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Preparation and Storage of Platelet Concentrates

II. STORAGE VARIABLES INFLUENCING PLATELET VIABILITY AND FUNCTION

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SUMMARY. Factors affecting the viability and function of stored platelet concentrates have been investigated in a blood component programme. It was found that platelets could be maintained for up to 72 h without bacterial contamination under the following conditions: (1) surgical skin preparation at venipuncture site; (2) blood collection in CPD or ACD anticoagulant in a closed bag system; (3) centrifugation of PRP at 3000 *g* for 20 min; (4) storage in Fenwal PL-146, Cutter CL-2383, or McGaw plastic bags; (5) resuspension of the platelet pellet in 70 ml residual plasma; (6) storage at 22 ± 2°C; and (7) constant gentle mixing throughout storage. Platelet viability as determined by recovery and survival is largely maintained, as is platelet function measured by template bleeding time. Both viability and function of concentrated platelets stored at 4°C are severely compromised.

Bleeding due to thrombocytopenia can be controlled by transfusing adequate numbers of functional, viable platelets. In a companion paper (Slichter & Harker, 1976), factors affecting the harvest of viable platelets from whole blood were investigated. This report analyses variables affecting platelet viability and function during storage. The purpose is to develop optimum storage procedures while at the same time minimizing risk of bacterial contamination.

MATERIALS AND METHODS

Laboratory Procedures

Blood from routine blood bank donors (450 ml) was collected in a closed bag system containing ACD or CPD anticoagulant. Five types of bags were used: Fenwal PA-20 with PL-130 or PL-146 transfer packs; McGaw double Hemo-Pak, T2250, ACD-A; Cutter PR2370A, ACD-A; and Cutter CL-2383 (new plastic bag material currently being evaluated for FDA licensing), CPD. Platelet concentrates were prepared with optimum platelet yield and labelled with ^{51}Cr as previously described (Slichter & Harker, 1976). Some concentrates

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were prepared using ACD-acidified PRP (pH 6.5). At specified times before and after storage, each concentrate was sampled for measurement of platelet count, radioactivity, pH and bacterial growth.

To vary platelet concentration, platelet pellets were resuspended in a plasma volume of 20, 50, 60 or 70 ml measured by syringe. For practical blood banking purposes, these volumes can be achieved by allowing the plasma extractor (BM-1, Fenwal Laboratories, Morton Grove, IL) to close completely (20 ml), by placing a $1 \times 1 \times 0.7$ (depth) cm stop in the two upper corners of the extractor (50 ml), a $1 \times 1 \times 0.8$ cm stop (60 ml) and a $1 \times 1 \times 0.9$ cm stop (70 ml). The bag and tubing receiving the platelet-poor plasma were elevated 4 cm above the extractor during transfer to prevent plasma syphoning.

For studies of storage at room temperature, the bags were maintained at $22 \pm 2°C$ in an air conditioned, thermostatically controlled room. For studies at 4°C, concentrates were allowed to rest 1½ h at room temperature in 20 ml or 50 ml residual plasma (24 and 72 h studies, respectively) following preparation. They were then resuspended and placed in a refrigerator or walk-in cold room. Continuous mixing during storage was accomplished by laying the bags unstacked on open wire racks mounted on a platform rotator (Eberbach, Ann Arbor, MI) adjusted to 40 cycles/min, the slowest speed at which platelets stayed in suspension. A fan blew air over the concentrates to prevent the heat of the rotator from increasing the temperature of the platelets.

Viability Measurements

Viability of the stored platelets was determined by measuring recovery and survival of autologous ^{51}Cr labelled platelets, details of the procedure have been presented (Slichter & Harker, 1976). A platelet viability index was calculated by expressing the average area under the survival curve as a proportion of the mean area obtained following autologous transfusion of 38 unstored platelet concentrates (Slichter & Harker, 1976). All unstored concentrates were injected within 6 h of collection.

