Section of Experimental Medicine and Therapeutics

President P L Mollison MD

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President's Address

The Reticulo-endothelial System and Red Cell Destruction

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The commonest way in which a red cell meets its end is to be engulfed by a cell of the reticulo-endothelial system (RES). In this paper I shall consider how the phagocytic properties of the RES, with respect to red cells, can be explored.

Red cells injected into the circulation are rapidly removed by the RES if they are incompatible or if they have been damaged in some way, such as by being heated or by being stored under adverse conditions. Their rate of removal from the circulation may be studied by labelling a small sample (less than 1 ml) with ^{51}Cr *in vitro* and re-injecting the cells into the circulation. The use of ^{51}Cr as a red cell label makes it possible to follow precisely the rate of elimination of the cells from the circulation and also to discover whether any hæmoglobin is released into the plasma, since the ^{51}Cr temporarily remains bound to hæmoglobin; furthermore, when intact red cells are removed by the RES, the ^{51}Cr temporarily remains at the site of destruction and can be detected there by surface counting.

The clearance of red cells which have been acted upon by incompatible antibodies may be studied either by injecting a small volume of red cells into a subject whose serum happens to contain an incompatible antibody, or by treating red cells with a suitable antibody *in vitro* and then re-injecting them into the circulation.

Opportunities for the first kind of study are limited since in most human subjects the only blood-group antibodies present in the serum are those of the ABO system. These antibodies are usually readily hæmolytic *in vitro* and bring about intravascular lysis *in vivo* so that the RES has no opportunity of removing intact red cells from the blood stream. In occasional subjects, particularly those who have had repeated blood transfusions, blood group antibodies other than anti-A or anti-B are present. By carrying out tests in such subjects it has been found that there is a close relationship between the serological characteristics of particular blood-group antibodies, as determined by tests *in vitro*, and the way in which red cell destruction is brought about *in vivo*. Thus complete antibodies, that is those which agglutinate red cells suspended in saline *in vitro*, and those incomplete antibodies which bind complement, bring about the removal of red cells predominantly in the liver. Probably these antibodies bring about the removal of red cells by all the cells of the RES, and the liver, being easily the largest site of such tissue, gets the largest share. Agglutinins of very low titre (Cutbush & Mollison 1958, Chaplin 1959) and those incomplete antibodies which are incapable of binding complement (Hughes Jones *et al.* 1957) bring about the removal of red cells predominantly in the spleen (*see below*).

As mentioned above, 'suitable' blood-group antibodies are present only in occasional subjects but the clearance of antibody-coated red cells can be studied in almost any subject by taking a small volume of red cells from the subject, treating them *in vitro* with a suitable blood-group antibody and re-injecting them into the circulation (Jandl *et al.* 1957, Cutbush & Mollison 1958, Mollison 1959, 1962). The advantages of this approach are twofold: (1) Given a supply of suitable antibody, for example anti-Rh, obtained from a particular donor, tests can be carried out in any normal subject whose red cells carry the corresponding (Rh) antigen. (2) The ratio of antibody to red cells can be varied at will.

The results of tests with antibody-coated red cells may be summarized as follows:

(1) Red cells coated with a sufficient amount of a suitable incomplete, complement-binding, antibody are cleared from the circulation with a half-time of 110–120 seconds, corresponding to the clearance of approximately 35% of the blood volume per minute (Mollison 1962); the main site of removal of the red cells is the liver (Mollison & Hughes Jones 1958). It is probable that under these circumstances the extraction rate by the

liver is near to 100% but direct proof of this, by demonstrating that the amount of labelled red cells in hepatic venous blood is negligible, has not yet been obtained.

(2) Red cells coated with a sufficient amount of incompatible antibody which does not bind complement (for example anti-Rh) are cleared from the circulation with a half-time of approximately twenty minutes, corresponding to the clearance of 3 to 4% of the blood volume per minute. The main site of removal of the red cells is the spleen (Mollison & Cutbush 1955, Hughes Jones *et al.* 1957, Jandl *et al.* 1957). It seems likely that this maximum rate of removal represents virtually complete clearance of the blood at a single passage through the spleen and therefore indicates splenic blood flow.

