BRITISH MEDICAL JOURNAL

LONDON SATURDAY NOVEMBER 8 1941

PREPARATION AND USE OF CONCENTRATED RED CELL SUSPENSIONS IN TREATMENT OF ANAEMIA

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The establishment of blood banks has greatly increased the use of blood transfusions, not only in the most urgent cases of haemorrhage and shock and for the more severe and resistant cases of chronic anaemia but also as a means of shortening the convalescence of patients suffering from anaemias of less severity, many of whom would probably recover ultimately without resort to such treatment. From the point of view of the individual patient as well as from that of national economy it may often be justifiable to utilize the blood of one healthy person to enable another to return to work at an earlier date than would otherwise be possible; but the growing tendency to substitute intravenous transfusion of blood for the oral administration of iron results in a constant drain on the supply of blood now urgently required for preparation of plasma. The transfusion habit now being established may also cause serious embarrassment to the blood transfusion services in peacetime, for such a useful therapeutic weapon will not willingly be surrendered at the conclusion of hostilities.

Large-scale preparation of plasma from stored blood, however, has made available considerable quantities of red cells in concentrated form. These red cells, amounting to about 50% of the original volume of whole blood, are usually discarded, which in view of their potential value in the treatment of anaemia is particularly regrettable.

Suspensions of red cells separated from the plasma have been used in the past for the treatment of anaemia, but with few exceptions these suspensions were diluted with saline to such an extent as to make them more dilute than normal blood (Robertson, 1918). The use of the red cells in concentrated suspensions has recently been described by MacQuaide and Mollison (1940). At the time their article was published a few cases of anaemia had been treated by the Liverpool Regional Blood Transfusion Service with similar concentrated suspensions, and since then these have been used in increasing amounts.

Preparation of the Suspensions

At the regional laboratory of our transfusion service plasma is withdrawn from all bottles of blood, irrespective of group, on the seventh day of storage, up to which time they have been available for transfusion as "whole blood" wherever required. The stocks of blood stored in each individual hospital, which under present conditions are considerably in excess of the normal transfusion requirements

of civilian medical practice, are similarly processed. The technique of plasma separation is the "closed" method described by Edwards and Davie (1940). In this process the plasma is aspirated through a long needle inserted through the perforated metal cap and rubber diaphragm which closes the bottle. After separation of the plasma (a process which can be performed aseptically) the aspirating needle is plunged to the bottom of the red cell layer and aspiration of the concentrated red cells is continued into a fresh sterile bottle. The layer of gelatinous material which normally lies on the surface of the red cells and consists of leucocytes, platelets, and fibrin is carefully left behind in the blood bottle. The last remaining drops of cells in the needle and tubing are transferred to a tube of citrate-saline solution for grouping and cross-matching. The red cells of another bottle of blood of the same group, and, if necessary, of a third bottle, are added to those of the first, a specimen of these cells being similarly retained for grouping, etc. A bottle of concentrated red cells will therefore consist of contributions from two or three donors. The citrated sample tubes of cells are attached by a rubber band to the neck of the bottle of concentrated cells. Cross-matching of each sample with the serum of the patient is occasionally undertaken by the clinician, but is generally performed in the laboratory before the bottle is issued for use. These concentrated suspensions are usually prepared only at the request of clinicians, but it is sometimes convenient to anticipate the demand by preparing a small stock of bottles of assorted groups. These are stored in the refrigerator for forty-eight hours, after which time those not used are discarded.

Nature and Composition of Concentrated Red Cells

Specimens of concentrated red cell suspensions prepared by the present method show considerable variations in their haemoglobin and cell content. This is mainly due to individual differences in the rate and extent of cell sedimentation in citrated blood under conditions of storage.

