	29	-	Pernicious anaemia.
		24/7/52	-	7.5	-	16	0.47	1	-	-	-	-	-	-	28.5	-	-	Oral therapy with Hog's
		1/8/52	1.5	9.5	1.6	21	0.45	20.4	-	58	53	5	7.40	-	28.5	28.5	-	stomach and vitamin B_{12}
		28/8/52	3.83	15.7	1.0	33	0.44	1	-	-	-	-	-	-	28.5	-	-	started on 25/7/52 and
	51	24/2/53	3.82	18	1.2	41.5	0.45	-	-	-	-	-	-	-	27	-	-	continued during period of study.
10	F	8/8/52	2.22	10	1.1	21.5	0.47	1	0	62	56	6	7.44	-	28.5	30	-	Pernicious anaemia.
		14/8/52	2.32	10.2	1.1	24	0.43	18.5	-	64	58	6	7.45	-	28.5	30.5	-	Parenteral vitamin B_{12}
	59	5/9/52	3.8	17	1.1	36.5	0.46	1	-	-	-	-	-	-	29	-	-	started on 9/8/53
11	F	19/1/53	2.91	13.3	1.2	-	-	14.8	0	59	54	5	7.42	-	31	31.5	-	Pernicious anaemia with subacute combined degeneration of cord. Parenteral vitamin B_{12} started on 9/1/53
		30/1/53	3.69	17.3	1.2	38	0.45	2.8	-	-	-	-	-	-	31	-	-	
	43	17/2/53	4.21	17.3	1.0	39	0.44	1	0.5	-	-	-	-	-	28.5	-	-	
12	F 40	6/3/53	4.11	18	1.1	39	0.46	1	0.5	57	48	9	7.40	7.22	27	27	27	All cases of pernicious anaemia in therapeutic remission for 3 months (case 12), 5 years (case 13) and 2 years (case 14).
13	F 78	27/1/53	4.83	20.5	1.0	47	0.44	-	0.5	55.5	45	10.5	7.38	7.20	27	26.5	26.5	
14	M 85	28/1/53	4.55	20	1.0	43	0.46	-	0.5	-	-	-	-	-	26	-	-	

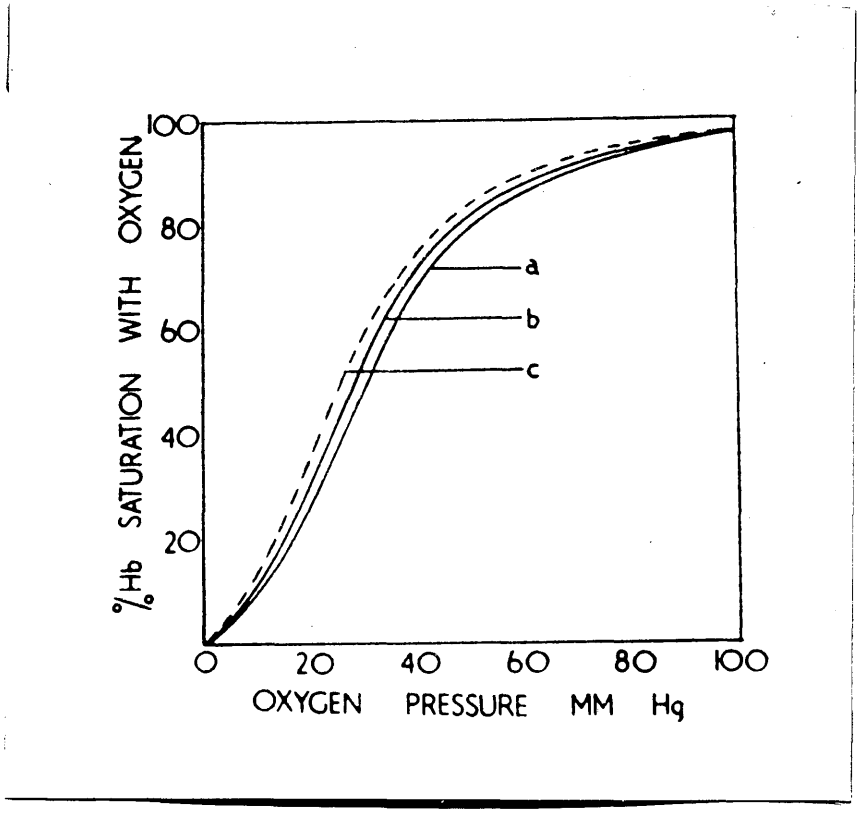

Figure 15 Oxygen dissociation curves from anaemic and normal subjects.

