BLOOD-GROUP ANTIBODIES AND RED-CELL DESTRUCTION*

BY

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In devising compatibility tests to precede transfusion, and in interpreting them, we need to know the precise relationships between the interactions of red cells and serum *in vitro* and the survival or destruction of the same red cells *in vivo*. An inquiry into this problem can be divided into the following questions:

1. Does any interaction between red cells and serum at 37° C. *in vitro*, however trivial, imply some interference with survival *in vivo*? If so, is the same true for 30° C. *in vitro*, and if not, what is the highest temperature of interaction *in vitro* associated with no effect *in vivo*?

2. Are the characteristics of blood-group antibodies *in vitro* exactly related to their effects *in vivo*? For example, if an antibody binds complement *in vitro* does it bring about the removal of red cells in a different way from that of an antibody which does not bind complement?

3. Is there a close relationship between the potency of an antibody, judged by tests *in vitro*, and the speed and extent of the red-cell destruction which it causes *in vivo*?

4. Do antibodies display precisely the same specificity *in vitro* and *in vivo*? This problem arises particularly in the selection of blood for transfusion to patients with acquired haemolytic anaemia.

5. Is it possible for red cells to be compatible with the recipient's serum *in vitro* and yet to be treated as incompatible after transfusion?

An attempt will be made to answer these questions in turn.

Antibodies Weakly Active in Vitro at 37° C.

It seems to be the rule that any degree of agglutination of a donor's red cells by the recipient's serum at 37° C. *in vitro*, due to a blood-group antibody, implies that there will be at least partial destruction of the red cells if they are transfused. For example, in a subject whose serum contained anti-Mi^a with a titre of 2 at 37° C. almost 100% of Mi(a+) cells were removed with a half-time of 20 minutes (Case 13 of Cutbush and Mollison, 1958). In another case, in which the recipient's serum contained anti-Lu^b giving only very weak agglutination of Lu^aLu^b cells at 37° C. (titre 1 in 1), about 20% of the cells were removed from the circulation with a half-time of about 20 minutes, the rest being removed only over a period of many weeks (Case 12 of Cutbush and Mollison, 1958).

Chaplin (1959) has reported that in some cases of hypogammaglobulinaemia in which the patient's serum agglutinated A or B cells only when undiluted, and then only weakly, A or B cells were sometimes removed with a half-time of a few minutes.

Antibodies which, at 37° C., agglutinate very weakly or not at all, but sensitize red cells to an antiglobulin serum, usually bring about red-cell destruction, even when their titre is very low. There seems to be a large difference between antibodies which bind complement and antibodies which do not. Thus, incomplete antibodies which do not bind complement, such as anti-c

or anti-E, when they are very weak (indirect antiglobulin titre about 1 in 4) bring about only very slow red-cell destruction, with a half-time of the order of four or five days (Case 8 of Mollison and Cutbush, 1955; Case 15 of Cutbush and Mollison, 1958). On the other hand, incomplete antibodies which bind complement and have a titre at 37° C. of only 1 in 4 may bring about removal of a proportion of injected red cells with a half-time of a few minutes. One such case, due to anti-Le^b, has been described elsewhere (Mollison, 1959b); Fig. 10 shows another case, in which anti-O was concerned. Since red-cell destruction due to anti-O has probably not been described before, a few details are given.

FIG. 10.—Survival of group O and subgroup A₂ red cells in a subject (subgroup A₁) whose serum contained anti-O. *In vitro*, at 25° C., the serum agglutinated O cells and A₂ cells; at 37° C. the reaction with O cells was so weak as to be doubtful and A₂ cells were not agglutinated. At 37° C. the serum, provided that complement was present, sensitized O cells but not A₂ cells to an antiglobulin serum. Most of the group O red cells were rapidly removed from the circulation, and this component is plotted separately as a broken line. The A₂ cells underwent no significant destruction during the hour following injection.

The patient herself belonged to subgroup A₁. Although her saliva inhibited anti-H (extract of *Ulex europaeus*), her red cells were scarcely agglutinated at all by the same extract; normal A₁ cells were agglutinated by the extract. At 25° C. the patient's serum agglutinated group O and A₂ cells but not A₁ cells. The reactions were not inhibited by H substance (secretor saliva). At 34° C. the serum very weakly agglutinated O cells; at 37° C. the reactions were so weak as to be doubtful; the reactions were not enhanced by suspending the cells in serum or albumin; A₂ cells were not agglutinated. At 37° C. the serum sensitized O cells to an antiglobulin serum, but only when the red cells were incubated with complement as well as with antibody. The reaction with A₂ cells was barely detectable.