Clinical Effectiveness

To evaluate the clinical effectiveness of transfused platelets, viability and function (bleeding time correction) were measured after transfusion of pooled random donor platelets into aplastic thrombocytopenic patients. Each transfusion consisted of four to 10 concentrates. All patients had pre-transfusion platelet counts of less than 10×10^9/l. and bleeding times greater than 60 min. The majority were on no medications except oxymethalone and were free from infection or other conditions known to compromise platelet viability and function (Harker & Slichter, 1972a, b). All showed a rise in platelet count following transfusion of unstored pooled concentrates. To measure platelet viability at least three blood samples were drawn in the first 4 h after transfusion, two or three samples in the next 24 h, two samples between 24 and 48 h, and daily thereafter for the next 3 days. Platelet recovery and survival were determined by ^{51}Cr techniques, electronic particle counting (Slichter & Harker, 1976) or both.

Platelet function. The function of infused platelets was determined by performing standardized template bleeding times (Mielke *et al*, 1969) before and at frequent intervals after the transfusion of concentrates into aplastic thrombocytopenic recipients until the platelet level

fell below 10×10^9/l. The normal bleeding time in minutes is predicted by the equation $30.5 - \left(\frac{\text{platelet count} \times 10^9/\text{l.}}{3.85}\right)$ at platelet counts in the range $10–100 \times 10^9$/l. (Harker & Slichter, 1972b). Prolongation of the observed bleeding time as compared to that expected indicates platelet dysfunction.

Effect of ASA. The effect of acetylsalicylic acid (ASA) ingestion on platelet function was investigated in a limited series of patients. Bleeding time was measured following infusion of platelets from donors who had ingested 1.2 g ASA twice daily for 3 d prior to and on the morning of plateletpheresis. Results were compared with those obtained when the same donors were not on an ASA regimen.

Bacteriologic Monitoring

For bacterial cultures of stored platelets, a sample was obtained by stripping some of the platelet concentrate into transfer tubing left on the satellite bag. After a segment of the tubing was sealed off, it was opened in a sterile manner, and a 1 ml sample was inoculated into thioglycolate and brain heart infusion broths and streaked onto blood agar media. Duplicate cultures were kept at both 22°C and 37°C for 3 weeks. If after 3 weeks there was no visual evidence of bacterial growth and a Gram stain was negative, samples were discarded.

Selection of Recipients

The thrombocytopenic patients receiving random donor platelets were selected because of their severe thrombocytopenia and recent episodes of serious bleeding which required platelet transfusions. All transfused blood products were negative for Australia antigen by radioimmunoassay (Abbott Laboratories).

RESULTS

VIABILITY MEASUREMENTS OF STORED AUTOLOGOUS PLATELETS IN NORMAL VOLUNTEERS

Mixing

Mixing was found to have a critical effect on viability of platelets stored at 22°C for 24 h. Platelets stored without mixing in Fenwal PL-146 bags had a viability index of 0.35 ± 0.05 (mean $\pm$ SE) compared to 0.93 ± 0.05 with sustained mixing (Table I). Mixing of concentrates stored at 4°C had no effect on platelet viability (see later).

Storage Bags

Platelets collected in five types of bags were stored for 24 h at 22°C under conditions of sustained mixing. For three of the five bags (McGaw, Cutter PR 2370A, and Fenwal PL-130), viability of stored platelets was significantly impaired (Table I). (Further data on platelet viability in McGaw and Cutter CL-2383 bags after 72 h storage is presented later.) For Cutter PR 2370A and Fenwal PL-130 bags, the loss in viability was due to platelet death, since recovery was 16–18%. The type of bag appeared to affect viability only after storage, since platelets in PL-130 bags that were transfused immediately had a recovery of $56 \pm 3\%$. Unless otherwise specified, Fenwal PL-146 bags with continuous mixing were used in all subsequent storage studies.

