The relation of the isoagglutinins in pooled plasma to the haemolytic anaemia of burns

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SYNOPSIS Patients showing red cell loss following severe burns have been considered in two groups, the more severe cases with late haemoglobinuria and the more numerous less severe cases without late haemoglobinuria. Patients who had late haemoglobinuria usually belonged to group A or AB, showed a normal or increased osmotic fragility of red cells, and had received more than three plasma volumes of pooled plasma. It is suggested that this haemolytic anaemia may have been caused by the anti-A isoagglutinins in pooled plasma or serum. Patients showing red cell loss but who had no late haemoglobinuria had a normal ABO group distribution and an increased osmotic resistance of their red cells. It is concluded that this more common type of red cell loss in burns is independent of transfused isoagglutinins. Isoantibodies present in pooled plasma have been investigated. Corresponding antibodies have been demonstrated in the serum of groups A and AB patients transfused with moderate quantities of pooled plasma. The same patients commonly also had a raised erythrocyte sedimentation rate, without there being a clinically important red cell loss.

The proportions of the groups of the bloods used in preparing dried pooled plasma are arranged to minimize the effects of isoagglutinins, and the resulting mixture can normally be safely infused without regard to the blood group of the recipient. There remains, however, the possibility that when such plasma is given in very large quantities sufficient isoagglutinins may be present to cause damage to the recipient's red cells. Such an occurrence has been reported by Keitel and Wich (1954) in patients receiving plasma transfusions, and other evidence has been reviewed by Mollison (1961). Large volumes of plasma are sometimes given in the treatment of burns, and Ebert and Emerson (1946) described increased osmotic fragility associated with transfusion of group O blood or plasma to seven patients, including one who developed haemoglobinuria and other signs of intravascular haemolysis and died.

In the present investigation the contribution of ABO isoagglutinins to red cell loss has been studied in a large series of burned patients treated with various plasma preparations, in many of whom red cell loss was measured by serial ^{51}Cr red cell volumes.

CLINICAL MATERIAL

Studies were made on 111 patients who received intravenous therapy for burns. The different régimes of treatment were: 1 Reconstituted dried pooled plasma given in amounts sufficient to maintain near normal circulatory volume (75 patients); 2, comparison in a formal trial of six patients treated with plasma of homologous ABO group and four comparable cases treated with dried pooled plasma; 3, comparison in another trial of a total of 26 patients treated with albumin, dried pooled serum, dried pooled plasma, or a combination of these.

Each pool of dried plasma or serum was derived from 10 donations of blood (four group O, four group A, two B or AB, or one B and one AB). Each bottle contained the dried solids from 400 ml. of acid-citrate-dextrose plasma or serum which were reconstituted with 400 ml. sterile distilled water. Each pool of homologous ABO group plasma was derived from 10 donations of blood.

INVESTIGATIONS

Red cell loss was calculated from ^{51}Cr or ^{32}P red cell volume measurements (Davies, 1960) and the average normal red cell volume for the patient's height and weight with suitable correction for volume of blood transfused

(Topley and Jackson, 1957). These determinations were made when possible about six hours after injury (range two to eight hours) and thereafter daily for two or three days. The red cell loss was expressed as a percentage of the normal circulating red cell volume.

Hourly urine samples were examined for haemoglobin during the period of colloid transfusion. The term 'late haemoglobinuria' was used for the occurrence of at least three haemoglobin-stained hourly specimens between 12 and 48 hours after injury. This should be distinguished from an earlier haemoglobinuria probably due to the direct effect of heat on red cells.

Serological studies to detect anti-A isoagglutinins were made on the patients in the trial of homologous plasma and on certain others.

Osmotic fragility, erythrocyte sedimentation rate, and plasma protein studies were made on patients in the trial of albumin, dried pooled serum, and dried pooled plasma.

Osmotic fragility was measured by adding 0.05 ml. quantities of heparinized blood to 5 ml. quantities of a range of concentrations of sodium chloride. The latter were prepared from a stock solution checked by titration with silver nitrate. After keeping the suspensions of red cells at room temperature for two hours they were centrifuged and the optical density of the supernatant read on a Spekker photoelectric colorimeter using a blue-green filter (Ilford 625). These optical densities were expressed as percentages of that of a total lysate in ammonia-water of an equivalent quantity of red cells.

