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BLOOD-GROUP ANTIBODIES AND RED-CELL DESTRUCTION*

BY

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In founding these lectures Dr. George Oliver not only wished to record his admiration for Professor William Sharpey—" the Founder of English Physiology "—but also intended " to promote physiological knowledge by observation and experiment, and to encourage the application of physiological knowledge to the prevention and cure of disease and the prolongation of life."

The study of blood transfusion in man has complied with some of these intentions, for it has led not only to knowledge of how to transfuse blood from one person to another without ill effects, but has also contributed in many ways to knowledge of physiological and pathological processes. For example, it was through the study of the survival of transfused red cells that the first correct estimates of red-cell life-span in man were obtained, and haemolytic anaemias were first divided into those due to an intrinsic red-cell defect and those due to a random destructive process.

The safety of blood transfusion depends very largely on the avoidance of serological incompatibility, and a detailed consideration of this problem forms the main subject of these lectures. In pursuing this problem a certain amount of new information about haemolytic processes has been obtained, and this will also be discussed.

I should like to begin by tracing the history of the idea that serological compatibility is essential to safe and effective transfusion: then to discuss the discovery of human blood groups and to consider briefly some of the characteristics displayed by blood group antibodies *in vitro*. Next I shall consider methods of estimating the survival of incompatible red cells *in vivo*, and discuss particularly the information that can be obtained using ^{51}Cr as a label. In the second lecture I shall consider how far results observed *in vivo*, when red cells are transfused, can be predicted from tests made beforehand *in vitro*.

The First Two Hundred Years of Blood Transfusion

The first successful cross-transfusion experiments were performed between dogs by Dr. Richard Lower (1665–6). His command of technique was impressive. He was able, by using a large dog (a " mastive ") as a donor and a little dog (a " curr ") as a recipient, to pass through the recipient an amount of blood equal to twice its total blood volume, and to control the transfusion so well that the recipient left the table in excellent condition: " After he was unty'd he ran away and shak'd himself as if he had been thrown into water." Serological incompatibility did not intrude in these particular experiments, since severe incompatibility in first transfusions in dogs is virtually unknown (Dr. Scott Swisher, personal communication).

Shortly after Lower had carried out his first successful transfusion, the first transfusion to a human was carried out by Denis (1667–8) in France, using lamb's blood. He performed several transfusions, using either a lamb or a calf as a donor, and some of these transfusions were accompanied by severe reactions: " As this second Transfusion was larger, so were the effects of it quicker and more considerable. As soon as the blood began to enter into his veins, he felt the like heat along his Arm and under his Arm-pits which he had felt before. His pulse rose presently, and soon after we observed a plentiful sweat over all his face. His pulse varied extremely at this instant, and he complain'd of great pains in his Kidneys, and that he was not well in his stomack, and that he was ready to choak unless they gave him his liberty." The following morning: " He made a great glass full of Urine, of a colour as black, as if it had been mixed with the soot of chimneys."

Shortly after this, transfusion was placed under an interdict in France, and little more is heard until the nineteenth century. Then Dr. Blundell, of the United Hospitals of St. Thomas and Guy, began to experiment with the injection of human blood into dogs. He found that a dog which had been bled could be revived by a transfusion of dog's blood, but that the transfusion to a dog of as little as 4 oz. (114 ml.) of human blood could be fatal (Blundell, 1824). Blundell, now convinced that only human blood was suitable for transfusion to humans, began very cautiously experimenting in man, usually not daring to attempt transfusion until his patient was beyond hope. Finally, he recorded a successful case in which 8 oz. (227 ml.) had been transfused by means of a special syringe, " the impellor " (Blundell, 1828–9).

By 1873 about 240 transfusions of all kinds had been given in England, according to Jennings (1883); in most of these it is probable that relatively small volumes of blood had been injected from some form of syringe.

Aveling (1873) carried out some cross-transfusions, inserting a cannula into the arm vein of the donor, connecting this to a small length of rubber tubing with a bulb, and then to a cannula in the arm vein of the recipient. Aveling's very first case—according to him only the fifth cross-transfusion ever performed in England—was a great success, and is recorded in a

*The first of two Oliver-Sharpey Lectures given at the Royal College of Physicians of London on March 10 and 12, 1959.

well-known picture, showing the family coachman acting as donor to the lady of the house.

Despite Blundell's earlier work, Aveling now turned to the use of lambs as donors, and published a case in which he had cross-transfused a few ounces of lamb's blood (Aveling, 1874). The transfusion was not a success, but this did not stop Aveling from recommending that when human blood was unobtainable one should use lamb's blood without hesitation: "No person should now be allowed to perish from loss of blood without transfusion in some form or other being attempted."

