STUDIES IN THE ANAEMIAS OF INFANCY AND EARLY CHILDHOOD

PART XIV.—THE FATE OF TRANSFUSED ERYTHROCYTES

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The tendency of late years has been to consider the results obtained by transfusion of blood as giving the most reliable indication of the life of red cells and to disregard such results as have been obtained by other methods if found inconsistent with these.

The first transfusion experiments made with this object were carried out almost a century ago by Marfels and Moleschott and by Brown-Séquard (Hunter, 1884-6). In these experiments donor and recipient were taken from different species so that their red cells might easily be distinguished by their shape or possession of a nucleus. In the first experiments in which blood from the same species was used the fall in the red cell count was the only criterion, certainly an unreliable one, of the disappearance of transfused cells (Worm-Müller, see Hunter, 1884-6). Todd and White (1912) were the first to introduce a method for the differentiation of the donor's cells in the circulation of a recipient of the same species. Isohaemolytic serum placed in contact with R.B.C. of the individual for which it has been made is a specific reagent for these corpuscles and by making use of this fact these authors were able to follow the fate of red cells transfused from one bull to another. The transfusion, however, was followed by the development of haemolysins for the donor's cells in the serum of the recipient, a fact which invalidates the result.

Credit for the introduction of a method by which the cells of donor and recipient can be differentiated in human beings belongs to Ashby (1919, 1921) who transfused group O blood into group A recipients and counted those red cells which were not agglutinated by α -agglutinin serum before and at intervals after transfusion. These were the first of a number of similar experiments which, though they have been adversely criticized, are still regarded by many authorities as being the most convincing contribution to the solution of the problem. It must be emphasized, however, that later authors have drawn conclusions from Ashby's results which are quite different from those drawn by the author himself. Ashby stressed the wide variation, from 30 to more than 100 days, in the time for which the transfused cells persisted in the recipient's cir-

ulation. She did not believe that the red cell died because of its age, but assumed a process of active destruction by the organism leading to a cyclic elimination of the donor's cells. In quoting her work, however, many authors have accepted the longest time for which transfused cells were found to persist as the normal life span of erythrocytes, regarding it as unlikely that red cells transfused into another organism should thereby have their life prolonged. In this connexion it is worth mentioning that the same consideration induced Görl (1926) to contest the reliability of Ashby's method, the life span of R.B.C. deduced from these experiments being so much longer than that obtained by other methods, e.g. bile pigment excretions. Some of Görl's criticisms, which are supported by observations of Wildegans (1926), are certainly not without foundation. The fact that the number of unagglutinated cells in a single blood sample may vary between 25,800 and 368,000 per c.mm. according to the length of time and vigour with which the pipette is shaken does not encourage confidence in the method. It seems, however, that in the hands of a single investigator comparable results may be obtained. The most convincing proof that the unagglutinable cells after transfusion of group O blood to an A recipient are transfused cells in fact is given by the observation of Maizels and Paterson (1940) that the unagglutinated cells, filtered off after preliminary agglutination with α -serum, corresponded in their reactions to anti-M and anti-N serum with the donor's corpuscles.

Results similar to those of Ashby have been obtained by Wearn, Warren and Ames (1922), Krjukof and Krilova (1935) and by Maizels and Paterson (1940); others, however, were able to trace the transfused cells for only a comparatively short time (Jerwell, 1924; Hotz, 1921; Kramarenko, 1928). A method which seems to be superior to Ashby's test has been introduced by Woronow (1936). By repeated small intravenous injections of group A or B blood into group O individuals a specific anti-A or anti-B haemolytic serum was obtained. This serum with added complement was used as a counting fluid after blood transfusions

from universal donors into A or B recipients. The serum haemolyzed the recipient's cells leaving only those of the donor. Unfortunately only two cases were reported. The survival of transfused cells was 19 and 35 days respectively. It is certain that the 'indirect differential agglutination test' of Ashby gives less reliable results than a 'direct differential agglutination test,' a method in which the transfused cells are detected by a serum which agglutinates these and leaves the recipient's cells unaffected. Using the four groups of human blood this test is only occasionally applicable in newborns in whom sometimes the serum does not contain agglutinins and blood of group A or B can therefore be used for a recipient of group O. Jerwell (1924) used this method in one case and followed the transfused cells for about six weeks. A wider field of application became available as the result of Landsteiner's discovery of M and N factors. With anti-M and anti-N serum Landsteiner, Levine and Janes (1928) followed the transfused cells for seven weeks. More extensive investigations with this method have been made by Martinet (1938), Dekkers (1939) and recently by Mollison and Young (1940). Dekkers was able to demonstrate the persistence of the donor's cells in the recipient's circulation by a macroscopic test for an average of $54\frac{1}{2}$ days and by a microscopic test for $75\frac{1}{2}$ days. Martinet considers that the macroscopic test only is reliable. The direct differential agglutination test allows the detection of very small amounts of transfused blood but it is possible only roughly to follow quantitatively the disappearance of transfused cells with this method. If, on the other hand, the indirect differential agglutination test is applied to the factors M and N the test for technical reasons becomes liable to the same objections as the original method of Ashby. Dekkers' figures indicate the time at which approximately all transfused cells had disappeared. It is probable, however, that the red cells produced on any day vary to some extent in quality and may be expected to survive for varying periods; observations supporting this view will be given later. If the standard deviation of the red cell population from this point of view be high, the time taken for the disappearance of all transfused cells may well be considerably longer than the average cell's life span. Apart from the intrinsic variations in the quality of R.B.C. the influence of environmental factors must be born in mind. The erythrocyte population may be compared with a human population in which a high percentage of the mortality is due to old age, yet 'if we were to view "natural death" as the termination of existence due to the uniform senescent atrophy of all the structures of the organism, leading to a uniform depression and ultimately simultaneous extinction of all its functions, then "natural death" in all probability never occurs' (Robertson). For the R.B.C. variations in the number of cells in the circulation on one and in the reservoirs on the other hand, variations in the oxygen consumption by the tissue (e.g. exercise) and formation of CO_2 leading