Curve a - Pernicious anaemia in relapse, hypochromic anaemia, secondary anaemia. At plasma pH 7.4

Curve b - Pernicious anaemia in relapse, hypochromic anaemia, secondary anaemia. At cell pH 7.22

Curve c - Normal blood at plasma pH 7.4 and cell pH 7.22

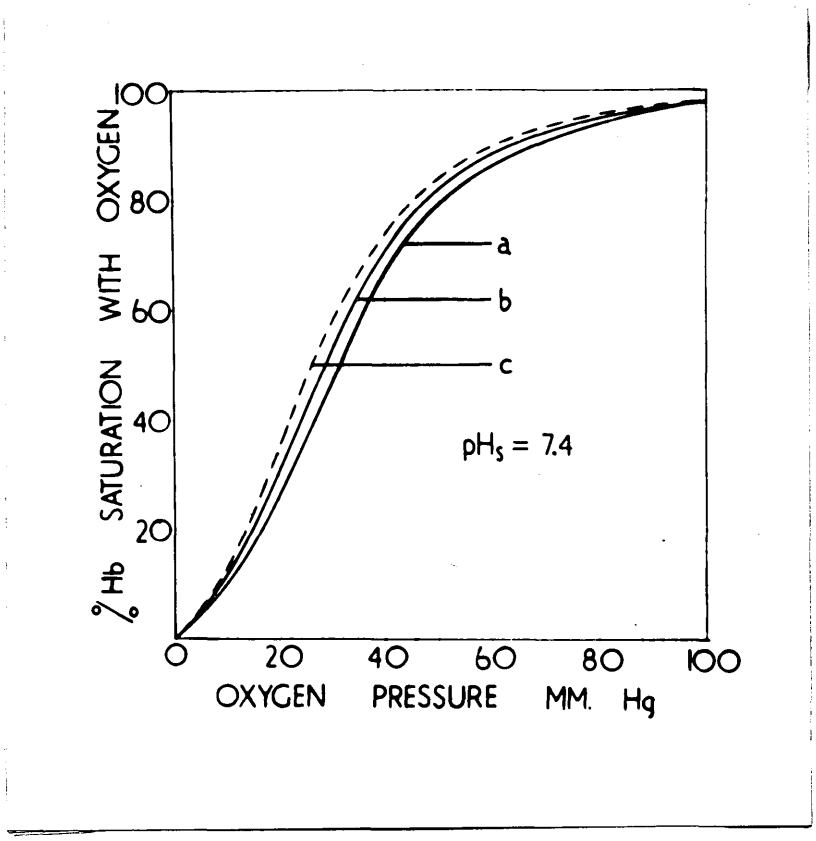

Figure 16 The effect of treatment on the oxygen dissociation curve in pernicious anaemia. All curves at plasma pH 7.4

Curve a - In relapse.

Curve b - Red cell level 3 - 4 million per cubic mm.

Curve c - In full therapeutic remission.

Other forms of anaemia

In Table 17 are shown the results obtained in patients with nutritional hypochromic anaemia, chronic post-haemorrhagic anaemia, secondary anaemia, aplastic anaemia, and haemolytic anaemia. With the exception of the case of anaemia associated with chronic nephritis (case 8), in which there was an acidosis, the plasma pH of the subjects was normal; in the majority of cases the values were in the lower range of normal.

The position of the oxygen dissociation curve at the plasma and cell pH of the subject was displaced to the right of normal in all the patients with the exception of one patient with post-haemorrhagic anaemia (case 5), one patient with aplastic anaemia (case 11), and all three patients with haemolytic anaemia (cases 13 - 15). The displacement to the right was still present after correction to the standard plasma pH of 7.4 (Figure 15) excepting the patient with chronic nephritis whose curve was to the right of normal at blood plasma pH but normal in position at standard plasma pH.

