THE SURVIVAL OF TRANSFUSED RED CELLS IN ACUTE RHEUMATIC FEVER WITH REFERENCE TO A LATENT HAEMOLYTIC MECHANISM

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Acute rheumatic fever is commonly associated with anaemia, and the degree of this anaemia and rapidity of its onset is proportionate to the severity of the disease. Osler (1892) described it as being the most severe of any anaemia associated with infection. Dyshaemopoiesis (Hubbard and McKee, 1939) and an increase in plasma volume (Cochran, 1951) have both been put forward as causes, and, although it has been suggested that haemolysis plays a part (Hubbard and McKee, 1939), there has hitherto been no satisfactory evidence for this hypothesis.

In the course of observing patients with acute rheumatic fever, the rapidity of the onset of anaemia has been most striking. This has given rise to the present investigation to detect whether a haemolytic process is operative in the acute stage of the disease.

Evidence of haemolysis has been sought in a study of the survival of transfused normal red cells in the circulation of patients with acute rheumatic fever using the Ashby technique. Previous personal observations on the osmotic and mechanical fragility of the red cells, the serial reticulocyte count and the serum bilirubin and faecal urobilinogen output have indicated that if haemolysis is present, it is not of marked degree. However, latent haemolytic mechanisms, where the process may only be revealed by special studies such as the survival of transfused red cells, are known to occur in a variety of conditions, and particularly with acute infections (Brown, Hayward, Powell and Witts, 1944; Emerson, 1948; Berlin, 1951; Loge, Lange and Moore, 1950).

The survival of transfused red cells in the circulation of normal recipients and of those with various diseases has been investigated to estimate the mean red cell life. Ashby (1919) introduced a method of differential agglutination using the ABO blood groups to count the donor cell population and she made estimations of the normal red cell life in certain haemolytic conditions. The Ashby technique was

subject to too many inaccuracies in its original form for it to come into common use as a method of studying haemolysis. The modifications of Mollison and Young (1940), and of Dacie and Mollison (1943) have done much to improve its accuracy in recent years. The normal mean cell life of 100 to 120 days, determined by this method, correlates well with that found by estimating the survival of the subject's own cells in his circulation by tagging them with N_{15} labelled glycine (Shemin and Rittenberg, 1946). The Ashby technique with its recent modifications is now widely accepted as a useful method for studying haemolytic mechanisms (Brown *et al.*, 1944; Berlin, 1951; Mollison, 1947; Young and Lawrence, 1946).

Method

The survival of transfused red cells was estimated by Dacie and Mollison's (1943) modification of the Ashby technique. In the author's hands, the coefficient of variation of this method was found to be 3.3%.

Material

The material included in this study was composed of eight cases of acute rheumatic fever. In addition six control cases were studied comprising cases of hypochromic anaemia, anaemia following haemorrhage and normal convalescent cases. In all of these conditions, normal survival of transfused red cells has been shown previously (Mollison, 1947; Callender, Powell and Witts, 1945; Kaplan and Zuelzer, 1950).

Control Series

It is known that the elimination of transfused normal red cells in the circulation of normal recipients occurs at a steady rate, 0.83% of the total introduced at transfusion being destroyed each day (Callender *et al.*, 1945). Thus the slope of elimination is linear (Mollison, 1947) and the average total

FIG. 1.—Survival of transfused red cells in six control cases.

survival time has been variously estimated at between 100 and 120 days.

In Fig. 1 points indicating percentage survival in the six control cases are plotted on one graph. A straight line has been fitted to these points by inspection and this gives an estimate of the normal elimination of donor red cells by the methods used in this investigation. It will be seen that the mean cell life is about 116 days.

Cases of Acute Rheumatic Fever

Eight cases were transfused in the acute stage of the disease and one was again transfused when the disease was considered to be inactive. She suffered a relapse of the condition 22 days after the second transfusion, and following this the transfused red cells were eliminated more rapidly than normal.

In all cases of rheumatic fever during the acute phase of the illness the elimination of transfused red cells was more rapid than normal and the slope was curvilinear. This indicates that the cells were being destroyed in a random manner irrespective of the normal process of ageing. This is demonstrated in Fig. 2, and is related to the normal slope of elimination derived from the control series. In three cases, undergoing an acute relapse in the course of elimination of donor cells from their circulation, the rate of elimination was further increased.

