SYMPTOMLESS INCOMPATIBLE TRANSFUSION AND RESULTANT CHANGES IN ISO-AGGLUTININ TITRE

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Incompatible blood transfusions in which studies of iso-agglutinin titre have been made are not numerous. The number of these mishaps reported in the literature is not a true reflex of their incidence. In the case here reported blood-grouping technique was impeccable. The mishap, mercifully without any consequences, occurred for a different reason—namely, scrupulous care was not taken to see that the correct bottle of blood was obtained from the blood bank. The circumstances were somewhat similar to those in Mollison's (1943) Case 10, in which effete group O blood was given to a group A recipient. After 19 days in cold storage the blood was left on a shelf at room temperature for four weeks. A nurse sent to fetch the blood selected this bottle instead of the correct one in the refrigerator. An hour after transfusion there was haemoglobinaemia but no surviving donor cells. A febrile reaction occurred, but no further sequelae were noted.

Previous Reports

Rö (1937-8) studied a group O recipient who recovered after receiving 500 c.cm. of group A blood. In preliminary tests the recipient's serum did not agglutinate the donor's cells. The biological test of Oehlecker (1921) was negative. Haemoglobinuria, jaundice, and oliguria occurred. Two days later the anti-A titre was 20. It rose to 200 in the fifth week and fell to 70 in the ninth week. A strong anti-A haemolysin was detected. Wiener (1939) and Wiener *et. al.* (1941) studied a group O recipient transfused with 500 c.cm. of group B blood. Rigor and haemoglobinuria resulted. In a few hours the anti-B titre was only 1, but on the thirteenth day it was 512. Wiener (1941) investigated a group B recipient who recovered after receiving 150 c.cm. of group AB blood. Rigor and haemoglobinaemia occurred. Shortly after transfusion the anti-A titre for A₁ cells was 4 and for A₂ cells 0. A peak of 512 was attained on the seventh day against A₁ and A₂ cells. Mollison and Young (1941) observed a group O air-raid casualty transfused with citrated group B blood (content, 360 c.cm. whole blood) and unpooled group A sera. Group O blood and pooled plasma were also given. Recovery occurred after uraemia. Eight hours after the incompatible transfusion the anti-A titre was 128, but some hours later it was only 32. The anti-B titre fell as low as 2. There was a subsequent colossal rise in titres. On the eighth day the anti-B titre was 500,000, and on the eleventh day the anti-A titre was over 65,000. Return to normal was slow. Rise in anti-A titre was due to group A substance in the transfused unpooled group A sera. Agglutinates were present in the recipient's blood for seven days after the incompatible transfusion. A haemolysin acting on A and B cells was observed from the seventh to twenty-fifth days. Anti-M and anti-N were not involved. Rises of anti-A titre to 16,384 and 262,000 were observed by Mollison (1943) in two group O recipients transfused respectively with 45 c.cm. and 1,000 c.cm. of group A blood; both recovered.

Certain workers, apart from those mentioned, used differential agglutination to study the *in vivo* survival of incompatible erythrocytes. Jervell (1924) reported the following cases. (i) A newborn child with melaena neonatorum. It was group O, but the α and β agglutinins were absent. It received 100 c.cm. of group A blood from the mother. The donor's cells survived six weeks. The mother's β agglutinin was detected in the child's serum for two weeks, and attained a titre of 10 on the second day. (ii) A group B female transfused with 600 c.cm. of group O blood and 600 c.cm. of group A blood. After being jaundiced she recovered. All

group A cells were eliminated in 12 hours. (iii) A group B female given 550 c.cm. of group O blood and 350 c.cm. of group A blood. She had a severe reaction, and died three hours later. The recipient's serum agglutinated the donor's cells to 1 : 4 and completely haemolysed them in 15 minutes. Blood taken by cardiac puncture 10 minutes after death showed marked haemoglobinaemia and no surviving group A cells. (iv) A 2-year-old child, group O, afflicted with leukaemia, given 200 c.cm. of group A blood. Drowsiness, vomiting, bloody urine, and jaundice occurred. Improvement followed transfusion of 200 c.cm. of group O blood. The group A cells survived three days. In Lauer's (1941) case, immunization of a group O person to cells of another person (group A) occurred in a skin-graft operation. The anti-A titre was 2048 and later fell to 2. Burnham's (1930) patient, group B, recovered after transfusion with 400 c.cm. of group A blood. Symptoms were mild. The recipient's serum agglutinated group A cells at room temperature but not at all at body temperature. The donor's cells survived three to five days. Grove and Crum (1930) investigated a group O recipient transfused with 300 c.cm. of group B blood. No reaction occurred, and this was attributed to inability of the recipient's serum to agglutinate 2% suspensions of A or B cells in test-tubes, after incubation at 42° C. for one hour. The donor's cells were surviving the day after transfusion. The recipient's blood was not further examined till the seventh day, when no donor's cells were found.

