THE INVESTIGATION OF HAEMOLYTIC TRANSFUSION REACTIONS*

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(Concluded from p. 532)

Interpretation of Tests

The large number of tests described above will not all have to be applied in the majority of cases. The procedure should be to test for the most likely cause first and only to apply other tests when the first ones are negative or inconclusive. For instance, preliminary regrouping of the blood of the donor and recipient may reveal a mistake in A-B-O grouping, and in such cases no other tests will be necessary (Cases 1 to 4). Alternatively, if no theoretical incompatibility according to the A-B-O system is revealed between the donor's cells and the recipient's serum, but the recipient is a woman who has given birth to an infant affected with erythroblastosis foetalis, the possibility that the reaction has been caused by previous immunization of the recipient to the Rh agglutininogen should be first considered and the appropriate means taken to test this possibility (Cases 5 and 6).

When a blood sample is taken from the recipient immediately after an incompatible transfusion it should be realized that it may contain an appreciable number of donor cells and that this may possibly lead to a mistaken diagnosis of the recipient's group. Thus, for instance, in Case 4, 24 hours after the transfusion of group B blood to a person of group O the recipient's circulation contained appreciable numbers of B cells, both agglutinated and unagglutinated. In the case of the A-B-O groups mistakes are not likely to be made, however, because the appearance of partial agglutination is so different from the usual complete agglutination of the A and B reactions. In the case of incompatibility due to Rh differences mistakes are more likely, however. When an Rh-negative person already sensitized to the Rh agglutininogen is transfused with Rh-positive cells the post-transfusion sample may contain appreciable numbers of Rh-positive cells although their destruction is proceeding rapidly, and this may lead to the impression that the recipient is Rh-positive (see Diamond, 1942, for instance). The use of powerful sera should enable this appearance of partial agglutination to be recognized with certainty from that of complete agglutination.

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

It must also be borne in mind that in recently delivered women immune agglutinins may be found in the serum owing to stimulation by foetal agglutinogens occurring during pregnancy. There is evidence that when the foetal red cells contain either the A or B agglutinogens and the mother's red cells lack the same agglutinogens, the mother's anti-A or anti-B agglutinins are increased in titre after delivery not only in a proportion of cases (Jonsson, 1936) but in the great majority, reaching a maximum titre 10 to 20 days after delivery (Boorman, Dodd, and Mollison, unpublished observations). In recently delivered women care must therefore be exercised in interpreting the results of serial titrations of agglutinins.

It may be added that agglutinins in the recipient's serum which are not active at 37° C. are in our experience not capable of causing the destruction of transfused red cells.

If stored blood has been used inquiries should be made as to how long it has been stored; inquiries should also be made as to whether the blood was allowed to stand at room temperature for an excessive length of time. The answers to one or other of these questions may suggest the most probable cause for signs of haemolysis following transfusion.

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

Case Records

The following cases illustrate some of the points referred to in the text and some of the methods of investigation. Cases 1 to 8 illustrate a few of the features of incompatible transfusions. In Cases 1 to 4 blood of incompatible A-B-O group was transfused; in Cases 5 to 8 the blood was of suitable A-B-O group but was Rh-positive and proved incompatible because the recipient had become sensitized to the Rh agglutininogen. In Cases 1 and 2 the early onset of the classical symptoms of severe lumbar pain caused the transfusion to be abandoned after only small amounts of blood had been given. In Case 3, however, an injection of morphine prevented the recurrence of this symptom after its early onset. In Cases 5 and 6 the onset of characteristic symptoms was delayed, and in Cases 4, 7, and 8 no characteristic symptoms developed at any time; in all these cases, therefore, considerable amounts of incompatible blood were transfused. Case 9 illustrates the fact that the occurrence of a rigor does not necessarily indicate incompatibility even when, as in this case, the recipient is Rh-negative and the donor Rh-positive.

In Case 10 the transfusion of grossly haemolysed blood produced the signs of a haemolytic reaction. In Case 11 the transfusion of heavily infected blood gave rise to signs which might have been mistaken for those of a true haemolytic reaction. In Case 12 the possibility of a haemolytic reaction due to incompatibility of the donor erythrocytes was excluded by a cell-survival test.

