THE SURVIVAL OF TRANSFUSED ERYTHROCYTES IN HAEMOLYTIC DISEASE OF THE NEWBORN*

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The survival in vivo of erythrocytes after transfusion to infants has apparently never been studied systematically. In fact, apart from cases reported by Jervell (1924) and Wiener (1943a) in which erythrocytes of a theoretically incompatible group were transfused and found to survive for several weeks, the literature does not appear to contain any records of cases in which the differential agglutination method of estimating the survival of transfused erythrocytes has been applied in an infant. It may be mentioned, however, that Lloyd (1941) has reported two cases in which survival was estimated by the method of resolution of Price-Jones curves.

Acquaintance with the survival time of transfused erythrocytes in normal infants is an essential piece of physiological knowledge. Not only does it provide important evidence of the life of the normal erythrocyte but it also supplies a base line for the comparison of survival in pathological conditions. For this reason alone, a study of the survival of transfused erythrocytes in infants seemed desirable. A further impetus was provided by the recent remarkable discoveries of Levine and his co-workers (1941) in connection with haemolytic disease of the newborn, previously known as erythroblastosis foetalis. These workers have postulated that the essential process in haemolytic disease of the foetus and the newborn is the destruction of the foetal erythrocytes by immune agglutinins formed in the mother's serum, in response to stimulation by an antigen contained in the foetal erythrocytes but not present in the maternal erythrocytes. This theory was supported by observations which showed that in approximately 92 per cent. of cases there was an antigenic difference between mother and foetus with respect to the recently discovered Rh factor. That is to say, in the majority of cases the mother was found to be Rh negative and the father and infant Rh positive. In many cases anti-Rh agglutinins could be demonstrated in the mother's serum. Confirmatory observations have been published by Wiener (1942) and by Boorman, Dodd and Mollison (1942). An obvious inference from these observations is that if the affected infant requires transfusion, Rh-negative blood should be given, since the destructive mechanism may only operate against Rh-positive erythrocytes. Levine et al. (1941, 1942)

have mentioned that better results are obtained from the use of Rh-negative blood, and Mollison (1943a) has reported a series of cases in which good results were obtained by using Rh-negative blood for transfusion. Gimson (1943) has also found this treatment strikingly successful. Nevertheless, from observations of red cell counts and haemoglobin values following transfusion, no exact idea of the survival rate of the transfused erythrocytes can be obtained. If the infant's own red cells are being destroyed rapidly at the time of transfusion, the haemoglobin may continue to fall despite transfusion and it is impossible to tell whether the fall is due to destruction of the infant's own erythrocytes or of the donor's erythrocytes or both. Furthermore, if successive transfusions of Rh-positive and Rh-negative blood are given, a better result attending the second transfusion might be due to a spontaneous slowing of the rate of destruction. Only a direct study of the survival rate of transfused erythrocytes of different types can provide the precise knowledge which is desirable.

Quantitative estimation of the survival rate of Rh-positive and Rh-negative erythrocytes in cases of haemolytic disease of the newborn seemed worth while then for the following reasons. Firstly, a demonstration that Rh-positive cells are more rapidly destroyed than Rh-negative cells would provide valuable confirmatory evidence of the correctness of the theory put forward by Levine et alia. Secondly, it should be possible to gain some idea of the length of time after birth for which the active destruction of erythrocytes persists. Thirdly, if the survival rate of Rh-negative cells were found to be constant from one case to the next, suggestive evidence of the life of the transfused erythrocyte in normal newborn infants would be obtained, although this would require to be confirmed by direct study of the normal infant. Fourthly, observation of the difference in survival time between Rh-positive and Rh-negative cells would help to decide how important it is to secure an Rh-negative donor rather than an Rh-positive donor for transfusion in cases of haemolytic disease of the newborn.

The differential agglutination method of estimating the survival of transfused erythrocytes in man was first introduced by Ashby (1919). The method is most simply explained by an example. Blood of group O is transfused to a recipient of

* The substance of these observations was presented in a paper read before the Medical Research Society in October, 1942.

group A. Red cell counts are made before and after transfusion using anti-A serum as a diluent. Whereas before transfusion only a small fraction of cells is unagglutinated, after transfusion the number is increased by the number of group O cells present. Estimations are repeated at intervals until the number of unagglutinated cells has fallen to the pretransfusion level. Unfortunately, when the technique originally suggested by Ashby is used, the number of unagglutinated recipient's cells may often be considerable (say 100,000 per c.mm.). When only 400 to 500 c.c. of donor blood are transfused to an adult, the recipient's unagglutinated cells then form an appreciable proportion of the total number of unagglutinated cells. For this reason the method has been severely criticised and has not been widely used. The method can, however, be very considerably improved. The number of unagglutinated recipient's cells can be reduced by adjusting the strength of the cell suspension used and by employing more potent sera (Jervell) and various other modifications can be made with advantage (Mollison and Young, 1940; Dacie and Mollison, 1943). With these modifications, the number of unagglutinated recipient's cells can often be reduced to 10,000 per c.mm. Moreover, this number is not appreciably increased by agitation of the mixtures. This small number of unagglutinated cells becomes insignificant when large volumes of donor blood are transfused and the method then becomes not only reliable but capable of yielding accurate quantitative results.

One objection to Ashby's original method was that it involved the transfusion of group O blood to recipients of other groups with the attendant possibility of destruction of some of the recipient's erythrocytes by high titre agglutinins in the donor's plasma. This can be overcome to a large extent by removing the bulk of the donor's plasma and using a pooled concentrated red cell suspension prepared from the blood of two donors. If the use of homologous blood is preferred, differentiation can be carried out by means of the anti-M and anti-N sera after choosing a suitable donor (Landsteiner, Levine and Janes, 1928; Wiener, 1934). For instance, blood of group A, type M can be given to a recipient of group A, type N, and the type M cells can be counted in suspensions of blood taken from the recipient after carrying out agglutination with anti-N sera. (The introduction of anti-M and anti-N test sera also made it possible to identify transfused erythrocytes by direct agglutination and some authors have preferred this method. For instance, in the example given above, the type M erythrocytes of the donor could be recognized by being directly agglutinated with anti-M serum in a sample taken from the recipient after transfusion.)