The erythrocyte sedimentation rate (E.S.R.) was measured by the method of Hardwicke and Squire (1952). Results were expressed both as distance of sedimentation in one hour and as maximum sedimentation velocity. The sedimentation rates of the patient's and normal cells in normal and patient's serum and plasma were also investigated. Fibrogen was determined by micro-Kjeldahl estimation on clots separated from oxalated plasma.

RESULTS

Severe red cell loss was found in a few patients and the evidence suggests that therapy with dried pooled

plasma was partly responsible for this loss. These cases will be considered first.

SEVERE RED CELL LOSS A loss of more than 40% of red cells was found in 10 patients. All had late haemoglobinuria and two developed jaundice (Table I). Nine of these 10 patients died as compared with an expectation of 7.5 based on area of burn and age (Bull and Fisher, 1954). Eight other patients had late haemoglobinuria. In many instances it was associated with a smaller but still serious (20 to 40%) loss of red cells; in a few cases the measurements of loss are inadequate to give a valid estimate of its degree.

Relation to ABO groups Nine of the 10 most severe cases belonged to group A or AB. When cases showing milder degrees of red cell loss were included as well as other cases with late haemoglobinuria, there were 13 instances of red cell loss known to have been greater than 20% in one day and 18 instances of late haemoglobinuria. These abnormalities occurred in 24 patients (Table II) only five of whom belonged to group O as against the 11 or 12 to be expected in a random sample of the population. This difference is statistically significant ($\chi^2 = 6.8$, $P < 0.01$).

Osmotic fragility The osmotic fragility curves of seven group A patients with late haemoglobinuria showed either near normal or decreased resistance to saline (Fig. 1). This may be compared with 31 patients selected for similarity in burn area and the absence of late haemoglobinuria. The red cells of these 31 cases often showed increased resistance to saline (Fig. 2). Five of the seven patients with late haemoglobinuria showed 50% haemolysis of cells in 0.45% or higher saline concentration whereas only one out of 31 comparable cases without late haemoglobinuria had this degree of osmotic fragility.

TABLE I

DETAILS OF 10 PATIENTS WITH LATE HAEMOGLOBINURIA AND LOSS OF MORE THAN 40% OF RED CELL VOLUME MEASURED WITH ^{51}Cr TWO TO THREE DAYS AFTER THE BURN

Year	Case No.	Age	Group	Area of Burn (%)		Plasma Volumes of Dried, Pooled Plasma, or Serum by 48 Hours	Hours of Haemoglobinuria	Maximum Recorded Red Cell Disappearance	
				Total	Full-thickness Loss			%	Hours after Burn
1954	11	4	A	70	70	7.1P	31-48	65	36
1955	12	5	O	80	70	9.3P	27-48	76	51
1955	13	10	A	85	80	11.2P	0-48	63	48
1956	14	2	A	35	10	5.1P	24-72	104	67
1958	15	11	AB	65	25	7.8P	23-36	49	49
1958	16	7	A	65	60	3.7 ¹	18-26	67	47
1958	17	7	A	65	60	6.6 ¹	0-30	43	31
1959	18	4	A	41	25	6.3 ⁸	11-48	45	26
1959	19	50	A	40	38	2.8 ⁸	Anuric	46	26
1960	20	15	A	65	50	5.5 ¹	13-36	60	50

Albumin replaced dried pooled plasma after haemoglobinuria had started.

P = Plasma only

S = Serum only

FIG. 1. Osmotic fragility curves of red cells 24 to 48 hours after injury in seven patients with late haemoglobinuria. $\bigcirc$ — — — $\bigcirc$ = average result on normal individuals.

FIG. 2. Saline fragility curve of red cells 24 hours after injury in two patients without late haemoglobinuria. $\bigcirc$ — — — $\bigcirc$ = average result on normal individuals.

Serology Serological studies were made on six patients who had late haemoglobinuria. One of the two who also had jaundice (No. 15) was investigated in greater detail. Spherocytes were seen in the blood film, and saline resistance of red cells was markedly reduced (Fig. 1), and the blood appeared to 'agglutinate' in the syringe during sampling. Serum taken two days after the burn, at the time when jaundice was developing, was titrated in AB serum. It agglutinated A cells to a dilution of 1:8, B cells to 1:8, and O cells to 1:2. After neutralization with secretor saliva there was still weak agglutination of A and B cells, though the indirect anti-globulin test using corresponding A or B cells was negative. No anti-A or anti-B was demonstrated in the eluate

prepared from the patients' red cells by heating aliquots in AB serum at 56°C. for five minutes. The serum after storage was tested against a wide range of O cells, in saline and albumin at 37°C., 20°C., and 4°C., and with cysteine-activated papain at 37°C. but no agglutination was found.