Case 1: Donor, Group AB; Recipient, Group O.—The patient was a woman aged 60; diagnosis, carcinoma of the colon. Her blood group was determined by the hospital as AB, and a transfusion of group AB blood was begun. No previous direct matching was carried out, owing to a misunderstanding. After a very small amount (about 10 c.cm.) of blood had been transfused she complained of very severe pain in the back. It was assumed that the patient, who had previously received a transfusion of group O blood, had become immunized to the Rh agglutininogen and that the reaction was due to the fact that the donor blood was Rh-positive. The Blood Transfusion Service was therefore asked to supply group AB Rh-negative blood. At this point the patient's blood was submitted for investigation and was found to belong to group O Rh-positive.

Case 2: Donor, Group A; Recipient, Group O.—Woman aged 35; diagnosis, duodenal ulcer with melaena. Blood group determined by hospital as A. Transfusion of group A blood started.

Graph showing serial titrations of serum iso-agglutinins of a patient of group O who received a transfusion of 45 c.cm. of group A blood (Case 2). The titre is expressed as the reciprocal of the greatest dilution in which agglutination of standard test cells occurred (judged microscopically). In cases in which larger amounts of incompatible blood are given the offending agglutinin is usually found to have a very low titre during the day or two after transfusion.

Almost immediately after the beginning of the transfusion the patient felt her face "flaming," then she had a very severe throbbing pain in her lumbar region and felt faint. The pain in the back was intense. The transfusion was stopped after 45 c.cm. had been given. Afterwards she shivered for about an hour, and then became very

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hot and complained of a headache. By the next morning she was perfectly well, but investigations were requested. The recipient's group was found to be O; during the next two weeks the anti-A agglutinin steadily increased in titre, reaching a figure of 16,384, whereas the titre of the anti-B agglutinin remained at 64 to 128 throughout (see Graph). These titrations, as well as the other serum titrations mentioned in this paper, were carried out by Miss K. E. Boorman and Miss B. E. Dodd, to whom my thanks are due for permission to include the results.

Case 3 : Donor, Group A; Recipient, Group O.—A woman aged 30; grouped as A by hospital, transfused with 1,000 c.cm. of group A blood. After receiving 5 c.cm. the patient complained of throbbing in the head and back, and the transfusion was stopped. The symptoms were considered to be neurotic, however, and omnopon gr. 1/3 was injected. The transfusion was restarted, 500 c.cm. being given, without the production of further symptoms, in one and three-quarter hours. At the end of this time the patient developed a rigor, but another 500 c.cm. of blood was transfused during the next 3 hours. At the end of that time a second injection of omnopon was given. The patient slept well, and on waking in the morning had no symptoms. No abnormality of the urine was noted and she did not become jaundiced. The blood urea was estimated on the sixth day after transfusion and found to be normal (30 mg. per 100 c.cm.).

The patient was first seen 9 days after transfusion, not because any suspicion of incompatibility had been aroused, but merely because a request had been received for a further transfusion. Investigations showed that her blood group was O and not A as had been previously believed. Her cells showed no trace of agglutination with a potent anti-A serum—demonstrating (as might have been expected) the complete elimination of the blood given at the first transfusion. Titration of the recipient's iso-agglutinins showed that, whereas the anti-B agglutinin had a titre of 1,000, the anti-A titre was 262,000. Recovery was uneventful.

Case 4 : Donor, Group B; Recipient, Group O.—A woman aged 63, suffering from carcinoma of the lung. Group determined by hospital as B. A transfusion of 600 c.cm. of group B blood was given in 7 hours. Only 250 c.cm. of blood was administered during the first 5 hours, but at the end of this period the patient passed 120 c.cm. of red urine. The transfusion was continued until 600 c.cm. of blood had been given, but investigations were requested in case the passage of red urine had had any untoward significance. Apart from a feeling of hotness towards the end of the transfusion the patient did not develop any symptoms, and considered that she had benefited by the transfusion.

Investigations carried out next day showed that the patient's blood group was O and not B as had been supposed. Examination of a saline suspension of her blood revealed the presence of small scattered agglutinates, the largest consisting of from 8 to 12 cells. These agglutinates could be increased in size by the addition of anti-B serum, showing that besides the agglutinated group B cells there were a number of unagglutinated group B cells remaining in the circulation. As anticipated, the titre of the anti-B agglutinin was found to be very low.