With the discovery of the Rh agglutinin further possibilities of differentiation have become available.

Using the Ashby method, several workers have estimated the survival of transfused erythrocytes in adults. Ashby herself (1919) obtained irregular results which is not surprising in view of the method used. She did, however, find evidence of survival for periods up to 100 days after transfusion. Wearn, Warren and Ames (1922) also found prolonged survival of transfused erythrocytes (average 83 days)

in primary and secondary anaemias. Wiener (1934) using anti-M and -N sera instead of anti-A and anti-B studied the survival in ten 'normal' recipients and found a steady rate of elimination, terminating 80-120 days after transfusion. Dekkers (1939) using a direct differential agglutination method found evidence of survival for an average of 75 days after transfusion, but stated that survival must be longer than the latter figure and might considerably exceed three months. Martinet (1938), using a rather elaborate technique, found evidence of survival for 78 to 108 days after transfusion in six cases. Mollison and Young (1942), using blood stored for periods up to four days in a citrate-glucose mixture, found, in good agreement with Wiener, that the transfused erythrocytes were eliminated at a steady rate and that elimination was not complete until 109 days after transfusion (on the average). Despite the good agreement between workers who have applied the differential agglutination method carefully, it is clear that these results are still not generally known, or if known are not generally accepted as applying to the survival of normal erythrocytes in normal subjects. For instance, in a recent article Baar and Lloyd (1943a) calculated that the life of the erythrocyte was 42 days and made no reference to the fact that this estimate conflicts with the results quoted above.

Cases studied: methods

The survival of transfused erythrocytes was estimated in twenty-one infants affected with haemolytic disease of the newborn of varying degrees of severity. The results of the serological tests made in some of these cases have been reported previously; others are to be reported later (Boorman, Dodd and Mollison, 1942, 1943). One normal newborn infant, one child aged 11 months and one aged 15 months were also studied.

Fresh blood or blood stored for two or three days in citrate-glucose was used in all cases. The amount transfused varied from 50 to 230 c.c. In many cases blood of two serological types (usually Rh positive and Rh negative) was given consecutively or simultaneously, so that the survival of the two types could be directly compared. The transfusions were administered at a drip rate (usually 8 to 15 drops a minute) via the internal saphenous vein. When blood of two different groups was given consecutively a small amount of sodium citrate was run through the cannula intermediately.

Blood samples were obtained by skin prick, using a glass pricker (Wright, 1942), from the warmed heel of the infant, before transfusion, immediately afterwards and then at suitable intervals. In many cases the infant was kept under observation for 4 months or more.

When Rh-positive and Rh-negative bloods were given simultaneously, the following plan was usually adopted. A mixture was prepared from two group O donors, one type N Rh positive, the other type M (or MN) Rh negative. Provided that the infant belonged to type M or MN (as it does in 80 per cent. of cases) and provided that it was Rh positive (as it is in almost every case of haemolytic disease), differentiation of the two types of donor blood could then be carried out with anti-M and anti-Rh sera respectively, for with anti-M serum all but the

type N Rh positive cells would be agglutinated and with anti-Rh serum all but the type M Rh negative cells. Agglutination tests were always carried out on a pre-transfusion sample so that an estimate of the recipient's own unagglutinated cells could be made. In using anti-A, -B or -Rh sera the centrifuge technique described by Dacie and Mollison was used and with anti-M and anti-N sera the technique described by Mollison and Young (1940) was used.

Because of the large amounts of blood used for transfusion the initial concentration of donor cells in the recipient's blood stream usually ranged from 1 to 4 million per c.mm. (average 2.2 million) and was only below 1 million in two instances and above 4 million in two others. Thus survival could be estimated satisfactorily even when the number of unagglutinated cells found before transfusion was as high as 100,000 per c.mm., as it was in a few cases when anti-A or anti-B sera were used. It may be added that in the majority of cases the number of unagglutinated cells attributable to the recipient was below 50,000 per c.mm. As Wiener (1943) has pointed out, the M, N and Rh reactions are more satisfactory in newborn infants, since these agglutinogens, unlike the A and B agglutinogens, appear to be fully developed at birth.

In expressing the results, it is clearly desirable to take as 100 per cent. survival the maximum concentration of donor cells reached in the recipient's circulation after transfusion. In cases in which there was no rapid destruction of the transfused erythrocytes, it was found, as in adults (Mollison and Young, 1942), that the concentration of donor cells was greater in a sample taken twenty-four hours after transfusion than in one taken immediately after transfusion, presumably due to blood volume changes and in several cases the concentration of donor cells in a sample taken later still after transfusion was even greater. This is perhaps not a surprising observation considering the large volume of many of the transfusions in relation to the size of the infant. For the sake of accuracy it must be added that when the maximum concentration of donor cells is not reached for twenty-four hours or more after transfusion, that this concentration will be slightly lower than the true 100 per cent. because of the small amount of destruction that must occur during this initial period of stabilization of blood volume.

In cases in which destruction of the transfused erythrocytes was rapid, the sample taken at twenty-four hours usually contained a considerably smaller concentration of donor cells than that taken immediately after transfusion. Clearly if destruction is rapid, even a sample taken immediately after transfusion may only contain a proportion of the transfused cells, but in such cases the accurate quantitative estimation of survival will be less important.

Below, the maximum concentration of donor cells found after transfusion has been taken as 100 per cent. in all cases and subsequent counts have been expressed as percentages of this figure.

Results

Cases 1-19. In all these cases a diagnosis of icterus gravis neonatorum was made from the presence of jaundice, anaemia and erythroblastæmia. All these infants were Rh positive, all the mothers

were Rh negative and in every case the mother's serum contained anti-Rh agglutinins.