Discovery of Serological Incompatibility

Meanwhile very important progress was being made in Germany. Landois (1875) discovered that when the red cells of one animal were mixed with the serum of another animal and incubated at 37° C. they might be lysed within two minutes. At about the same time Professor Ponfick, of Rostock, reported the results of cross-transfusion between animals of many species. In all, 16 combinations of donor and recipient were tried. He found that when donor and recipient belonged to the same species as much as 60 ml./kg. of blood could safely be given, but that when they were of different species the transfusion of as little as 4 ml./kg. might be fatal. He also found that animals transfused with the blood of another species developed haemoglobinuria, not haematuria as had previously been supposed. He further showed that if blood was haemolysed *in vitro* by freezing and thawing and then injected into an animal of the same species haemoglobinuria could also be produced. Moreover, the amount of lysed blood which would produce haemoglobinuria (about 1.2 ml./kg. of the recipient's body weight) was almost exactly the same as the amount of intact blood of a foreign species which would produce haemoglobinuria (Ponfick, 1875).

Thus, by 1875 it was clear that when the blood of one species was transfused to another the donor's cells were promptly lysed by the serum of the recipient. Despite this, some people continued to be enthusiastic advocates of lamb's blood, and in the textbook of medicine (Fagge and Pye-Smith, 1891) which my father used at Guy's at the turn of the century it was felt necessary to emphasize that in transfusion only human blood should be used.

Discovery of the ABO Blood-group System

It is interesting that Sharpey's pupil, E. A. Schäfer, came somewhere near discovering the existence of serological incompatibility between human bloods, for in 1880 he published a paper in which he described the lysis of human red cells when mixed with animal serum. Had he carried out tests between a few samples of human red cells and a few samples of human serum he would certainly have found agglutination in some of the mixtures.

This discovery, however, remained for Landsteiner to make. In the account which he published in 1901 he made it clear that he fully understood the significance of his discovery and its relevance to blood transfusion.

Landsteiner occupies a unique place in the field of blood groups: for the 40 years after his discovery of the ABO system he continued, with various colleagues, to make most of the important discoveries in the field. At the same time, of course, he made other contributions to immunology of the greatest importance. After qualifying as a doctor in 1891 he had had five years' training as a chemist, and thus equipped himself for the contributions he was to make on the nature of serological specificity.

After the publication of Landsteiner's discovery of the blood groups many years elapsed before the idea of matching blood before transfusion was accepted. In 1909 Crile, in his book on transfusion, wrote, "In the author's opinion agglutination may be disregarded." Ottenberg and Kaliski (1913) were among the first to advocate cross-matching, but even they were inclined to underestimate the significance of agglutination *in vitro*, as opposed to haemolysis.

One may well wonder why there were not more accidents. One reason is that if transfusions are given between humans without any preliminary tests whatever only one out of three will be incompatible (see Table I). Moreover, even when blood of the wrong ABO group is transfused the outcome will seldom be fatal, and may even appear to be favourable. For example, in one case reported elsewhere a patient of group A was admitted to hospital suffering from melaena; her haemoglobin concentration was 5 g./100 ml. and her pulse was thready. Immediate transfusion seemed desirable, and the blood of two donors was cross-matched by a house-physician. The donor blood was of group B, but the patient rapidly improved during the transfusion. Subsequent tests showed that the anti-B in the recipient's serum was completely absorbed by the transfusion (of 1,000 ml. of group B blood), and that the bulk of the injected cells survived normally for five days after transfusion. There was then an immune reaction, and the transfused cells were eliminated. However, this produced only a mild jaundice, and a fall in haemoglobin concentration which the recipient was now well able to tolerate (Mollison, 1956, p. 345).

If all the other blood-group systems were ignored in transfusion their antibodies would cause trouble only very seldom. Some examples of the approximate risks involved are given in Table II. Thus the risk of incompatibility from anti-Le^a if no tests were made would be 1 in 1,000, and the risk from anti-Rh would be similar. Probably not more than 1 in 5 or 1 in 10 of such incompatible transfusions would cause a serious haemolytic reaction. Some antibodies, such as anti-Wr^a, occur relatively commonly in human sera, but the

TABLE I.—*Chance of Giving Incompatible Blood if ABO Groups of Donor and Recipient are Ignored in Transfusion*

Recipient's Blood Group		Blood Groups of Incompatible Donors		Chance of Incompatible Transfusion
	(i) Frequency		(ii) Combined Frequencies of Donors	(i) × (ii)
O	0·47	A, B, AB	0·53	0·25
A	0·42	B, AB	0·11	0·05
B	0·08	A, AB	0·45	0·04
AB	0·03	None	0	0
				0·34

TABLE II.—*Risk of Incompatibility from Some Antigens Outside the ABO System*

	Frequency of:		Risk
	(i) Antigen	(ii) Corresponding Antibody	(i) × (ii)
Le^a	0·2	0·005	0·001
Rh (D)	0·8	0·001	<0·001
Wr^a	0·002	0·01	0·00002

corresponding antigen is very infrequent, so that the risk of an incompatible transfusion is minute.