The following figures are quoted as being fairly typical of the results obtained from examination of a sample of concentrated red cells:

Haemoglobin (Haldane)	..	150%
Red cells	..	8,500,000 per c.mm.
White cells	..	3,000 per c.mm.
Corpuscular volume (Wintrobe)	..	85% (normal for whole blood, 45%)

The most important difference between stored blood and fresh blood is the increased corpuscular fragility of the former. This is mainly due to the fact that the anticoagulant solution of 2.5% sodium citrate is hypertonic and produces in the blood corpuscles a state of increased intracellular osmotic tension. When such red cells are placed in contact with isotonic saline solutions they exhibit greatly increased fragility, since by comparison with their contents their new surroundings are now hypotonic (Maizels and Whittaker, 1939). Another possibility is that venous blood, by reason of its high CO₂ content, is somewhat more fragile than either capillary or arterial blood (Whitby and Hynes, 1935).

It was suggested by MacQuaide and Mollison that a small amount of hypertonic (1.1%) sodium chloride solution should be added to the suspensions in order to reduce their viscosity and thus facilitate their administration. Before adopting this procedure ourselves a few preliminary experiments were performed to determine the resistance of blood cells to 1.1% saline solution after they had been stored for seven days. Red cell counts of specimens of stored blood were made, using as a diluting fluid normal human serum of the same blood group. Duplicate counts were also performed on the same specimens, using 1.1% saline solution as the diluent. Any reduction of the red cell count in the saline compared with that in serum would indicate the degree of haemolysis that had occurred in the former solution. The counting pipettes, containing the red cells diluted 1 in 200 with the respective diluents, were left at room temperature for fifteen minutes before being finally shaken and counted. In one such experiment the discrepancy between the results of the two counts amounted to as much as 1.7 millions per c.mm., or approximately 20% of the total. This represented a degree of *in vitro* haemolysis that would be incompatible with safety in a bottle of blood intended for transfusion. It was therefore decided that the addition of 1.1% saline to suspensions of concentrated red cells as prepared by us would be inadvisable. In any case, this dilution has not been found necessary, as no insuperable transfusion difficulties have been experienced on account of increased viscosity of the concentrated suspensions.

The apparent increase in the fragility of stored red cells does not appear to have the clinical significance that one might be led to expect. There seems to be no direct relation between the fragility of the red cells, as measured by the usual test of resistance to hypotonic solutions of sodium chloride of varying concentrations, and the rate of their destruction in the body of the recipient. This contention is supported by recent investigations into the survival time of stored blood cells after transfusion experiments which have shown only slightly longer survival of fresh cells as compared with those stored for a week, in spite of the greatly increased fragility of the latter (Mollison and Young, 1940).

Administration

Although the increased viscosity of suspensions of concentrated red cells slows the flow through the delivery apparatus, no modification of the standard blood transfusion outfit as supplied by E.M.S. transfusion centres is necessary beyond lengthening the transfusion rubber tubing to provide a greater head of pressure. When giving concentrated red cell suspensions in any quantity it is important that the flow be regulated to approximately 100 c.cm. an hour to avoid the risk of cardiac embarrassment by rapid addition of a viscous fluid to the circulating blood, particularly as the myocardium of an anaemic person may have undergone pathological changes which impair its efficiency.

The indications for the administration of concentrated red cell suspensions are such that it should seldom be necessary or even advisable for them to be given as an urgent or emergency measure, since supplies of plasma and Group IV (O) whole blood are available to every hospital for emergency transfusions. Concentrated suspensions should therefore be used only for cases of chronic anaemia which require rapid replacement of haemoglobin and red cells with minimal increase of circulating blood volume.

Results of Treatment

Material for over 150 transfusions of concentrated red cells has been issued in the past eight months. Of these transfusions over sixty have been administered to two patients suffering from aplastic anaemia, but owing to certain special features these cases have been omitted from the present series. Information regarding responses to transfusions, in terms of elevation of haemoglobin percentages and of changes in the red cell counts, has been procurable in only 38 transfusions, administered to 35 patients. These results for convenience of comparison have been tabulated, together with other data relevant to the cases, in Table I. Fair comparison of these results is