As Fig. 10 shows, when a small sample of group O cells was injected, 80% of the cells were removed with a half-time of 3.5 minutes; there was no significant destruction of A₂ cells during the 60 minutes for which their survival was followed.

Antibodies Active in Vitro only at Temperatures below 37° C.

It will be generally agreed that an antibody which is inactive above 25° C. *in vitro* is without effect *in vivo*; some examples of tests in such cases have been published

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(Mollison and Cutbush, 1955; Cutbush and Mollison, 1958). What is difficult is to find cases in which the antibody is inactive at 37° C. *in vitro* but is active at some slightly lower temperature, such as 33° C., so as to discover as precisely as possible the relationship between thermal range of activity *in vitro* and activity *in vivo*. However, one such subject was studied by Cutbush and Mollison (1958). This patient's serum contained an anti-M which agglutinated MM cells at 34° C. but not at 37° C., and agglutinated MN cells at 31° C. but not at 34° C. At 37° C. no reaction of any kind could be detected between MM cells and the patient's serum, either in albumin or using the indirect antiglobulin test. When MM cells were injected, about 60% of the cells were removed with a half-time of a few minutes; when MN cells were injected there was no destruction whatever during the following hour, but the red cells were removed at a definitely accelerated rate (half-time of several days).

This case suggests the following conclusions: first, a cold antibody whose thermal range *in vitro* extends almost but not actually to 37° C. may bring about very rapid destruction of red cells *in vivo*. This evidently implies that, in cross-matching, an agglutination test performed strictly at 37° C. may not be so reliable as a test performed at a slightly lower temperature. Second, a cold antibody whose thermal range *in vitro* extends to 31° C. or thereabouts but not higher is probably incapable of bringing about rapid destruction of red cells—that is, with a half-time measured in minutes—but may be capable of bringing about the slow destruction of transfused red cells. This latter conclusion agrees well with the observation that in acquired haemolytic anaemia of the cold antibody type the thermal range of the antibody *in vivo* may extend only to 30° C. or 31° C. (Dacie, 1957).

Relationship between Characteristics of Antibodies *in Vitro* and their Effects *in Vivo*

On the basis of their reactions *in vitro* antibodies may be divided into the following classes: (a) agglutinating and readily haemolytic *in vitro*; (b) agglutinating (but not haemolytic); (c) incomplete, binding complement; and (d) incomplete, not binding complement. The effects which these antibodies produce *in vivo* may be summarized thus:

(a) Antibodies which are readily haemolytic *in vitro* bring about intravascular lysis of the majority of injected red cells. Examples have already been given (see Fig. 7 in Lecture I).

(b) Antibodies which agglutinate at 37° C. but do not bind complement—that is, are non-haemolytic *in vitro*—except when they have a very low titre, bring about removal of red cells in the liver (see case of anti-c with a titre of 128 in saline described by Cutbush and Mollison, 1958), and when they are weak bring about the removal of red cells in the spleen (see case of anti-Mi^a with a titre of 1 in 2 in saline described by Cutbush and Mollison, 1958).

(c) Incomplete antibodies which bind complement bring about removal of most or some of the injected red cells from the circulation with a half-time of between two and six minutes (see, for example, Fig. 11 and various cases described by Cutbush and Mollison, 1958). It can be shown by surface counting that most of the red cells are removed from the circulation by the liver.

The pattern of destruction produced by this type of antibody can also be studied by taking red cells from a normal subject, incubating them with serum containing antibody, and then reinjecting them into the donor's circulation and studying their rate of removal from the circulation (using ⁵¹Cr as a red-cell label).

Fig. 11 shows the results of two experiments in the same subject, using different amounts of incomplete, complement-binding antibody. As the figure shows, with a larger dose of antibody about 85% of the cells were rapidly removed and with a smaller dose about 30%. These components have been plotted separately in Fig. 12. This shows that the smaller dose of antibody not only caused the destruction of a smaller proportion of cells but also caused a slower rate of destruction.