to transient changes in pH are factors of which it can rightly be assumed that they exert a considerable influence on the life span of R.B.C. In experiments described in the previous paper (part XIII) it has been shown that the majority of R.B.C. formed at one time die at approximately the same time; old age is therefore the main cause of the erythrocyte's death. But if it were the only cause a linear disappearance of transfused cells would always be found. This was not so in most of Ashby's experiments. In experiments described below, a linear disappearance was observed only for a certain length of time.

Although for these and other reasons, which will be discussed below, transfusion experiments can only demonstrate the fate of transfused cells in various conditions, not the life span of normal cells, they afford a useful way of investigating many questions connected with the production and destruction of R.B.C. It was thought desirable therefore to follow the fate of transfused cells with other methods than the differential agglutination.

Two methods have been used in the course of these investigations: (1) the analysis and decomposition of Price Jones' curves in cases where the donor's and recipient's red cells formed distinguishable groups; (2) quantitative determinations of alkali-resistant and alkali labile haemoglobin after blood transfusions in newborns. It is obvious that each method has only a restricted field of application.

The simplest way to follow the fate of transfused cells is encountered in the case in which the recipient's R.B.C. population is completely replaced by the donor's cells. One such case has been observed: a baby with erythronoclastic anaemia of the newborn (already mentioned in part XIII). In chart I the solid curve represents the baby's red cell population before transfusions, the dotted curve the donor's R.B.C. population. After three transfusions the baby's Price Jones' curve (dashed curve) was found to be identical with that of the donor and at the same time reticulocytes practically disappeared from the peripheral circulation. In this aregenerative period there was a steady fall in the red cell count for six days at which time a fresh reticulocytosis occurred. Two days later the Price Jones' curve, which had remained unchanged up to the time of reticulocytosis, showed distinct signs of heterogeneity (chart II, dotted curve). The drop in the six days was from 3,520,000 to 2,300,000 or 34.7 per cent. (the figures for four counts in this period fell on a straight line), from which an average persistence of the transfused R.B.C. in the recipient's circulation of 17.3 days may be deduced. This conclusion is quite consistent with the appearance of the Price Jones' curve 15 days after transfusion for this fairly closely resembles the patient's original curve (dashed curve).

In order to compare the length of life of the transfused cells with that of the recipients' cells it is necessary only to estimate the time taken for the complete replacement of the recipient's cells by those of the donor. Unfortunately the second Price Jones film was taken six days after transfusions had been started. As this curve already corresponds to that of the donor's R.B.C. population it may be concluded that the baby's cells had a life span of

CHART I.—R.B.C. population of a case of erythronoclastic anaemia before transfusion (solid curve), after three blood transfusions (dashed curve) and this of the donor (dotted curve).

CHART II.—R.B.C. population of a case of erythronoclastic anaemia of the newborn during an aregenerative phase following 3 blood transfusions (solid curve), two days (dotted curve) and six days (dashed curve) after the start of reticulocytosis.

at the most six days. A further limitation of this time is possible if the figures for mean corpuscular volume are taken in account. The mean corpuscular volume decreased from $92\mu^3$ to $69.5\mu^3$ within three days, that of the donor's cells being $68.1\mu^3$ (two determinations: 68.0 and $68.2\mu^3$ respectively). A life span of about three days for the baby's R.B.C. can therefore be assumed. No estimate of the reticulocyte maturation time having been made before transfusion it was not possible to form an estimate by this means until a fortnight

after the last transfusion when red cell and reticulocyte counts were fairly constant for four days. The average reticulocyte count at this time was 13 per cent. and the maturation time eleven hours. The daily regeneration rate therefore was 28.4 per cent. and the life span 3.55 days. In this case, therefore, the transfused cells survived some five times longer than the recipient's cells yet their life was considerably shorter than that of normal cells in normal circumstances.* A similar selective

* The donor was the Rh-negative mother.

destruction affecting the recipient's cells to a higher degree than those of the donor was observed in a case of acholuric jaundice, mentioned in part XII, when two blood transfusions were followed by an aregenerative phase of six days. The replacement of the recipient's R.B.C. population by that of the donor was, however, in this case not complete.