The serum contained 1.3 mg. of bilirubin per 100 c.cm. but no methaemalbumin. (Specimen kindly examined by Mr. R. J. Bromfield.) This latter finding seems important because it is not generally recognized that this pigment may be absent from a sample of the recipient's plasma after the transfusion of a considerable quantity of incompatible blood even when the sample is obtained within 20 hours after transfusion.

A sample of urine obtained 24 hours after transfusion showed no free haemoglobin, but there were a few haemoglobin casts in the centrifuged deposit. The reaction of the urine was acid. The blood urea on the fifth day after transfusion was 35 mg. per 100 c.cm. (after a meal of fish). Death occurred on the sixth day, following signs and symptoms suggestive of pulmonary embolism. Unfortunately permission for necropsy could not be obtained.

Note on Cases 1 to 4.—The grouping mistakes that were responsible for the giving of these four incompatible transfusions were directly due to the use of infected test serum in at least two of the cases; but the fundamental errors in all four cases were the failure to use controls of any kind, presumably owing to over-confidence in the technique used, and the failure to carry out direct matching before transfusion.

Cases 5 to 8 : Donor, Rh-positive; Recipient Rh-negative, Sensitized to the Rh Agglutinin :

Case 5.—The patient was a woman aged 25 who five days previously had given birth to twins affected with hydrops foetalis. Her group was O Rh-negative, and her serum contained anti-Rh agglutinins. She was transfused with a concentrated cell suspension prepared from the blood of two donors, both of whom were thought at the time to be Rh-negative. In one hour 250 c.cm. of cell suspension was given without the occurrence of any reaction. Shortly

afterwards, however, while the transfusion was still running, the patient started to complain of violent lumbar pain, difficulty in breathing, and generalized discomfort. A violent rigor followed. The transfusion was stopped after 300 c.cm. of the suspension had been given. A venous sample was obtained three hours after beginning the transfusion. The serum contained no free haemoglobin but was intensely icteric. The serum bilirubin was found to be 4 mg. per 100 c.cm., compared with the pre-transfusion figure of 0.2 mg. Moreover, the post-transfusion sample of serum gave a "direct" van den Bergh reaction.

Investigations with potent test sera showed that one of the donors was in fact Rh-positive and that all the cells of this donor had been eliminated within three hours of starting transfusion. From the clinical point of view this reaction might well have been considered non-haemolytic, since there was no haemoglobinuria or jaundice.

(Some of the details of this case have been reported previously by Boorman, Dodd, and Mollison.)

Case 6.—(The details of this case are being reported separately elsewhere.) The patient was a woman aged 47. She had had four previous pregnancies, the last 14 years ago. She was admitted to hospital with a diagnosis of menorrhagia. A transfusion of 500 c.cm. of group O blood was given. No characteristic symptoms occurred during the transfusion, although a rigor followed it. Next day she was jaundiced, complained of pain in the loins, and passed only 10 oz. of urine. A sample of urine obtained 36 hours after transfusion contained albumin, haemoglobin, bile pigments, and a trace of sugar. Investigations carried out 18 days after the transfusion showed that the patient's group was O Rh-negative and that the donor group was O Rh-positive. Her serum contained moderately strong anti-Rh agglutinins.

The most interesting feature of this case seems to be the persistence of sensitivity to the Rh agglutinin for at least 14 years, and, moreover, a sensitization of sufficient degree to give rise to a severe haemolytic reaction.

Case 7.—The patient was a woman aged 30 who was given a transfusion of 1,000 c.cm. of group O blood after a severe post-partum haemorrhage. Investigations were requested a week later because the haemoglobin was only 39%. The transfusion itself had been symptomless, and was not followed by jaundice or by any other noteworthy signs. Investigations showed that the patient was group A Rh-negative and that her blood contained a negligible number of group O cells, despite the fact that it was only 7 days since the transfusion. Her serum was found to contain weak anti-Rh agglutinins. The infant's blood was examined for evidence of a haemolytic anaemia, but it appeared to be perfectly normal and the haemoglobin was 120%.