TABLE I. Effect of storage bag and mixing on platelet viability

No. of observations	Storage conditions: Interval (h)	Storage conditions: Plastic bag (material)	Storage conditions: Mixing	Platelet: Recovery (%)	Platelet: Survival (d)	Platelet: Viability index
7	0	Fenwal (PL-130) ACD	No	56±3	8.2±0.2	1.00±0.02
31	0	Fenwal (PL-146) ACD or CPD	No	53±2	8.2±0.1	
16	24	Fenwal (PL-146) ACD	No	23±3	6.7±0.5	0.35±0.05†
4	24	Cutter (CL-2383)‡ CPD	Yes	55±2	8.1±0.3	1.00±0.02
15	24	Fenwal (PL-146) ACD	Yes	51±3	8.2±0.2	0.93±0.05
8	24	McGaw ACD	Yes	49±4	7.5±0.6	0.81±0.08*
4	24	Cutter (PR2370A) ACD	Yes	18±2	8.7±1.1	0.34±0.06†
8	24	Fenwal (PL-130) ACD	Yes	16±2	7.4±1.2	0.27±0.06†

Platelets prepared in 20 ml plasma and stored at 22°C. Results are expressed as the mean±1 SE. Significantly different from unstored concentrates: * $P<0.05$; † $P<0.01$. ‡ Investigational plastic bag.

The Effect of pH

ACD concentrates. The effect of pH on platelet viability was seen in seven concentrates stored for 48–72 h. Recovery, survival and viability index dropped to 10±5%, 2.5±1.3 d and 0.05±0.02, respectively. For six of the seven concentrates, the pH dropped below 6.0. In contrast, the pH of all concentrates stored for 24 h was above 6.0.

Further studies of pH were undertaken. An inverse relationship was found between the pH and the platelet count after storage for 24 h (Fig 1a). Since a lower platelet concentration yielded a higher pH, concentrates were diluted in a larger volume of plasma (50 ml) and the pH measured after storage for 72 h. Again, an inverse correlation was found between pH and platelet count (Fig 1b). However, the data showed that pH could be maintained above 6.0 if platelet counts were kept below 1.7×10^{12}/l. Concentrates diluted in 50 ml plasma measured after storage for 48 h showed no change in pH.

Platelet viability was measured for concentrates with a pH after storage greater than 6.0 (i.e. diluted in 50 ml plasma so that platelet counts were less than 1.7×10^{12}/l.). After 48 and 72 h, platelet recovery and survival remained high (Table II).

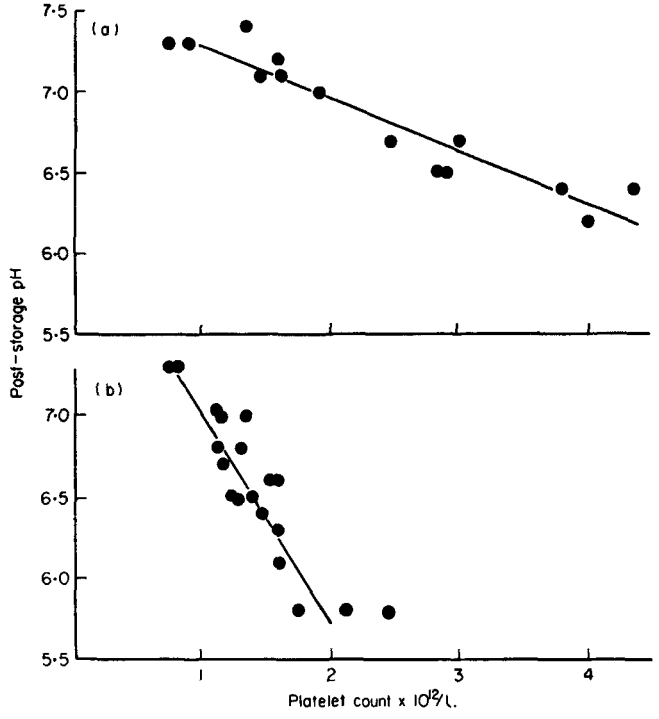

FIG 1. Inverse relationship between post-storage pH of ACD concentrates and platelet count. (a) Concentrates prepared in 20 ml plasma and stored for 24 h at 22°C. Correlation coefficient (r) = 0.95; regression line, $y = -0.33x+7.61$. (b) Concentrates prepared in 50 ml plasma and stored for 72 h at 22°C. $r = 0.87$; $y = -1.33x+8.38$. The slope of the line was much steeper at 72 h and pH fell below 6.0 at platelet counts above 1.7×10^{12}/l.