Except in cases with complete replacement of recipient's cells by those of the donor such a simple analysis of Price Jones' curves is not a feasible way to follow the fate of transfused R.B.C. If the donors and recipient's cells differ considerably in mean diameter, however, a transfusion results in a heterogeneous distribution curve and for quantitative determination of the components representing the cells of donor and recipient in such cases a somewhat laborious decomposition is necessary. The mathematical foundation for decomposition of distribution curves has been laid by Pearson but a satisfactory and fairly simple way for a graphic decomposition has only recently been elaborated by Mogensen (1938). As some observations concerning the application of Mogensen's method for the decomposition of Price Jones' curves after blood transfusions can be only understood with the knowledge of the procedure, this method will now be briefly described.

A preliminary adjustment precedes the decomposition. For this purpose the sum of the numbers of cells present in two neighbouring classes of the original Price Jones' curve is taken as the number of cells belonging to one class, the diameter of which is the midpoint between the two classes. The result is a new distribution curve with 1000 cells instead of the original 500. The reason for making such an adjustment has been given by Pohle (1927): the width of the classes in a Price Jones' curve is 0.25μ but the measurements are made to the nearest of 0.5μ . If all R.B.C. were perfectly circular the resulting distribution curve would represent a series of peaks separated by classes without any cells.

The adjusted curve is copied on tracing paper, the copy inverted and moved to and fro over the original till one covers the other as completely as possible, especially in the region of the peak. The midpoint of that part of the abscissa common to both curves represents the mean diameter of the main component. As the figures on the abscissa for the non-inverted curve run from left to right and for the inverted one from right to left the figure for this midpoint is not always identical for both abscissae and the arithmetic mean of the two figures must be taken in such a case. A graph is now constructed by plotting the squared differences between the mean diameter of the main component and the diameter of each class $(x_1 - M_0)^2$ against the logarithm of the number of cells found in the corresponding class ($\log n'$) (chart VI). A series of points is obtained in this way and that line is drawn which touches most of the points in the lower right hand part of the chart, i.e. the points corresponding to the classes with the greatest numbers of cells. The standard deviation of the main component is proportional to the slope of this line. If, as in Mogensen's charts and in chart VII, the scale on the x-axis is twice the scale on the y-axis (0.5 abscissa = 1.0 ordinate) the slope of the line is $2 \tan \alpha$. If the slope is b , the squared standard deviation

$s^2 = 0.217 \times b$. If a is the antilogarithm of the piece cut off the abscissa the number of cells belonging to the main component is calculated from the formula: $N = 5.013 \times a \times s$ or $5.013 \times \sqrt{0.217b} \times a$. Having now obtained the main characteristics of the main component the number of cells expected in each class of an ideal distribution curve having the mean diameter and the standard deviation of the main component can be calculated with the aid of a table of areas of the normal distribution curve: $Q(x) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^x dx e^{-\frac{x^2}{2}}$. In Charlier's book this table is

found on p. 121. The procedure is best explained by an example given by Mogensen. The number of cells expected in the class interval with the midpoint 8.50 is to be calculated for a population with a mean diameter of 8.850 and a standard deviation of 0.737 . The limits of this class are 8.375 and 8.625 . The deviation of these points from the mean diameter (M_0) are -0.475 and -0.225 respectively. These deviations are expressed as multiples of the standard deviation $\left(\frac{x_1 - M_0}{S_0}\right)$ which in this case gives $\frac{8.375 - 8.850}{0.737}$ and $\frac{8.625 - 8.850}{0.737}$ or -0.644 and -0.305 .

Two figures are now looked up in the table of areas: the area of the distribution curve to the left of $-0.644 = 0.2597$ and the area to the left of $-0.305 = 0.3801$. The difference between these two figures (0.1204) is the area between the two limits, and this when multiplied by the number of cells belonging to the main component (N_0) gives the number of cells which out of the total number (N_0) on average are placed in the class with the diameter 8.5 . In normal homogeneous curves the agreement between the expected number and that found by actual cell measurement is very close, whilst in cases with two components, e.g. a minor component with a mean diameter smaller than that of the main component agreement is only found near to the mean diameter of the main component and to the right of this. In the classes with smaller diameters the original Price Jones curve shows more than the expected number of cells; by subtraction an approximate distribution curve of the minor component can be constructed.

Applying this method to the decomposition of bi-apical Price Jones' curve, such as may occasionally be encountered after transfusion, only the higher peak, usually corresponding to the recipient's curve, should be taken into account in covering the original adjusted curve with the inverted one.