SURVIVAL OF RH-POSITIVE BLOOD (table 1). The survival of Rh-positive cells was estimated in ten of these infants, receiving a total of thirteen transfusions of Rh-positive blood (in one instance the group of the donor blood was not determined, see below). Of these thirteen transfusions, nine were given when the infant was fourteen days old or less and in these nine elimination was complete by the end of ten days after transfusion in all but one case (within three days of transfusion in three instances). In the ninth case, elimination was not complete until approximately thirty days after transfusion. Of the remaining four transfusions of Rh-positive blood, two were given to infants (1 and 11) who had previously received Rh-positive blood and had eliminated it rapidly. In each of these cases the donor cells were eliminated less rapidly on the second occasion (see case 1, fig. 1, for instance). The last two transfusions were given to infants aged 31 and 35 days respectively and in these cases survival was prolonged.

FIG. 1.—Survival of four consecutive transfusions given to an infant (case 1) affected with icterus gravis neonatorum. Second and third transfusions and probably first, Rh positive; fourth transfusion Rh negative.

Case 1 was of special interest because it received repeated transfusions and because it was studied before the first paper by Levine et alia on the relationship between iso-immunisation and the causation of haemolytic disease of the newborn had become available. Apart from the four transfusions of group O blood whose survivals were estimated (see table 1 and fig. 1) a transfusion of group A blood was given after the second transfusion of group O blood. When the work of Levine and his colleagues was published, all the donors except the first of the five were traced and their Rh groups were determined. It was found that the second and third group O donors were Rh positive, whereas

TABLE 1

SURVIVAL OF RH-POSITIVE AND RH-NEGATIVE ERYTHROCYTES AFTER TRANSFUSION

Case No.	Groups of Infant Mother		Age at time of transfusion (days)	Rh-positive transfusions per cent. survival at				Rh-negative transfusions per cent. survival at					
				Amount given c.c. (ABO group)	7 days	30 days	60 days	Amount given c.c. (ABO group)	7 days	30 days	60 days	Per cent survival at 75 days or more	
<i>Cases of Icterus Gravis. Mother Rh negative ; Infant Rh positive. Anti-Rh in Mother's Serum</i>													
I	ARh+	ARh-	3	1.50 (O)	0	(12 per cent. at 2 days)							
			6	2.160 (O)	20	0	—	—	—	—	—		
			43	3.155 (O)	72	0	—	—	—	—	—		
II	ORh+	ORh-	65	—	—	—	—	4.120 (O)	87	64	46	6 at 100 days.	
			9	—	—	—	—	1.120 (O)	92	71	54	20 at 88 days.	
			10	2.100 (O)	61	2	0	—	—	—	—		
III	ORh+	ARh-	8	—	—	—	—	95 (O)	100	80	36	16 at 86 days.	
IV	ORh+	ORh-	2	—	—	—	—	1.120 (O)	88	50	—		
V	ARh+	ARh-	35	2.50 (O)	90	26	0	2.50 (O)	—	—	—	30 at 76 days.	
			11	1.200 (A)	0	0	—	—	—	—	—		
			14	—	—	—	—	2.200 (O)	77	—	—		
VI	ARh+	ARh-	14	1.50 (O)	0	(39 per cent. at 24 hours)							
			21	—	—	—	—	1.90 (A)	77	60	28	3 at 98 days.	
			21	—	—	—	—	2.125 (O)	99	48	23	8 at 91 days.	
VII	ARh+	ARh-	3	—	—	—	—	150 (O)	—	54	—		
			14	1.60 (O)	0	(at 3 days)		1.60 (O)	100	(at 3 days)			
			17	—	—	—	—	2.200 (O)	98	73	37	22 at 80 days.	
IX	ARh+	ARh-	20	—	—	—	—	3.200 (O)	98	78	50		
			3	—	—	—	—	120 (O)	88	78	50		
			X	ARh+	ABRh-	5	50 (O)	5	0	—	—	—	—
XI	ARh+	ARh-	9	1.90 (O)	12	0	—	1.90 (O)	91	—	—		
			17	2.100 (O)	79	60	26	2.100 (O)	84	65	29	15 at 83 days.	
			31	60 (O)	65	49	29	120 (O)	90	67	30		
XII	ORh+	ORh-	5	1.50 (O)	3	0	—	1.170 (A)	28	—	—		
XIII	ARh+	ARh-	14	—	—	—	—	2.200 (O)	83	66	28		
			18	—	—	—	—	90 (O)	92	73	40	11 at 87 days.	
			13	—	—	—	—	300 (O)	100	74	40	2 at 110 days.	
XIV	ORh+	ORh-	15	—	—	—	—	100 (O)	96	64	28		
XV	ORh+	ARh-	18	—	—	—	—	200 (O)	—	—	—	6 at 95 days.	
XVI	ARh+	ARh-	20	—	—	—	—	210 (O)	91	65	34	19 at 78 days.	
XVII	ORh+	ORh-	11	—	—	—	—	120 (O)	—	—	—	7 at 97 days.	
XVIII	ORh+	ORh-											
XIX	ORh+	ORh-											
<i>Case of Icterus Gravis. Mother and Infant ARh+. Anti-Rh₂ agglutinin in Mother's Serum</i>													
XX	ARh+	ARh+	13	60 (A)	73	24.5	—	60 (A)	97	80	—		
					(9 per cent. at 38 days)		(75 per cent. at 38 days)						
<i>Case of Congenital Anaemia of the Newborn. Mother Rh negative ; Infant Rh positive. Anti-Rh in Mother's Serum</i>													
XXI	ARh+	ARh-	4	1.115 (O)	94	62	49	2.100 (A)	91	69	40	36.5 at 77 days.	
<i>Case of acute haemorrhage from the cord in a newborn normal infant</i>													
XXII	ARh+	ORh+	few hours	110 (O)	—	—	—	—	—	76	47	10 at 107 days.	
<i>Case of Nutritional Anaemia in a child aged 12 months</i>													
XXIII	ORh+	ORh+	—	120 (O)	95	73	38	120 (O)	96	79	44	35 at 76 days.	
<i>Case of Aplastic Anaemia in a child aged 15 months</i>													
XXIV	ARh+	ARh+	—	200 (O)	91	68	40	—	—	—	—	16 at 100 days.	

the fourth was Rh negative. Because the first transfusion of group O blood was eliminated even more rapidly than the second, and because in any case Rh positive persons are five times as common as Rh negatives, it is very probable that this first donor was also Rh positive. The group A donor was found to be Rh negative and it was interesting

to note from the records that a far more sustained rise in haemoglobin followed this transfusion than either the preceding or following Rh-positive transfusions. Moreover, the jaundice which had remained extreme up to the time of this transfusion completely disappeared during the following three days.