TABLE II. Viability of platelets at pH > 6.0

	Storage conditions		*Platelet*		
No. of observations	*Interval (h)*	*Residual plasma (ml)*	*Recovery (%)*	*Survival (d)*	*Viability index*
38	0	20.0	54±2	8.2±0.2	1.00±0.05
15	24	20.0	51±3	8.2±0.2	0.93±0.05
8	48	50.0	49±3	7.6±0.3	0.83±0.05*
11	72	50.0	40±3	7.9±0.2	0.71±0.06†
4	96	50.0	25±9	4.1±0.3	0.24±0.10†

Storage at 22°C; ACD anticoagulant. Results are expressed as the mean±1 SE.
Significantly different from unstored concentrates: *$P<0.05$; †$P<0.01$.

CPD concentrates. Similarly, the pH of 78 CPD concentrates prepared in 50 ml plasma was measured after 72 h. All concentrates with a platelet concentration less than 1.7×10^{12}/l. had a pH greater than 6.0. By increasing the residual plasma volume, pH could be maintained above 6.0 while allowing for increased platelet content. Since the normal platelet count is $250 \pm 40 \times 10^9$/l. (± 1 SD) (Harker & Finch, 1969) the residual plasma volume needed to maintain a platelet concentration of less than 1.7×10^{12}/l. can be calculated to be 70 ml based on a 450 ml blood donation and an 86% average platelet yield in the concentrate (Slichter & Harker, 1976). This ability to adjust for increased platelet content is important since concentrates prepared from female donors have been found to have a significantly higher platelet content than those from males. In tests of concentrates prepared from 23 female donors, platelet content was $14 \pm 5\%$ greater than that from 98 males.

Anticoagulant

Viability of ACD-anticoagulated platelets (pH 7.0) was measured in Fenwal PL-146 and McGaw bags after 24 and 72 h (Table V). No significant difference was found in viability of platelets collected in either bag after the same period of storage ($P > 0.05$). When the viability of ACD acidified platelets (pH 6.5) was compared with that of nonacidified ACD platelets after 24 h, the average viability indexes were 0.88 ± 0.05 and 0.93 ± 0.05, respectively (Table III). However, concentrates evaluated after 72 h had viability indexes of 0.38 ± 0.12 and 0.65 ± 0.08, respectively. These values included three acidified and one nonacidified concentrate with pH less than 6.0. (The four concentrates had platelet counts ex-

TABLE III. Effect of anticoagulant on platelet viability

	Storage conditions		*Platelet*		
No. of observations	*Interval (h)*	*PRP (anticoagulation)*	*Recovery (%)*	*Survival (d)*	*Viability index*
8	24	Acidified ACD	48 ± 3	8.3 ± 0.2	0.88 ± 0.05
15	24	ACD	51 ± 3	8.2 ± 0.2	0.93 ± 0.05
8	24	ACD (McGaw)	49 ± 4	7.5 ± 0.6	0.81 ± 0.08*
5‡	72	Acidified ACD	36 ± 6	6.8 ± 0.5	0.57 ± 0.11†
11§	72	ACD	40 ± 3	7.9 ± 0.2	0.71 ± 0.06†
8	72	ACD (McGaw)	36 ± 2	7.9 ± 0.6	0.65 ± 0.07†
8	72	CPD	46 ± 3	7.9 ± 0.3	0.81 ± 0.06*
4	72	CPD (Cutter CL-2383)¶	44 ± 4	7.6 ± 0.3	0.76 ± 0.07*
4	96	ACD	25 ± 9	4.1 ± 0.3	0.24 ± 0.10†
4	96	CPD	35 ± 5	4.3 ± 1.4	0.32 ± 0.10†

Initial pH of ACD acidified, ACD unacidified, and CPD platelets was 6.5, 7.0 and 7.2, respectively. The pH of all concentrates following storage was greater than 6.0. Results are expressed as the mean ± 1 SE.