Discovery of Other Blood-group Systems

The way in which the various blood-group systems were discovered is summarized in Table III. After the discovery of the ABO system in 1901 no new blood-group systems were found for 25 years. Landsteiner

TABLE III.—*Subjects in Which the Antibodies of the Eleven Main Blood-group Systems Were First Found*

Blood-group Systems	Antibodies First Found in
ABO, Le	Normal subjects
MN, P	Rabbits injected with human red cells
Rh	Rabbits injected with rhesus monkey red cells
Rh, K, Jk, Di	Mothers of infants with haemolytic disease
Lu, Fy, Js	Transfused patients

examined human sera for other antibodies, but could find only weak agglutinins, active at low temperatures. It occurred to Landsteiner and Levine that they might be able to reveal other antigens in human red cells by making immune antibodies against them in rabbits, and accordingly they carried out experiments in which several different samples of human red cells were injected into rabbits. Antibodies identifying these new human antigens were obtained (Landsteiner and Levine, 1928), and to the first of these the letter M was given, to show that the antigen had been identified with immune serum—the letter I was avoided because of possible confusion with the numeral 1 (Levine, 1944).

Landsteiner and Wiener (1941) adopted a slightly different approach when they injected the red cells of rhesus monkeys into rabbits. They hoped that in this way they might reveal the existence of an antigen present both in human cells and in rhesus cells, and they were successful. At about the same time Levine and his colleagues were making their highly important discoveries relating to iso-immunization in pregnancy. Levine and Stetson (1939) had found an antibody in the serum of a recently delivered woman whose foetus had died *in utero*. They discussed the possibility that the mother had been immunized by cells of her foetus which carried an antigen inherited from the father that was foreign to the mother. This was the beginning of the fascinating work which led to the discovery that iso-immunization in pregnancy is the usual cause of haemolytic disease of the newborn (Levine *et al.*, 1941).

"Incomplete" Blood-group Antibodies

Anti-Rh differs from anti-A and anti-B in being more difficult to detect *in vitro*. In many cases a serum containing Rh antibody fails to agglutinate Rh-positive red cells suspended in saline. Race (1944) and Wiener (1944) independently discovered one method of demonstrating these "incomplete" types of antibody, and in the ensuing few years many other methods were described. Certainly the most important of all these methods was the antiglobulin test (Coombs, Mourant, and Race, 1945), which is discussed in some detail later. By means of the indirect antiglobulin test several more blood-group systems were discovered—for example, K, Jk, and Fy. In some cases the antibodies were first found in the serum of a mother whose infant had had haemolytic disease of the newborn, or were found attached to the red cells of the infant; in other cases they were found in the serum of a patient who had received transfusions and so been immunized.

Complement-binding Blood-group Antibodies

Coombs and Mourant (1947) showed that the reaction between an anti-human-globulin serum and red cells sensitized with anti-Rh could be inhibited by adding human gamma-globulin to the antiglobulin serum, and they concluded that incomplete anti-Rh must consist predominantly of gamma-globulin. For several years it was thought that anti-gamma-globulin was the only important constituent in antiglobulin serum. Then Dacie (1951) found that an antiglobulin serum to which gamma-globulin had been added would still react with the red cells of patients with the "cold antibody" type of haemolytic anaemia, and others found that anti-globulin sera which had been absorbed with red cells sensitized with one kind of antibody might still react with red cells sensitized with other kinds of antibody (Crawford and Mollison, 1951; Renton, 1952).

It was found that an antiglobulin serum completely lacking anti-gamma-globulin might react with red cells sensitized with certain antibodies (for example, anti-Le^a), and it was concluded that some antibodies consist of "non-gamma"-globulin—that is, beta- or alpha-globulin (Cutbush, Crawford, and Mollison, 1955). Some antibodies of this kind were found to be detectable in the antiglobulin test only when fresh serum was used to sensitize the cells (Mollison and Cutbush, 1955). It was then demonstrated that in certain cases the antiglobulin serum was reacting with complement adsorbed on to the sensitized cells rather than with the antibody itself (Dacie, Crookston, and Christenson, 1957). It was now realized that, at least in most cases, the antibody itself was a gamma-globulin and the "non-gamma"-globulin was complement.

The binding of complement to red cells sensitized with antibody can be demonstrated in other ways—namely, either by showing that under suitable conditions haemolysis occurs or by the use of a serum containing conglutinin (Streng, 1930).

A few human blood-group antibodies will haemolyse untreated red cells in the presence of human complement—namely, many examples of anti-A and anti-B, anti-Le^a, and perhaps all examples of anti-P + P_1 (anti-Tj^a). Other antibodies will lyse enzyme-treated cells: probably all examples of anti-A, anti-B, and anti-Le^a, many examples of anti-Le^b and anti-Jk^a, and some examples of anti-P_1. If rabbit serum rather than human serum is used as a source of complement all these antibodies and several others (some examples of anti-S, anti-K, and anti-Fy^a) will haemolyse untreated red cells (Mollison and Thomas, 1959).

The binding of human complement (or animal complement) to red cells sensitized with all the complement-binding antibodies referred to in the preceding paragraph can also be demonstrated by testing the cells with a rabbit serum containing immuno-conglutinin (Mollison and Polley, 1959).