Occasionally the application of Mogensen's original method is impossible. Charts III and IV show two plateau-type curves, both cases of acholuric jaundice, that in chart III after a haemolytic crisis, that in chart IV after blood transfusion. In chart III there is a definite peak corresponding to the 'normal' microcytic component, whereas in chart IV (solid curve) there is no definite peak.

If in the first case $(x_1 - m_0)^2$ is plotted against $\log n$, a line can be drawn, the slope of which is proportional to the standard deviation of the main component, as seen in chart VI. In the second case, however, a series of points is obtained on the right side of the chart lying on a line which forms an angle of 90° with the x-axis and would correspond to an indefinitely great standard deviation (chart VII).

This difficulty has been overcome in the following way: It was assumed that the main component

CHART III.—Adjusted distribution curve of R.B.C. in a case of acholuric jaundice (R.D.) after a haemolytic crisis.

CHART IV.—Adjusted Price Jones' curves of a case of acholuric jaundice before transfusion (---) . . . few hours after transfusion (—) and 17 days after transfusion (.....).

CHART V.—Adjusted Price Jones' curve of a case of acholuric jaundice after blood transfusion. ——— Non-inverted, --- inverted curve.

immediately after transfusion was identical with that of the recipient's R.B.C. population before transfusion. The mean diameter and standard deviation of the main component can therefore be taken from the Price Jones' curve (or its main component) before

CHART VI.—R. D. Acholuric jaundice after transfusion. Mo=mean diameter of the main component=6.50, x'=diameter of any class interval in the adjusted distribution curve, N'=the corresponding number of cells.

CHART VII.—V. M. Acholuric jaundice after blood transfusion. Decomposition of the erythrocyte distribution curve.

transfusion. The figure *a* is usually so close to the actual number of cells at the peak of the adjusted Price Jones' curve that the highest number of cells met in any of the classes after transfusion can be taken with but little error as being identical with *a*. Having thus the figures for *s* and *a* the number of cells in the main component can be calculated using the same constants as those given by Mogensen.

In the case represented in chart IV the accuracy of this procedure has been checked, the number of cells in the main component (*N*₀) being almost identical with that expected from the amount of transfused blood and the child's blood-volume. Approximately one-third of the patient's red cells after transfusion have been those added by transfusion which would correspond to 167 cells out of 500. The decomposition gave 175 cells in the minor component.

The method of following the fate of transfused cells by changes in the Price Jones' curve is subject to more limitations than that of the necessary difference in the size between the cells of the donor and recipient. Except in the ideal case when regeneration is such that an exactly constant level of red cells is maintained throughout the experiment a correction must be applied for any changes in red cell level, for the minor component is expressed as the number of cells per five hundred of the whole; moreover any significant change in the distribution curve (mean diameter and standard deviation) of the recipient's cells make any conclusions on the disappearance of the minor component unreliable. Normally, apart from some degree of diurnal variation, the distribution curve shows a most striking constancy. This is probably true also in acholuric jaundice in periods of equilibrium between blood formation and destruction. Even in normal babies in the neonatal period on the other hand a change in the distribution curve takes place (van Creveld, 1932) which may be more marked in cases of macrocytic erythronoclastic anaemia (Hawksley and Lightwood, 1934). Exact matching of the mean diameter of major and minor components with those of the recipient's and donor's cells is not absolutely necessary. It can be assumed that changes in the numerical values of major and minor components run parallel to the disappearance of transfused cells as long as the main characteristics of the major component do not change during the period of observation.

TABLE I
NUMBER OF CELLS IN THE MAJOR AND MINOR COMPONENT OF THE RED CELL DISTRIBUTION CURVE AT VARIOUS DAYS AFTER TRANSFUSION OF NORMOCYTIC BLOOD IN TWO CASES OF ACHOLURIC FAMILY JAUNDICE

Case	Days after transfusion	Number of cells	
		Major component	Minor component
M.M.	0	324	176
	11	452	48
	29	494	6
V.M.	0	325	175
	7	395	105
	11	448	52
	17	492	8

Table I shows the figures for major and minor components in two cases of acholuric jaundice after transfusion with normocytic blood. The figures suggest that the transfused cells disappear within

three weeks. The life span of the cells of the recipient calculated from the reticulocyte count and maturation time was five and four days respectively. As in the case of erythronoclastic anaemia of the newborn described above, the red cell destruction was selective and the transfused cells evidently survived much longer than those of the recipient, though apparently not so long as the average normal cell. In a third case of acholuric family jaundice recently under observation, when two large transfusions were given in a haemolytic crisis and Price Jones' curves made daily for ten days and every second day for another three weeks, the transfused cells survived

in the recipient's circulation just for three weeks. The finding of a short survival of transfused R.B.C. in acholuric jaundice suggests an abnormal activity of the blood destroying mechanism and is in agreement with the observations of Dekkers (1939), Placeo (1928) and Dedichen (1932), who by means of both Ashby's method and the differential agglutination test with anti-M and anti-N serum observed the disappearance of transfused cells from the blood of acholuric family jaundice in periods varying from a few to twenty-three days.