Fig. 12 suggests that removal of red cells from the circulation did not begin for more than 60 seconds after the injection. This apparent lag is in all probability due simply to the method of injecting the cells and of obtaining blood samples; thus, the red cells were injected into a vein in one arm and samples were withdrawn from a superficial vein in the other arm. The rate of mixing observed under these circumstances can be studied by injecting compatible red cells (see Fig. 13), and is found to be distinctly slower than is observed when samples are obtained from the arterial side of the circulation.

(d) Incomplete antibodies which do not bind complement bring about removal of red cells predominantly in the spleen. When the antibody is sufficiently potent (titre about 64 or more), blood passing through the spleen may be completely cleared of red cells, so that the rate of removal of cells from the blood stream is equal to the proportion of circulating blood passing through the spleen in a given time. Even when

FIG. 11.—Rate of disappearance from the circulation of a subject's own (Fy(a+)) red cells sensitized *in vitro* with a complement-binding anti-Fy^a. ●—●=One volume of red cells incubated with one volume anti-Fy^a. x—x=One volume of red cells incubated with 2.5 volumes anti-Fy^a. Both curves have two components.

FIG. 12.—“Rapid” component of the curves shown in Fig. 11. The arrows indicate the time after injection at which removal of red cells from the circulation appeared to begin.

the titre of antibody is as high as 2,000 there is no increase in the rate of removal of red cells (Mollison and Cutbush, 1955). On the other hand, when the titre is about 1 in 16 the half-time of clearance falls to about 60 to 70 minutes (Cutbush and Mollison, 1958), and when the titre is about 4 the half-time may be several days. Further studies on the quantitative aspects of this type of removal are described in the following section.

In the preceding paragraphs several references have been made to removal of red cells in the liver and spleen. In some of these cases actual surface-counting measurements were made, using apparatus calibrated

FIG. 13.—Concentration of injected red cells found in blood samples from a superficial vein in one arm at different times after the injection of compatible cells into the other arm. 100% = the concentration attained after complete mixing, based on samples taken 10–20 minutes after injection. Two different populations of red cells (● and ×) labelled with ^{51}Cr and ^{32}P respectively, were injected at an interval of about 30 seconds; in each case the times were expressed as seconds from the midpoint of injection to the midpoint of the sample.

predominantly in the spleen. Some examples are shown in Table V.

When red cells are fully saturated with complement-binding antibody the half-time of removal (when the volume of red cells injected is of the order of 0.7 ml.) is about 115 seconds (unpublished observations; see also Fig. 12). With knowledge of the total blood volume, the volume of blood cleared of incompatible cells per minute can be calculated. Assuming that incompatible red cells are being removed only by the liver and that the blood is completely cleared of incompatible cells by a single passage through the liver, the volume of blood flowing through the liver can be

TABLE V.—Examples of Rate of Removal and Main Sites of Destruction Associated with Complement-binding and Non-complement-binding Antibodies. In Each of These Examples Red Cells Were Sensitized with Antibody and then Reinjected Into the Circulation

Red Cells Sensitized with	Main Component of Curve		Percentage† in	
	Half-time (min.)	%	Liver	Spleen
Anti-Fya (complement-binding) . .	2.0	86*	69	13
Anti-Rh (non-complement-binding)	15.0	90†	30	78

* The remaining cells were removed much more slowly.

† 10% of the cells were removed with a half-time of approximately three minutes.

‡ Estimated by surface-counting.

calculated. In five cases in which calculations were made the average was 1,790 ml. a minute. This is higher than the figure usually accepted for the blood flow through the liver, and suggests that some reticulo-endothelial tissue outside the liver is also removing complement-coated red cells.

Relationship between Potency of Antibody in Vitro and Rate and Extent of Red-Cell Destruction which it Produces in Vivo

This question has been answered partially by examples already given. It can be explored further by incubating samples of red cells from normal subjects with varying amounts of anti-body and reinjecting them, and measuring their rate of removal from the circulation. This can be done conveniently with Rh antibody, and Fig. 14 shows the results of some tests.

A surprising feature of these results is that in some cases at least the rate of destruction does not diminish with time, as would be expected if most of the antibody were coming off the red cells and being distributed amongst the entire (Rh-positive) population. The shape of the curve suggests that, on the contrary, most of the antibody remains attached to the original small sample of red cells for a period of at least 60 minutes after reinjection. The transfer of antibody from sensitized to unsensitized red cells was studied in a series of subsidiary experiments.