The gradually increasing survival rate of the erythrocytes of successive Rh-positive transfusions is of interest, but it should be noted that Rh-positive erythrocytes were still being eliminated rapidly in this case 43 days after birth.

It will be observed that in the other case receiving successive transfusions of Rh-positive blood (case 11), although the erythrocytes of the first transfusion (given nine days after birth) were rapidly eliminated, those of the second transfusion, given on the 17th day of life, survived very well. The protocol of the tests made in connexion with the first transfusion is set out in table 2.

TABLE 2

SIMULTANEOUS ESTIMATION OF THE SURVIVAL OF RH-POSITIVE AND RH-NEGATIVE ERYTHROCYTES IN A CASE OF ICTERUS GRAVIS NEONATORUM

(Protocol of Experiment in Case 11, First Transfusion)

DONOR BLOOD

Mixture prepared from citrated blood of 2 donors :

(1) OMN Rh⁻, (2) ON Rh⁺ : approximately 3 parts (1) added to 2 parts (2)

Control analysis of mixture by agglutination with (1) anti-Rh and (2) anti-M serum

(1) with anti-Rh serum, 3,870,000 free cells per c.mm. (group OMN Rh⁻ cells)

(2) with anti-M serum, 2,690,000 free cells per c.mm. (group ON Rh⁺ cells)

i.e. mixture contains approximately 3 Rh⁻ erythrocytes to 2 Rh⁺ erythrocytes

RECIPIENT'S BLOOD GROUP : OM Rh⁺

	Count of free cells after agglutination with :			
	(1) anti-Rh serum		(2) anti-M serum	
	Total No. of unagglutinated cells	No. of OMN Rh ⁻ cells	Total No. of unagglutinated cells	No. of ON Rh ⁺ cells
Before transfusion	98,000	—	3,000	—
Immediately after transfusion	1,956,000	1,858	1,117,000	1,114,000
1st day after transfusion	(not estimated)		923,000	920,000
4 days after transfusion	2,351,000	2,253,000	277,000	274,000
7 days after transfusion	2,128,000	2,030,000	132,000	129,000
8 days after transfusion	(not estimated)		69,000	66,000

Note that ratio of $\frac{\text{Rh}^-}{\text{Rh}^+}$ donor erythrocytes in infant's circulation immediately after transfusion approximates quite closely to the 3/2 ratio expected from an analysis of the donor mixture.

It may be mentioned that in one case (case 6) the number of surviving Rh-positive cells found in a sample taken a few hours after transfusion was much smaller (approximately 300,000 per c.mm.) than that expected (approximately 800,000 per

c.mm.) compared with the number of Rh-negative cells. Evidently, therefore, a proportion of them had already been destroyed.

SURVIVAL OF RH-NEGATIVE CELLS (table 1 and fig. 2). In eight instances a transfusion of Rh-negative blood was given at the same time as (or within three days of) a transfusion of Rh-positive blood so that the survival of the two types could be directly compared (see table 2, for instance). In all except one of these cases (case 13) prolonged survival of the Rh-negative blood was observed, and even in this exceptional case the survival of the Rh-negative cells was distinctly longer than that of the Rh-positive cells transfused simultaneously (see fig. 3). The survival of a subsequent transfusion of Rh-negative erythrocytes given to this same infant was only slightly below the average for the other cases. In fourteen other instances the survival of transfused Rh-negative cells was estimated. Excluding the first transfusion given to case 13, the average survival rate of Rh-negative cells seven days after transfusion in the eighteen cases in which an estimate was made, was 90 per cent. In sixteen cases survival was estimated thirty days after transfusion and the average figure was found to be 66 per cent. In fourteen cases in which estimations were made at sixty days, the average figure was 36 per cent. In three other cases no intermediate observations were made but an appreciable number of donor cells was recognized in the recipient's circulation 76-97 days after transfusion. In the case of these figures and the figures in table 1 for survival at 75 days or more after transfusion, survival has only been considered 'appreciable' when the concentration of unagglutinated cells was at least 100,000 per c.mm. greater than the concentration found in a later sample.

Reference to fig. 2 shows that in the majority of cases the survival of the Rh-negative cells was approximately proportional to the time since transfusion, i.e. the curve of elimination, was linear.

In at least two instances, however (case 6, first and second Rh-negative transfusions), the slope of elimination was curved, that is to say, the rate of elimination was at first rapid but then slowed progressively as the concentration of surviving erythrocytes diminished. In three other instances (cases 4, 5 and 7) the initial rate of destruction was distinctly greater than the rate observed in the rest of the cases, but survival was not followed to completion. In case 13, elimination of the erythrocytes of the first Rh-negative transfusion was both rapid and linear.

In a few cases it was observed that a sample taken from the infant a few days after a large Rh-negative transfusion consisted almost exclusively of donor erythrocytes. For instance, in case 8 (A Rh positive) three days after the third transfusion (of Rh-negative blood), the number of O Rh-negative erythrocytes was estimated as 3,940,000 and 3,840,000 with anti-A and anti-Rh serum respectively (allowing for the small number of unagglutinated recipient's erythrocytes), whereas the total count (of O Rh-negative plus A Rh-positive erythro-

FIG. 2.—Survival of Rh-negative erythrocytes after transfusion in 14 cases of icterus gravis neonatorum (19 separate transfusions)

cytes) was only 4,110,000. The mixtures with anti-A and anti-Rh serum both contained a few small clumps confirming that there were still a small number of A Rh-positive cells present. In case 5 (A Rh positive) a sample taken from the infant twelve days after the O Rh-negative transfusion appeared to contain no A cells at all on testing with anti-A serum.