Schiff (1924) produced a specific precipitin in rabbit serum against agglutininogen-A substance in human serum. Aubert, Boorman, and Dodd (1942) extended Schiff's work. They injected Seitz-filtered human A or B sera into guinea-pigs or human volunteers, and produced anti-A or anti-B antibodies, some of quite high titre. Alternate responses were produced in a group O subject by injecting first group A and then group B serum. Rises in iso-agglutinin titre after incompatible transfusion, while principally due to agglutininogen of incompatible cells, may therefore in part also be due to agglutininogen substance in solution in the donor's plasma.

Case Report

In the case here recorded the iso-agglutinin changes were studied and an attempt was made to find an *in vitro* explanation for absence of symptoms.

A man aged 54 was admitted to hospital on Feb. 19, 1943, for melaena and loss of weight. He was crippled by chronic rheumatoid arthritis, which was still active. Seventeen months ago he had a course of gold salt injections. Seven years ago he underwent an operation for gastric ulcer. Latterly pain had occurred in the epigastrium after meals and was relieved by taking food. Weight, 8 st. 10 lb.; blood pressure, 120/60; blood count—red cells 4,000,000, leucocytes, 7,600; haemoglobin, 40%; blood group, B and Rh-positive. On March 22 the haemoglobin was 60%.

On March 23 the patient was inadvertently transfused with group A blood under the following circumstances. The local issuing blood depot was requested to supply group B concentrated red cells. After cross-matching tests the blood was issued and placed in the hospital blood bank. The medical officer in charge of the case instructed a nurse to fetch the blood from the refrigerator. A bottle of blood was selected and given to the medical officer. The bottle label was not examined, and transfusion was started at 6 p.m. at a very slow drip rate. Some 5½ hours later it was noticed that the bottle was labelled group A. The patient had complained of no symptoms whatever. Transfusion was immediately stopped. Approximately 460 c.cm. of blood had been transfused. The blood mixture consisted of 120 c.cm. of 2.5% sodium citrate solution, 20 c.cm. of 10% glucose solution, and 400 c.cm. of whole blood.

The blood had been stored for seven days under proper conditions. The remnant of blood in the bottle was checked and found to be group A, and the cells were agglutinated by the recipient's serum, a specimen of which was collected at the time. The recipient's group was confirmed as B. The group B blood supplied for the recipient was still in the hospital blood bank.

No febrile reaction occurred during transfusion, when the pulse was 76 and temperature 97° F. Six hours later the pulse rose to 102 and the temperature to 100° F. However, the patient felt well. The temperature was normal within 12 hours, and thereafter remained normal or subnormal. In 24 hours the pulse dropped to 76. The recipient had run an occasional pyrexia since admission, and the day before transfusion the pulse was 100. On March 24, some hours after transfusion, 300 c.cm. of 2.5% sodium citrate solution was injected intravenously. Potassium citrate gr. 60 was given hourly by mouth till the urine was alkaline. Intravenous glucose-saline was given at a slow drip rate. General condition satisfactory. Over 24 hours the fluid intake was 94 oz. and output 123 oz. Serum bilirubin, 0.1 mg. per 100 c.cm.; icterus index, 3.3. Trace of urobilin in urine.

March 25.—Condition satisfactory. Temperature and pulse normal. Urine normal and no casts or erythrocytes in centrifuged deposit. No haemoglobinuria. The serum of the venous blood was clear; there was neither bilirubinaemia nor haemoglobinaemia. During 24 hours the fluid intake was 149 oz. and the output 125 oz. Intravenous glucose-saline discontinued.