Analysis of Material

The results in acute rheumatic fever are compared below with the normal findings in the six cases in this control series and with the normal controls in the series of Mollison (1947) and of Callender *et al.* (1945) in adults, and that of Kaplan and Zuelzer (1950) in children.

FIG. 2.—Survival of transfused red cells in acute rheumatic fever.

Where transfused red cells are being destroyed in a random manner irrespective of their age, an estimate of mean cell survival becomes an inexact method of expressing results. Some workers have preferred the half-life of the donor cells as a way of expressing results (Young and Lawrence, 1946), while others estimate the time after transfusion at which 50% survival occurs (Berlin, 1951). In expressing results in these cases of acute rheumatic fever, where the greatest activity of the disease occurred in the first 20 to 30 days after transfusion, the percentage survival at 10 and 20 days after transfusion seems most likely to give the maximum information.

Mollison's Normal Series. The percentage survival of transfused red cells has been estimated at 10 and 20 days after transfusion in 11 of the control cases from this series. They consisted of cases of hypochromic anaemia, convalescent cases and of one healthy subject.

Case No.	Percentage Survival at 10 Days	Percentage Survival at 20 Days
1	85	73.5
2	88	72.5
3	91	76.5
4	91	83.5
5	94	82.5
6	95.5	85
7	95.5	86.6
8	96.0	87.5
9	97.0	91.0
10	97.5	95
11	99.0	87
Mean	93.6 ($\sigma = 4.33$)	83.7 ($\sigma = 7.06$)

Callender *et al.* (1945), in an investigation into the life-span of the red cell, concluded that the normal mode of destruction of erythrocytes acts so as to remove in unit time a fixed fraction of the total introduced at transfusion. They estimated this fraction at 0.83% per day. On this assumption, survival 10 days after transfusion would be 92.7% and 20 days after transfusion would be 83.4%.

Kaplan and Zuelzer's Normal Series in Children. These investigators studied the survival of normal transfused red cells in the circulation of five "normal" infants and children. Their "normal" controls consisted of children in hospital convalescent from various medical or surgical illnesses and free of any haematological abnormality save for iron deficiency anaemia in some cases. The mean survival at 10 and 20 days after transfusion in this series has also been calculated.

Case No.	Percentage Survival at 10 Days	Percentage Survival at 20 Days
1	81	73
2	88	88
3	96	94
4	96	81
5	99	88
Mean	92 ($\sigma = 7.4$)	85 ($\sigma = 8.1$)

Control Cases in the Present series. These findings for percentage survival 10 and 20 days after transfusion compare very closely with those in the other control studies described above.

Case No.	A Percentage Survival at 10 days	B Percentage Survival at 20 days
1	95.2	81.5
2	90.7	81.5
3	89	82
4	97	90
5	90	87.5
6	97	88
Mean	93.2 ($\sigma = 3.66$)	85.1 ($\sigma = 3.84$)

Percentage Survival at 10 and 20 Days in Acute Rheumatic Fever. This has been estimated in eight cases 10 days after transfusion and in seven cases 20 days after transfusion. In view of the relatively

Case	A1 Percentage Survival at 10 Days	B1 Percentage Survival at 20 Days
E.D. (1)	83.5	67.5
C.C. ..	82	67.5
A.F. ..	81	75.5
P.B. (1) ..	78.5	67.2
D.D. ..	78.0	68.5
B.T. ..	76.0	70
D.N. ..	76.0	66
B.R. ..	79.0 (at 7 days)	—
Mean	79.3 ($\sigma = 2.73$)	68.9 ($\sigma = 3.16$)

small number of cases in the control and rheumatic fever series, Student's *t* test was applied to the results to estimate the significance of the differences observed. Comparing the mean percentage survival at 10 days after transfusion in the control series (A) and in the rheumatic fever series (A1), $t = 8.17$. Therefore the differences observed would occur by chance less than once in a thousand times. Similarly comparing survival at 20 days after transfusion (B and B1), $t = 8.34$, and therefore the observed differences would again occur by chance less than once in a thousand times. From this it can be stated that these results are statistically highly significant.