FIG. 14.—Rate of removal from the circulation of Rh-positive red cells sensitized *in vitro* with Rh antibody. The red cells of different normal subjects were incubated with varying amounts of a single batch of gamma-globulin containing a potent incomplete antibody; the figure at the end of each curve refers to the ratio of gamma-globulin to red cells—for example, 0.09 means that 0.09 vol. of gamma-globulin was incubated with 1 vol. of red cells.

Experiments on Transfer of Rh Antibody from Sensitized and Unsensitized Red Cells

The plan of the experiment was to sensitize one volume of Rh-positive red cells (group O) with Rh antibody, then to mix these sensitized red cells with 40 volumes of unsensitized Rh-positive group A red cells. After incubating the mixture for one hour at 37° C. the O cells were separated out by agglutinating the group A red cells. It was found that incubation of the sensitized cells with unsensitized cells for one hour at 37° C. did not greatly diminish the degree of sensitization.

In a second experiment, group A, Rh-positive red cells were sensitized with Rh antibody and then, after thorough washing, mixed with an equal volume of group O, Rh-positive cells. After one hour's incubation at 37° C. the group O cells were extracted, and their degree of sensitization was determined by the direct antiglobulin test. As Table VI shows, when the A cells

TABLE VI.—Results of Incubating One Volume of Group A, Rh-positive Red Cells, Sensitized with Varying Amounts of Antibody, with an Equal Volume of Unsensitized Group O Rh-positive Red Cells, then Extracting the Group O Cells (Using Anti-A), and Testing them With an Antiglobulin Serum

Original Reaction of Group A Cells	Final Reaction of Group O Cells
+++	++
+++	+
++	-

were strongly sensitized, the O cells after being incubated with them acquired a positive direct antiglobulin test, but when the A cells were only moderately strongly sensitized the O cells did not acquire any detectable amount of antibody.

These experiments thus show that antibody tends to stay on the red cells with which it originally combines, and that it comes off readily only when it is present in excess. Fig. 15 shows the results of two experiments *in vivo* in one subject. In the first experiment, 0.25 ml. of red cells was labelled with ^{51}Cr , then incubated with 0.95 ml. of gamma-globulin prepared from a serum K, containing Rh antibody. In the second

FIG. 15.—Effect of incubating sensitized red cells with an excess of unsensitized red cells on the rate of removal of the sensitized red cells after reinjection. ●—● 0.25 ml. of red cells labelled with ^{51}Cr , then sensitized *in vitro* with 0.95 ml. of gamma-globulin containing Rh antibody before being reinjected. x—x Red cells labelled and sensitized exactly as above, but then incubated with 5 ml. (20 vols.) of unsensitized red cells before being reinjected.

experiment, 0.25 ml. of red cells was treated in exactly the same way, but was then incubated with 5 ml. (20 volumes) of unsensitized cells before reinjection. As Fig. 15 shows, there was only a very slight diminution in red-cell destruction when the sensitized cells were incubated with unsensitized cells before reinjection.

Effect of Sensitization of Red Cells with Different Amounts of Antibody

When the red cells of different subjects were incubated with varying amounts of anti-Rh (preparation K of gamma-globulin) it was found that the rate of destruction varied with the dose over the range 0.1–1 volume of gamma-globulin to 1 volume of red cells (see Fig. 14). Increasing the dose from 1.2 to 3.8 volumes of gamma-globulin to 1 volume of cells

produced no further increase of red-cell destruction—presumably because the rate of red-cell destruction was limited by the rate of blood flow through the spleen (Mollison and Cutbush, 1955; Hughes Jones *et al.*, 1957).

As already mentioned, a striking finding is the steady destruction of the injected cells for a period of one hour or more after reinjection. When the observations were extended for a longer period some change in this pattern was apparent. Thus, Fig. 16 shows that the rate of destruction slows about 5 hours after injection, suggesting that by the end of this time an appreciable amount of antibody has come off the red cells. The red cells which are left in the circulation at the end of this time may survive normally, suggesting that the dose of antibody on their surface has now fallen to a harmless level (see Fig. 17).