The/

The cell pH was low in six of the seven cases in which it was determined and normal in the remaining case (case 14), a patient with haemolytic anaemia. After correction to the standard cell pH the position of the oxygen dissociation curve was normal in the two patients with haemolytic anaemia (cases 13 and 14) and in one patient with aplastic anaemia (case 11) but was still slightly to the right (Figure 15) in the patients with hypochromic anaemia (cases 1 and 4), secondary anaemia (case 9), and the remaining patient with aplastic anaemia (case 12).

TABLE 17

TABLE 17.

Results in hypochromic anaemia, secondary anaemia, aplastic anaemia, and haemolytic anaemia.

CASE NO.	SEX AGE (YRS.)	DATE OF ESTIMATION	RED CELL LEVEL mill./cu. mm.	HbO_2 vol.%	COLOUR INDEX	PACKED CELL VOLUME	$\frac{HbO_2}{P.C.V.}$	RETICS. %	CO vol.%	$(CO_2)_s$ vol.%	$(CO_2)_o$ vol.%	ΔCO_2 (s-o) vol.%	BLOOD pH_s	BLOOD pH_c	PO_2 for $Hb=HbO_2$ at BLOOD pH_s and pH_c	PO_2 for $Hb=HbO_2$ at pH_s 7·4	PO_2 for $Hb=HbO_2$ at pH_c 7·22	COMMENTS
1	M 68	27/2/53	3.77	8	0.53	21	0.38	2	0.5	57.5	51	6.5	7.4	-	29	29	-	Nutritional hypochromic anaemia.
		After concentration	-	15	-	39	-	-	-	-	42	15.5	-	7.16	29	-	27	
2	F 41	13/2/53	2.71	7	0.65	18	0.40	2	0	54	47	7	7.37	-	30	29	-	Nutritional hypochromic anaemia.
3	M 70	16/3/53	2.92	10	0.85	23	0.43	2	0.2	58	50	8	7.41	-	32	32	-	Nutritional hypochromic anaemia.
4	F 51	23/2/53	3.1	9	0.73	25	0.36	2	0.2	52	47	5	7.36	-	32	30	-	Hypochromic anaemia secondary to heriditary telangiectasis.
		After concentration	-	15	-	37.5	-	-	-	-	42	10	-	7.12	31.5	-	28	
		18/3/53	3.58	13.5	-	33	-	-	-	58	50	8	7.4	-	34	34	-	
		24/3/53	3.79	15.2	-	-	-	-	-	55	47	8	7.38	-	32	31	-	
5	M 33	24/2/53	3.10	8.5	0.70	22	0.40	2	0.2	52	47	5	7.35	-	27	25	-	Hypochromic anaemia secondary to haemorrhoids.

TABLE 17

TABLE 17 - Continued

CASE NO.	SEX AGE (YRS.)	DATE OF ESTIMATION	RED CELL LEVEL mill./cu. mm.	HbO_2 vol.%	COLOUR INDEX	PACKED CELL VOLUME	$\frac{HbO_2}{P.C.V.}$	RETICS %	CO vol.%	$(CO_2)_s$ vol.%	$(CO_2)_o$ vol.%	ΔCO_2 (s-o) vol.%	BLOOD pH_s	BLOOD pH_c	Po_2 for Hb = HbO_2 at BLOOD pH_s and pH_c	Po_2 for Hb = HbO_2 at pH_s 7·4	Po_2 for Hb = HbO_2 at pH_c 7·22	COMMENTS
6	M 42	15/9/53	3.42	7	0.51	19	0.36	1.3	0	59	54	5	7.41	-	32	32.5	-	Hypochromic anaemia secondary to haemorrhoids.
7	M 73	5/3/53	2.15	9.5	1.11	24	0.40	12	0	56	52	4	7.39	-	28.5	28.5	-	Severe scurvy due to dietary inadequacy.
8	M 28	19/2/53	1.85	7	0.95	13	0.54	5	0	34	31	3	7.14	-	32	25	-	Chronic glomerulo-nephritis. Blood urea 240 mg.%
9	M 73	24/4/53	2.17	9	1.03	20	0.45	5	0.2	56	48.5	7.5	7.39	-	32	31.5	-	Reticulosis.
		After concentration	-	16	-	36	-	-	-	-	44	12	-	7.15	32	-	28.5	
10	M 16	7/4/53	1.51	7.5	1.25	14	0.53	1	0	63	57	6	7.44	-	29.5	31	-	Acute leukaemia.
11	M 16	4/3/53	1.76	9	1.27	20	0.45	1	0.2	53	48.5	4.5	7.37	-	28	27	-	Aplastic anaemia of recent onset.
		After concentration	-	18.5	-	40	-	-	-	-	43	10	-	7.17	28	-	26	