Some of these reactions are summarized in Table IV. Red cells sensitized with anti-Le^a in the presence of complement are not agglutinated by an anti-gamma-globulin serum, but they are agglutinated by an anti-"non-gamma"-globulin serum. Similarly, they are agglutinated by a serum containing immuno-conglutinin. Sera containing anti-Le^a and complement will often lyse untreated red cells. More rapid and complete lysis is observed if rabbit complement is added or if the cells are first treated with an enzyme.

TABLE IV.—*Tests for Complement-binding by Human Blood-group Antibodies*

Red Cells Incubated with Antibody*	Agglutination by Anti-human Globulin Sera		Agglutination by Rabbit Immuno-con-glutinin Serum	Haemolysis with	
	Anti-gamma-globulin	Anti-"non-gamma"-globulin		Human Complement	Rabbit Complement
Anti-Le^a ..	−	++	++	±	++
Anti-Fy^a (some†)	+	++	++	−	++
Anti-Rh ..	+++	−	−	−	−

* And human complement.
† Only some examples of anti-Fy^a bind complement.

Red cells sensitized with some examples of the anti-Fy^a behave similarly, the only differences being that some reaction is obtained between sensitized red cells and an anti-gamma-globulin serum and that no reaction is obtained with enzyme-treated cells (since the Fy^a antigen is destroyed by the enzyme).

Red cells sensitized with incomplete anti-Rh are strongly agglutinated by anti-gamma-globulin, but they give none of the reactions which would be expected if complement were bound.

This difference between the behaviour of antibodies *in vitro*—some binding complement and some failing to bind complement—is reflected in differences in the way in which the same antibodies bring about the destruction of red cells *in vivo*, as is discussed later.

Survival of Transfused Red Cells

One criterion of a successful red-cell transfusion is that the survival of the red cells should be maximal—that is, not curtailed by serological incompatibility. The first quantitative estimates of red-cell survival following transfusion were made by Todd and White (1911) in cattle. By absorption, they prepared a serum which would lyse the red cells of one animal but not those of another. After cross-transfusion between the two animals they analysed samples of blood and determined the percentage of transfused red cells at different intervals after transfusion. They found that the number of surviving cells fell progressively, and reached zero after four or five days. On the fifth day a haemolysin active against the donor's red cells was first detected, and this increased in titre to reach a peak about ten days after transfusion. Todd and White concluded: "The implications for human blood transfusion are obvious," but fortunately this interpretation was wrong.

In 1919, Winifred Ashby, working at the Mayo Clinic, applied differential agglutination (rather than differential lysis) to the investigation of red-cell survival in man. After transfusing group O blood to group A recipients she took samples and incubated them with anti-A. It was thus possible to make estimates of the survival of the group O (unagglutinated) cells.

For 20 years or so little use was made of Ashby's method, perhaps mainly because, owing to incomplete agglutination of A cells, it was difficult to obtain quantitative estimates. At the beginning of the second world war the method was used to estimate the survival of red cells after storage, and this called attention to its value. Then followed the era of application of the method to the investigation of haemolytic anaemias, which yielded a spate of information and altered ideas of the classification of this large group of diseases.

Application of the method of differential agglutination to the investigation of blood-group incompatibility began with the studies of Wiener (1941). These studies were essentially qualitative. Thus in most cases Wiener made use of differences between donor and recipient with regard to MN type and simply tested for the presence or absence of cells of the donor's type. Others made attempts to obtain quantitative information, but this could be done only when the antibody was very weak, since relatively large volumes of incompatible red cells had to be detected to obtain useful results. Even when 50 ml. of blood was transfused to an adult, only very crude quantitative data could be obtained (see Mollison, 1951, p. 301).

The availability of radioactive isotopes in the past decade has altered the position. Not only can relatively minute volumes of red cells (less than 1 ml.) be labelled, but results of great precision can be obtained. Two convenient labels are available: ^{51}Cr and ^{32}P. ^{51}Cr is the more useful, since the rate of "elution" from labelled red cells in the circulation is relatively slow. Thus, about 3% leaks out in the first three to five hours and 5–6% in the first 24 hours; thereafter the rate of loss is much slower. By contrast about 10% of ^{32}P leaves the red cells in the first hour after injection, and more than 50% in the first 24 hours. ^{32}P can thus be used as a label for incompatible cells only when the investigation is to be limited to a period of about 60 minutes after injection. However, ^{32}P is very suitable as a label for compatible cells to be injected as a control; the cells are best obtained from the subject in whom the test is to be made. Thus, in a typical test, 2–3 ml. of the subject's own red cells are labelled with ^{32}P (as phosphate), and 0.1–1 ml. of the donor's red cells are labelled with ^{51}Cr (as chromate). After labelling, the red cells are mixed and injected as a single suspension.

Validity of Results Obtained with Very Small Transfusions

The advantage of using 1 ml. or less of red cells for a test of compatibility is mainly that the development of symptoms of any kind is unlikely. The potential disadvantage is that the pattern of destruction of 1 ml. of red cells may differ considerably from that of 500 ml. or 1,000 ml.

The destruction of a relatively large volume of red cells may be limited in two ways. Firstly, if a sufficient volume of blood is transfused, all the circulating antibody (and/or complement) may be adsorbed on to the transfused red cells, and red-cell destruction may then be virtually arrested until the recipient produces more antibody. An example of this is given in Fig. 1.