These findings may be used to explain and reconcile some previous observations. For example, Loutit and Mollison (1946) transfused blood from some patients with acquired haemolytic anaemia to normal subjects and found substantially normal survival of the transfused red cells. The donors were only mildly affected, and presumably, therefore, the rate of destruction of transfused cells during the few hours immediately after transfusion was only slightly more rapid than normal. Thereafter one may suppose that by slow redistribution of antibody the amount on the transfused cells fell below the critical amount needed to bring about red-cell destruction, and the remaining cells survived normally. A similar explanation may be applied to the findings of Mollison and Paterson (1949),

FIG. 16.—Some of the observations shown in Fig. 14 continued over a longer period of time. There is evidence of a change in the rate of destruction four to five hours after injection.

FIG. 17.—Survival of Rh-positive red cells incubated *in vitro* with relatively small amounts of Rh antibody. K 0.09=one volume of red cells incubated with 0.09 volume of gamma-globulin from donor K; the titre of this preparation was 1 in 1,000. E 4.0=one volume of red cells incubated with 4 volumes of gamma-globulin from donor E; the titre of this preparation was 1 in 64.

who transfused normal Rh-positive red cells which had previously been incubated with Rh antibody and found a substantially normal survival, but they added that "although the bulk of the sensitized erythrocytes survived normally" after transfusion "there may have been a fraction which was destroyed more rapidly." Re-examination of their data shows that the greatest amount of incomplete Rh antibody used in any experiment was 120 ml. of serum with a titre of 256, incubated with 260 ml. of red cells. This might be equivalent to about 0.15 ml. of serum with a titre of 1,000 added to 1 ml. of red cells; if so, it would be expected (see Fig. 14) that roughly 20% of the cells would be destroyed within the first hour after transfusion and that thereafter the rest would survive normally.

Similarly, in infants with a positive indirect antiglobulin test but no haematological evidence of red-cell destruction Mollison and Cutbush found that transfused Rh-positive red cells might survive normally even though they took up antibody and gave a positive direct antiglobulin test (quoted in Mollison, 1951, p. 382).

Finally, there are cases in which an apparently normal subject is found to have a positive direct antiglobulin test (of the type inhibited by adding gamma-globulin to the antiglobulin serum) and yet to have a normal red-cell survival (see Mollison, 1956, p. 165). In such cases it may well be that the amount of gamma-globulin on the red cells is below that found in typical cases of acquired haemolytic anaemia. Red cells which have adsorbed sufficient gamma-globulin in the form of Rh antibody to give a moderately strong direct antiglobulin test may still have adsorbed an amount insufficient to bring about an accelerated rate of destruction.

Owren (1948) and Selwyn and Hackett (1949) reported that when red cells were taken from patients severely affected with acquired haemolytic anaemia and transfused to normal subjects 50% or more of the transfused red cells were destroyed within the first day or two, and the remaining cells survived normally. This finding may be compared with some of the curves shown in Figs. 16 and 17; the slowing of the rate of destruction after the first few hours is presumably accounted for by redistribution of antibody.

The data in Fig. 14 give some indication of the amount of antibody which can be expected to produce appreciable red-cell destruction if transfused to an Rh-positive subject. Thus, supposing that an Rh-positive recipient with a red-cell volume of 1,000 ml. is transfused with a bottle of blood containing 200 ml. of plasma with a titre of 256, the amount of red-cell destruction will be expected to be approximately equivalent to that observed if 50 ml. with a titre of 1,000 was injected—that is, a ratio of 0.05 of Rh antibody with a titre of 1,000 to 1 ml. of red cells. As Fig. 14 shows, this will be expected to produce only about 10% of red-cell destruction, but this might be enough, when applied to the whole red-cell mass, to produce transient bilirubinaemia, etc. The conclusion agrees well with observations published by Jennings and Hindmarsh (1958) based on actual transfusions of plasma containing anti-Rh to Rh-positive patients. They found that when 250 ml. of plasma was transfused there was a definite fall in the recipient's haemoglobin concentration only when the titre of Rh antibody was 1 in 128 or more.

Some Practical Applications of a Test *in Vivo* for Compatibility

Doubts about the interpretation of tests *in vitro* may arise on two counts: (1) doubts about the thermal range of the antibody, and (2) doubts about the specificity of the antibody.