Discussion

The survival of transfused red cells has been extensively studied in the various forms of haemolytic

anaemia and these investigations have done much towards clarifying the aetiology of these conditions. For instance, fundamental abnormality of the erythron in familial haemolytic anaemia has been demonstrated by showing that, whereas blood from normal donors survives normally in the circulation of these patients, their red cells have a greatly diminished survival rate in normal recipients (Loutit and Mollison, 1946).

In idiopathic acquired haemolytic anaemia, normal donor red cells are very rapidly destroyed, in extreme cases only 50% surviving after three to five days (Loutit and Mollison, 1946). This abnormal destruction of donor cells may occur either in a linear fashion (Mollison, 1947) or by means of a curvilinear slope of elimination indicating random destruction of cells irrespective of their age (Brown *et al.*, 1944); the latter appears to be by far the more common finding in such cases. Such results have been reported by many authorities (Young and Lawrence, 1946; Mollison, 1947; Selwyn and Hackett, 1949; Brown, 1950), and the value of transfused red cell survival experiments in the evaluation of haemolytic mechanisms is now generally recognized.

Acquired haemolytic anaemias associated with other diseases have been grouped together under the title of the symptomatic haemolytic anaemias. Haemolysis in these conditions is often easily demonstrated. In some instances evidence of haemolysis may be less obvious, and in an increasing number of diseases the presence of a latent haemolytic mechanism is being detected by transfused red cell survival studies and other methods.

Latent haemolytic anaemia has been described in acute infections and in neoplastic conditions. Brown *et al.* (1944) have detected it in various types of acute sepsis by demonstrating increased elimination of transfused red cells. Brown (1950) has confirmed this finding in six further cases of anaemia of sepsis by transfused red cell survival experiments and has emphasized the latent character of the haemolysis by showing an absence of reticulocytosis or increased faecal urobilinogen excretion. In chronic leukaemia, also, the presence of a latent haemolytic mechanism has been shown (Berlin, 1951) by transfused red cell survival studies on 17 patients; and similar observations on a case of myelomatosis are recorded by Brown and his associates (1944).

In several conditions that may be aetiologically allied to rheumatic fever, evidence of a latent haemolytic mechanism has also been discovered. Brown *et al.* (1944) found a markedly diminished survival rate of transfused red cells in a case of disseminated lupus erythematosus with arthritis.

Emerson (1948) described elimination of donor erythrocytes which was three times the normal rate in a case of acute nephritis and this was associated with a gross fall in the haematocrit readings and in the red blood count. Gardner (1952) has made a similar observation in a case of chronic nephritis. Loge *et al.* (1950) have also demonstrated a latent haemolytic mechanism in patients with chronic nephritis and azotaemia. This was similar to the haemolytic mechanism described in the present investigation in that it was disclosed by the means of studies on the survival of transfused red cells, although the serum bilirubin and faecal urobilinogen output were normal, and the Coombs test was negative. In two cases of rheumatoid arthritis increased destruction of donor cells has been reported (Mollison and Paterson, 1949), 50% being destroyed in 22 days in one case.

Studies on the survival of transfused red cells show a significant difference between the survival at 10 and 20 days after transfusion of donor red cells in cases of rheumatic fever compared with a control series having normal survival times. Moreover, the slope of destruction of these cells in cases of rheumatic fever is curvilinear, indicating the presence of a haemolytic mechanism destroying red cells irrespective of their age. It might seem possible that these changes could be accounted for by changes in plasma volume, and that in fact the anaemia is merely due to haemodilution. Reid, Watson and Sproull (1950) claim that there is an increase in plasma volume in the acute stage of rheumatic fever. In seven cases that they investigated the plasma volume was higher than would be expected from the body weights of the patients. On salicylate therapy there was a sharp increase in the plasma volume followed eventually by a fall in plasma volume as recovery took place. No study of the serial changes in plasma volume was made in untreated cases. This seems to cast some doubt on their contention that the untreated disease, when in the acute phase, is associated with an increase in plasma volume. Six out of their seven cases had in fact been treated with sodium salicylate before their investigation in hospital; and as the serum salicylate level was not estimated on these cases at the time of the initial plasma volume estimation, it is possible that the initial raised reading might have been due to a sodium effect. Hecht and Jager (1947) have shown a 22% increase in plasma volume due to the ingestion of large doses of sodium salicylate. Moreover, in many of Reid's patients, as was almost always the case in the patients studied in this investigation, the acute phase of the disease was accompanied by a fall in body weight, and this