The serum of three patients with late haemoglobinuria but without jaundice did not agglutinate saline suspensions of untreated or papainized O, A₁, or A₂ cells at 37°C., 22°C., or 4°C. The serum of one patient with late haemoglobinuria was examined for the presence of haemolysins at 32°C. and the results compared with those of normal serum. These tests with and without complement, using patients' and normal cells, failed to show any haemolysis.

TABLE II

PATIENTS SHOWING SOME EVIDENCE OF EXCESSIVE RED CELL DISAPPEARANCE (1954 TO 1960)

Evidence of Excessive Red Cell Disappearance	Day after Burn	Blood Group					Total
		A	B	AB	A, B, or AB	O	
Loss of more than 20% of red cell volume (⁵¹ Cr)	0-1	4	0	0	4	1	5
	1-2	4	1	1	6	2	8
Haemoglobin in at least three urine specimens	$\frac{1}{2}$ -2	12	1	1	14	4	18
Patients showing one or more of these findings		16	2	1	19	5	$\left\{ \begin{array}{l} 24 \\ \chi^2 = 6.8 \\ P < 0.01 \end{array} \right.$
Expected from normal ABO distribution					12.6	11.4	

FIG. 3. Erythrocyte sedimentation of blood taken at varying times after injury in case 17.

- 1 Two and a quarter hours after injury
- 2 Three and a half hours after injury
- 3 Seven and a half hours after injury
- 4 Sixteen hours after injury
- 5 Forty hours after injury

E.S.R. and plasma proteins Many of the patients with severe loss of red cells had high erythrocyte sedimentation rates (Table III). The most marked increase was in patient no. 15. The maximum sedimentation velocity was 600 mm./hour when tested 48 hours after injury. Repeated estimations of E.S.R. in case 17 (Fig. 3) showed a maximum sedimentation velocity rising to 350 mm./hour by 40 hours after injury.

Changes of plasma protein concentration likely to affect sedimentation rate were studied. The most

severe case (no. 15) showed the highest fibrinogen level (1.3 g. %) but a level as high as 1 g. % was found in a patient showing no haemoglobinuria. Conversely late haemoglobinuria occurred in the presence of fibrinogen levels similar to those of 17 patients who did not have late haemoglobinuria. To test the possible effect of fibrinogen given in the transfusions on E.S.R., red cell loss, and haemoglobinuria some patients were treated with pooled serum only. Among these patients who received no extraneous fibrinogen there were two (nos. 18 and 19, Table I) who developed late haemoglobinuria.

Serum factors affecting the E.S.R. were tested by preparing mixtures of serum or plasma and whole blood to give a final haematocrit of 30% to 35%. In the most serious case (no. 15) serum alone produced a marked rise in sedimentation rate in addition to that due to the high level of fibrinogen. In two cases the serum was examined in the ultracentrifuge; the severe case (no. 15) (Table III) showed a marked elevation of the S 19 fraction, whereas the other case (no. 16) did not. Gel diffusion of the sera of three cases showed levels of α_2 globulin raised to about twice the normal values but similar levels were also found in some patients having no late haemoglobinuria. Haptoglobins were studied by paper electrophoresis followed by staining for haemoglobin with O-toluidine; haptoglobin was usually present, the findings being similar to those reported below in patients without late haemoglobinuria.

Bacteraemia Positive blood cultures were found during the shock stage in one of three patients studied who had haemoglobinuria.

COMMONLY OBSERVED LESS SEVERE RED CELL LOSS AFTER BURNS NOT ASSOCIATED WITH LATE HAEMOGLOBINURIA In the remaining patients treated with dried pooled plasma the average rate of disappearance of red cells for group O patients was not

TABLE III

PLASMA PROTEIN FINDINGS IN PATIENTS WITH LATE HAEMOGLOBINURIA

Case No.	Days after Burn	Fibrinogen (g. %)	Immunophoresis				Ultracentrifuge			Comment	Sedimentation Rate				O-toluidine Staining after Electrophoresis	
			1 γ Globulin	2 α_2 Globulin	2 β Lipoprotein	2 Albumin	% S 19	% S 7	% S 4.5		Maximum Velocity (mm./hr.)	One Hour (mm.)	Plasma Effect	Serum Effect	Albumin	α_2
15	2	1.3					9.4	21.2	69	S19 about 4 times normal	600	70	+++	+++		0
16	1	0.5					2.7	8.2	89.1	S19 peak possibly sharper than normal	120	55			+	++
17	1	Failed to clot		200						—	350	70				
18	1	0.15	2,280	200	100	37				—	80	30			+	+
19	1	0.25	1,560	175	100	125				—	140	55	++	+		++
20	1	0.6								—	160	55				
	2	0.8								—	160	60				

1 = mg. %   2 = % of standard serum

significantly different from the average for group A patients whether measured two to eight hours, one day, or two days after injury. This remains true if the cases are subdivided according to severity of burn or other feature which might be relevant.