Doubts About Thermal Range of Antibody

As already described, antibodies exist which *in vitro* react with red cells only up to a temperature of 34° C., but are capable of bringing about destruction of transfused red cells. Moreover, antibodies which are active *in vitro* only up to a temperature of approximately 31° C. may bring about destruction of transfused red cells, though at a slower rate. Therefore, when an antibody is encountered which is definitely active at a temperature of 30° C. but only dubiously active at higher temperatures there is bound to be some doubt whether or not the antibody will be capable of bringing about the destruction of transfused red cells.

It might seem that the proper solution in such cases would always be to select for transfusion red cells lacking the relevant antigen, but there are circumstances in which this is difficult to arrange. For example, in patients undergoing certain heart operations in the course of which perfusion with the "heart-lung" machine is to be used, large amounts of fresh blood are required. If a cold agglutinin in the patient's serum is to be taken into account in the selection of donors, preliminary tests on a large number of donors will have to be made, and this may be almost impossible to arrange. Indeed, in such circumstances the only practicable way of obtaining the necessary donors may be to call upon members of the "National Panel" whose full blood groups are known. One should be fairly certain that it is really necessary to take a particular cold agglutinin into account before using such donors. That this is not a hypothetical situation is shown by the fact that in a period of two months at one particular hospital three patients due to undergo heart operations involving perfusion were encountered with anti-P in their serum, active *in vitro* at 30° C. or higher. In each case a test with ⁵¹Cr-labelled P-positive cells was performed, using selected "strong" P-positive red cells. In two cases the survival of the cells was absolutely normal, and P-positive blood was used for transfusion without any untoward effects. In the third case there was slow destruction of a proportion of the injected P-positive red cells (15–20% in the first few hours), and it was deemed necessary to use known P-negative donors for transfusion during operation.

In these particular cases hypothermia was not employed. In patients who are to be cooled to about 30° C. it may be necessary to take into account specific cold agglutinins which are active *in vitro* only at temperatures up to 25° C. or 30° C.; but this is speculation, and no experience has yet been obtained in such circumstances.

Doubts About Specificity of Antibody

Two types of case are known in which it is difficult to predict the specificity which an antibody will display *in vivo*. The first is some patients with acquired haemolytic anaemia. Sometimes in these patients the serum displays clear blood-group specificity, as in the case described by Weiner *et al.* (1953). In some of these cases at least it is possible, by carrying out tests with the

patient's serum *in vitro*, to find red cells which will survive very well *in vivo*; that is, much better than the patient's own red cells. An example is shown in Figs. 18 and 19. In this case, when red cells having the same Rh antigens as those of the patient's own red cells were injected, 25% were removed in the first 120 minutes (Fig. 18) and 90% were removed in 24 hours (Fig. 19).

FIG. 18.—Survival of two different types of red cells in the circulation of a patient with acquired haemolytic anaemia. The patient's Rh phenotype was CcDEe, and his serum contained anti-e and anti-C.

FIG. 19.—Further observations in the patient referred to in Fig. 18. The results for the survival of CcDEe cells injected on December 21, 1956, are simply a continuation of the results shown in Fig. 18. The results for the CcDEE cells refer to an injection given 10 days later.

example, a combination of differential agglutination for one population and ^{51}Cr labelling for another. An example of this application has been published elsewhere (Mollison, 1959a).

When the serum of a patient reacts with all samples tested it may be worth while to determine by a preliminary test with very small volumes of ^{51}Cr -labelled red cells whether the red cells are not very rapidly destroyed *in vivo* before embarking on a full-scale transfusion.

One other circumstance in which the precise specificity displayed by an antibody cannot readily be determined by tests *in vitro* was described by Cutbush *et al.* (1956). In a patient whose serum contained anti-Le^a group O, Le(a-b+) cells were found to undergo partial destruction (see Figs. 4 and 5 in Lecture I). *In vitro* an interaction between such cells and the patient's serum could be revealed only by the technique of first treating the red cells with an enzyme and then carrying out an indirect antiglobulin test.

Incompatibility without Demonstrable Antibodies

In occasional recipients whose own red cells survive normally, transfused normal red cells fail to survive for the normal length of time. It seems reasonable to describe this as a kind of incompatibility, even though no antibody can be demonstrated. This phenomenon

Red cells lacking the critical antigens were injected at the same time and survived normally for the first 120 minutes; their survival could not be followed satisfactorily for longer because they were labelled with ^{32}P . Ten days later a sample of red cells from the same donor was labelled with ^{51}Cr and the cells were found to survive very well (Fig. 19). It was difficult to assess this second result because the patient was improving rapidly as a result of steroid therapy. In such circumstances more decisive results can be obtained by using methods permitting the simultaneous estimation of the survival of two populations — for

was first described by Loutit, Mollison, and Young (1943), who encountered 4 examples in 35 transfusions. A later survey suggested that the incidence of the phenomenon was about 1 in 20 transfusions (Mollison, 1954).