Case 20. In this case of 'icterus gravis,' although the mother, father and infant were all group A Rh positive, the mother's serum contained an agglutinin active at 37° C. which acted upon the father's and infant's cells and upon approximately 30 per cent. of group O bloods (almost all Rh positive). The serological findings are being described more fully elsewhere* (Boorman, Dodd and Mollison, 1943). This infant was given a transfusion consisting of a mixture of two group A bloods, one of which was agglutinated by the mother's serum and the other of which was compatible with the mother's serum. For convenience, the results have been tabulated as if the first transfusion had been Rh positive and the second Rh negative (see table 1). As shown in the table, an estimate thirty-eight days after transfusion showed that only 9 per cent. of the 'incompatible'

cells contrasted with 75 per cent. of the 'compatible cells' were surviving.

Case 21. This infant was never jaundiced, but was very anaemic from the time of birth and a diagnosis of 'congenital anaemia of the newborn' was made; the serological findings were the same as in cases 1-19. As shown in table 1, Rh-positive and Rh-negative erythrocytes transfused on the fourth and fifth days of life respectively both survived for long periods in the infant's circulation.

Case 22. This normal, full-term infant suffered an acute haemorrhage within thirty minutes of birth when the ligature slipped off the umbilical cord. A transfusion was given approximately two hours later. As shown in fig. 4, the survival rate of the transfused erythrocytes was similar to that observed in cases 21, 23 and 24 and survival in this group was indistinguishable from that found in a group of adult recipients (see table 3).

Case 23. This infant, aged eleven months at the time of transfusion, had a history of pallor since the age of two weeks. A blood examination showed: R.B.C. 3,610,000; Hb, 37 per cent.; C.I. 0.5. A blood film showed many megaloblasts, occasional normoblasts and punctate basophilia, polychromasia, poikilocytosis and anisocytosis. A diagnosis of nutritional anaemia was made. A mixture of

* This agglutinin, first called *k*, has been identified as anti-Rh₂; see Wiener, 1943b and Race et al. 1943.

FIG. 3.—Survival of Rh-negative and Rh-positive erythrocytes given simultaneously to a severe case of icterus gravis neonatorum on the fifth day of life. Note rapid destruction of Rh— blood.

TABLE 3

A COMPARISON OF THE SURVIVAL RATE OF TRANSFUSED ERYTHROCYTES (1) IN A GROUP OF 14 ADULTS, (2) IN A GROUP OF 4 INFANTS (AGES : FEW HOURS, 4 DAYS, 12 MONTHS AND 15 MONTHS RESPECTIVELY) NOT AFFECTED WITH ICTERUS GRAVIS NEONATORUM, AND (3) IN A GROUP OF 16 INFANTS AFFECTED WITH ICTERUS GRAVIS NEONATORUM AND TRANSFUSED WITH RH-NEGATIVE BLOOD

Cases	Per cent. survival at		
	7 days	30 days	60 days
(1) Adults	94.4	70.5	41.8
(2) Miscellaneous infants	93.4	71.2	43.0
(3) Infants with icterus gravis (Rh-negative erythrocytes transfused)	90.0	66.0	36.0

FIG. 4.—Survival of transfused erythrocytes in four infants not affected with icterus gravis neonatorum (cases 21, 22, 23 and 24, see table 1). In cases 21 and 23 Rh-positive and Rh-negative erythrocytes were transfused simultaneously and the survival of the two types is shown.

Rh-positive and Rh-negative erythrocytes was transfused and survival of the two types estimated as a control on the above cases. No appreciable difference between the two was observed and the rate of elimination was approximately 1 per cent. per diem.

Case 24. This child, aged fifteen months, had received many previous transfusions after very full investigations (including marrow biopsy) had established the diagnosis of aplastic anaemia. Again the rate of elimination was approximately 1 per cent. each day.

Clinical observations. It has already been mentioned that in case 1 the jaundice appeared to diminish rapidly after an Rh-negative transfusion. This occurred in case 9 also. This case merits a brief description because of the favourable response that occurred as a result of an early Rh-negative transfusion.

The mother of this infant had had two previous children. The first was healthy at birth but died later from intercurrent disease. The second child died at the age of twelve days from icterus gravis neonatorum. The present infant became jaundiced within forty-eight hours of birth and was found to have a blood picture characteristic of haemolytic disease of the newborn. A transfusion of 120 c.c. of Rh-negative blood was given on the fourth day of life. By the next day the jaundice had faded and it had practically disappeared within forty-eight hours of transfusion. Presumably the filling of the circulation with Rh-negative erythrocytes diminishes the rate of production and, therefore, the rate of destruction of Rh-positive erythrocytes. Although this infant required a further transfusion later, it never looked ill after the first transfusion, and in view of the previous family history it appears that the early transfusion of Rh-negative blood may possibly have been life-saving. The value of this form of treatment is again discussed below.

Discussion

Survival of Rh-positive erythrocytes. It has been shown that during the first fourteen days of life in the majority of cases of 'icterus gravis neonatorum' Rh-positive erythrocytes are far more rapidly destroyed in the circulation than are Rh-negative erythrocytes. Of the cases transfused later than the fourteenth day of life the rate of destruction of Rh-positive erythrocytes was found to have become as slow as that of Rh-negative erythrocytes by the seventeenth day in one case, having been far more rapid between the ninth and sixteenth days; in another case it was found to be still rapid between the forty-third and fifty-eighth days after birth. In two other cases transfused on the thirty-first and thirty-fifth days respectively, survival was distinctly shorter than that of Rh-negative blood in one case, but equally long in the other. In the one case of 'congenital anaemia of the newborn' (case 21) Rh-positive erythrocytes transfused on the fifth day of life were not destroyed any more rapidly than Rh-negative erythrocytes.