TABLE I.—Summary of Results of Transfusions

Case No.	Amount given (c.cm.)	Hb% Before	Rise in Hb %	R.B.C.s before Transfusion (millions per c.mm.)	Rise in R.B.C.s (millions per c.mm.)	Rise in Hb % per 500 c.cm. conc. R.B.C.s	Condition
2	900	50	30	—	—	17	Hodgkin's disease
"	500	80	(-5)	—	—	-5	Severe exacerbation of the disease at time of transfusion
3	900	45	20	2.56	0.89	11.1	Septic abortion
4	800	44	18	2.67	1.2	11.25	Haemorrhage
5	500	18	14	—	—	14	"
6	800	56	6	3.32	0.39	3.75	Recurrent peptic ulcer
8	900	48	18	2.8	0.61	10	Septic abortion
11	900	26	24	2.17	—	13.3	Anaemia and pyrexia
12	1,000	40	20	2.5	1.6	10	Haemorrhage
14	1,000	40	7	1.75	0.45	3.5	Pernicious anaemia
15	500	35	35	—	—	35	Chronic haemorrhage from cervical polyp (operation was performed on same day as the transfusion)
17	1,000	30	6	2.53	1.3	3	Carcinoma ventriculi; haematemesis
18	1,000	35	0	—	—	0	Aplastic anaemia (fatal)
19	1,000	28	17	2.43	0.75	8.5	Septicaemia; secondary haemorrhage
21	1,000	35	23	2.75	1.25	11.5	Hysterectomy
22	700	35	10	2.02	0.38	7.1	Amputation (septic)
24	800	26	14	2.12	1.08	8.8	Severe anaemia
25	1,000	58	26	5.29	(-14)	13	Gastric ulcer; partial gastrectomy
26	1,000	56	24	3.24	—	12	Puerperal anaemia
27	950	44	30	2.87	1.92	15.8	Uterine fibroid
29	2,000	24	65	—	—	16.25	Pernicious anaemia
30	800	37	23	2.46	1.02	14.25	Leukaemia; haemorrhage
33	350	28	0	2.22	(-31)	0	Anaemia (post-natal)
"	1,000	48	18	2.92	0.74	9	Anaemia
36	1,000	20	18	0.87	0.56	9	Severe anaemia
37	500	52	12	2.9	0.62	12	Haemorrhage—source not specified
39	1,000	55	22	3.55	0.55	11	Sepsis
42	500	50	8	3.15	0.53	8	Gastric ulcer
45	1,000	40	30	3.14	2.42	15	Carcinoma ventriculi
47	900	28	14	1.01	2.34	7.8	Pernicious anaemia
"	900	49	16	2.35	0.7	8.9	"
48	850	45	33	2.65	2.21	19.4	Haemorrhage (haematemesis)
52	900	30	25	3.28	—	13.9	Ulcerative colitis
56	500	30	35	—	—	35	Haemorrhage (metropathia haemorrhagica)
57	1,000	40	15	2.0	1.1	7.5	Anaemia
58	500	60	10	—	—	10	Septicaemia
59	600	60	10	—	—	8.3	"
61	1,000	23	23	—	—	11.5	Pernicious anaemia

unfortunately rendered difficult by the fact that blood counts and haemoglobin estimations, although in most cases performed by experienced pathologists, include readings taken by several different standards and under varying circumstances. These individual findings are therefore more than usually liable to error, and are of somewhat limited value as data upon which scientifically accurate conclusions may be based. They are presented here simply as evidence from which our own clinical impressions were formed.

Owing to the wide variety of conditions which have been treated with concentrated red cells these results have been classified into four main groups, as follows:

- Group I: Post-haemorrhagic anaemias (7 cases).
- Group II: Anaemias associated with severe infection (6 cases).
- Group III: Anaemias of pregnancy and the puerperium (4 cases).
- Group IV: Dyshaemopoietic anaemias (pernicious, aplastic, and idiopathic hypochromic anaemias) (7 cases, 8 transfusions).