Whenever it is found that 'altered' red cells are being removed predominantly by the spleen it must be concluded that the liver is relatively very inefficient in removing them, since the blood flow through the liver is probably eight to ten times as great as that through the spleen. This relative inefficiency of the liver can be demonstrated by injecting Rh-sensitized red cells into a splenectomized subject, using an amount of antibody which would bring about complete clearance at a single passage through the spleen. In a splenectomized patient Rh-sensitized cells are cleared with a half-time of approximately five hours (Jandl *et al.* 1957, Mollison 1961) corresponding to the clearance of less than 1% of the blood at each passage through the liver.

In a normal subject, when Rh-positive red cells sensitized with a suitable amount of antibody (Table 1) are injected, most of the cells are cleared by the spleen within an hour and during this period only about 10% of the cells are taken up by the liver. Nevertheless, as Jandl & Kaplan (1960) showed, if the ratio of incomplete Rh antibody to Rh-positive red cells is high enough the proportion of red cells destroyed in the liver may be almost as great as that destroyed in the spleen. I have recently had an opportunity of confirming this; the subject was a patient whose serum contained an exceptionally potent incomplete antibody (titre 10,000); the antibody did not bind any trace of complement as judged by test with an anti-β_1 (complement) globulin serum. By a fortunate chance the patient's spleen had been removed ten years previously (for hereditary spherocytosis). Following the injection of 0·5 ml of Rh-positive red cells, the cells were cleared from the circulation with a half-time of 14 minutes (*see* Fig 1). Surface counting confirmed that the liver was the main site of destruction. Although this experiment shows that incomplete antibody of the type which does not bind complement can bring about relatively rapid destruction by the liver, it should be noted that the antibody can have brought about only partial clearance at each circulation compared with the virtually complete clearance associated with complement-binding antibodies of relatively low titre.

Table 1

Clearance of Rh-sensitized red cells from the circulation in 8 normal subjects

Subject	*Ratio of anti-Rh to red cells* ●	*Time taken to clear 50% of red cells (min)*
A	3·8 : 1	17
B	1·65 : 1	15
C	1·6 : 1	32
D	1·25 : 1	13·5
D■	1·25 : 1	14
E	0·5 : 1	55
F	0·4 : 1	73
G	0·2 : 1	240
H	0·1 : 1	(more than 240 min to clear 10%)

● 1 volume of the subject's own (Rh+) red cells was incubated at 37° C for 15–30 min with between 0·1 and 3·8 volumes of γ-globulin solution containing anti-Rh. The indirect antiglobulin titre of the γ-globulin solution was 1,000 and it was prepared from a donor, Mrs K.

■ Second test on subject D, two months after first test

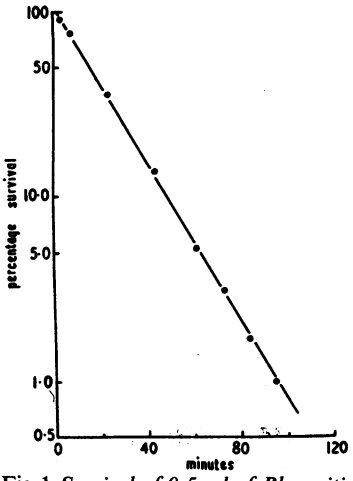

Fig 1 *Survival of 0·5 ml of Rh-positive red cells in the circulation of a splenectomized patient whose serum contained an exceptionally potent, incomplete Rh antibody (indirect antiglobulin titre 10,000); the antibody did not bind complement. Surface counting confirmed that destruction occurred predominantly in the liver. Less potent Rh antibodies bring about only very slow destruction in the liver and in normal subjects the cells are destroyed predominantly in the spleen*

Removal of Heated Red Cells by the Spleen

The spleen is also very much more efficient than the liver in removing red cells damaged by heating (Harris *et al.* 1957). If red cells are labelled with ^{51}Cr, then heated to 50° C for 20 minutes and re-injected into the circulation, approximately 80% are removed from the circulation in the first

Table 2
Rate of clearance of Rh-sensitized red cells in 6 patients with rheumatoid arthritis, before and after receiving cortisone or prednisone