TABLE 17

TABLE 17 - Continued

CASE NO.	SEX AGE (YRS.)	DATE OF ESTIMATION	RED CELL LEVEL mill./cu. mm.	HbO_2 vol.%	COLOUR INDEX	PACKED CELL VOLUME	$\frac{HbO_2}{P.C.V.}$	RETICS %	CO vol.%	$(CO_2)_s$ vol.%	$(CO_2)_o$ vol.%	ΔCO_2 (s-o) vol.%	BLOOD pH_s	BLOOD pH_c	PO_2 for Hb=HbO_2 at BLOOD pH_s and pH_c	PO_2 for Hb=HbO_2 at pH_s 7·4	PO_2 for Hb=HbO_2 at pH_c 7·22	COMMENTS
12	F 53	25/5/53	1.82	7.5	1.04	16	0.47	1	0.2	59.5	55	4.5	7.42	-	30	31	-	Aplastic anaemia of several years' duration. Numerous transfusions.
		After concentration	-	17	-	36	-	-	-	-	47	12.5	-	7.17	30	-	28	
13	F 56	6/7/53	2.24	10	1.0	22	0.45	12.5	0.2	59	52	7	7.41	-	28	28.5	-	Congenital acholuric jaundice. Microspherocytosis prominent.
		After concentration	-	17.5	-	39	-	-	-	-	45	14	-	7.16	-	28.5	26	
14	M 8	10/4/53	3.86	17.5	1.14	39	0.45	10	0	75	47.5	27.5	7.4	7.21	27	27	26.5	Congenital acholuric jaundice. Microspherocytosis prominent.
15	F	8/6/53	1.06	5.5	1.34	11	0.50	20	0.25	52	50	2	7.36	-	27	25.5	-	Acquired haemolytic anaemia. Microspherocytosis prominent.

Table 18 given below shows that the oxygen dissociation curves of haemoglobin solutions (buffered solutions of laked blood) from patients with pernicious anaemia and hypochromic anaemia are the same as that of haemoglobin solutions of normal blood.

TABLE 18

Oxyhaemoglobin saturation of laked blood solutions derived from normal blood and from patients with anaemia.

	Normal blood	Pernicious anaemia in relapse (Table 16, Case 8)	Hypochromic anaemia (Table 17, Case 4)	Pernicious anaemia after treatment (Table 16, Case 10)
pO_2 mm. Hg.	HbO_2 volume per cent			
15	28	29	29	28
20	50	50	48	50.5
30	78	80	78	79
40	86	87	85	85

CHAPTER 10

THE OXYGEN DISSOCIATION CURVE IN ANAEMIA

DISCUSSION

Previous studies

Observations on the oxygen dissociation curve in anaemia have been presented previously by Odaira (1923), Stadie and Martin (1925), Richards and Strauss (1927), Dill et al (1928) and Isac et al (1938). Odaira states that in anaemia there is a marked acidosis and that as a result there is a shift to the right of the oxygen dissociation curve. The techniques employed by Odaira are open to criticism, however, and many authors (Dautrebande 1925, Dill et al 1928, Connery and Jolliffe 1931, Emerson and Helmer 1935, and Isac et al 1938), have failed to demonstrate an acidosis in pernicious anaemia but have, in fact, shown a tendency to an alkalosis. Stadie and Martin obtained a normal oxygen dissociation curve in a single patient with pernicious anaemia but they do not give the plasma pH at which the curve was performed. Richards and Strauss reported that the oxygen dissociation curves in six patients with pernicious anaemia and five patients with secondary anaemia/