To compare the individual responses, the percentage rise in haemoglobin for each case was calculated for a "standard transfusion" of 500 c.cm. (approximately one bottle) of concentrated red cell suspension. The mean response to a standard transfusion was obtained for each group of cases, and graphs were prepared by plotting these individual results in terms of percentage rise of haemoglobin per 500 c.cm. of suspension. These, with the mean in each group, are illustrated in Figs. 1 to 4.

Graphs showing Calculated Percentage Rise of Haemoglobin per 500 c.cm. of Concentrated Red Cell Suspension. (The mean of each series is represented by a horizontal broken line at the appropriate level. Figures along base line refer to case numbers.)

FIG. 1.—Group I: Post-haemorrhagic anaemias.

FIG. 2.—Group II: Anaemias associated with severe infection.

FIG. 3.—Group III: Anaemias of pregnancy and puerperium.

FIG. 4.—Group IV: Dyshaemopoietic anaemias.

In the first, or post-haemorrhagic, group the results show a wide range of scatter about the mean. Allowance for 5% error in haemoglobin estimation, however, places all except four of the readings within reasonable limits of variation. The four readings which fall outside these limits include two cases with very low responses (Nos. 6 and 17), both of which were suffering from chronic gastric complaints, one being a carcinoma and the other a recurrent peptic ulcer that proved fatal two weeks after transfusion. The other two cases (Nos. 15 and 56) showed abnormally high responses to transfusion. These were both cases of chronic uterine haemorrhage of non-malignant origin which were transfused immediately before operation, and the second haemoglobin readings of which were performed soon after operation. Omission of these four cases from the series

would reduce the mean of this group from 14.3% to 12.8% haemoglobin per 500 c.cm. of concentrated blood.

The second group of post-infective anaemias, although too small to provide conclusive evidence, shows a surprisingly high average response to transfusion. From the subsequent histories of these few patients it appears that cases of septicaemia associated with anaemia are very much improved by transfusion therapy. We now therefore recommend, for severe cases of this kind, repeated transfusions of concentrated red cell suspensions until the haemoglobin is over 60%. When this level has been attained fresh whole blood, which is richer in antibodies and contains active leucocytes, is of greater benefit, and transfusion with this should be continued until the haemoglobin is at least 90%. This treatment should, however, be additional to rational anti-infective measures.

The results of four cases of anaemia associated with pregnancy and the puerperium are shown in Fig. 3. Little may be deduced from our findings in these cases, but clinicians have been impressed with the value of concentrated red cell transfusions in rapidly rehabilitating patients who might otherwise remain in a state of chronic debility and vague ill-health as a result of nutritional or post-haemorrhagic anaemia.

The graph illustrating the dyshaemopoietic group (Fig. 4) shows a considerable variation in the responses of the individual cases. Two cases showing very poor results (Nos. 14 and 18) were respectively severe pernicious anaemia with subacute combined degeneration of the cord (which ultimately made a good recovery) and a case of aplastic anaemia. The latter patient failed to respond to transfusion treatment, and died a few weeks later. There is reason to believe that in both these cases the haemoglobin estimations were faulty. Their omission from the series would have raised the mean increase of haemoglobin per 500 c.cm. transfusion from 8% to 10% for this group.

From these results we are able to conclude that, irrespective of the nature of the condition causing the anaemia, transfusion with one bottle of 500 c.cm. of concentrated red cell suspension raises the haemoglobin between 10% and 12%. Exceptions to this occur, and are usually low responses due to a rapid exacerbation in the factors causing the anaemia. Unexpectedly high results have in some cases been attributable to operations which have not only removed the cause of the anaemia but may also have produced some degree of haemoconcentration.

Reactions

Reports of the incidence of reactions following the use of concentrated red cell suspensions were obtained regarding 77 transfusions which were given to 61 patients. In all, fifteen reactions of all grades were noted, the gross reaction rate thus being 19.5%. Reference to Table II shows that this series consisted of five Grade I reactions (slight elevation of temperature), two Grade II reactions (pyrexia with slight shivers but no definite rigor), and seven Grade III reactions (definite rigors associated with rise of temperature).