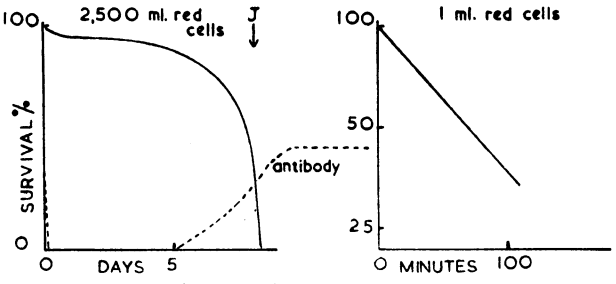

FIG. 1.—Contrast between the survival of a very large volume and a very small volume of incompatible red cells. After the transfusion of 2,500 ml. of red cells, all antibody (anti-Fy^a) disappeared from the circulation for about five days, and during this period there was no destruction of incompatible red cells. Then antibody reappeared, the incompatible red cells were rapidly destroyed, and the patient became jaundiced (J). When a test was made later with only 1 ml. of red cells the cells were cleared from the circulation with a half-time of about 70 minutes.

(The limitation of red-cell destruction by exhaustion of antibody was demonstrated in dogs by Christian *et al.* (1951).)

In man the transfusion of 500–1,000 ml. of incompatible blood will absorb all circulating antibody only when the antibody is very weak. For example, in one case in which the patient's serum contained Rh antibody with a titre of only 2, and 500 ml. of Rh-positive blood was transfused, antibody could not be demonstrated in the serum during the following 48 hours (Mollison and Cutbush, 1955, Case 7). In a patient whose serum contained an anti-B agglutinin with a titre of 32 the transfusion of 1,000 ml. of blood led to the disappearance of antibody from the circulation for about five days. Probably, incomplete antibodies are less easily absorbed than agglutinins. Thus, in a patient whose serum contained anti-c with a titre of 8, the transfusion of 500 ml. of incompatible blood failed to remove all free antibody, and the incompatible red cells underwent steady destruction after transfusion (see Fig. 2).

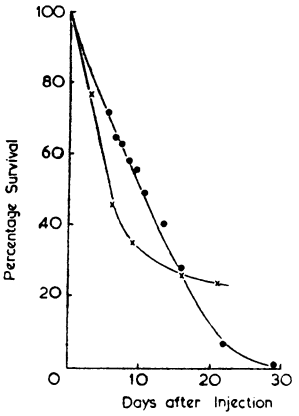

FIG. 2.—Contrast between the survival of a moderately large volume and a very small volume of incompatible red cells. After the transfusion of approximately 200 ml. of red cells the titre of antibody (anti-c) fell slightly; red-cell destruction, though slow, was continuous (●——●). Presumably the rate of red-cell removal was not limited by the capacity of the reticulo-endothelial system in this case. Five weeks later, when 1 ml. of red cells was injected, the rate of destruction was only slightly more rapid (×——×). (Redrawn from the data of Mollison and Cutbush, 1955.)

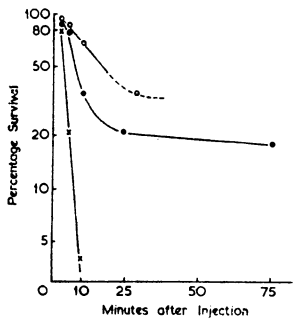

FIG. 3.—Survival of Le(a+) red cells from a particular donor (H. L.) after injection into three recipients whose serum contained anti-Le^a; in each case the titre of the serum was estimated by the indirect antiglobulin technique. ×——×=titre 32; ●——●=titre 8; O——O=titre 1. (From Mollison and Cutbush, 1955.)

When the patient's serum contains a potent antibody the transfusion of even 2,000 ml. of blood may fail to absorb all circulating antibody (see a case described by Mollison, 1956, as Case A in Fig. 2 on page 347, in which 2,000 ml. of Rh-positive blood was transfused to a patient whose serum contained potent incomplete Rh antibody).

The rate of destruction of amounts of incompatible blood which are too small to absorb all circulating antibody, or even to reduce its titre, may be limited by the capacity of the reticulo-endothelial system. Thus, according to Dr. C. A. Finch (personal communication) the reticulo-endothelial system can remove from the circulation only about 30 ml. of red cells per hour. Therefore, for example, after the transfusion of 2,000 ml. of Rh-positive blood (equal very approximately to 1,000 ml. of red cells) the elimination of red cells from the circulation would be expected to take more than 24 hours; this is in fact what has been observed (see Case A referred to in the preceding paragraph).

When the antibody concerned is capable only of slow red-cell destruction, so that the capacity of the reticulo-endothelial system to take up cells is not exceeded, small amounts of red cells may not be removed any more rapidly than large amounts. Fig. 2 shows a case in which a patient whose serum contained anti-c (titre 8) received a transfusion of 500 ml. of c-positive blood. After the red cells had been eliminated (at a very slow but steady rate) an injection of 1 ml. of c-positive red cells was given. The red cells were eliminated only slightly more rapidly than on the first occasion. This suggests that the slower elimination of the larger amount was due solely to the slight lowering of the titre of anti-c, and was not due to overloading of the capacity of the R.-E. system.