anaemia were in the normal position at the normal plasma pH of the blood (7.4), and that only at the abnormal plasma pH of 7.64 was there a shift to the right. Several of the cases studied by Richards and Strauss had received transfusions of whole blood within a few days prior to the determination of the oxygen dissociation curves. In discussing the cause of the shift observed at plasma pH 7.64, they suggest that it could be due, in the patients with pernicious anaemia, to an increased concentration of haemoglobin within the red cell, which would have the result, at this high plasma pH, of increasing the value (- log r) as developed by Van Slyke et al 1923, and Van Slyke 1926, and so increasing the plasma and cell pH. The hypothesis is admitted to be invalid in hypochromic anaemia in which there is a shift of the oxygen dissociation curve to the right. Dill et al report that in one patient with pernicious anaemia the oxygen dissociation curve performed before treatment was normal but the subsequent curves during treatment with liver orally, and single curves in four other cases of pernicious anaemia during treatment, were displaced to the right of normal. No curves were determined after/

after recovery from anaemia. Dill and his co-workers by indirect methods found the value rH to be smaller than normal and so conclude that the difference between plasma and cell pH is increased, the cell pH being relatively more acid than normal. The patient most fully studied by these authors is further reported on by Henderson (1928). Both Dill et al and Henderson were unable to relate the observed shift in the oxygen dissociation curve to altered osmotic or electrolytic relationships between cell and plasma. Isac et al found that the oxygen dissociation curves in four patients with pernicious anaemia, four patients with secondary anaemia, one patient with aplastic anaemia, and one patient with haemolytic anaemia were displaced to the right of normal. These workers, using indirect methods like Dill and his associates, were unable to demonstrate any decrease in the cell pH.

The present study

The present observations confirm that there is a shift to the right of the oxygen dissociation curve at standard plasma pH in the majority of patients with hypochromic anaemia, secondary anaemia, and megaloblastic anaemia/

anaemia in relapse, thus providing for the anaemic patient an increased yield of oxygen to the tissues. The time taken for the shift in the curve to lessen following treatment was in the patients with megaloblastic anaemia some four to six weeks but it should be noted that many of these patients were the subjects of an investigation of the value of oral vitamin B_{12} in the therapy of pernicious anaemia and were not always showing optimal haematological responses. It is not apparent why two of the patients with megaloblastic anaemia had a normal oxygen dissociation curve initially but developed a shift to the right during the course of treatment. A similar development was observed by Dill and his co-workers. The present investigation does not confirm that there is a shift to the right of the oxygen dissociation curve in haemolytic anaemia (Isac et al 1938).

Causation of the rightward shift

The beneficial effect of the displacement of the oxygen dissociation curve is obvious but the mechanism responsible for it is not apparent. A reduction of the plasma pH of the subject would displace the curve to the/

the right but there is no evidence that acidosis is present and, moreover, the displacement of the curve is still present after correction to standard plasma pH. The presence of carboxyhaemoglobin causes a shift to the left of the oxygen dissociation curve (Stadie and Martin 1925) and the position of the oxygen dissociation curve in the normal subject may be influenced to a small extent by the carboxyhaemoglobin present (Riley et al 1946). Smaller amounts of carboxyhaemoglobin were found in the anaemic subjects in the present study than in normal subjects but the quantities involved are so small that they could not produce the observed displacement of the oxygen dissociation curve. Alteration in the concentration of haemoglobin within the red cell also cannot be the factor responsible for the displacement of the oxygen dissociation curve since the displacement is demonstrable in both pernicious anaemia and haemoglobin deficiency anaemia. The effect of the increased plasma volume in anaemia can likewise be excluded as the cause of the displacement since artificial dilution with fresh plasma from the same individual does not influence the position of the oxygen dissociation curve (Chapter 5).

It/

It is now accepted (Eastman et al 1933, Darling et al 1941) that many different kinds of haemoglobin, at least some of which may influence the position of the oxygen dissociation curve, may be present in pathological states. It is unlikely, however, that an abnormality of haemoglobin can be responsible for the observed displacement of the oxygen dissociation curve in anaemia since the oxygen dissociation curves of haemoglobin solutions of anaemia blood are the same as those of normal blood (Table 18).