in itself is surprising if there were a progressive increase in plasma volume over this period, although it might be accounted for by changes in distribution of the body water. York and Fischer (1947) showed no increase in plasma volume in six untreated cases of rheumatic fever, but when sodium salicylate was given five cases had an increase in plasma volume; the authors suggest that this was a direct result of sodium ingestion. It has been suggested that an increase in plasma volume in rheumatic fever is the sole cause of the anaemia, by means of haemodilution (Cochran, 1951). This would not account for the findings in the survival studies of transfused red cells. The immediate post-transfusion donor cell count would be artificially low if this were related to an increase in plasma volume. Now in all in the present series save for E.D. (2), blood transfusion was given at the peak of activity of the disease, and in six cases there was progressive improvement with a fall in the sedimentation rate in the ensuing 20 days. As it is suggested that the plasma volume diminishes as the sedimentation rate falls and recovery takes place, there should be an increased concentration of donor red cells during this period, rather than a diminishing donor cell population.

It will be noticed from the curves of elimination of transfused red cells that, in those cases that were treated with a short course of salicylate, there was no related increase in the elimination of transfused red cells. If York and Fischer's suggestion is correct and changes in plasma volume in cases of rheumatic fever treated with salicylate are due to a sodium effect, this observation is understandable, because nearly all these patients were given salicylate in the form of calcium aspirin. It seems very unlikely, therefore, that the slope of elimination of transfused red cells bears any relation to changes in plasma volume, if indeed such changes occur in cases of rheumatic fever not treated with sodium salicylate.

The finding that normal red cells are destroyed by an abnormal haemolytic mechanism in the circulation of patients with acute rheumatic fever makes it unnecessary to postulate that the anaemia is due to increased destruction of faulty red cells produced by a bone marrow damaged by the disease. To prove this contention conclusively it would be necessary to transfuse blood from rheumatic patients into normal recipients and to show that its mean red cell survival was normal; such an experiment was considered to be unjustifiable in view of our ignorance of the cause of the disease and the possibility of transference of some abnormal process by this means.

The degree of increased red cell destruction, as shown by the slope of elimination of donor red cells,

is in no instance very great; therefore gross signs of haemolysis are not likely to be present and the process may be defined as a latent haemolytic mechanism. Although investigations of the Coombs test have not identified any immune body reaction in rheumatic fever, some such mechanism would seem to be the most likely cause for the haemolysis. The recent work of Glynn and Holborow (1952) has demonstrated the presence of auto-antibodies in rabbits that have developed arthritis and rheumatic fever-like lesions following injection with a polysaccharide-streptococcus vaccine. This observation may be related to the mechanism of the production of haemolysis in patients with acute rheumatic fever.

Summary

The rapidity of the onset of anaemia in acute rheumatic fever suggests an underlying haemolytic process.

The survival of transfused red cells has been followed in six control cases and in eight children with acute rheumatic fever. In the control cases disappearance of donor red cells from the recipient's circulation occurred at a steady rate with a linear slope of elimination. In acute rheumatic fever there was an increased rate of disappearance of donor red cells with a curvilinear slope of elimination indicating random destruction unrelated to the normal process of ageing.

It is concluded that the anaemia in acute rheumatic fever is due, at least in part, to a haemolytic process.

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APPENDIX

Case 1. D.D., a boy, aged 14 years, was admitted to hospital on July 2, 1949, with a history of joint pains and fever for one week and of pleuritic chest pain for three days. His illness started suddenly with pains in the feet, and these rapidly spread to involve the knees, hands and elbows, all joints being involved simultaneously. He had a previous history of three attacks of acute rheumatism involving the joints, apparently without carditis and with no residual joint changes. There was a family history of acute rheumatism.