In the controlled trial of homologous and pooled plasma, the six cases treated with homologous plasma and the four patients receiving dried pooled plasma in the same trial (Table IV) showed no significant difference of red cell loss, nor were the losses different from average findings after the administration of dried pooled plasma (Topley, Jackson, Cason, and Davies, 1962).

None of the sera of the six patients receiving homologous plasma contained any abnormal anti-A or anti-B isoagglutinins during transfusion. In the period of five to 45 hours after injury the serum of

one of the four patients receiving pooled plasma (case 1) who was of group A showed traces of agglutination of A cells when the serum was diluted with AB serum (Table V). The direct antihuman globulin test was weakly positive and two red cell eluates showed a trace of anti-A activity by the indirect antihuman globulin test. The anti-A level remained virtually unchanged, but one eluate showed a trace of anti-B activity by the indirect antihuman globulin test. The results suggest that small traces of isoantibodies may be demonstrable following large transfusions of pooled dried plasma even when there is no late haemoglobinuria. It is moreover of interest that there was in this case an unusual loss of red cells around this time.

Direct and indirect antihuman globulin tests were made on a further 12 patients and agglutination

TABLE IV
DETAILS OF PATIENTS IN TRIAL, COMPARING RESULTS OF TRANSFUSION
OF RECONSTITUTED DRIED HOMOLOGOUS PLASMA WITH THOSE OF TRANSFUSION
OF RECONSTITUTED DRIED SMALL POOL PLASMA

Case No.	Pooled Plasma				Homologous Plasma					
	1	2	3	4	5	6	7	8	9	10
ABO blood group	A	A	O	O	A	A	O	O	B	B
Age (years)	62	65	70	60	36	45	6	74	54	6
Area of burn (% total body area)										
Total burn	40	40	35	30	55	80	15	18	45	30
Full-thickness loss	30	35	30	20	50	30	7	12	30	20
Microcytes on admission blood film (%)	7	20	5	13	3	<2	18	5		2
Plasma transfused in 48 hours (litres)	8.3	3.9	4.0	3.8	13.5	9.7	1.4	2.7	4.8	3.1
Normal plasma volume ¹ (litres)	3.0	1.5	2.6	2.3	2.4	1.8	0.8	1.7	2.3	0.8
... Plasma transfused in plasma volumes	2.8	2.6	1.5	1.8	5.6	5.4	1.8	1.6	2.1	3.9
Haemoglobinuria 12 to 48 hr.	—	—	—	—	—	+	—	—	—	—
Red cell disappearance (% of normal red cell volume) ¹										
By 5 to 8 hours	7	Gain 17	6	Gain 3	13		Gain 2	13		Gain 6
By day 1	6	5	8	21	29	17	Gain 4	22	55	Gain 5
By day 2	19			Gain 9						31
By day 3, 4, or 5	21		20		25	31			68	
... More than 20% in one day	—	+	—	+	—	—	—	—	+	+

¹Taken from average normal figures for height (Topley and Jackson, 1957) except in short adults. (In case 3 weight was used and in case 8 weight and height were used.)

²Two different urine specimens only

TABLE V
FINDINGS IN CASE 1 AFTER 8.3 LITRES OF DRIED POOLED PLASMA
(NORMAL PLASMA VOLUME FOR HEIGHT 3.0 LITRES)

	Time after Burn					
	1 Hour	5 Hours	18 Hours	45 Hours	56 Hours	3 Days
Plasma transfused (litres)	0	3.0	6.0	7.4	8.3	
Plasma volumes	0	1	2	2.5	2.8	
Anti-B titre in saline	32	16	16	8	8	8
In AB serum	32	16	32	16	32	16
Anti-A titre in saline	0	0	0	0	0	0
In AB serum	0	Trace	Trace	Trace	0	0
Direct anti-human globulin test	0	Weak	Weak	Weak	0	0
Eluate, indirect anti-human globulin test	0	α and β	0	α	0	0
Total red cell disappearance by ⁵¹ Cr estimation		7%	6%	30%		32%

FIG. 4. Relationship between maximum erythrocyte velocity and fibrinogen level 24 to 48 hours after injury.