This apparently high rate of rigor production (amounting to 9.1% of all transfusions) may be attributable to the peculiar circumstances in which the Liverpool Regional Transfusion Service was placed during three months of the period under review. Enemy action had destroyed the apparatus necessary for the production of distilled water, and the supply of 2.5% sodium citrate solution had therefore to be obtained from other sources, under circumstances that made it impossible to guarantee the exclusion of pyrogens. To this was attributed the fact that during this period the reaction rate (all three grades) among patients receiving stored whole blood had risen to an exceptionally

high figure—more than double the incidence before this period. Of the 77 transfusions of concentrated red cells 21 were given during this three-month period, and accounted for four out of the seven Grade III reactions and three others of less severity. The exclusion of all transfusions administered during this period gives a Grade III reaction (rigor) incidence of 3 in 56 transfusions, or 5.35%.

TABLE II.—Details of Cases showing Reactions to Transfusion

Case No.	Sex	Age	Group	No. of Transfusions	Hb % Before	Amount of C.B. given (c.cm.)	Hb % After	Details of Reaction	Diagnosis and Results
<i>Grade I Reactions (T. 100° or over, Without Symptoms)</i>									
16	F	—	2	1	58	1,000	—	Temp. 101° following day	Post-natal anaemia
29	M	63	2	2	24	2,000	89	Temp. 100.2° after first transfusion	Pernicious anaemia
48	M	65	2	2	45	850	78	Temp. 100° after second transfusion	Haematemesis
50	M	28	4	1	—	500	56	Temp. 101°	Haematemesis. Ordinary blood given, with no reaction
52	F	32	4	1	30	900	55	Temp. 100°	Ulcerative colitis
<i>Grade II Reactions (T. over 100°, with Slight Shivering)</i>									
63	M	39	4	2	50	400	—	Slight rigor after first transfusion	Haematemesis
47	F	46	4	2	28	900	42	Slight rigor after first transfusion	Aplastic anaemia
<i>Grade III Reactions (Definite Rigors, with Pyrexia)</i>									
1	M	—	4	2	50	500	—	Rigor : temp. 101° after first 100 c.cm.	Papilloma vesicae. Remainder of transfusion given next day
15	F	43	4	1	35	500	70	Rigor : temp. 101° following day	Cervical polyp
22	M	—	4	1	35	700	45	Rigor (reaction)	Sepsis and amputation
33	F	30	3	2	28	350	28	Rigor with first transfusion	Post-natal anaemia. No reaction with second transfusion
43	F	37	3	2	35	500	—	Rigor with first transfusion	Severe septicaemia. Rigor ? not due to transfusion. Given old suspension on second occasion and became jaundiced
55	F	54	4	1	50	1,000	—	Rigor : temp. 102°	Carcinoma ventriculi; pyrexial before transfusion
60	M	37	2	1	—	400	—	Rigor : temp. 103°	Haematemesis. Improved
<i>Grade IV Reactions (Anaphylactic Phenomena, etc.)</i>									
31	F	26	—	1	34	500	—	Urticarial oedema	Complete abortion. Oedema subsided with adrenaline

Our gross reaction rate thus appears to have been considerably higher than it might have been in the absence of any disturbance of our laboratory routine. Corrected for this, the impression is confirmed that, as stated by MacQuaide and Mollison, the incidence of reactions to transfusions of concentrated red cell suspensions is no higher, and probably is lower, than that produced by the use of stored whole blood.

In addition to the febrile reactions discussed above there remains a group of reactions which appear to be due to some hypersensitivity on the part of the recipient to some component of the transfused fluid. They are not peculiar to concentrated red cells, for they occur also after plasma transfusions. These reactions would seem to be of an allergic nature, as is illustrated by the case reported in Table II under the heading "Grade IV Reactions." In this case no harm was done, as the oedema that resulted subsided after the administration of adrenaline. One fatal reaction, which appeared to have been due to a similar mechanism, occurred following one transfusion of con-

centrated blood, and owing to its importance the case is here reported in full.