Case no.	*Date of test*	*Details of corticosteroids administered*	*Ratio of anti-Rh to red cells*●	*Time taken to clear 50% of red cells (min)*	*Reaction of patient's serum with Rh-sensitized red cells* in vitro▲
1	6.5.57	None	1·25 : 1	19·0	—
	17.5.57	Cortisone 100 mg/day from 11.5.57	1·25 : 1	21·5	—
2	6.5.57	None	1·9 : 1	14·0	—
	20.5.57	Cortisone 100 mg/day from 15.5.57	1·95 : 1	18·0	—
3	14.5.57	None	1·45 : 1	18·5	—
	29.5.57	Cortisone 100 mg/day from 24.5.57	1·5 : 1	19·5	—
4	2.12.59	9.12.59–13.12.59 Prednisone 10 mg/day	0·4 : 1	20·5	trace
	16.12.59	14.12.59–15.12.59 Prednisone 30 mg/day	0·4 : 1	30·5	trace
5	30.11.59	None	0·4 : 1	87 (30% clearance)	trace
	14.12.59	7.12.59–11.12.59 Prednisone 10 mg/day 12.12.59–13.12.59 Prednisone 30 mg/day	0·4 : 1	130 (30% clearance)	trace
6	28.3.58	None in previous four weeks	2·1 : 1	6·5	++
	2.7.58	Cortisone 50 mg/day for previous two months	2·0 : 1	14·8	++

●As in Table 1 ▲Red cells sensitized with anti-Rh from donor Mrs K, i.e. as used to sensitize the red cells injected

hour in a normal subject but only about 20% are removed in the first hour in a splenectomized subject (unpublished observations).

Effect of Steroids on the Clearance of Rh-sensitized Red Cells

In acquired hæmolytic anæmia the administration of steroids frequently produces some improvement in the hæmatological condition within a few days. The most likely mechanism for this seems to be an interference with the removal of antibody-coated red cells by the RES, particularly in view of the fact that it is known that the administration of cortisone and other steroids reduces the phagocytic index of the RES, as estimated by measuring the rate of clearance of carbon particles in mice (Nicol *et al.* 1956, Bilbey & Nicol, 1958).

In an attempt to demonstrate this effect in man, Rh-sensitized red cells were injected into 6 patients before and after they had received steroid therapy (usually for five days) and the rate of destruction of the cells was estimated. All 6 patients were in an active stage of the disease; in each case the serum gave a positive Rose-Waaler test and agglutinated Rh-positive red cells sensitized with the anti-Rh serum 'Ripley', which appears to react with all 'rheumatoid' sera (Waller & Vaughan 1956, Fudenberg & Kunkel 1961). Only one serum (from Case 6) strongly agglutinated Rh-positive red cells sensitized with the anti-Rh serum Mrs K which was used to sensitize red cells for the test injections. Sera from two other patients (Cases 4 and 5) weakly agglutinated red cells sensitized with Mrs K's serum (Table 2).

The method of treating red cells with a carefully measured amount of γ-globulin prepared from antibody-containing serum has been described elsewhere (Mollison 1962). With the particular preparation of γ-globulin from donor Mrs K, used in this work, which had an anti-Rh titre of 1,000, a maximal rate of clearance was observed in normal subjects when the ratio of γ-globulin to red cells exceeded about 1·2:1 (Table 1). The particular antibody appeared to elute very slowly from the red cells, judged by results *in vivo*; and subsequent tests *in vitro* with ^{131}I-labelled antibody have confirmed this (Dr N C Hughes Jones, personal communication). The point is mentioned here to emphasize the importance of choosing a suitable antibody; other examples studied have been found to elute relatively rapidly, both *in vitro* and *in vivo* (unpublished observations with Dr N C Hughes Jones).