When ^{51}Cr was introduced as a red-cell label and cross-transfusions of relatively small volumes of red

FIG. 20.—Survival of red cells from a normal donor (L.F.) ×—× = Survival of L.F.'s red cells in her own circulation, estimated by ^{51}Cr survival and corrected for Cr elution. ●—● = Survival of L.F.'s cells in recipient I.S., estimated in 1952 by differential agglutination. ○—○ and ■—■ = Survival of L.F.'s cells in recipient I.S., estimated on two occasions (in 1955) with ^{51}Cr -labelled cells; estimates corrected for Cr elution.

cells were given, the phenomenon was encountered more often (see Adner and Sjölin, 1957). Indeed, it appears that one must expect a diminished survival in about one-third of cases. Thus, Drs. P. L. Adner and S. Sjölin (personal communication) have now found a diminished survival in 13 out of 40 cross-transfusions. In 4 out of

FIG. 21.—Survival of red cells from L.F. (x—x) and P.M. (o—o) in recipient I.S. (cf. Fig. 20, and see also Table VII). The broken line is the curve of normal survival.

labelled red cells than with ordinary transfusions; it is not found when a sample of a subject's own red cells is labelled with small amounts of ^{51}Cr and reinjected into his circulation.

Observations in two recipients in whom normal red cells failed to survive normally are shown in Figs. 20–24,

and blood group results in the same cases are shown in Table VII. The first recipient (Figs. 20 and 21) received her first transfusion in 1952—for severe post-partum anaemia. Transfused red cells (from two donors, H.T. and L.F.) were all removed from her circulation in 40 days. Three years later, when she was in normal health, she was given a small injection of red cells from one of the same donors (L.F.), labelled with ^{51}Cr . The red cells were again removed abnormally rapidly, and a third transfusion gave the same result (see Fig. 20). On a further occasion the patient was given red cells from a different donor (P.M.), and these survived distinctly better than those of the first donor (see Fig. 21), although their survival was not strictly normal (possibly because of menorrhagia). The subject's own red cells

FIG. 22

FIG. 23

FIG. 24

FIG. 22.—Survival of red cells from a donor (D.B.) after injection into a patient (P.K.) suffering from thalassaemia/haemoglobin C disease (see Table VII). Three tests were made with ^{51}Cr -labelled red cells; the estimates in the figures have been corrected for elution of ^{51}Cr . ●—● = May, 1954. ×—× = August, 1954. ○—○ = 1957.

FIG. 23.—Further red-cell-survival tests in patient P.K. ●—● = W.R., 1954. ×—× = W.R., 1958. ○—○ = D.B., 1954. All estimates were made with ^{51}Cr -labelled red cells and corrected for Cr elution.

FIG. 24.—A further red-cell-survival test in patient P.K. Figure shows the survival of red cells from donor J.M. (see Table VII).

survived normally in her own circulation. No blood-group antibody, apart from anti-B, could ever be demonstrated in this subject's serum.

In Table VII the blood groups of the recipient and of the various donors are shown. The only antigen possessed by L.F. which was foreign to the recipient was the Rh antigen E, and it will be seen that this antigen was also possessed by the donor H.T., whose cells also survived badly, and was not possessed by P.M., whose red cells survived well. It is thus tempting to

suppose that in this case the red-cell destruction was due to a very weak anti-E, although repeated attempts to demonstrate this antibody in the recipient's serum failed.

Figs 22, 23, and 24 show observations in a patient (P.K.) with thalassaemia/haemoglobin C disease who was severely anaemic. Some details of this case have been published previously (Mollison, 1956, p. 355, Fig. 55). The red cells of one donor (D.B.) survived poorly on several occasions: sometimes survival was normal or almost normal for the 10 to 15 days after transfusion, and the cells were then rapidly eliminated (see Fig. 22). Red cells from another donor (W.R.) survived well on the first two occasions on which they were used (in 1954), but when used again (in 1958) they were eliminated very rapidly (Fig. 23). No antibodies against the cells of any of the donors could ever be demonstrated in the patient's serum. Some of the survival curves were distinctly convex, suggesting the possibility that an immune response developed 10 to 20 days after transfusion (see Figs. 22–24).