It appears from fig. 1 and 3 that when destruction is rapid the slope of elimination is approximately linear as it is in most of the cases in which destruction only occurs slowly, although there are scarcely sufficient points on these graphs to settle the question decisively. Reference to the protocol of case 11 (table 2) suggests that in this case the rate of elimination of the Rh-positive erythrocytes of the first transfusion was proportional to their concentration. For example, the count of donor cells fell from 1,114,000 to 274,000 in the first four days, but only from 274,000 to 66,000 in the next four days. In this case, however, Rh-positive erythrocytes given immediately afterwards in the second transfusion were found to be destroyed slowly, so that a spontaneous slowing of the rate of destruction may have been responsible for the change in rate observed during the elimination of the first transfusion. In haemolytic anaemias in adults it can often be demonstrated that the slope of elimination is not linear but curved, the rate of destruction of transfused erythrocytes being more rapid at first and then becoming slower as the concentration of surviving cells diminishes: types of haemolytic anaemia in which the slope of elimination is linear have, however, been encountered (Brown, Hayward, Powell and Witts, 1943; Mollison, 1943b).

Correlation of the survival time of Rh-positive cells with other features of the cases. In attempting to correlate the rate of destruction of transfused erythrocytes with other criteria of severity, one is faced with the difficulty of obtaining any reliable guide to the latter from clinical or pathological data. For instance, the time of onset of the jaundice is usually regarded as some guide to the severity. Case 6, however, which appeared very ill, became very intensely jaundiced and developed the syndrome of kernicterus, did not become jaundiced until the third day of life, whereas the remaining cases all became jaundiced within the

first twenty-four hours of life. In this case the clinical impression of severity was supported by the observation of a short survival time of Rh-positive erythrocytes. Similarly, the degree of anaemia seems quite unreliable as a guide. For instance, in the case just quoted, the haemoglobin had only fallen to 76 per cent. by the fourteenth day. As a contrast, case 21 may be quoted in which the haemoglobin had fallen to 50 per cent. by the fourth day and yet in which an approximately 'normal' survival of Rh-positive erythrocytes was observed. Case 20 is of interest in this connexion. This infant was only moderately jaundiced from the first day of life onwards and the jaundice had practically disappeared by the ninth day of life. The haemoglobin was then 86 per cent., although another estimation on the eleventh day gave a value of 72 per cent. The blood smear showed no nucleated red cells. From the clinical point of view the diagnosis appeared to be doubtful. However, as mentioned above, the mother's serum was found to contain an atypical agglutinin incompatible with the foetal erythrocytes. Moreover, donor blood, also incompatible with the mother's serum, was eliminated quite rapidly as compared with blood that was compatible with the mother's serum. In this case, as in several others in the series, although the haemoglobin had only fallen to approximately 70 per cent. by the tenth day of life, it was found by about the third or fourth week after birth to be as low as 50 to 60 per cent. despite transfusion.

The degree of erythroblastemia is notoriously unreliable as a guide to severity (Parsons, Hawksley and Gittins, 1933). Case 13, for example, one of the most severe in this series from the clinical point of view, had practically no nucleated red cells in the peripheral blood from the fifth day onwards.

It might be expected that some correlation would be found between the titre of anti-Rh agglutinins in the mother's serum and the rate of destruction of Rh-positive erythrocytes and in fact the highest titres were found in the sera of the mothers of cases 6 and 8 in whose infants the most rapid destruction was observed. This correlation, however, was not found in other cases.

Survival of Rh-negative erythrocytes. As shown in table 3, the survival of Rh-negative cells in the majority of cases investigated is but little different on the one hand from that observed in a small group of infants not affected with icterus gravis neonatorum, and on the other from that found in a group of adults. The fact that the survival rate of transfused erythrocytes in infants appears to be similar to the rate observed in adults does not necessarily imply that the life of the normal erythrocyte is the same in the two, because in each case erythrocytes from adults were transfused and if erythrocytes taken from infants had been transfused instead the results might have been different.

The case (case 13) in which a rapid destruction of Rh-negative cells was found deserves further comment. This infant became jaundiced within a few

hours of birth. It was first seen on the fourth day of life, by which time it had become intensely jaundiced, and it was noted that its stools were pale and its urine dark. The haemoglobin was found to be 76 per cent. and a blood film showed the presence of a few nucleated red cells. The infant's group was A Rh positive, that of its mother A Rh negative and her serum contained weak anti-Rh agglutinins. On the next day a transfusion of 50 c.c. group O Rh-positive blood was followed by 170 c.c. of group A Rh-negative blood, a small volume of sodium citrate solution being run in between the two to prevent their mixing outside the body. As noted above, a rapid destruction of the group O Rh-positive cells was observed and the destruction of the group A Rh-negative cells was only slightly less rapid.

The diminished survival of the Rh-negative cells in this case might be ascribed to at least three possible causes. (1) Some abnormality of the donor blood. To this it can only be answered that the donor appeared to be normal and that, in a series of survival tests carried out in adults, no similar rapid destruction of cells from an apparently normal donor has been observed. (2) The A cells might have been sensitized by high titre agglutinins introduced in the plasma of the group O blood. The titre of the anti-A agglutinins was determined, however, and found to be only moderate. Moreover, only a small amount of group O blood was transfused. Even after the injection of high titre incompatible agglutinins it is usually impossible to detect them in plasma taken from the opposite arm (Thalhimer 1942). Moreover, in other cases in which this type of experiment has been carried out a similar rapid destruction of the A cells has never been observed. (3) The A 'Rh-negative' cells may have been 'Rh-positive' with the mother's serum. It is well known now that some 'Rh-positive' bloods react with some anti-Rh sera but not with others. The donor blood was, however, tested with seventeen different anti-Rh sera and, more important, with the mother's serum on different occasions and was always negative.