Chart VIII indicates the rate of disappearance of transfused normal erythrocytes in one case of acholuric jaundice. The observed points follow a straight line, thus indicating that in spite of an increased activity of the destructive mechanism the cells are neither destroyed indiscriminately nor in proportion to their concentration. The most plausible explanation of this straight line is that while the duration of life of normal cells transfused to a case of acholuric jaundice is shortened, nevertheless, the cells die in relation to their age.

One of us (T.W.L.) has described elsewhere (1941) in detail a series of experiments in which blood from both an active and from cured cases of acholuric jaundice was transfused into normal persons. The results of the decomposition of the Price Jones' curves in one of these experiments are given in charts IX and X in which the major and minor components of a curve constructed shortly after transfusion are compared with the original curves of recipient and donor (chart IX) and the gradual disappearance of the minor component is shown (chart X).

The bulk of the transfused cells disappeared in about seven days when the donor was an active case and in some ten days when the donor had been completely relieved of symptoms by splenectomy some years before. It was observed that in the latter case the transfused cells became more spheroidal a few days after transfusion. It is apparent from these results that the red cell of active

CHART VIII.—Elimination of transfused R.B.C. in a case of acholuric jaundice (V.M.)

CHART IX.—(a) ——— distribution curve of the donor's (acholuric jaundice) red cell population, - - - - minor component of the recipient's red cell population after transfusion. (b) ——— recipient's red cell population, - - - - major component of the recipient's red cell population after blood transfusion.

acholuric jaundice has but a brief span of life whether in the patient's body or after transfusion into normal persons. Moreover, though after splenectomy abnormally rapid destruction ceases in this condition, the cells are still readily susceptible of destruction when transfused into a normal person.

The donor, who had active acholuric jaundice, had 14 per cent. reticulocytes with a maturation time of six to eight hours. The life span of his cells was therefore two to three days. It would seem that these cells were less readily destroyed by the normal individual than by the patient.

In these experiments the bulk of the transfused red cells disappeared rapidly and, in two cases, the figures for the minor component fell upon a straight line: in all, however, there remained a small microcytic minor component which persisted without change for some days. It seemed probable that a few of the transfused cells were considerably more resistant than the average, or for some other reason had persisted longer in the recipient's circulation than might be expected if the life span of all cells were identical. Similar observations are reported by Ashby (1919, 1921) and Jerwell (1924). They afford a firm basis for argument against the general assumption that the last of the donor's cells to disappear are always the youngest and that the time of their disappearance is equivalent to the average life of the red cell.

macrocytic population in icterus gravis and the donor's normocytic population is often not great enough to make the heterogeneity of the resulting distribution curve obvious. It must be realized that a distribution curve which appears homogeneous by Mogensen's analysis may yet be heterogeneous in fact. We have constructed from a series of distribution curves with different mean diameters summation curves which appeared to be homogeneous by Mogensen's decomposition. Even a curve practically identical with a Gauss-curve may result from a mixture of populations. If, however, at least with a great number of elements, the state of affairs appears to be such that the frequency curves hardly differ appreciably from the normal shape this is by no means due to a small number of types in the statistical subjects but appears to depend rather upon the fact that the number of such types is very large (Charlier, 1931).

Haemoglobin method

In the neonatal period another method can be used to differentiate between blood of the recipient and donor, i.e. the quantitative estimation of alkali resistant and alkali labile haemoglobin. Since Koerber's discovery of differences in the alkali resistance of the haemoglobin of various animals and its confirmation by Krüger (1925) a number of

CHART X.—Changes in the major and minor component of the R.B.C. population following transfusion of microspherocytic blood into a normal recipient. The curves result from a decomposition of Price Jones' curves constructed in two-day intervals in the following order: solid curve, dashed curve, dashed and dotted curve, dotted curve.

All these experiments showing the short duration of life of normal R.B.C. transfused into a case of acholuric jaundice and the rapid destruction of red cells from acholuric jaundice transfused into a normal recipient afford support for the opinion (Baar, 1928) that whilst a hereditary defect of R.B.C. in acholuric family jaundice is undoubtedly the main feature of the disease, at least in some cases, some abnormality in the mechanism of blood destruction (spleen) must be assumed.

Decomposition of Price Jones' curves in cases of icterus gravis neonatorum after blood transfusions is usually less satisfactory than in acholuric jaundice. The difference in the mean diameter between the

investigations have been published on this subject. A considerable difference between the resistant foetal and labile adult haemoglobin, first discovered in human blood, has also been found in goats, cattle and rabbits (Barcroft, 1933, 1935; Brinkman and Jonxis, 1936). Krüger's hypothesis, that, the prosthetic group being the same in all haemoglobin, the variability in resistance to alkali is due to difference in the globin component, is now generally accepted.