On admission he had a temperature of 103° F., and sleeping pulse of 125; there was arthritis in the shoulders, wrists and small joints of the hands and feet, generalized lymph node enlargement and enlargement of the liver. There were also signs of consolidation in the right upper zone of the chest and a discrete maculopapular rash on the extensor surfaces of both forearms. The sedimentation rate was 125 mm. in one hour, QTc. 0.452 sec., red blood count 4.5 million, haemoglobin 12.3 g. His

blood was group A Rh positive. He exhibited a remittent fever with a high sleeping pulse rate and gross elevation of the sedimentation rate for the first 10 weeks in hospital. During this time he developed a moderately severe anaemia and had a markedly elevated white cell count; his QTc remained prolonged during this period. Nodules appeared over the left elbow two weeks after admission and he developed pericarditis on August 9. Shortly after this, mitral and aortic diastolic murmurs became audible. He was transfused with 450 ml. of group O Rh-positive packed red cells, two days old, on August 8 with no untoward reaction. At this time the disease was still very active, the sedimentation rate was grossly elevated, and arthritis and pericarditis were all present shortly after transfusion. From the tenth week, however, the disease started to subside, the arthritis disappeared, the QTc became normal and the fever and sedimentation rate settled, but on October 12 he had a severe relapse with arthritis and olecranon bursitis, a sharp rise in sedimentation rate accompanied by slight fever, and prolongation of the QTc. This episode subsided quite rapidly but he continued to run a course of chronic low grade activity and he was in this condition when the investigation of his transfused cell survival rate was completed. A direct and indirect Coombs test on August 5 was negative; serum bilirubin 0.4 mg. %, reticulocytes 1%, osmotic fragility of red cells (Creed's technique), trace of haemolysis at 0.48% NaCl, but otherwise normal.

The percentage survival of transfused red cells in this case, plotted against the erythrocyte sedimentation rate, can be seen in Fig. 3. The slope of elimination during the first 20 days after transfusion was considerably steeper than normal but following this period the slope tended to flatten out until the fiftieth day after transfusion when it again took on a steeper slope only to flatten out again from 70 days until the time of total elimination at 94 days.

Case 2. B.T., a boy, aged 12 years, was admitted to hospital on October 15, 1949, with a one-day history of severe pain in the legs and right arm, and swelling of the right knee. He had a sore throat three weeks before the onset of these symptoms.

On admission he had a fever of 103° F. and severe arthritis involving the knees, ankles and small joints of the right foot; the spleen was enlarged and firm, 2 cm. below the left costal margin. The sedimentation rate was 96 mm. in one hour; QTc 0.426 sec.; red blood count 4.0 million per c.mm.; haemoglobin 12.6 g.; packed cell volume 35 c.cm. %; white blood count 26,000 per c.mm. (94% polymorphonuclear leucocytes). His blood was group A, Rh positive. A Coombs test, direct and indirect, was negative. The arthritis continued for 17 days and then subsided spontaneously. On the fifth day of admission a basal early diastolic and an apical mid-diastolic murmur were heard for two days and there was considerable prolongation of the QTc. He was febrile for the first three weeks and had a high sedimentation rate, and the haemoglobin had fallen to 11.1 g. by the sixth day. On October 20, six days after

FIG. 3.—Survival of transfused red cells in Case 1.

the estimated onset of the disease, he was transfused with 500 ml. of group O Rh-positive packed red cells without any untoward reaction. From the third week

FIG. 4.—Survival of transfused red cells in Case 2.

onwards he showed signs of recovery with subsidence of fever and of the raised sedimentation rate, which returned to normal at the end of the seventh week of his stay in hospital. He also started to gain weight, and the diastolic murmurs in the heart became inaudible. The percentage survival of transfused red cells in this case is shown in parallel with the sedimentation rate (Fig. 4). During the first 15 to 20 days after transfusion the rate of donor cell destruction was considerably increased above the normal, corresponding to the period of greatest activity of the rheumatic fever with arthritis, carditis and a high sedimentation rate. Faecal urobilinogen excretion over the three-day period (October 26

to 28) gave a mean value of 130 mg. per 24 hours and the haemolytic index was raised to 41. Repeated estimation when the disease was inactive gave a mean 24 hour excretion of 26 mg. and a haemolytic index of 5.5.

Case 3. D.N., a boy, aged 12 years, was admitted to hospital on November 18, 1949, with an eight-day history of pains in the right ankle, left knee and in both

FIG. 5.—Survival of transfused red cells in Case 3.

wrists, associated with fever. This illness was preceded by scarlet fever which started on October 2, 1949.