○ = group O
● = group A
· = Results on artificial mixtures of fibrinogen

tests of saline suspensions of normal and trypsinized red cells on 30 patients at 37°C. In each case the patient's serum and normal serum were tested against the patient's cells and normal cells of the same ABO group. Neither the red cells nor serum of the patients showed any significant abnormality and there was no difference between patients with less than average and those with more than average red cell disappearance.

Group A patients showed a higher erythrocyte sedimentation rate than group O patients when tested 24 to 48 hours after injury. The relationship between the erythrocyte sedimentation rate and fibrinogen level at this time is shown for group O and group A patients (Fig. 4). Each point represents a different patient, the results nearest to 24 hours being selected for all patients with tests 24 to 48 hours after injury. Mixtures of fibrinogen in normal plasma were prepared and their fibrinogen content assayed by the same method as for the patient's plasma. The line shown in Fig. 4 represents the maximum erythrocyte sedimentation velocity for these mixtures. The group O patients showed maximum sedimentation velocities only slightly greater than those expected for the fibrinogen level while the group A patients had higher sedimentation rates. This difference was therefore possibly due to some

factor in the patient's serum. The role of serum factors was investigated in six patients by making mixtures of the patients' serum with normal plasma and red cells. The serum of the three group O cases contributed less to the sedimentation than that of any of the three group A patients. One of these group A patients with a partial thickness skin loss burn received 1.1 plasma volumes of dried pooled plasma. His serum markedly increased the erythrocyte sedimentation rate when tested one, two, and seven days after injury. The effect was absent on the twenty-first day. He showed no red cell loss and no haemoglobinuria during the plasma transfusion.

The rise of fibrinogen level in 17 patients was deliberately limited by transfusion of albumin or serum instead of plasma, and the results compared with those from patients receiving pooled plasma. In eight patients whose fibrinogen levels were low as a result of receiving albumin or serum transfusion measured red cell losses were not significantly reduced.

The γ and α_2 globulins, β lipoprotein, and albumin were measured in 10 patients by gel diffusion. There was no apparent relation with the degree of red cell loss.

Haptoglobins were present in 10 or 11 patients studied at various periods during the first 48 hours. O-toluidine staining was often present in the albumin and β globulin as well as in the α_2 globulin regions.

These findings in cases without late haemoglobinuria demonstrate the increased erythrocyte sedimentation produced by the serum of group A patients receiving pooled plasma, and have failed to define any association between red cell loss and abnormality of plasma proteins studied.

Blood cultures were taken during the shock stage in 25 patients without late haemoglobinuria. The blood of 11 of these patients showed some bacteraemia due to recognized pathogens during the first 48 hours after injury. This bacteraemia was not significantly associated with any particular blood group nor with the degree of measured red cell loss.

ISOANTIBODIES IN POOLED DRIED PLASMA Samples of reconstituted dried pooled plasma were defibrinated. The anti-A and anti-B isoagglutinin titres were then measured in dilutions in saline or group AB serum (Table VI). In serum, titres of 1 in 16 to 1 in 32 were fairly common against group A cells and slightly less common against group B cells. After neutralization with secretor saliva of the corresponding blood group, the serum agglutination titre against group A cells fell, though antibodies were still detectable (Tables VI and VII).

In saline, no titres of more than 1 in 64 against group A or B cells were found (Table VI).

TABLE VI
TITRES OF DRIED PLASMA POOLS

Titre	Against Group A Cells				Against Group B Cells			
	Initial		After Neutralization with Group A Secretor Saliva		Initial		After Neutralization with Group B Secretor Saliva	
	In Saline	In AB Serum	In Saline	In AB Serum	In Saline	In AB Serum	In Saline	In AB Serum
0-2	67	30	74	76	70	38	73	81
4-8	5	23	0	6	4	33	1	2
16-32	2	26	0	1	0	10	0	0
64	0	3	0	0	0	2	0	0
128	0	1	0	0	0	0	0	0
Total pools	74	83	74	83	74	83	74	83

TABLE VII

INDIRECT ANTI-HUMAN GLOBULIN TESTS AFTER
NEUTRALIZATION OF DRIED PLASMA POOLS WITH
SECRETOR SALIVA OF CORRESPONDING GROUP

Test	Group A Cells	Group B Cells
Negative	59	71
Granular or weak	6	2
+	2	1
++ or +++	7	0
Total pools	74	74

These titres are somewhat lower than those described by Ebert and Emerson (1946) for American dried pooled plasma.