Credit for the introduction of a spectrophotometric method for following the denaturation of oxyhaemoglobin by alkali is due to Haurowitz (1929, 1930). This method permits quantitative estimation of the process and of the relative amounts of resistant

and labile haemoglobin in a mixture of the two, and allows study of the kinetics of the reaction. It has, however, the disadvantage of depending on a visual comparison and requires a very expensive apparatus. The photoelectric method of Brinkman, et al. (1933, 1935, 1936) has other drawbacks which will be discussed in a later communication. In our experiments we have adapted the method of Haurowitz to use with the Evelyn photo-electric step-photometer (Baar and Hickmans, 1941).

Method. After accurate estimation of the haemoglobin in the blood sample to be examined a 0.12 per cent. haemoglobin solution is prepared in distilled water. The solution is centrifuged and the supernatant fluid poured into another tube, and shaken to ensure complete oxygen saturation. 1 c.c. of the clear solution is placed in each of two cups of Evelyn's microapparatus. 0.2 c.c. of distilled water is added to one cup thus making the solution 0.1 per cent. The extinction (L) * of this solution is determined using filter 540M; this should not differ much from 0.676, the extinction of an oxyhaemoglobin solution of exactly 0.1 per cent. in the spectral region of filter 540M at 1 cm. depth. This cup is now replaced by the other, 0.2 c.c. of a N/4 NaOH solution is quickly added, stirred with a glass rod and half a minute after the addition of NaOH the first reading is taken. Further readings are taken after 1, 1½, 2, 2½, 3, 4, 6, 8, 10, 15 and 30 minutes. When the denaturation of an accurate 0.1 per cent. oxyhaemoglobin solution is complete the extinction of the resulting compound (alkaline globin-haematin) is 0.395. As oxyhaemoglobin and alkaline globin-haematin both follow the Beer-Lambert law the oxyhaemoglobin solution need not be exactly 0.1 per cent. All readings can easily be calculated for an accurate 0.1 per cent. solution. The drop in the extinction ($L_{540M}^{1cm.}$) from 0.676 to 0.395 corresponds to 100 per cent. denaturation of a 0.1 per cent. oxyhaemoglobin solution at 1 cm. depth. The difference between these two figures is 0.281. If the extinction at any known time is calculated for an exact 0.1 per cent. solution and this figure subtracted from 0.676 the resulting difference, expressed as a percentage of 0.281, gives the percentage of denaturated oxyhaemoglobin at that time. For example if the extinction of an approximate 0.1 per cent. solution of oxyhaemoglobin is 0.695 and after five minutes denaturation 0.520 the corresponding values for an accurate 0.1 per cent. solution would be 0.676 and 0.505 respectively. The difference between these two figures is 0.171 which is 60.8 per cent. of 0.281: 60.8 per cent. of the oxyhaemoglobin has therefore been denaturated in five minutes.

For graphic representation the procedure of Brinkman and his collaborators is more correct than that of Haurowitz. The logarithms of the haemoglobin concentrations expressed in percentage of the original concentration are plotted against the time in minutes. In the case of a mixture of labile and resistant oxyhaemoglobin the points follow a straight line for a short time and then turn fairly sharply to another straight line with a much milder slope than the first. A typical curve is seen in chart XI. The first linear slope corresponds to the rapid denatura-

tion of labile haemoglobin, the second to the slow denaturation of resistant O_2Hb . If this second linear part of the curve be extrapolated to zero time the antilogarithm of the corresponding ordinate gives the percentage of resistant oxyhaemoglobin in the specimen examined. The mean error of the method has been determined by making a series of denaturation curves in duplicate, taking the amount of denaturated oxyhaemoglobin after two minutes in each determination and calculating on the basis of the formula $e = \sqrt{\frac{\sum d^2}{2n}}$, where e is the mean error, d the difference between duplicates and n the number of estimations. The error so calculated was 2.3 per cent.

In the neonatal period blood is always a mixture of resistant and labile haemoglobin. The amount of labile haemoglobin varies considerably but

CHART XI.—Denaturation of oxyhaemoglobin in N/4 NaOH. — Baby B., age 4 weeks, --- normal adult. The initial haemoglobin concentration (0.1 per cent.) is taken as 100.

changes slowly in any individual if no transfusion is given, even in cases with a high rate of blood destruction and regeneration.

Chart XII shows the change in the denaturation curve following blood transfusion in a baby with icterus gravis neonatorum. The amount of labile oxyhaemoglobin rose in this case from 52 per cent. before to 74.2 per cent. after transfusion.

The spontaneous replacement of resistant haemoglobin by the labile form takes place slowly but must not be neglected if the fate of transfused cells is to be followed for several weeks. The rate of change, however, shows considerable individual variations. The method therefore is impracticable for observations over a long time and gives an accurate account of the fate of transfused cells only if considerable change in the relation of resistant and labile haemoglobin is produced by transfusion.