On admission the patient had fever up to 102° F. at night, arthritis in the left knee, a long, early rather loud systolic murmur at the apex and a short early basal diastolic murmur. The spleen was palpable 2 cm. below the left costal margin for the first month and it was firm in consistency. An apical mid-diastolic murmur developed on the fourth day after admission to hospital and was heard constantly after this. The sedimentation rate was 107 mm. in one hour; QTc 0.401 sec.; red blood count 4.0 million per c.mm.; haemoglobin 11.5 g.; white

cell count 8,250 per c.mm. (66% polymorphonuclear leucocytes), packed cell volume 30 c.cm. %. He was treated with salicylates, 6 g. daily, for 10 days with subsidence of fever and dispersal of arthritis. The apical and basal diastolic murmurs remained constant and the QTc was prolonged beyond the limits of normal, on several occasions. On the fifteenth day after admission four days after stopping salicylate therapy, there was a recurrence of arthritis and fever and a slight rise in sedimentation rate and QTc. This relapse lasted for eight days after which it subsided spontaneously with a rapid fall in sedimentation rate. On November 28, 18 days after the estimated onset of the disease, he was transfused with 500 ml. of group O Rh-positive packed red cells, two days old, without any reaction.

The survival of donor cells was only estimated for 42 days after transfusion, but by this time the patient's condition was almost normal. There was considerably increased destruction of transfused red cells in the first 15 to 20 days corresponding to the period when the sedimentation rate was high and the patient had severe arthritis and active carditis (Fig. 5).

Case 4. C.C., a boy, aged 11 years, was admitted to hospital on November 4, 1949, with a history of joint pains involving both knees and the left ankle for four days, the left knee being swollen and immobile. There was a past history of rheumatic fever with carditis one year previously.

On admission his temperature was 102° F. and he had arthritis of both knees and both ankles. There were no diastolic murmurs on auscultation but he had a pericardial friction rub which was present for two days. The spleen was just palpable. His sedimentation rate was 98 mm. in one hour; red blood count 4.0 million per c.mm.; haemoglobin 11.3 g. and white cell count 12,000; QTc 0.402 sec. His blood was group B Rh-positive. Polyarthritis, fever and tachycardia persisted for the first four days and on November 8 calcium aspirin therapy, g. 5 daily, was started. Following this, there was rapid relief of arthritis with subsidence of fever and of tachycardia. On November 9 a mitral diastolic murmur was heard for the first time and a few days later an aortic diastolic murmur also became audible. On November 12, 12 days after the estimated onset of the disease, he was transfused with 300 ml. of group O Rh-positive packed red cells with no subsequent reaction. Salicylates were stopped on November 17 and two days later he had a recurrence of fever, tachycardia and arthritis and his sedimentation rate rose from 75 to 106 mm. in one hour. There was a fall in haemoglobin accompanying this relapse. He remained febrile with persistent arthritis and a grossly prolonged sedimentation rate for the next six weeks, although there was gradual improvement in his condition towards the end of this period. Survival of transfused red cells was studied in this case for 48 days after transfusion. The case is unique in this series in that during the whole period of the experiment the rheumatic fever ran a course of acute activity and only showed some tendency to recovery in the past fortnight. The slope of elimination of donor cells was

fairly steep throughout the whole 48 days (Fig. 6) although in the last 14 days there appeared to be some flattening out of the curve. Thus the complete slope of elimination is again curvilinear.

FIG. 6.—Survival of transfused red cells in Case 4.

Case 5. A.F., a girl, aged 6 years, was admitted to hospital on May 27, 1949, with a history of transient pain in one ankle six days previously, followed by substernal pain on breathing three days later and pains in the feet and legs on the day before admission. On the day after admission she developed arthritis in both knees, erythema marginatum and chorea with a high fever and sedimentation rate of 87 mm. in one hour. Three days

later an apical mid-diastolic murmur was first heard and the QTc was prolonged to 0.437 sec. She was treated with calcium aspirin, g. 3 daily, for nine weeks, and this rapidly controlled the fever and joint pains. On admission the red blood count was 4.8 million, haemoglobin 12.1 g. and her blood was group A Rh-positive. She was transfused on June 13, 23 days after the estimated onset of the disease, with 400 ml. group O Rh-positive packed red cells with no reaction. The fever, QTc and sedimentation rate had settled to normal five weeks after admission and the apical mid-diastolic murmur and chorea disappeared two weeks later. Following this the disease ran an uneventful course to complete recovery.