As Table VII shows, red cells from three donors were transfused in 1954. The red cells of donors A.G. and W.R. survived well, whereas those of D.B. survived poorly. The only red-cell antigen which D.B. was known to have which was not present in the red cells of the other two donors or the recipient was the Rh antigen C. However, the rapid destruction of transfused red cells by this recipient could not have been due solely to a very weak anti-C, since the red cells of donor M.S., who was C-negative, were rapidly eliminated.

The occurrence of this form of incompatibility hardly constitutes a danger in transfusion, since the red cells are destroyed too slowly to cause any untoward effects.

TABLE VII.—Blood Groups of Donors and Recipients in Two Cases in Which Transfused Red Cells Failed to Survive Normally, although no Blood-group Antibody could be Demonstrated

		Blood Groups*							Red-cell† Survival (Days)	Year of Study	Volume of Red Cells Injected (ml.)
Case 1 (Figs. 20 and 21)	Recipient I.S.	A ₂	CeDee	MN	S+s+	Le(a+b-)	Fy(a+)	Jk(a+b+)	(Own cells normal)		
	Donors {	O	ccDEe	MN	S+	Le(a-b+)	Fy(a-)		40	1952	110
		A ₁	CeDE	N	S-s+	Le(a-b+)	Fy(a+)		40	1952	70
		P.M.	A ₂	CeDee	MN	S+	Le(a-b-)	Fy(a-b+)	80-100	1955	6
Case 2 (Figs. 22, 23, and 24)	Recipient P.K.	O	ccDEe	N	S+	Le(a-b+)	Fy(a-)		(Own cells reduced)‡		
	Donors {	D.B.	O	CeDEe	N	S-	Le(a-b+)	Fy(a+)	30	1954	10
									50	1954	8
									60	1957	3
		A.G.	O	ccdde	MN	S+	Le(a-b+)	Fy(a+)	120	1954	200
									120	1954	200
		W.R.	O	ccdde	MN	S-	Le(a-b+)	Fy(a+)	120	1954	6
									10	1958	4
		M.S.	O	ccDEe	MN	S-	Le(a-b+)	Fy(a+)	40	1958	7
	J.M.	O	CCDEe	MN	S+	Le(a+b-)	Fy(a-)	Jk(a-)	35	1958	7

* All donors and recipients were P+, Lu(a-), K-. † Approximate time for elimination of all injected red cells. ‡ Patient affected with thalassaemia/haemoglobin C disease.

Summary

We are now very near to being able to predict from tests *in vitro* whether a given sample of red cells will undergo rapid destruction if transfused to a particular patient and, if so, how rapid destruction will be, whether it will involve all or only some of the transfused red cells, whether it will be predominantly intravascular or extravascular, and, if extravascular, whether it will occur predominantly in the liver or the spleen.

Further observations, particularly in patients whose serum contains "cold" specific iso-agglutinins, are needed to define still more clearly the relationship between the thermal range of an antibody *in vitro* and its power to bring about red-cell destruction *in vivo*. The more academic problem of the relatively slow destruction, in particular subjects, of transfused red cells in the absence of demonstrable antibodies still awaits solution.

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CASE OF CARBON-MONOXIDE POISONING WITH COMPLICATIONS SUCCESSFUL TREATMENT WITH AN ARTIFICIAL KIDNEY

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The inhalation of carbon monoxide is a common method of attempted suicide, but nerve damage and muscle necrosis with acute renal failure are a rare complication. Reports have been published of mental and neurological sequelae from carbon-monoxide poisoning; among records of cases that survive the usual fatal long exposure to the gas (Shillito *et al.*, 1936; Adams *et al.*, 1953) we have been unable to find any mention of myonecrosis and wasting due to neuropathy occurring in the same patient.

Table I gives a summary of the cases with muscle necrosis and renal failure we have traced in the literature.

The following case is published in view of its rarity and because, in spite of many serious hazards, treatment with an artificial kidney (Kolff twin-coil) was successful. As a result of the carbon-monoxide poisoning the patient had muscle necrosis, acute renal failure, mental changes, a radiculomyelitis of L 2 to 5, and probably myocardial damage.