The position of the oxygen dissociation curve is dependent basically upon the cell pH and it is unfortunate that there is no entirely satisfactory method by which the cell pH can be determined directly. Hampson and Maizels (1927-1928) using a direct method at room temperature (glass electrode determinations on red cells haemolysed by repeated exposure to low temperature) found that the difference between the cell pH and plasma pH in pernicious anaemia was greater than normal while in hypochromic anaemia it was less than normal. Maizels (1943), in referring to cell pH determinations, expresses doubts about his results partly because of the many manipulations involved and partly/

partly because the determinations were made at room temperature in conditions far removed from those in which red cells exist in the body. The indirect method for determining cell pH, in which the value "r" as developed by Van Slyke et al (1923) ($- \log r = pH_s - pH_c$) is determined, or the nomogram developed by Dill (quoted by Keys et al 1936) is used, is superior to the direct method particularly as regards comparative studies, although the Henderson-Hasselbalch equation is not a strict physical chemical equation. If the indirect method, however, is applied to anaemic blood where the cell phase is small, there is the disadvantage that experimental errors are multiplied. To avoid this disadvantage the cell pH was determined after concentrating the anaemic blood samples by removal of plasma under oil to bring the packed cell volume into the normal range. This manipulation permits more accurate study.

Using this indirect method the cell pH was low relative to the plasma pH in cases of pernicious anaemia in relapse and cases of hypochromic anaemia which showed a displacement of the oxygen dissociation curve to the right. On the other hand, in cases of pernicious/

pernicious anaemia in therapeutic remission having the oxygen dissociation curve normal in position, the cell pH was normal. The cell pH was also normal in the case of pernicious anaemia in relapse which showed a normal oxygen dissociation curve. In the cases in which the cell pH was low, correction of the oxygen dissociation curve to the standard cell pH largely, but not completely, corrected the displacement to the right, suggesting that while a low cell pH is the main factor in producing the displacement there is also a further factor. In both cases of aplastic anaemia the cell pH was also found to be low. Correction of the position of the oxygen dissociation curve in these cases to the standard cell pH wholly eliminated the displacement of the curve in the case of recent onset and partly corrected it in the case of long standing. This patient had received numerous blood transfusions over several years and it would be unwise to draw any conclusions from the examination of this individual's oxygen dissociation curve since it would appear that one was in fact examining the oxygen dissociation curve of many donors. The cell pH was low in one of the two cases of haemolytic anaemia in which the cell pH

was/

was determined and normal in the other. In the former case, correction of the position of the curve to the standard cell pH wholly corrected the slight displacement of the oxygen dissociation curve that was present at the cell pH of the subject. The cases of pernicious anaemia studied during recovery showed that the oxygen dissociation curve is still slightly abnormal when the red cell count is about the 4 million per cubic mm. level, at which time there is still a degree of macrocytosis. In the cases in complete therapeutic remission, where there is no macrocytosis, there is no displacement of the oxygen dissociation curve.

While a lowered cell pH thus accounts for the major part of the displacement of the oxygen dissociation curve the results suggest that there is in addition a further factor. Measurements of the red cell diameter thickness ratio were not made in this study but the relationship between displacement of the oxygen dissociation curve and the shape of the red cell in stored citrated blood reported in this thesis indicates that such measurements might usefully be done in further studies in gas transport in anaemia.

SUMMARY OF STUDIES IN ANAEMIA

The oxygen dissociation curves in twenty-nine cases of anaemia have been studied. In ten of the eleven cases of megaloblastic anaemia in relapse and in nine of the ten cases of hypochromic anaemia and secondary anaemia there was a shift to the right of the oxygen dissociation curve. No shift to the right was present in three cases of haemolytic anaemia or in one of two cases of aplastic anaemia.

The displacement of the oxygen dissociation curve persisted during the period of recovery in the cases of megaloblastic anaemia but was not present in three cases in full therapeutic remission. The position of the curve was not influenced by the presence of reticulocytes.

The major part of the displacement of the oxygen dissociation curve appears to be due to an alteration in the cell pH (cell pH low relative to the plasma pH).

REFERENCES

Adair, G.S. (1925a). J. biol. Chem. 63,529.

Adair, G.S. (1925b). Proc. roy. Soc., London. A 109,299.

Agote, L. (1915). An. Inst. Clin. méd., B.Aires, Jan., quoted by Keynes, G. (1949). Blood Transfusion. Wright. Bristol.

Ammundsen, E. (1941). J. biol. Chem. 138,563.

Aste-Salazar, H., Hurtado, A. (1944). Amer. J. Physiol. 142,733.