Thus, it seems probable that this infant had the capacity of destroying Rh-negative erythrocytes almost as rapidly as Rh-positive erythrocytes. As noted above, in four other cases the initial destruction of Rh-negative erythrocytes was distinctly more rapid than that observed in the majority of cases. It may be significant that in these cases the transfusion had either been given within the first three days of life (cases 4 and 7), when the destruction of Rh-positive cells was probably proceeding very rapidly, or had been given to an infant in which the destruction of Rh-positive cells is known to have been proceeding very rapidly at the time of transfusion (cases 5 and 6). In case it may appear that the figures from which these deductions are drawn are not outside the limits of experimental error, those for case 5 may be quoted. In this case, the concentration of donor cells immediately after transfusion was 4.8 million per c.mm.; twelve days later, the figure had fallen to 2.9 million per c.mm. If elimination had occurred at the rate of only 1 per cent. per day, as it

appeared to in many of the other cases, the count of donor cells on the twelfth day should have been approximately 4.4 million per c.mm. Since the error of this method is similar to that of ordinary blood counting, provided that satisfactory agglutination is secured, it is clear that the figure of 2.9 million is outside the likely error and, therefore, that the rate of destruction must have been greater than 1 per cent. per day in this case.

It thus appears that in some cases, probably those in which the destruction of Rh-positive cells is proceeding very rapidly, that there may be some overlap in the destructive mechanism, so that Rh-negative cells, though not destroyed as rapidly as Rh-positive cells, are destroyed more rapidly than they are in the circulation of the normal infant.

Nevertheless, it seems clear that in the majority of cases Rh-positive erythrocytes are far more rapidly destroyed than Rh-negative erythrocytes in cases of haemolytic disease of the newborn, and thus the theory of destruction by immune specific agglutinins is strongly supported. The observations in case 20 are particularly interesting in demonstrating that it is not Rh-positive erythrocytes as such which are destroyed rapidly, but erythrocytes containing the antigen to which the mother has become sensitized.

Value of transfusions of Rh-negative blood. In an earlier report (Mollison, 1943a) the results of treating cases of haemolytic disease of the newborn with transfusions of Rh-negative blood were referred to and it was pointed out that the favourable recovery rate (seventeen out of seventeen cases transfused) exaggerated to some extent the value of this form of treatment, because the infants transfused included many mild cases that might have recovered without transfusion, whereas the infants that had not been transfused and that died before a transfusion could be given (ten out of ten) included some more severe cases that transfusion might not have saved. Although the value of this form of treatment in the moderately severe cases cannot be doubted, an estimate of its power to save life in the seriously ill cases, that would otherwise die within a few days of birth, will only be possible when the results of treating a large series of the latter are available. As mentioned above, the amount of Rh-positive erythrocytes produced is probably diminished by massive Rh-negative transfusions and this doubtless accounts for the rapid disappearance of jaundice observed in some of the cases. On theoretical grounds, early massive Rh-negative transfusions might, therefore, be expected to lower the incidence of kernicterus.

Further observations. It may be mentioned that variations in the haemoglobin level between one infant and another before and after transfusion did not appear to affect the survival time of the (Rh-negative) erythrocytes.

For instance, in case 15, the red cell count rose to 6,530,000 per c.mm. (Hb 136 per cent.) after

transfusion and six days after transfusion the figures were 6,440,000 per c.mm. and 128 per cent. Nevertheless, the survival time was just as long as in case 14 for instance, in which the haemoglobin never rose above 98 per cent. In two other instances no increased destruction was observed, although the haemoglobin was raised above 110 per cent., namely, case 21 in which the haemoglobin was 130 per cent. one day after transfusion, 118 per cent. six days after and 110 per cent. at thirteen days and case 11, in which the haemoglobin was 114 per cent. after transfusion, 118 per cent. at six days and 110 per cent. at thirteen days.

The observation that no increased rate of destruction occurs when the infant is rendered plethoric (Hb greater than 110 per cent., Haldane) is of some interest in view of the generally accepted theory of physiological jaundice, which is that jaundice results from a physiological process designed to destroy the excessive red cells which, though needed in foetal life, are unwanted after birth. If this theory were correct, it would be expected that an increased destruction would be observed when the haemoglobin was raised above, say, 110 per cent. The following case may be quoted since it demonstrates even more forcibly than the above cases that the infant may tolerate a high haemoglobin level.

A woman who had previously given birth to an infant with icterus gravis neonatorum was delivered of a full-term infant which appeared normal at birth. Nevertheless, as a precaution it was decided to start a drip transfusion of Rh-negative blood while the infant's blood was taken to a laboratory for examination. When it was found that the infant was Rh-negative and evidently normal, the transfusion was stopped, some 80 c.c. having been given. The haemoglobin which had been 134 per cent. before transfusion was raised to over 150 per cent two days later. Ten days after birth the haemoglobin was 134 per cent. These haemoglobin estimations were carried out with a Haldane haemoglobinometer standardized at the National Physical Laboratory. During these ten days the infant never exhibited the slightest tinge of jaundice.

It is difficult to reconcile these findings with the existence in the normal infant of an active destructive mechanism.

Principle of selection of blood donors for infants affected with haemolytic disease of the newborn

When there is time to carry out serological tests upon the mother's and infant's bloods before choosing a donor, it is clear that the principle should be to discover the nature of the immune agglutinin causing the destruction of the foetal erythrocytes and then to select a donor whose red cells lack the corresponding agglutinin. In most cases this will mean selecting an Rh-negative donor of a compatible ABO group.

Anti-Rh agglutinins occurring in human sera do not give exactly parallel reactions *in vitro*; in fact it is now apparent that several different varieties occur, evidently corresponding to different subtypes of Rh agglutinin. For this reason, and because

there are certain other difficulties in Rh testing, the erythrocytes of prospective donors should always be tested against more than one powerful anti-Rh serum and at least one of these sera should be of the type that gives the lowest proportion of negative reactions with random human bloods. The most reliable test of all, when the mother's serum contains anti-Rh agglutinins which give good reactions *in vitro*, is to test the donor's erythrocytes directly against her serum. This direct test becomes essential when the mother's serum contains atypical anti-Rh agglutinins (as in case 20 in this series) or when her serum contains atypical agglutinins unrelated to anti-Rh.