In Cases 1–3 the ratio of anti-Rh to cells was in the range (1·25, or more, to 1) expected to give a maximal rate of clearance. Before treatment with cortisone 50% of the sensitized cells were cleared in 14–19 minutes; after five days' therapy with cortisone (100 mg/day) clearance was very slightly slower in all three cases, whereas in a

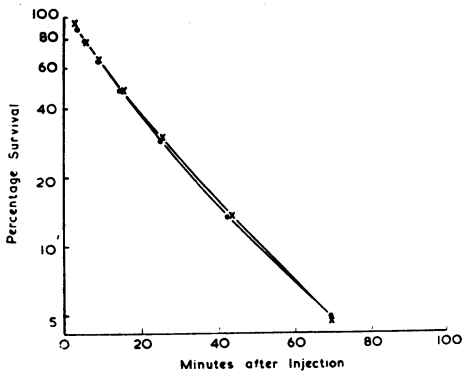

Fig 2 *Survival of Rh-sensitized red cells in a normal subject (D, Table 1), on two occasions about two months apart. The same dose of anti-Rh was used to sensitize the subject's own red cells on each occasion*

single normal subject not receiving cortisone two tests at about an interval of two months gave virtually identical results (Table 1; Figs 2 & 3). In Case 4, although the ratio of anti-Rh to cells was only 0·4 : 1, the rate of clearance of Rh-sensitized red cells before treatment was almost maximal (Table 2). After six days' treatment with prednisone, clearance appeared to be slower (Fig 3). In

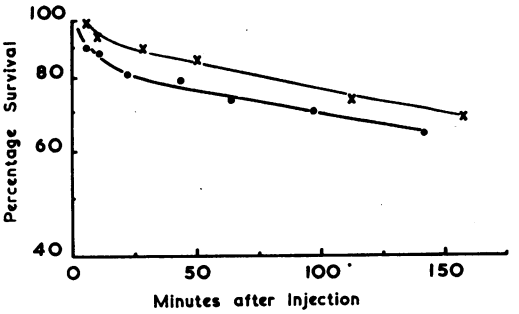

Fig 4 *Survival of Rh-sensitized red cells before (●–●) and after (x–x) six days' treatment with prednisone in Case 5*

Case 5 the effect of prednisone was even more pronounced (Fig 4).

In summary, after five to six days' treatment with corticosteroids the rate of clearance of antibody-coated red cells from the circulation appeared to be slowed although this effect was shown really clearly in only 2 out of the 5 cases (Cases 4 and 5). Perhaps in the remaining 3 cases (Cases 1–3) the fact that the red cells were treated with a relatively large dose of antibody provided unfavourable conditions for showing up relatively