March 26.—I was asked to make investigations. The patient's complexion was ruddy and his general condition good. No jaundice. Closely interrogated as to whether he had felt any untoward symptoms during the transfusion, he replied confidently in the negative. Indeed, he asserted he felt better for it. Ever since admission to hospital he had had a more or less constant dull aching pain in the dorsal region of the back, and in the ankle- and knee-joints. These symptoms had been present for months. Backache was present all day before transfusion and was in no way worse during or after it. The backache was still present months later. Urine normal and nothing abnormal in the centrifuged deposit. Blood urea, 40 mg./100 c.cm.; icterus index, 4; serum bilirubin, 0.1 mg./100 c.cm. Temperature and pulse normal. Although untoward symptoms were absent, it was decided to take anticipatory measures. Transfusion of compatible blood as recommended by Hesse and Filatov (1932) and Hesse (1936) was advised in combination with preliminary phlebotomy of the recipient: Von Deesten and Cosgrove (1933-4) employed phlebotomy with good result for uraemia due to haemolysis after blood transfusion. Withdrawal of 500 c.cm. of recipient's blood was made by venepuncture and promptly followed by transfusion of 540 c.cm. of group B whole blood (stored in mixture already alluded to). No febrile reaction. During 24 hours the fluid intake was 152 oz. and output 125 oz.

March 27.—The patient had some slight transient pain on breathing. Withdrawal of 420 c.cm. of recipient's blood and transfusion of 540 c.cm. of group B blood. No reaction. Blood urea, 32 mg./100 c.cm.; serum bilirubin, 0.3 mg./100 c.cm.; icterus index, 5. Fluid intake 150 oz. and output 103 oz. **March 28:** No untoward symptoms. Withdrawal of 420 c.cm. of recipient's blood and transfusion of 540 c.cm. of group B blood. No febrile reaction. Fluid intake 104 oz. and output 97 oz. It was noted that the plasma of the recipient's blood withdrawn on the previous day had a peculiar slightly greenish tinge. **March 29:** Withdrawal of 420 c.cm. of recipient's blood and transfusion of 540 c.cm. of group B blood. No reaction. Fluid intake 110 oz. and output 107 oz. Urine normal. Haemoglobin, 66%; blood urea, 31 mg./100 c.cm.; serum bilirubin, 0.05 mg./100 c.cm. **March 31:** Condition satisfactory; haemoglobin, 68%. **April 1:** Urine contained a few granular casts and trace of urobilin. **April 2:** Blood urea, 40 mg./100 c.cm. **April 22:** Haemoglobin, 74%; patient left hospital.

He was observed for several months, and except for the arthritis remained well. There are no grounds for assuming that the bleeding of the recipient and transfusions of homologous blood contributed to the favourable outcome.

Special Investigations of Case

Regrettably, in the 48 hours immediately after incompatible transfusion no investigation for methaemalbumin, Schumm's test, or spectroscopy for serum haemoglobin was made. Fortunately a pre-transfusion specimen of the recipient's serum was available at the local issuing blood depot. This specimen was collected the day before the incompatible transfusion. This serum strongly agglutinated the conserved red cells of the donor's blood (now 10 days old). The technique was to mix equal volumes (0.4 c.cm.) of saline, citrated red cell suspension (strength 0.5%), and serum in tubes of 3/8 in. diameter. After standing five minutes at room temperature tubes were centrifuged at 2,000 r.p.m. for two minutes. The groups of recipient and donor were confirmed as B and A respectively. Tests were performed in tubes, and groups were checked on cells and serum. The donor's plasma agglutinated the recipient's cells

weakly. The above results likewise obtained at 37° C., whether tubes were stood five hours or centrifuged. The recipient's serum was diluted 1 : 25 (i.e., 1 c.cm. serum to 24 c.cm. saline) and titrated against cells of a standard A donor (subgroup A₁); the titre was 1 : 100 at room temperature.

The titration technique was similar to that of Challinor, Ives, and van Rooyen (1939). Volumes of 0.4 c.cm. were used. Serum dilutions were doubled in saline from tube to tube. Tubes were 3/8 in. by 2 in. The washed citrated fresh red cell suspension was packed by the centrifuge to a strength of 0.5% in terms of cell sediment. The titre was stated as the greatest dilution in which agglutination was observed. Dilution caused by addition of the volume of red cells was taken into account, though many workers ignore this. Tubes, including controls, were stood five hours at room temperature and then examined for agglutination after adequate flicking with the index finger. The end-point observed by the naked eye was noted, and in succeeding tubes agglutinates were sought for by microscopy. Clumps of 3 to 5 cells were taken as evidence of agglutination. The end-point observed by microscopy was invariably one or two tubes, occasionally three, higher than that noted by the naked eye. Tubes were adequately shaken so as to avoid false positive readings. This precaution is necessary, since if a suspension of red cells in citrate or saline is allowed to settle and is then microscopically examined after inadequate shaking, agglomerates of cells may sometimes be observed. Such agglomerates may be quite large and resemble clumps of agglutinated corpuscles. Too vigorous agitation may, however, eliminate evidence of agglutination in the higher dilutions. Titrations were done in duplicate.