It may be pointed out that in newborn infants, testing of the donor's erythrocytes against the mother's serum is a more desirable precaution as a preliminary to blood transfusion than is direct testing against the infant's serum. This is because for some time after birth the infant's serum only contains agglutinins derived from the mother's serum (see Polayes, Lederer and Wiener, 1929) and because the titre of these agglutinins is on the average about ten times lower in the infant's serum (Wiener and Silverman, 1940). This method of selection, if employed alone, would sometimes lead to the choice of a donor of theoretically incompatible group. For instance, when the mother is group A and the infant O, an A donor would be compatible with the mother's serum, although theoretically incompatible with the infant's serum. However, there is reason to believe that in such cases the donor erythrocytes survive for long periods in the infant's circulation, for at least six weeks in one case (Jervell, 1924) and at least fifty days in another (Wiener, 1943). At the same time it is probably sounder to transfuse blood of a theoretically compatible group until more exact knowledge of the time of development of iso-agglutinins in the infant has been acquired.

For the reasons stated in the preceding paragraph, the use of the mother's erythrocytes, washed free from plasma (Lloyd, 1943), has much to commend it from the theoretical point of view. It is doubtful, however, whether this will often prove the most convenient procedure in practice.

The following case is a reminder that Rh-negative blood of the same group as the infant is not always the most suitable for transfusion.

Infant H., a firstborn male, became jaundiced within a few hours of birth and developed a definite anaemia by the end of the first week of life. It was found that the infant's and father's blood group was B Rh positive, whereas the mother was group O Rh positive and her serum contained tremendously potent immune anti-B agglutinins but no atypical agglutinins. In this case, therefore, it seemed extremely probable that destruction of foetal erythrocytes by anti-B agglutinins was occurring and that group B cells, whatever their Rh group, would be eliminated more rapidly than group O cells. Accordingly, group O blood was used for transfusion—with satisfactory results. This case

again demonstrates the wisdom of testing the donor's erythrocytes against the mother's serum.

In emergency, and when it is impossible to carry out serological tests, group O Rh-negative donors will be found suitable in the great majority of cases. It may be noted, however, that the causation of haemolytic disease of the newborn by an atypical agglutinin Hr acting for preference on Rh-negative bloods has been described (Levine et alia, 1942)* so that in exceptional cases Rh-negative blood may prove unsuitable. However, if the donor cells are tested against the mother's serum, such exceptional cases should be detected.

Summary

(1) In eight out of nine cases of 'icterus gravis neonatorum' transfused during the first fourteen days of life Rh-positive erythrocytes were eliminated from the infant's circulation within ten days of the transfusion. In the ninth case elimination was not complete until thirty days after transfusion. In one further case, in which the destruction of the foetal erythrocytes was due to an atypical anti-Rh agglutinin, erythrocytes containing the corresponding agglutino-gen were destroyed rapidly.

In four cases in which Rh-positive erythrocytes were transfused after the fourteenth day of life, destruction was less rapid and in two instances was as slow as that of Rh-negative erythrocytes in two instances.

(2) Rh-negative erythrocytes transfused to twenty-one infants with haemolytic disease survived for not less than approximately eighty days in all but one case. In the majority of the cases the rate of elimination appeared to be uniform, so that approximately 1 per cent. of the erythrocytes was eliminated each day and the total time of survival appeared to be approximately one hundred days. In five instances, however, the initial rate of destruction appeared to be distinctly greater and in the one exceptional case mentioned above only 28 per cent. of the donor cells were found to be surviving seven days after transfusion. Because of the absence of any obvious cause for this more rapid destruction in certain cases, it seems possible that there may be some overlap in the destructive mechanism in this condition, that is to say, it may not always be directed exclusively at Rh-positive erythrocytes.

(3) In one normal infant transfused within a few hours of birth, in one infant aged eleven months and in one aged fifteen months, the survival of transfused erythrocytes was estimated and found to correspond closely with that of Rh-negative erythrocytes in the majority of cases of haemolytic disease of the newborn. This rate of survival is similar to that which has been found in adults, i.e. approximately 1 per cent. of the total number of transfused erythrocytes are eliminated from the recipient's circulation each day after transfusion. In all these experiments blood from adult donors was used for transfusions.

(4) When an infant with haemolytic disease of the newborn has to be transfused, Rh-negative blood of its own group is recommended when serological tests have been made and it is fairly certain that destruction is due to anti-Rh agglutinins. Group O, Rh-negative blood is recommended when no tests have been made and group O blood should be used when destruction is thought to be due to anti-A or anti-B agglutinins. When destruction is due to agglutinins other than atypical anti-Rh, group O blood compatible with the mother's serum is probably the safest. In every case direct matching of the donor erythrocytes against the mother's serum seems a desirable precaution before transfusion.

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* See also Race and Taylor, 1943.

ADDENDUM

Whilst this paper was in the press, a further article was published by Baar and Lloyd (1943b) in which considerable criticism was directed against the Ashby method, and stress was placed upon the irregular results obtained by Ashby. From an analysis of cell-diameter distribution curves following transfusion and from other studies it was concluded that ' . . . the conception of an "intrinsic" longevity of the red cell is purely relative. . . . ' Finally, it was concluded that 'transfusion experiments . . . are useless for the estimation of the normal life span of the erythrocyte.' There is only room here for the following comments.

Ashby's irregular results have not been confirmed

by recent workers who have had the advantage of using improved methods. Contrary to the supposition of Baar and Lloyd, the disappearance curve of transfused erythrocytes, as estimated by the differential agglutination method, in subjects not affected with a haemolytic anaemia, is normally linear and, moreover, almost always extends for about a hundred days after transfusion [see references to Wiener (1934) and to Mollison and Young (1942), and see table 3 in the present paper; see also Brown, Háyward, Powell and Witts (1943)]. These observations strongly suggest, firstly, that the erythrocyte has a normal 'span of life,' and secondly, that this survival time is approximately one hundred days.