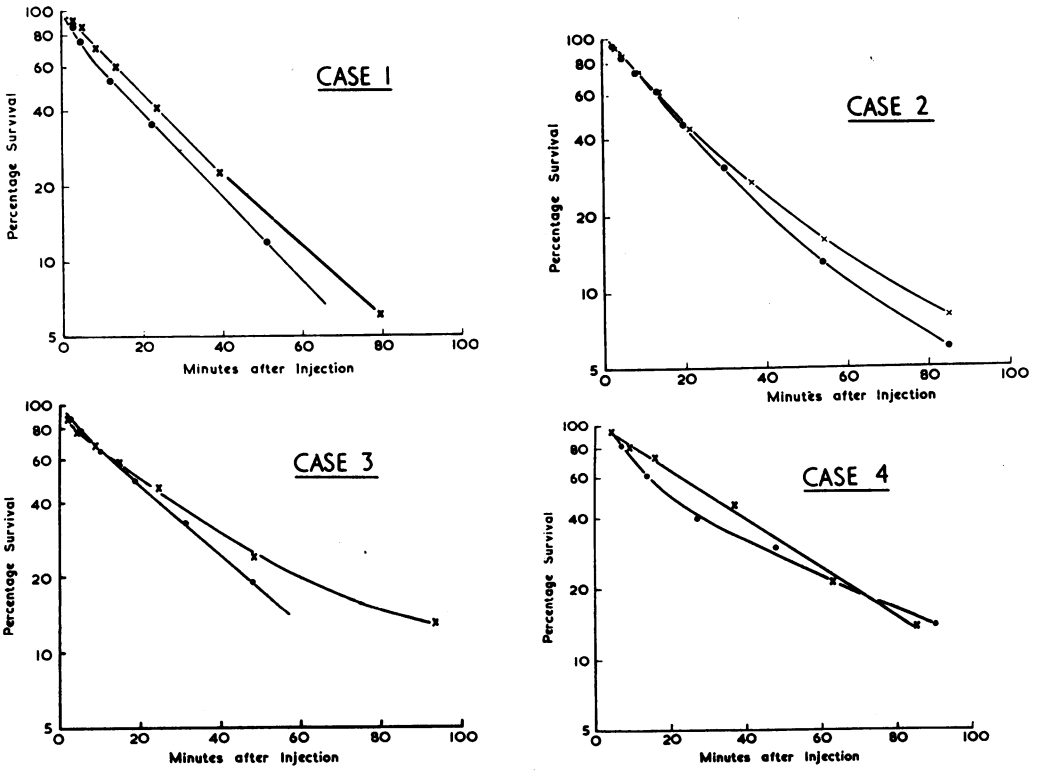

Fig 3 *Survival of Rh-sensitized red cells in 4 patients with rheumatoid arthritis (Cases 1–4). In each case the test was made before (●–●) and after (x–x) five to six days' treatment with corticosteroids; the same amount of anti-Rh was used to sensitize the subject's own red cells on each occasion (Table 2)*

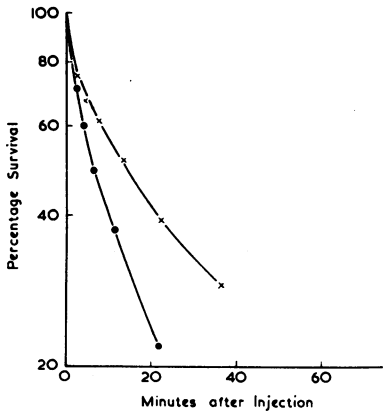

Fig 5 *Survival of Rh-sensitized red cells before* (●–●) *and after* (x–x) *two months' treatment with cortisone in Case 6. In this case, alone amongst those studied, the patient's serum* in vitro *strongly agglutinated the Rh-sensitized red cells used for injection*

small changes in the phagocytic property of the RES.

In Case 6 the second test was made after the subject had received cortisone for two months. In this subject the clearance of Rh-sensitized red cells in the first test was extremely rapid, probably because the patient's serum agglutinated red cells sensitized with anti-Rh from donor Mrs K, a reaction considered by Dr Sylvia Lawler (personal communication) to have anti-Gm^b specificity; after two months' treatment with cortisone, the rate of clearance of Rh-sensitized red cells in this case was very much slower (Fig 5), despite the fact that the patient's serum still strongly agglutinated red cells sensitized with Mrs K's serum. The rapid clearance in this case (previously mentioned by Mollison, 1959), recalls the rapid clearance of Rh-sensitized red cells observed in a patient with myelomatosis by Jandl *et al.* 1957. In this latter case, in which the patient's serum globulin concentration exceeded 12 g/100 ml, Rh-sensitized red cells had a half survival time of about 5 minutes compared with about thirty minutes in control subjects. Presumably in both these cases the Rh-sensitized red cells were agglutinated in the circulation and were therefore removed predominantly by the liver.

There seem to have been at least two previous attempts to demonstrate the effect of steroids, administered for at least a few days, on the rate of destruction of antibody-coated red cells. Dr J R Anderson (personal communication) produced a severe chronic hæmolytic anæmia in rabbits by giving repeated injections of an immune anti-red-cell serum; administration of cortisone did not appear to diminish the severity of the hæmolytic process. On the other hand Kaplan & Jandl (1961) injected antibody-coated red cells into rats and showed that in animals which had received steroids for only three days the rate of clearance was diminished. They estimated that the effect of steroids was maximal after about six days. They showed that the rate of hepatic sequestration was significantly reduced although the rate of splenic sequestration appeared to be unaffected. It seems very probable that in man the beneficial effects produced in patients with hæmolytic anæmia within a few days of starting steroids are due to interference with the phagocytic properties of the RES and that this is likely to include an effect on the spleen since in some cases splenectomy produces a striking improvement.

If the effect produced by steroids in hæmolytic anæmia were due primarily to interference with the phagocytic efficiency of the RES it would be expected that steroids would have some beneficial effect in all types of hæmolytic anæmia except those associated with intravascular destruction. Evidence that the administration of large doses of steroids does diminish red cell destruction in congenital red cell disorders has in fact been produced. Thus, Coleman & Finch (1956) found a fall in urobilinogen excretion and in reticulocyte count in patients with hereditary spherocytosis treated with steroids and Dr A Marmont (personal communication) has found a prolongation of red cell survival in patients with thalassæmia major after cortisone therapy. Nevertheless, the effects produced in syndromes due to congenital red cell abnormalities are certainly less striking than those observed in acquired hæmolytic anæmia.