The recipient's serum taken the third day after incompatible transfusion agglutinated the conserved donor cells only to a titre of 1 : 16. Identical results were obtained with fresh cells from the incompatible donor and from a standard A donor (subgroup A₁). Hereafter the titre rose rapidly and attained a peak of 1 : 25,600 on the fifteenth day. The titre fell slowly, and eight months later had not quite approximated the pre-transfusion level (see Fig. 1). In

FIG. 1.—Transfusion of a group B recipient with group A blood. The curve shows the initial fall, rapid steep rise, and slow fall of the anti-A titre.

FIG. 2.—Agglutinates of group A cells surviving in the blood of the group B recipient on the third day after incompatible transfusion.

all titrations the same group A donor was used as standard. It was not possible to obtain blood regularly from the incompatible donor. No haemolysis was detected in the recipient's serum; this is in contrast to the observations of Jervell, Rø, and Mollison.

On the third day after the incompatible transfusion 2 c.cm. of the recipient's blood was mixed with 1 c.cm. of citrate solution and allowed to stand till sedimentation was complete. Using a red cell counting pipette, some red cell sediment was drawn up to the mark 0.5, and then citrate solution up to the 101 mark. After mixing, a

few drops were expelled on to a glass slide and examined with a microscope. Agglutinates consisting of 20 to 30 cells were seen lying free among large numbers of unagglutinated cells and were hardly affected by a strong anti-A serum. The red cell sediment, however, was solidly agglutinated by a strong anti-B serum. These observations were confirmed by Prof. J. B. Duguid, who kindly took the photomicrograph (Fig. 2). Four days after incompatible transfusion only a few small agglutinates were found among the recipient's cells, but none were found on the fifth day.

In an attempt to find an *in vitro* explanation for the absence of symptoms, the following investigations (Tests I to IV) were made about eight months after the incompatible transfusion (Nov. 18).

TEST I.—The Incompatible Donor's Serum (anti-B) was Tested for Potency by Titration against the Cells of the Recipient and Cells of Random Group B Persons

Serum	Cells of Group B Persons	Titre					
		2	4	8	16	32	64
Incompatible group A donor	Recipient 3200 ..	++	++	++	++	+	0
	11623 ..	++	++	++	++	+	0
	11624 ..	++	++	++	++	+	0
	11625 ..	++	++	++	++	+	0
	11626 ..	++	++	++	++	+	0
	11647 ..	++	++	++	++	+	0
	11652 ..	++	++	++	++	+	0

++ = Agglutinates visible to naked eye. + = Agglutinates visible only on microscopy. 0 = No agglutination.

TEST II.—The Recipient's Serum, Diluted 1:10, was Tested for Potency by Titration against the Incompatible Donor's Cells and Cells of Random Group A Persons

Serum	Cells of Group A Persons	Titre							
		20	40	80	160	320	640	1280	
Recipient group B	Incompatible donor	++	++	++	+	?	0	0	0
	Standard donor ..	++	++	++	+	0	0	0	0
	11628 ..	++	++	++	+	0	0	0	0
	11633 ..	++	++	++	+	0	0	0	0
	11635 ..	++	++	++	+	0	0	0	0
	11636 ..	++	++	++	++	+	+	+	+
	11640 ..	++	++	++	+	0	0	0	0
	11646 ..	++	++	++	++	+	0	0	0
	11654 ..	++	++	++	++	+	0	0	0

TEST III.—The Incompatible Donor's Cells (Group A) were Tested for Sensitivity against Sera of Random Group B Persons

Sera of Group B Persons	Cells	Titre								
		2	4	8	16	32	64	128	256	512
3200 ..	Incompatible group A donor	++	++	++	++	++	++	+	0	0
11623 ..		++	++	++	++	++	++	+	0	0
11624 ..		++	++	++	++	++	++	+	0	0
11625 ..		++	++	++	++	++	++	+	0	0
11626 ..		++	++	++	++	++	++	+	0	0
11647 ..		++	++	++	++	++	++	+	0	0
11652 ..		++	++	++	++	++	++	+	0	0

TEST IV.—The Recipient's Cells (Group B) were Tested for Sensitivity against Sera of Random Group A Persons