Austin, J.H., Cullen, G.E., Hastings, A.B., McLean, F.C., Peters, J.P., Van Slyke, D.D., (1922). J. biol. Chem. 54,121.

Aylward, F.X., Mainwaring, B.R.S., Wilkinson, J.F. (1940). Lancet. 1,685.

Bagdassarow, A. (1937). Sang. 11,466.

Barcroft, J. (1914). The Respiratory Function of the Blood, University Press, Cambridge.

Barcroft, J. (1925). The Respiratory Function of the Blood, Pt. I. Lessons From High Altitude. University Press, Cambridge.

Barcroft, J. (1928). The Respiratory Function of the Blood, Pt.II. Haemoglobin. University Press, Cambridge.

Barcroft, J., Dryerre, H., Meakins, J.C., Parsons, T.R., Parsons, W. (1926). Quart. J. Med. 19,257.

Belk, W.P., Henry, N.W., Rosenstein, F. (1939). Amer. J. med. Sci. 198,631.

Best, C.H., Taylor, N.B. (1950). The Physiological Basis of Medical Practice, Baillière, Tindall and Cox, London.

Blundell, J. (1824). Researches Physiological and Pathological, London.

Blundell, J. (1829). Lancet. 1,431.

Brooks, J. (1935). Proc. roy. Soc. B, 118,560.

Chambers's Encyclopedia (1950). Vol.2, p.378, Newnes, London.

Christiansen, J., Douglas, C.G., Haldane, J.S. (1914). J. Physiol. 48,244.

Connery, J.E., Jolliffe, N. (1931). Amer. J. med. Sci. 181,830.

Coryell, C.D., Pauling, L., Dodson, R.W. (1939). J. Physiol. Chem. 43,825.

Crosbie, A., Scarborough, H. (1941). Edinb. med. J. 48,253.

Darling, R.C., Roughton, F.J.W. (1942). Amer. J. Physiol. 137,56.

Darling, R.C., Smith, C.A., Asmussen, E., Cohen, F.M., (1941). J. clin. Invest. 20,739.

Dautrebande, L. (1925). C.R. Soc. Biol., Paris. 93,1031.

De Gowin, L.E., Harris, J.E., Plass, E.D. (1940). J. Amer. med. Ass. 114,850.

Denstedt, O.F., Osborne, D.E., Roche, M.N., Stansfield, H. (1941). Canad. med. Ass. J. 44,448.

Denys, J. (1667). Philos. Trans. 2, No.27,489.

Dill, D.B. (1928). In Henderson, L.J. Blood, a Study in General Physiology. New Haven, Conn.

Dill, D.B., Bock, A.V., Van Caulaert, C., Folling, A., Hurxthal, L.M., Henderson, L.J. (1928). J. biol. Chem. 78,191.

Dill, D.B., Daly, C., Forbes, W.H. (1937a). J. biol. Chem. 117,569.

Dill, D.B., Edwards, H.T., Consolazio, W.V. (1937b). J. biol. Chem. 118,635.

Dill, D.B., Graybiel, A., Hurtado, A., Taquini, A.C. (1940). Ztschr. Altesfoschung. 2,220.

Douglas, C.G., Haldane, J.B.S., Haldane, J.S. (1912). J. Physiol. 44,275.

Drew, C.R., Edsall, K., Scudder, J. (1939). J. Lab. clin. Med. 25,240.

Duran-Jorda, F. (1939). Lancet. 1,773.

Eastman, N.J., Geiling, E.M.K., De Lawder, A.M. (1933). Bull. Johns Hopkins Hosp. 53,246.

Emerson, C.P., Jr., Helmer, O.M. (1935). Arch. Int. Med. 55,254.

Evelyn, K.A., Malloy, H.T. (1938). J. biol. Chem. 126,655.

Ferguson, J.K.W., Roughton, F.J.W. (1935). J. Physiol. 83,87.

Frazer, J.G. (1922). The Golden Bough (abridged). McMillan. London.

Gibson, J.G., Evans, R.D., Aub, J.C., Sack, T., Peacock, W.C. (1947). J. clin. Invest. 26,715.

Haldane, J.S. (1912). Respiration, Yale University Press, New Haven.

Hampson, A.C., Maizels, M. (1927-1928). J. Physiol. 64,XX.