It might be thought that when only 1 ml. of incompatible cells was injected even a very weak antibody would be able to bring about their immediate destruction; but this is not the case. Thus, Fig. 3 shows the survival of Le(a+) cells in three recipients whose serum contained varying amounts of anti-Le^a. In the first, who was a patient who had developed haemoglobinuria after the transfusion of a bottle of Le(a+) blood and whose serum had an indirect antiglobulin titre of about 32, practically all the injected red cells were removed with a half-time of about two minutes.

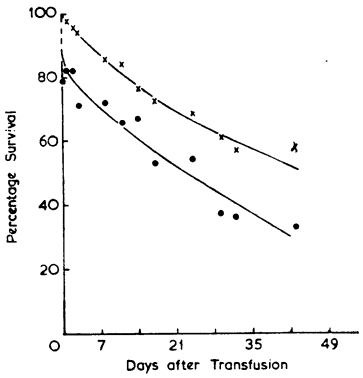

FIG. 4.—Survival of A$_2$, Le(a−b−) red cells (×——×), estimated by ^{51}Cr-labelling and corrected for Cr elution, and of O, Le(a−b+) red cells (●——●) estimated by differential agglutination, in the circulation of a patient whose serum contained anti-Le^a. (From Cutbush *et al.*, 1956.)

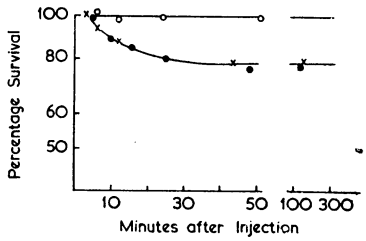

FIG. 5.—Survival of 1 ml. of O, Le(a−b+) red cells on two separate occasions (●——● and ×——×) after injection into the patient referred to in Fig. 4. The donor was one of two who gave blood for the transfusion illustrated in Fig. 4. O——O= Survival of red cells from a group A$_1$, Le(a−b+) donor. (From Cutbush *et al.*, 1956.)

In the second recipient, who was a patient who had developed jaundice after a bottle of Le(a+) blood and whose serum had an indirect antiglobulin titre of about 8, approximately 80% of the cells were removed with a half-time of three minutes. In the third, who was a normal subject whose serum reacted only very weakly at 37° C. (titre 1), 65% of the red cells were removed with a half-time of six minutes and the remainder were removed very much more slowly.

A further example of the similarity of results observed with small and large volumes of incompatible red cells is provided by Figs. 4 and 5. A patient whose serum contained anti-Le^a was transfused with 500 ml. of group O, Le (a−b+) blood (250 ml. from each of two donors). The number of red cells transfused was very carefully

estimated, as was the concentration of transfused cells in the recipient's circulation after transfusion. The red cells were transfused in 80 minutes; a sample was taken a few minutes later. This sample contained only 80% of the expected number of transfused cells, as Fig. 4 shows. Later, several tests were made using only 1 ml. of group O, Le(a – b +) red cells, including red cells from one of the two donors previously used. As Fig. 5 shows, 20% of the injected red cells were removed during the 20 minutes following injection, the remaining cells surviving for a much longer period.

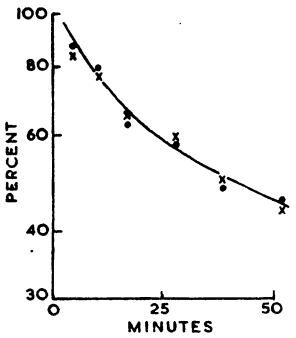

FIG. 6.—Estimates of haemoglobin (×) and ^{51}Cr (●) in the plasma after the injection of ^{51}Cr-labelled haemoglobin into a rabbit. The estimates are shown as percentages of values predicted from the amount of haemoglobin injected and the known plasma volume.

It may be concluded that a test using 1 ml. of incompatible red cells gives reliable information about the proportion of red cells which will be destroyed, although the rate of destruction of the large volume will usually be slower.

^{51}Cr as a Label for Haemoglobin.—When ^{51}Cr-labelled sodium chromate ($Na_2{}^{51}CrO_4$) is added to red cells, chromate rapidly passes into the red cells and is then apparently reduced to the trivalent cationic form and labels haemoglobin (Gray and Sterling, 1950). If the red cells are now haemolysed, and the haemoglobin is separated from the stroma, virtually all the radioactivity is found to be associated with the haemoglobin. If this haemoglobin is injected into the circulation, the ratio of haemoglobin to radioactivity remains constant for a period of approximately one hour after injection (see Fig. 6). Thereafter haemoglobin is cleared from the circulation more rapidly than ^{51}Cr, evidently because ^{51}Cr exchanges between haemoglobin and plasma proteins (Jandl *et al.*, 1957). Thus over a period not exceeding one hour ^{51}Cr is a useful label for haemoglobin in the plasma.