Sera of Group A Persons	Cells	Titre									
		2	4	8	16	32	64	128	256	512	1024
11628 ..	Recipient, group B	++	++	++	++	+	+	0	0	0	0
11633 ..		++	++	++	++	++	++	+	+	+	+
11635 ..		++	++	++	++	++	++	+	+	0	0
11636 ..		++	++	++	++	++	++	+	+	0	0
11640 ..		++	++	++	++	++	++	+	+	0	0
11646 ..		++	++	++	++	++	++	+	+	0	0
11654 ..		++	++	++	++	++	++	+	+	0	0
35896 ..		++	++	++	++	++	++	+	+	0	0

Whereas the donor's serum agglutinated the recipient's cells only to 32 (see Test I), yet random A sera agglutinated these cells on an average of 128 to 256, the range being 8 to 1024 (see Test IV). Elsdon-Dew (1936) found the anti-B titre of six A sera to be respectively 512, 16, 64, 32, 128, and 64. Aubert, Boorman, Dodd, and Loutit (1942) found that 22.4% of O sera had anti-B titres of 64, and 13.6% a titre of 256; if the dilution factor caused by adding the volume of red cells in the titrations be included, the figures become 128 and 512. Clearly the donor's anti-B titre was comparatively low. Aubert, Boorman, Dodd, and Loutit (1942) transfused conscious group A recipients with O sera of high anti-A titre and did not produce dangerous or fatal reactions. Very high titre sera did, however, cause some destruction of erythrocytes. It seems reasonable to assume that the low titre anti-B iso-agglutinin of the donor in no way harmed the recipient's cells, and absence of

symptoms may in part be attributed to this. For instance, the haemoglobin did not fall, being 60% before and 66% the sixth day after incompatible transfusion. The recipient's anti-A iso-agglutinin was but little weaker than the average (compare Tests II and III). Elsdon-Dew (1936) found the anti-A titre of five B sera to be respectively 2048, 32, 256, 2048, and 64. Aubert, Boorman, Dodd, and Loutit (1942) found 26% of O sera had anti-A titres of 256, and 40% had titres of 512 or higher, though, as noted above, these figures do not include the dilution factor. The red cells of donor and recipient were of average sensitivity (see Tests III and IV). In contrast to the findings of Burnham and of Grove and Crum, absence of symptoms was not attributable to inability of the iso-agglutinins to function at 37° C.

As pointed out by Aubert, Boorman, and Dodd (1942), plasma or serum agglutinin may function as an *in vivo* neutralizer of iso-agglutinins in transfused blood, plasma, or serum. However, Aubert, Boorman, Dodd, and Loutit (1942) observed that even if the recipient's plasma has considerable agglutininogen content, this will not completely protect the recipient's cells from transfused incompatible iso-agglutinins. To assess agglutininogen content of plasma and sera, and therefore their inhibiting power, Aubert, Boorman, and Dodd (1942) devised the "inhibition index" test. Briefly, A or B sera were titrated in saline and the end-point noted. The sera were then mixed in equal proportions and again titrated. Certain sera reduced or inhibited the titre of other sera—an observation also made by Elsdon-Dew (1936). Thus if a titre was reduced from 128 to 16 the inhibition index of the inhibiting serum was expressed as 8. It was indicated that the test was only an approximate guide to the amount of inhibiting substance present. The presence of group-specific substances A and B in solution, partly explains reduced titres of pooled plasma or serum.

Seven months after the incompatible transfusion, when the anti-A titre had approximated the pre-transfusion level, the inhibition indices of the recipient's and donor's sera were investigated. It was 0 for each. The titration technique was that already described. The pooled sera were incubated for one hour at 37° C. In titrating the pooled sera the dilution caused by mixing the sera was taken into account and the titre of the first tube was reckoned as 4 instead of 2. There was thus no *in vitro* evidence that the recipient's plasma protected its erythrocytes from the donor's iso-agglutinins, and vice versa. Presumably the donor's iso-agglutinins were absorbed by the tissues of the recipient. Aubert, Boorman, and Dodd (1942) found no evidence that the agglutininogen substance in transfused serum neutralized the recipient's iso-agglutinins.

Conclusion

In vitro tests did not fully explain absence of symptoms, though the donor's cells were eliminated in five days. Absence of symptoms may be due to the fact that the incompatible blood was injected at a very slow drip rate. Mollison (1943) mentioned that reactions to incompatible transfusion seemed to be less severe than formerly, and that this might be due to the fact that transfusions are now commonly given at a drip rate. The danger of transfusing compatible blood rapidly into chronically anaemic recipients has been previously referred to by me (Drummond, 1943) and by others. If the blood is incompatible the effects are almost certain to be aggravated. It is possible that the favourable outcome in the case here reported is attributable to the very slow rate of injection of the incompatible blood.

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