DISCUSSION

The red cell loss following burns has been known and studied for many years (Sevitt, 1957). It is known that during the first day after injury some red cells are lost due to the direct effect of heat, while others are lost in the immediate area of the burn (Topley, 1962). Later red cell loss may be due to the local and sometimes systemic infection that is so often associated with large burns. Superimposed on these factors is the possible effect of anti-A isoagglutinins in the pooled plasma commonly transfused in the first two days to avoid serious oligæmia.

The present investigation was stimulated by the case of a patient (kindly brought to our attention by Dr. M. M. Pickles) who had developed a haemolytic anaemia following the transfusion of large volumes of reconstituted dried pooled plasma from 14 different pools. Of the eight pools available for examination, one pool had an α isoagglutinin titre (in AB serum) of 16 and altered the osmotic fragility of group A but not of group B or group O cells exposed to it. Group A cells exposed to this pool and then washed and resuspended in a range of concentrations of sodium chloride lysed completely in 0.7% saline.

Serial measurements of red cell volumes in burned patients combined with clinical observations have

suggested that the loss in these patients can be conveniently considered as being of two types, one a severe haemolysis associated with 'late haemoglobinuria' and the other a less severe red cell loss without 'late haemoglobinuria' (Topley *et al.*, 1962). The severe red cell loss associated with late haemoglobinuria occurs at a time (12 to 48 hours after injury) when the maximal effect of antibodies transfused in pooled plasma is likely to be attained. The present study shows a preponderance of A blood groups among the patients who suffered the excessive red cell loss (Tables I and II) and the blood of these patients showed a relative increase in osmotic fragility at the time of late haemoglobinuria. Ebert and Emerson (1946) showed by differential agglutination that the increase of osmotic fragility during the transfusion of group O blood to patients with burns affected the patients' group A cells, but not the donor group O cells. Muir (1961) also noted increased osmotic fragility of red cells at the time of excessive red cell loss in two patients. These cases belonged to group A. These findings are all consistent with the excessive red cell loss, often associated with haemoglobinuria 12 to 48 hours after a burn, being predominantly due to the anti-A transfused in dried pooled plasma.

In the second type of red cell loss, which is less severe, not associated with haemoglobinuria, and considerably more common than the first type, there is no suggestion that the amount of blood loss is greater in group A than in group O patients, and there is no associated relative increase in osmotic fragility. The red cell loss is therefore probably due to factors other than the anti-A in pooled plasma. Any blood loss due to isoantibodies is therefore small enough to be masked by this other blood loss. It must therefore be less than 20% of the circulating blood volume in most cases. Within these limits therefore the results on 79 burned patients suggest that transfusion of up to 2 to 3 plasma volumes of dried pooled plasma does not cause excessive loss of red cells.

We have also confirmed the observations of

earlier workers that anti-A isoagglutinins are present in reconstituted dried pooled plasma (Table VI) and can sometimes be demonstrated in the blood of patients who have received plasma transfusion. The finding that among such patients, group A patients have a higher erythrocyte sedimentation rate than group O patients (Fig. 4) suggests that isoagglutinins may contribute to the sedimentation rate *in vitro*.

Other changes in severely burned patients which might cause red cell loss, such as raised erythrocyte sedimentation rate, raised fibrinogen or bacteraemia, cannot be the only cause, as excessive red cell loss has been found in patients transfused with serum instead of plasma, and also in patients with erythrocyte sedimentation rates no higher than those occurring in patients showing no red cell loss. The incidence of bacteraemia was the same in the cases with and without excessive loss of red cells.

The evidence therefore strongly suggests a haemolytic anaemia superimposed on other causes of red cell loss in some burned patients receiving more than 3 plasma volumes of dried pooled plasma. This effect might be reduced by excluding donors showing an appreciable amount of so-called 'immune' anti-A. The same end might be achieved by reducing plasma transfusion to the minimum required to avoid dangerous oligoemia, and by using other colloids in the rare cases where more than 2 or 3 plasma volumes of pooled plasma need to be given to a

group A, B, or AB patient. Such alternatives include homologous plasma, albumin, and homologous blood.

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