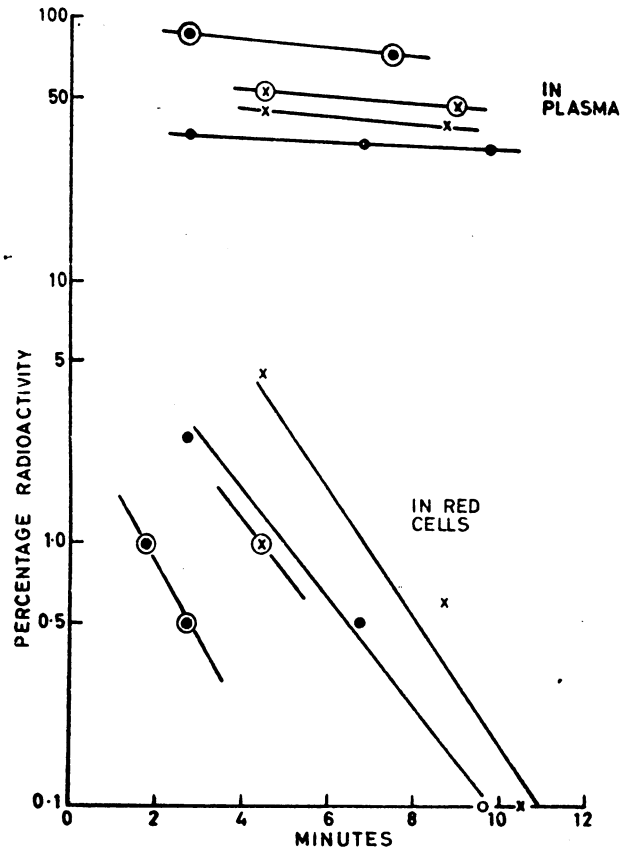

FIG. 7.—Amount of radioactivity found in plasma and red cells after injecting approximately 0.5 ml. of ^{51}Cr-labelled red cells of incompatible ABO group into two human subjects (●——●=A_2 cells injected into a group O recipient; ⊙=B cells injected into a group A recipient) and after injecting human group O red cells into two rabbits (× and ⊗).

Intravascular Lysis

Fairley (1941) suggested that there were two major modes of red-cell destruction: intravascular and extravascular. Studies with ^{51}Cr confirm this, although they show that in cases in which lysis is predominantly intravascular some red cells are usually removed intact from the circulation and that in destruction which is predominantly extravascular some haemoglobin is liberated into the plasma. Intravascular haemolysis of incompatible red cells is seen characteristically when antibodies which are readily haemolytic *in vitro* are involved. Thus Fig. 7 shows two examples of the removal of human red cells from the circulation of humans by haemolytic anti-A and anti-B, and also shows two examples of the removal of human red cells from the circulation of a rabbit by haemolytic anti-human red-cell antibody. In each of the four cases more than 90% of the cells were removed from the circulation within five minutes of injection and 40–90% of the injected radioactivity was found in the plasma.

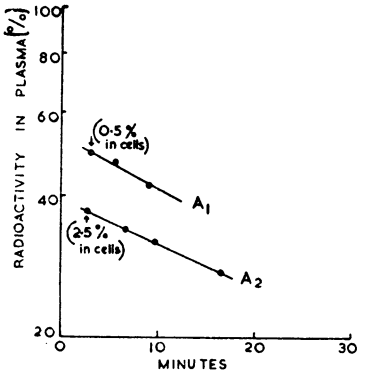

FIG. 8.—Amount of radioactivity found in plasma and red cells after injecting approximately 0.5 ml. of cells of subgroups A_1 and A_2 respectively into a group O recipient.

In this type of destruction the weaker the haemolysis *in vitro* the smaller is the percentage of cells destroyed intravascularly. Fig. 8 shows the results in a group O subject infected with A_1 and A_2 cells on different occasions. *In vitro*, the A_2 cells were lysed to a lesser extent than the A_1 cells, and *in vivo* the proportion of haemoglobin appearing in the plasma after the injection of A_2 cells was less—that is, more cells were removed intact from the circulation.

Extravascular Destruction

Practically all human blood-group antibodies other than some examples of anti-A and anti-B are not readily lytic *in vitro*, and they bring about predominantly extravascular destruction. Fig. 9 shows, for example, the rate of clearance of Rh-positive red cells sensitized with Rh antibody. The cells are cleared from the circulation with a half-time of approximately 20 minutes, indicating removal predominantly in the spleen (Mollison and Cutbush, 1955; Hughes Jones *et al.*, 1957), and the maximal level of radioactivity in the

plasma (indicating approximately the level of free haemoglobin in the plasma: see above) reached about 3% of the total amount of haemoglobin injected in the form of intact red cells (Fig. 9).