Survival of transfused red cells was studied in this case until complete elimination occurred at 94 days. Although the disease settled down more rapidly in this case than in those previously described, there was increased donor cell destruction initially followed by a flattening out of the slope of elimination (Fig. 7).

FIG. 7.—Survival of transfused red cells in Case 5.

Case 6. P.B., a girl, aged 13 years, was admitted to hospital on March 31, 1949, with a history of pains in the wrists, knees and small joints of the feet for five days

associated with fever and marked pallor. On admission she had a fever of 103° F. at night which persisted for 10 days, severe arthritis in the knees, ankles and fingers, and a significant apical systolic murmur with apical and

FIG. 8.—Survival of transfused red cells in Case 6.

basal diastolic murmurs developing on the fifth day after admission. Sedimentation rate was 107 mm. in one hour, QTc 0.441 sec., red cell count 4.0 million per c.mm., and haemoglobin 11.5 g. and white cell count 20,000 (85% polymorphonuclear leucocytes). Her blood group was A Rh-positive.

The apical mid-diastolic murmur disappeared in the third week and was only heard subsequently for a short time from the thirteenth to the seventeenth week in hospital; the early basal diastolic murmur was heard constantly. The sedimentation rate fell to normal in the twelfth week, but rose again sharply in the next week with an attack of diaphragmatic pleurisy. This was thought to be non-rheumatic as there was conversion of the Mantoux reaction from negative to positive following this episode. There were no signs of rheumatic activity

from this time onwards. The patient was transfused on April 22, 26 days after the estimated onset of the disease, with 450 ml. of group O Rh-positive packed red cells with no subsequent reaction. Immediately before transfusion the red blood count was 3.6 million; haemoglobin 10.3 g.; packed cell volume 31 c.cm. %. Two days after transfusion the red blood count was 4.9 million, haemoglobin 13.7 g.; packed cell volume 41 c.cm. %, and reticulocytes 3%.

The survival of donor cells was followed for 95 days after transfusion. Initially there was increased donor cell destruction for the first 20 days after transfusion, after which the slope of elimination became flatter and conformed with the normal. It is interesting that when the patient developed tuberculous diaphragmatic pleurisy 60 days after transfusion with fever and a sharp rise in sedimentation rate, there was no apparent increase in the rate of elimination of donor cells. This is in contrast to the increased elimination of donor cells in rheumatic relapse (Fig. 10). The slope of elimination is curvilinear initially with the steepest portion of the curve corresponding to the period of acute rheumatic activity.

Case 7. E.D., a girl, aged 6 years, was admitted to hospital on December 15, 1948, with a history of severe joint pains in the right hip and right knee of one day's duration following a sore throat three weeks earlier. An apical mid-diastolic murmur and an early basal diastolic murmur were heard on the first day of admission and persisted throughout her illness. She was choreic and developed rheumatic nodules over the right elbow and there was low grade pyrexia and a persistently raised sedimentation rate for the first 15 weeks in hospital. On March 27, 1949, she had a relapse with a raised temperature and sleeping pulse rate, erythema marginatum and an increase in activity of carditis as indicated by prolongation of the QTc from 0.444 sec. to 0.474 sec. She developed arthritis in the left hip on March 30. Three days later, at the height of this relapse, she was transfused with 500 ml. of fresh group O Rh-positive packed red cells over a period of eight hours. During the relapse and for the next three weeks she was treated with calcium aspirin g. 2 daily. On May 2 she developed tonsillitis with a rise in temperature and sleeping pulse rate and with elevation of the sedimentation rate. Salicylates were given for a further two weeks, but seven days after they were stopped, on May 17, she again relapsed with fever and arthritis in the left elbow and in both wrists. Following this the disease settled uneventfully whilst the patient was on continuous aspirin therapy. A further transfusion of 400 ml. of fresh group O Rh-positive packed red cells was given on July 29 with no reaction. Twenty-one days later, soon after stopping salicylate therapy, she again had a rise in temperature and sleeping pulse, a steep rise in sedimentation rate, a recurrence of chorea and of erythema marginatum and a rise in QTc from 0.404 sec. to 0.434 sec. After this, the sedimentation rate slowly returned to normal, but she had an occasional rise in evening temperature and the QTc remained elevated above normal for several weeks.