A woman aged 46 (Case 35) was admitted to hospital with a history of menorrhagia, and complaining of dyspnoea and palpitations; there were also signs of anaemia. Blood examination shortly after admission showed hypochromic anaemia, the haemoglobin being 35% and the red cell count 2.3 millions per c.mm. The menorrhagia was found to be due to a cervical polyp, and as a preliminary to operation the patient was given a transfusion of concentrated red cells, starting at 6.45 p.m. on April 2, 1941. Three hours later she was rather restless, but felt drowsy. Six hours after the transfusion was begun she was pale and cyanosed, with deep stertorous respirations. The heart rate was 160 per minute and the radial pulse not palpable. The transfusion, almost completed, was then stopped, and the patient given morphine, grain 1/6, oxygen, and coramine. She died in coma at 1.45 a.m., seven hours after the start of the transfusion. Post-mortem examination revealed a large, pale, flabby, and grossly dilated heart, with numerous ecchymoses, averaging about 5 mm. in diameter, scattered upon the surface of the interventricular septum. Some of these petechiae appeared to involve the left branch of the bundle of His. There was no lesion of the heart valves or of the coronary arteries, and no evidence of thrombosis of the saphenous vein. The spleen was enlarged to about double its normal size. Incompatibility of blood was excluded by direct cross-agglutination tests performed before transfusion and repeated afterwards, while the slow rate at which the transfusion was given excludes the possibility of primary myocardial failure such as may have resulted from sudden loading of the right heart with transfused blood. The presence of petechial haemorrhages in the myocardium renders it probable that death was due to sudden disturbance of the conducting mechanism of the ventricles. While these changes may have been due to severe anaemia alone, in view of the circumstances under which death occurred it is possible that they were due to an anaphylactoid reaction following the transfusion.

This case emphasizes the importance of avoiding the use of blood likely to produce reactions of an allergic nature, but unfortunately we are not yet in a position to detect the sensitive patient in advance. The only precautions at present available against such accidents consist in scrupulously excluding from the panel of donors all those with a history of allergic manifestations and, where practicable, performing intradermal skin tests upon prospective recipients with a similar history, using a few minims of the transfusion fluid as an antigen.

Summary

The preparation and properties of concentrated red cell suspensions are described, and the advantages of their use in preference to whole blood in certain cases are discussed.

Results of 77 transfusions are reported, special reference being made to the percentage rise of haemoglobin per 500 c.cm. of suspension, and to the effect, if any, of the patient's clinical condition upon this result.

The frequency of reactions to this form of transfusion is calculated, and found to be considerably lower than that occurring when stored whole blood is used. One fatal reaction is described, and its aetiology briefly discussed.

We are indebted to numerous physicians and surgeons on the staffs of Merseyside hospitals, and to the medical superintendents of municipal hospitals in Liverpool, Birkenhead, and Wirral, for their permission to consult their case records, and to those resident medical officers and house-surgeons who have themselves contributed much valuable information about their patients. We are also grateful to Drs. Joy Cronin-Lowe, Sheila Kenny, and J. C. Millar for having provided us with their own personal observations of several cases. Finally, we are glad to record our appreciation of the work of Mr. J. Stainton and other members of the laboratory and secretarial staff of the Regional Blood Transfusion Centre, without whose assistance the present work could not have been completed.

REFERENCES

- Edwards, F. R., and Davie, T. B. (1940). *British Medical Journal*, **2**, 73.
 MacQuaide, D. H. G., and Mollison, P. L. (1940). *Ibid.*, **2**, 555.
 Maizels, M., and Whittaker, N. (1939). *Lancet*, **2**, 1219.
 Mollison, P. L., and Young, I. M. (1940). *Lancet*, **2**, 420.
 Robertson, Oswald H. (1918). *British Medical Journal*, **1**, 691.
 Whitby, L. E. H., and Hynes, M. (1935). *J. Path. Bact.*, **40**, 219.