Apparently the proportion of haemoglobin liberated into the plasma depends upon the amount of red cells injected. Thus, Jandl *et al.* (1957) found that when

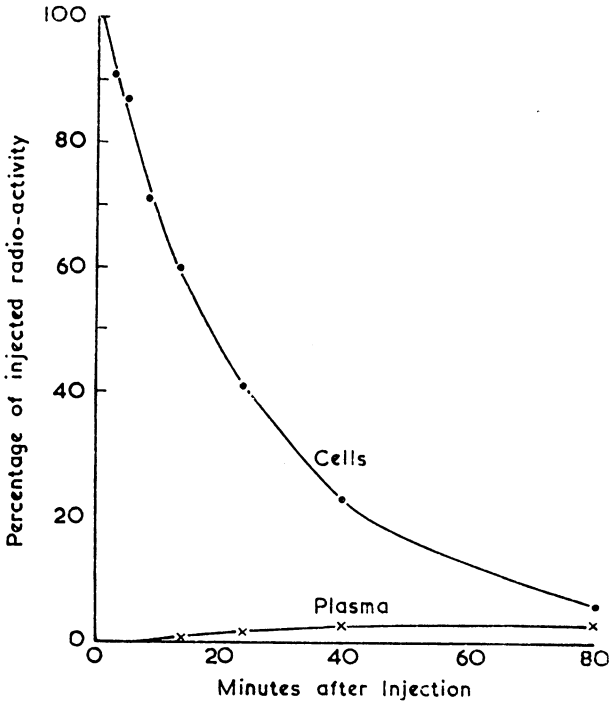

FIG. 9.—An example of predominantly extravascular destruction. 0.4 ml. of Rh-positive red cells was labelled with ^{51}Cr, then sensitized *in vitro* with Rh antibody and reinjected into the donor. The red cells were removed from the circulation with a half-time of approximately 20 minutes; the amount of radioactivity in the plasma never exceeded 3% of the total amount injected in the form of labelled red cells.

amounts of Rh-positive red cells equivalent to 3 g. of haemoglobin were injected into subjects whose serum contained anti-Rh the maximal level of plasma haemoglobin was usually equivalent to about 5% of the total dose injected, but when the amount of red cells was equivalent to about 7 g.—that is, about 50 ml. of blood—the maximal plasma haemoglobin level was equivalent to about 10% of the injected dose. Presumably when larger doses, of the order of 500 ml. of incompatible blood, are transfused an even higher proportion of the haemoglobin from destroyed red cells is likely to spill into the circulation, since haemoglobinuria does occasionally occur after haemolytic transfusion reactions due to anti-Rh (Vogel *et al.*, 1943).

When destruction is predominantly extravascular, the localization of the ^{51}Cr-labelled cells can be determined by surface counting—that is, counting over the surface of the body with a scintillation counter.

Surface Counting.—The gamma rays emitted by ^{51}Cr are very readily absorbed by tissues, and thus, owing to differences in bodily configuration, it is not possible to make precise quantitative estimates of the distribution of ^{51}Cr in different organs. However, it is possible to make rough quantitative estimates, and it is often possible to conclude that the greater part of the activity is localized in some particular organ—for example, the spleen. Interpretation of the findings is made easier by calibrating the particular scintillation counter used with models of the liver and spleen containing known amounts of radioactive chromium. Thus, Mollison and Hughes Jones (1958) made models out of agar, incorporating known amounts of ^{51}Cr, placed the models in a dead body, and then carried out surface counting. They found that when the liver and spleen were of a normal size a given amount of radioactivity in the spleen gave a counting rate which was three times higher than that for the same amount of radioactivity in the liver. By counting different models in different bodies they arrived at average figures expressed as counts per second per microcurie for ^{51}Cr in the normal liver and normal spleen, and they used these figures to make estimates of the amount of red cells trapped in the liver and spleen after injecting incompatible red cells. These estimates are referred to below.

Summary of the Capabilities of ^{51}Cr

Radioactive chromium is thus an extremely useful label for incompatible red cells. Volumes of less than 1 ml. can be easily and rapidly labelled *in vitro*. If the red cells are destroyed intravascularly the amount of haemoglobin set free in the plasma can be estimated. If they are destroyed extravascularly their main sites of destruction can be identified, and an approximate estimate made of the proportion of red cells destroyed there. Finally, ^{51}Cr can easily be counted in the presence of ^{32}P, which can be used to label a control sample of compatible red cells. This makes it possible to detect the destruction of even 5% of incompatible red cells.

With such a test system the effects of different antibodies on the destruction of injected red cells can be accurately determined.

[*The second lecture, with a list of references, will appear in our next issue.*]

MAXIMUM FORCED EXPIRATORY FLOW RATE AS A MEASURE OF VENTILATORY CAPACITY

WITH A DESCRIPTION OF A NEW PORTABLE INSTRUMENT FOR MEASURING IT

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The use of a single forced expiration as a method of assessing ventilatory capacity is becoming increasingly popular, mainly because, as pointed out by Kennedy (1953), it is a much simpler and less tiring procedure than the maximum voluntary ventilation (M.V.V.).

The basis of most of the various single-breath methods is the same: the volume of air expired is measured against time by means of a spirometer with either a recording drum or a timing device. There are some differences of opinion about the most suitable interval of time over which to measure the volume and about the relative merits of a recording drum or a timing device, but it is generally agreed that methods of this kind are clinically valuable and give results which are comparable