The Capacity of the RES

It is evident that if the removal from the circulation of red cells or other particles depends on phagocytosis, then the capacity of the system must be limited by the number of phagocytically-competent cells. It has been shown for a number of different types of particles, injected into a number of different animals, that the rate of removal is approximately inversely proportional to the dose; this has been shown, for example, for pigeon (nucleated) red cells injected into mice (Halpern *et al.* 1957) and for stored red cells injected into guinea-pigs (Miescher 1956).

Dr Hughes Jones and I have studied the effect of injecting large volumes of stored red cells into rabbits (Hughes Jones & Mollison 1962). In most experiments the red cells were stored at 37° C for about forty-five hours to render them 'non-viable'. We found that a small dose of red cells, of the order of 0·1 ml or approximately 0·03 ml/kg, was completely removed from the circulation within a few minutes but that much larger amounts were removed very much more slowly. The rate of removal of the larger doses was not uniform.

Instead, a proportion of the cells was removed within a few minutes and the remainder was removed very slowly. It was found that when approximately 10 ml of red cells was injected (equivalent to about 25% of the red cell volume of a rabbit) about 2·5 ml of the red cells were removed very rapidly. In a man this would be equivalent to the rapid removal of approximately 50 ml of red cells.

Estimation of the subsequent slow removal of red cells could not be made satisfactorily with red cells stored in this way because they tended to hæmolyse spontaneously. There is evidence from other sources that the maximum rate of clearance of 'non-viable' red cells is about 0·2 ml of red cells per kg per hour. For example, this was the rate observed by Bowman *et al.* (1961) in an Rh-positive subject injected with plasma containing an extremely potent Rh antibody, and was also approximately the rate observed in a rabbit treated with phenylhydrazine (*see* Noyes *et al.* 1960).

Knowledge of the maximal rate of clearance of antibody-coated or otherwise-damaged red cells helps in the understanding of various clinical phenomena. For example, after the accidental transfusion of Rh-positive blood to a subject whose serum contains anti-Rh, it is common to find some surviving Rh-positive red cells in the circulation on the following day. Suppose two bottles of blood, containing a total of 400 ml of packed red cells, have been transfused to a subject weighing 60 kg. Approximately 50 ml of the cells will be removed within the first hour or two and the rate of removal will then become approximately 10 ml/hour (0·2 ml/kg/hour). Thus thirty-five hours will be needed to clear the remaining 350 ml of cells.

As another example, in hæmolytic disease of the newborn, although the infant's red cells may be heavily coated with Rh antibody, the mean life span of the cells is never less than two or three days. Suppose the infant is maintaining a packed cell volume of 25% or approximately 15 ml of red cells per kg body weight; if the rate of destruction of cells is 0·2 ml/kg/hour, mean life span will be 15/0·2 or seventy-five hours.

These examples are only very approximate but they serve to show that the concept of a limited rate of red cell destruction by the limited capacity of the RES for phagocytosis is quite helpful.

Summary

(1) The relationships between the serological characteristics of different blood-group antibodies and their effects on red cells *in vivo* are briefly reviewed. Whereas incomplete, complement-binding antibodies may be responsible for complete clearance of a small dose of incompatible red cells in a single passage through the liver, those incomplete antibodies which do not bind complement as a rule cause only very slow clearance by the liver although they bring about complete clearance at a single passage through the spleen. Exceptionally potent incomplete non-complement-binding antibodies produce relatively rapid destruction in the liver although the rate of clearance is still much less than that brought about by moderately potent complement-binding antibodies.

(2) It is confirmed that red cells heated to 50° C for twenty minutes are removed from the circulation predominantly by the spleen.

(3) Some aspects of the effect of cortisone on the RES are reviewed and some new evidence is presented which strongly suggests that after five to six days' treatment with corticosteroids, the rate of clearance of Rh-sensitized red cells from the blood stream is diminished.

(4) The limited capacity of the RES to phagocytose red cells is illustrated by some recent experiments in rabbits and some clinical implications of the findings are discussed.

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