Survival of donor red cells from the first transfusion was estimated until complete elimination occurred 90 days after transfusion (Fig. 9). This period was marked

Fig. 9.—Survival of transfused red cells in Case 7, following first transfusion.

by three episodes: at the time of the transfusion the patient was undergoing a severe relapse, 30 days after transfusion she had tonsillitis and 45 days after transfusion she had a further relapse of a milder nature. Immediately before transfusion the red blood count was 4.3 million per c.mm.; haemoglobin 12.7 g.; packed cell volume 41%; reticulocytes 0.8%; serum bilirubin 0.2 mg. %. Direct and indirect Coombs tests were negative and osmotic fragility of the red cells in saline was normal.

There was increased destruction of donor cells in the first 12 days after transfusion, after which there was some flattening of the slope of elimination coinciding with clinical improvement in the patient's condition. The attack of tonsillitis was apparently not accompanied by any increased destruction of transfused red cells, but the relapse at 45 days after transfusion caused a further

increase in steepness of the slope of elimination, which subsequently flattened out again from 50 days onwards. The complete slope of elimination thus forms two curvilinear curves coinciding with the two rheumatic relapses and indicating increased donor cell destruction accompanying these episodes.

The second transfusion was given when the disease had apparently become inactive, with a view to estimating the normal transfused red cell survival in this case, and the first three readings obtained up to 21 days after transfusion appeared to follow the normal line, although they were rather higher than those usually found (Fig. 10). Twenty-two days after transfusion, however, the disease relapsed and an inagglutinable count taken six days later, together with two further counts in the next six days, all showed evidence of considerably increased donor cell destruction coinciding with this episode. Following recovery from this relapse, the slope of elimination flattened out again to conform with the normal. The slope of elimination coinciding with the clinical relapse is curvilinear.

FIG. 10.—Survival of transfused red cells in Case 7, following second transfusion.

This case is of particular interest for, following both transfusions there was a relapse, and accompanying each relapse there was increased elimination of transfused red cells.

Case 8. B.R., a girl, aged 10 years, was admitted to hospital on February 22, 1949, with a four month's history of recurrent joint pains following a sore throat.

FIG. 11.—Survival of transfused red cells in Case 8.

She had a past history of several attacks of rheumatic fever, the first occurring at the age of 4, and from August 1, 1947, until February 29, 1948, she was in hospital with rheumatic carditis and mitral and aortic valve disease.

On admission she had large nodules on the elbows,

knees and occiput. There were loud apical systolic and diastolic murmurs and a loud early diastolic murmur was audible over the base of the heart. The sedimentation rate was 31 mm. in one hour and the QTc was 0.428 sec. She had a moderate anaemia with 4.0 million red cells, haemoglobin 11.1 g. and her blood was group A Rh-positive; osmotic fragility of red cells was normal. Three days after admission she had a severe relapse with arthritis, a high temperature and sleeping pulse rate, pericarditis and severe epistaxes; her sedimentation rate rose to 56 mm. in one hour. The QTc rose to 0.436 sec., but with the onset of pericarditis it fell progressively to 0.303 sec. The degree of anaemia rapidly increased, and three weeks after the onset of the relapse the red cell count was 2.9 million, haemoglobin 7.3 g. and packed cell volume 23%. The reticulocyte count was 2.2%; serum bilirubin 0.8 mg. %; and there was excess of urobilinogen in the urine. Osmotic fragility of red cells, trace of haemolysis in 0.48 per cent. saline. On March 23, 1949, 26 days after the relapse, she was transfused with 300 ml. of group O Rh-positive packed red cells,

four days old, in nine hours. On March 26, she had a further relapse with arthritis and pericarditis and she died on April 4 in congestive cardiac failure. Necropsy revealed severe rheumatic carditis with gross involvement of the aortic valve, which was incompetent, and lesser involvement of the mitral valve; there was also evidence of acute rheumatic pericarditis with effusion. The spleen weighed 102 g. and the sectioned surface looked normal. It was only possible to follow the survival of transfused red cells for seven days after transfusion in this case owing to the death of the patient. In this period, however, there was marked increased destruction of donor red cells, 79.1% surviving after seven days (Fig. 11). During this time the patient was severely ill and in cardiac failure so that only two inagglutinable counts were obtained following the initial donor cell count. The more rapid fall in the donor count in this case compared with the previous cases might be accounted for to some extent by an increase in plasma volume associated with cardiac failure.