Harris, J.E. (1941). J. biol. Chem. 141,579.

Heilmeyer, L. (1943). Spectrophotometry in Medicine. London.

Henderson, L.J. (1928). Blood. Yale University Press, New Haven.

Hicks, J.Braxton. (1869). Guy's Hosp. Rep. S,3,14,1.

Hustin, A. (1914). J. méd. Brux. 12,436.

Isac, C., Matthes, K., Yamanaka, T. (1938). Arch. exp. Path. Pharmak. 189,615.

Jeanneney, G., Servantie, L. (1938). C.R. Soc. Biol., Paris. 129,1189.

Keys, A., Hall, F.G., Barron, E.S.G. (1936). Amer. J. Physiol. 115,292.

King, E.J., Gilchrist, M., Wootton, I.D.P., O'Brien, J.R.P., Jope, H.M., Quelch, P.E., Peterson, J.M., Strangeways, D.H., Ramsay, W.N.M. (1948). Lancet. 1,478.

Lewishon, R. (1915). Surg. Gynec. Obstet. 21,37.

Litarczek, G., Aubert, H., Cosmulesco, J. (1931). C.R. Soc. Biol., Paris. 106,973.

Loutit, J.F. (1945). J. Path. Bact. 57,325.

Loutit, J.F., Mollison, P.L., Young, I.M. (1943). Quart. J. exp. Physiol. 32,183.

Lower, R. (1666). from Hollingsworth M.W. (1928). Ann. M. Hist. 10,213.

Maizels, M. (1941). Unpublished report to M.R.C. quoted by Mollison, P.L. (1954). Proc. roy. Soc. 47,64.

Maizels, M. (1943). Quart. J. exp. Physiol. 32,143.

Maizels, M. (1949). J. Physiol. 108,247.

Maizels, M., Paterson, J.H. (1940). Lancet. ii,417.

Mettler, F.A. (1947). History of Medicine, Blakiston Co. Phil.

Mollison, P.L. (1954). Proc. roy. Soc. 47,64.

Mollison, P.L., Young, I.M. (1941). Brit. med. J. ii,797.

Mollison, P.L., Young, I.M. (1942). Quart, J. exp. Physiol. 31,359.

Odaira, R. (1923). Tohoku J. Exper. Med. 4,243.

Pauling, L. (1935). Proc. Nat. Acad. Sci., U.S. 21,186.

Peters, J.P., Van Slyke, D.D. (1932). Quantitive Clinical Chemistry. Baltimore.

Rapoport, S. (1947). J. clin. Invest. 26,591.

Rappaport, F. (1949). Rapid Microchemical Methods for Blood and C S F Examinations. New York.

Richards, D.W. jun., Strauss, M.L. (1927). J. clin. Invest. 4,105.

Riley, R.L., Lilienthal, J.L. jun., Proemmel, D.D., Franke, R.E. (1946). J. clin. Invest. 25,139.

Rimington, C. (1951). Lancet. 2,551.

Robertson, O.H. (1918). Brit. med. J. 1,691.

Roughton, F.J.W. (1935). Physiol. Revs. 15,241.

Roughton, F.J.W., Darling, R.C., Root, W.S. (1944). Amer. J. Physiol. 142,708.

Rous, P., Turner, J.R. (1916). J. exp. Med. 23,219.

Roussel, J. (1877). Transfusion of Human Blood. Translated from the French and German by C.H.C. Guiness. Churchill. London.

Scarborough, H., Thompson, J.C. (1940). Edinb. med. J. 47,567.

Sjostrand, T. (1951a). Acta physiol. scand. 22,137.

Sjostrand, T. (1951b). Acta physiol. scand. 22,142.

Stadie, W.C., Martin, K.A. (1925). J. clin. Invest. 2,77.

Van Slyke, D.D. (1926). Factors affecting the distribution of electrolytes water and gases in the animal body. J.B.Lippincott. Philadelphia and Boston.

Van Slyke, D.D., Sendroy, J. jun., Hastings, A.B., Neill, J.M. (1928). J. biol. Chem. 78,765.

Van Slyke, D.D., Wu, H., McLean, F.C. (1923). J. biol. Chem. 56,765.

Wurmser, R., Filitti-Wurmser, S., Briault, R. (1942). Rev. canad. Biol. 1,372.