Serial determinations of the denaturation curve in twelve cases of icterus gravis neonatorum and erythronoclastic anaemia of the newborn after blood transfusions showed two types of reaction. The first is represented by charts XIII and XIV. The amount of labile haemoglobin increased immediately after

* L corresponds to E in the usual nomenclature of spectrophotometry.

transfusion but thereafter remained almost unchanged for a considerable time. The curves in chart XIII indicate a decrease in the percentage of labile haemoglobin from 89 to 83.4 per cent. within thirteen days; but as in this period there was a spontaneous rise in haemoglobin from 11 to 12.3

a life span of about ten days as calculated from the number of reticulocytes and their maturation time.

The denaturation curves in chart XIV indicate an almost unchanged relation of alkali resistant and alkali labile haemoglobin in the time from 15/6/1940 to 2/7/1940. There was, however, a drop in total

CHART XII.—Denaturation curves in a case of icterus gravis neonatorum, ——— before transfusion (52 per cent. alkali labile oxyhaemoglobin) - - - - - 1½ hours after blood transfusion (72.4 per cent. labile oxyhaemoglobin) and 18 hours after transfusion (74.2 per cent. oxyhaemoglobin).

gm. per cent. the absolute figures are 9.61 and 10.2 gm. per cent. The slight increase corresponds exactly to the percentage of labile haemoglobin in the baby's own blood. There was in this case therefore no noticeable destruction of transfused cells within thirteen days. The baby's own cells had

haemoglobin from 14.2 to 10.4 gm. per cent. The additional labile haemoglobin was diminished from 5.6 to 3.6 gm. per cent. in seventeen days, which would correspond to a survival of 68 days if the rate of disappearance be linear. The baby had in this period an average of 3.4 per cent. reticulocytes which

CHART XIII.—Denaturation curves of oxyhaemoglobin in a case of erythronoclastic anaemia of the newborn, before transfusion, immediately and 13 days after blood transfusion. L.H.C=alkali labile haemoglobin.

corresponds to a life span of twelve to thirteen days for his own R.B.C.

The second type is represented by charts XV and XVI. In chart XV the oxyhaemoglobin denaturation curves of a baby with acute erythronoclastic anaemia of the newborn are seen. The solid curve before transfusion (2/7/40) corresponds to a mixture of 55.35 per cent. labile and 44.65 per cent. resistant

and 43.65 per cent. resistant haemoglobin, almost the same as before transfusion; the transfused cells, therefore, must have disappeared within two days. The life span of the baby's own cells, which in cases where the haemoglobin level is unsteady can only be roughly estimated from the number of reticulocytes and their maturation time, was about two days. It is apparent that in this case destruction of the

CHART XIV.—Denaturation curves of oxyhaemoglobin in a case of icterus gravis neonatorum before transfusion, immediately after transfusion and 17 days later.

oxyhaemoglobin. The second solid curve (3/7/40) was made immediately after blood transfusion. This shows 77.09 per cent. labile and 22.91 per cent. resistant haemoglobin. Forty-eight hours later the denaturation curve indicates 56.35 per cent. labile

red cells was taking place irrespective of their age and quality. Cases with such indiscriminate destruction of R.B.C. of which two have been seen had all the haematological signs of a severe haemolytic process (charts XV and XVI).

CHART XV.—Baby Pr. Alkali denaturation of oxyhaemoglobin before and after blood transfusion.

These observations throw some light on the pathogenesis of icterus gravis neonatorum and erythronoclastic anaemia of the newborn. Cases with indiscriminate destruction of the cells both of donor and recipient within so short a time as two days afford incontestable proof of the presence of an 'haemolytic' * process and confirm the thesis put forward by Parsons and his school (1933, 1935, 1938). On the other hand cases in which the abnormal destruction is largely confined to the patient's own cells suggest that these may be of inferior quality. We have, however, encountered several cases of intermediate type of R.B.C. destruction which serve to link the two extreme types already described and it seems probable therefore that the agent which causes violent haemolysis in some cases may in others chiefly cause injury to the bone marrow and result in the production of erythrocytes of inferior quality. The anaemia being in fact rather erythronoclastic than haemolytic in origin.

It will be seen that it has been possible by both decomposition of Price Jones' curves and esti-

* 'Haemolytic' is used here in the widest sense of the word meaning a rapid destruction of R.B.C. whether this is due to a circulating poison, to a haemolysin, or to a pathological function of tissue normally concerned with the destruction of erythrocytes.

mations of alkali resistant and alkali labile haemoglobin to demonstrate cases in which there was selective destruction of the red cells either of the recipient or donor. Review of the literature reveals several observations which can only be interpreted in the same way. As early as 1857 Brown-Séquard showed that the red cells of a fowl when transfused into a dog or rabbit disappeared within an hour, while those of dog or rabbit survived in the fowl's circulation for a month. In Ashby's experiments also the number of transfused erythrocytes often remained unchanged for weeks though there can be