It seems possible that in this case the mother's serum did not actually contain anti-Rh agglutinins at the time of the transfusion but that they were rapidly evoked by the transfusion and that the woman had been sensitized by her pregnancy (the infant was found to be Rh-positive). If this theory is correct this case should not strictly be included, since the definition of haemolytic reactions has been confined here to cases in which destruction starts immediately after transfusion. However, it illustrates well how easily this type of reaction may be overlooked and the features of these reactions merge imperceptibly into those in which destruction starts more rapidly after transfusion.

Case 8.—The patient was a woman who gave birth to her second infant on Aug. 23, 1942. The infant became jaundiced within 24 hours of birth, but a blood count on Aug. 25 showed that its red cell count was 5,320,000 and that its haemoglobin was 105%, so that the diagnosis of haemolytic disease was considered to be excluded. On Aug. 27 a transfusion of 500 c.cm. of a concentrated suspension of red cells prepared from 1,000 c.cm. of group A blood was administered to the mother with a view to correcting a mild secondary anaemia. After transfusion the patient developed a rigor but no other signs or symptoms, and no investigations were carried out at the time.

The infant remained jaundiced, and was seen on Sept. 1. At that time its haemoglobin had fallen to 64% and a diagnosis of haemolytic disease was made. Serological tests showed that the mother was group A Rh-negative and that her serum contained anti-Rh agglutinins. It was noted that no trace of agglutination occurred when the mother's blood was tested with two very potent anti-Rh sera. Since both donors were found to be Rh-positive it was clear that all the donor cells had been eliminated.

Case 9 : Donor, Group O Rh-positive; Recipient, Group O Rh-negative, not apparently Sensitized to the Rh Agglutinin.—The patient was a woman with three normal children; she was now pregnant for the fourth time and only two weeks from term. Her haemoglobin was found to be 52%, and she was given a transfusion of approximately 750 c.cm. of a concentrated suspension of group O

red cells. After the transfusion the patient had a mild rigor, and investigations were requested. These showed that she was group O Rh-negative, but that her blood contained a considerable number of Rh-positive cells, while her serum contained no anti-Rh agglutinins. All four donors who had contributed to the red cell suspension were Rh-positive. A normal infant was delivered two weeks later. One month after the transfusion the patient's blood stream still contained a considerable proportion of Rh-positive cells. It seemed clear, therefore, that the rigor following transfusion was not associated with an abnormal destruction of the donor cells.

Case 10: Haemolytic Reaction simulated by the Transfusion of Haemolysed Blood.—This patient was an elderly woman with carcinoma of the stomach. It was decided to give a blood transfusion because of her anaemia, and a bottle of a concentrated suspension of red cells was ordered from the Blood Transfusion Service. When the bottle arrived it was placed in the ward refrigerator. A few hours later the doctor arrived to carry out the transfusion and called for this bottle. The probationer went into the room in which the refrigerator was situated, saw a bottle of blood standing on a shelf, and assumed it was the right one. The transfusion of this bottle was completed in two and three-quarter hours, and although no alarming signs or symptoms developed it was noted that the patient was rather flushed and that her temperature had risen to 101° and her pulse rate to 130 to 140. Investigations were requested.

The patient was visited approximately three-quarters of an hour after the end of the transfusion. She was rather flushed and sweaty and had a rapid pulse. The bottle of blood had unfortunately been washed out and only a portion of the label remained. From the data on this portion, however, it was possible to establish that the bottle was seven weeks old. It was then discovered that it had been sent to the hospital after it had already been stored for 19 days in the depot refrigerator, and that it had stood in the ward pantry at room temperature for over four weeks.

A venous sample was obtained from the patient and showed considerable haemoglobinaemia (approximately 100 mg. per 100 c.cm.). Her blood group was A, and differential agglutination showed no trace of surviving group O cells. No urine was passed from the beginning of transfusion until about an hour after the end; then a small amount of highly coloured acid urine was passed. This specimen, however, contained no albumin, no haemoglobin, and no casts. The patient was given large doses of alkali, and the next specimen of urine passed was strongly alkaline. A further venous sample was obtained two hours after the first one. The plasma still contained free haemoglobin, although the degree was less and there was much bilirubin. The patient never became obviously jaundiced, and no further sequelae were noted.