CHART XVI.—Baby Pr. ——— gm. per cent. haemoglobin in the peripheral blood, ----- gm. per cent. alkali labile haemoglobin, -.-.- alkali labile haemoglobin in per cent. of total haemoglobin.

no question but that some of the recipient's own cells were destroyed and replaced during this period. In one instance quoted in part XII two transfusions of group O blood were given to a patient with pernicious anaemia after a transfusion with group A blood, the recipient being also group A. Seventeen days after the transfusion of group A and twenty-four hours after the second group O transfusion the only cells demonstrable in the recipient's circulation were not agglutinable, that is to say all R.B.C. of the recipient and of the group A donor had been destroyed leaving only those of the group O donor. This phenomenon is comparable with that in the case of erythronoclastic anaemia described on p. 125 when Price

Jones' curves revealed the complete replacement of the recipient's R.B.C. by those of the donor.

In most observations of selective cell destruction in which the course of events could be followed for a sufficient length of time the transfused cells outlived those of the recipient. The chief exceptions were those cases in which abnormal blood, that is blood from cases of alcholoric jaundice, was given to normal persons. In almost all instances although the donor's R.B.C. had a longer life span than those of the recipient they did not survive so long as does the average red cell in normal persons. Observations such as that in chart XIII and XIV in which the amount of alkali labile haemoglobin remained unchanged for thirteen days or a life span of 68 days was calculated for the transfused cells suggest the possibility that selective destruction of the recipient's cells may even lead to a prolongation of the life span of the transfused cells beyond the normal. It has been pointed out above, however, that this method is susceptible to some error if observations are continued over long periods and have therefore no certainty about this point. All our studies have been made on cases of haemolytic anaemia. If under these conditions indications of such abnormal survival of transfused cells are detectable it seems probable that this will be far more frequent if the recipient is not subject to a haemolytic condition; this suggestion finds some confirmation in the findings of Ashby and others.

It has been shown above that the conception of an 'intrinsic' longevity of the red cell is purely relative, depending as it does entirely upon environmental circumstances. Transfused cells may disappear from the circulation at a regular rate, indicating that the principal cause of death is old age, yet their life span may be much diminished. The life span of erythrocytes is determined by three factors, the intrinsic properties of the red cell, the activity of the blood destroying mechanism which may include the presence of an haemolytic poison, and in the case of transfusion the adaptation of this mechanism to the transfused cells. The first factor is proven by observations of the destruction of transfused cells at a different rate from the recipient's cells. The second is made clear by the shortening of the red cell's life span when transfused to cases of haemolytic anaemia. For the third factor we have no evidence in our own investigations but it is strongly suggested by the observation of Ashby in which both the cells of the recipient and one donor were destroyed and only those of group O survived.

Analysis of the fate of transfused erythrocytes by two different methods applied for the first time to this problem and comparison with the results of differential agglutination tests reported in the literature shows the action of various factors on the time for which transfused cells survive in the recipient's circulation. Indiscriminate destruction, selective destruction of the cells of the recipient or donor and shortening of the life span of transfused cells have been demonstrated and it seems probable that the life span of such cells may in some cases actually be

prolonged. Transfusion experiments, therefore, give valuable information about what Josephs (1932, 1934) calls the 'mechanism of anaemias', but are useless for the estimation of the normal life span of the erythrocyte.

Summary

1. The fate of transfused red blood corpuscles has been followed by decomposition of Price Jones' curves and by quantitative estimation of alkali-resistant and alkali-labile oxyhaemoglobin. A method for the latter estimation with the use of Evelyn's photoelectric step-photometer is described.

2. The life span of transfused cells is usually different from that of the recipient's cells. The disappearance of transfused cells, both as the result of old age and of indiscriminate destruction, have been demonstrated. A diminution in the average life span is usual, but prolongation may occur.

3. Transfusion experiments have demonstrated the inferior quality of erythrocytes and the increased activity of the destructive mechanism in acholuric jaundice and the presence of an erythronoclastic factor in icterus gravis neonatorum and haemolytic anaemia of the newborn.

4. No conclusions as to the normal life span of erythrocytes may justifiably be drawn from transfusion experiments.

Grateful thanks must be expressed to Professor L. G. Parsons for the interest with which he has followed these investigations and for his criticism and suggestions, to the Honorary Staff of The Children's Hospital, Birmingham, for access to their cases, to Miss Finch, M.Sc., assistant biochemist, for potassium determinations, to Mr. Brain for the photographs and charts and to the Board of the Children's Hospital for a personal grant to one of us (H.S.B.).

The investigations reported in this article were completed early in 1941. The recent discoveries concerning the rôle of anti-Rh agglutinins in the blood destruction in icterus gravis neonatorum could, therefore, not have been taken into consideration. A few quantitative estimations of alkali resistant and alkali labile haemoglobin after transfusions of Rh-negative blood, made in the past months, suggest a selective destruction of baby's cells. This is in agreement with the results obtained by Mollison with the Ashby method.

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