Case 11: Fatal Reaction due to Transfusion of Infected Blood.—The patient was a woman aged 46 with a history of menorrhagia due to uterine fibroids. As a preparation for operation she was given a transfusion for the relief of her anaemia. Her blood group was O, and the transfusion of two bottles of concentrated cell suspension prepared from group O blood was begun. The transfusion was started at a rapid drip. After one to two minutes the patient said that her face was feeling hot; a few seconds later she complained of generalized discomfort. Her face was found to be very flushed, so the transfusion was stopped after only about 40 c.cm. had been given. The patient next complained of dyspnoea and developed a cough. She said that her head felt as if it were bursting. At this point a venous sample was taken because it was thought that incompatible blood might have been given. Soon afterwards she became worse. She developed abdominal pain and diarrhoea, and then started to shiver. Later she became very collapsed and developed a high pulse and temperature. Her condition deteriorated, and she died 12 hours after the transfusion.

Examination of the donor blood showed that it was grossly haemolysed and heavily infected with bacteria. It is important to note that this haemolysis was revealed only after a mixed sample had been centrifuged. Before transfusion, when the bottles of stored blood were inspected, the supernatant plasma appeared perfectly clear. It is well known that when haemolysis occurs in stored blood the diffusion upwards of haemoglobin into the plasma may only take place slowly. Examination of the venous sample obtained immediately after stopping the transfusion revealed the presence of a small quantity of free haemoglobin in the plasma, which could be accounted for by the amount of free haemoglobin injected.

Case 12: Incompatibility Excluded by Cell-survival Test.—The patient was a male who had been severely burnt two days previously. He was transfused with 1,000 c.cm. of reputed group O blood. No direct matching was carried out. No donor blood remained for test, and the donors could not be traced. After transfusion the patient had a temperature of 109° F.; he died 10 hours later. A sample of blood was obtained from the body after death and found to be group A. The survival test indicated that there were approximately one million group O cells per c.mm. Thus incompatibility of the donor's cells could be excluded.

Summary and Conclusions

The diagnosis of a haemolytic reaction is based on evidence of increased blood destruction following transfusion and on the demonstration of a cause for this destruction. The commonest causes of severe haemolytic transfusion reactions at the present time are: (1) mistakes in blood grouping; (2) the use of Rh-positive blood for transfusion to recipients sensitized to the Rh agglutinin; and (3) failure to maintain adequate standards in blood storage.

The investigation of haemolytic transfusion reactions is greatly facilitated if a sample of donor blood is kept available for investigation for at least 24 hours after the transfusion, and is further facilitated if a pre-transfusion sample from the recipient is also kept available for testing. When the patient is seen within 12 or 24 hours of transfusion the increased destruction can often be demonstrated by simply obtaining a venous sample from him and showing that it contains oxy-haemoglobin, methaemalbumin, or an increased concentration of bilirubin. When the patient is seen later the application of the method of differential agglutination is often the only way of demonstrating that the donor's red cells have been rapidly destroyed. Valuable additional evidence can often be obtained by demonstrating the presence of immune agglutinins in the recipient's serum.

It should be possible to diagnose the cause of practically all haemolytic transfusion reactions provided that investigations are initiated within a short time after transfusion, and provided that a sample of the transfusion fluid is available for testing. In practice, however, these conditions are often not fulfilled, and the cause of many haemolytic reactions is therefore never determined. It is considered that the full investigation of these reactions would be one of the most effective ways of raising the standard of blood transfusion.

I should like to thank Dr. J. F. Loutit for helpful criticisms and for kindly allowing me to publish the details of Case 10, which was chiefly investigated by him. I should also like to thank the various medical officers in charge of the other cases investigated for giving me access to their cases and for permission to publish the data reported here.

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Under arrangements made by the Ministry of Labour with the governors of Queen Mary's (Roehampton) Hospital the facilities of the latter organization are to be available for the supply of artificial limbs to adults needing them for useful employment. At the same time the Board of Education has given general sanction to education authorities, and the Ministry of Health to welfare authorities, to provide any necessary surgical treatment and artificial limbs for school children. The facilities of Roehampton and of other Ministry of Pensions hospitals are to be at the disposal of local authorities, whose expenditure will rank for grant. This information and the addresses of regional medical officers and of hospitals of the Ministry of Pensions are contained in a circular issued jointly by the Board of Education (No. 1624) and the Ministry of Health (No. 2796).