Significantly different from unstored concentrates: *$P < 0.05$; †$P < 0.01$.

‡ Three additional samples excluded from calculations because pH < 6.0.

§ One additional sample excluded because pH < 6.0.

¶ Investigational plastic bag.

ceeding 1.8×10^{12}/l. in a 50 ml volume and a viability index of less than 0.20.) When these concentrates were excluded from calculations, the viabilities of acidified and nonacidified concentrates became 0.57 ± 0.11 and 0.71 ± 0.06, respectively (Table III). The difference is of marginal significance ($0.10 > P > 0.05$).

Comparison of the viability indexes of ACD-acidified, ACD and CPD concentrates is shown graphically in Fig 2. There was a moderately progressive loss of viability from 0 to 72 h storage, primarily due to reduced recovery (Table III). After 96 h, both recovery and survival declined markedly. CPD concentrates had the highest viability index at all storage intervals but they were not statistically different from ACD concentrates ($P > 0.05$). The initial pH of ACD-acidified, ACD and CPD platelets was 6.5, 7.0 and 7.2, respectively. A significant positive correlation ($r = 0.99$) existed between initial pH of the concentrate and the post-storage platelet viability index (Fig 3).

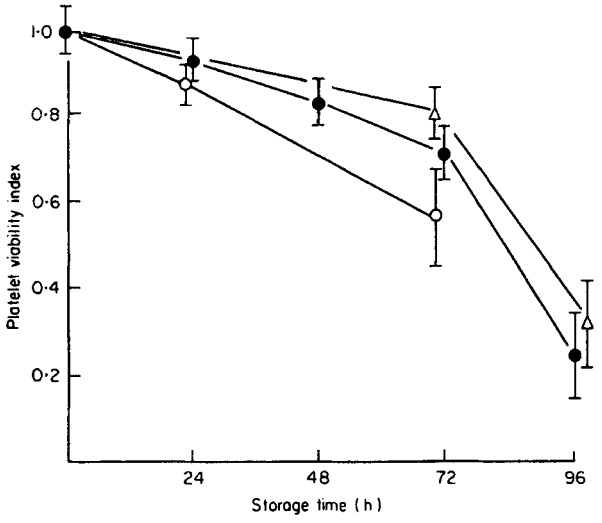

FIG 2. Platelet viability indexes (mean ± 1 SE) of ACD (●), ACD-acidified(○) and CPD (△) concentrates after storage. Final pH of all concentrates is above 6.0.

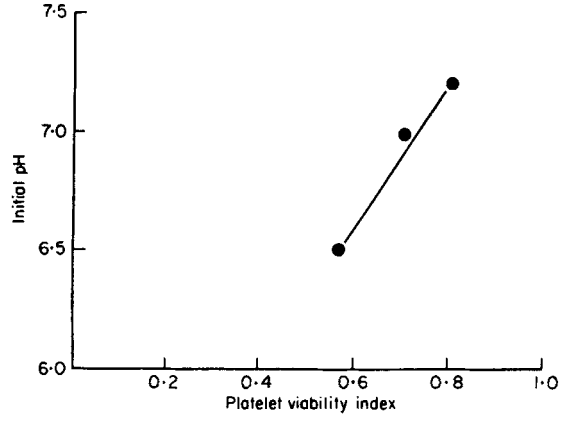

FIG 3. Direct correlation between initial pH of platelet concentrate and viability index after 72 h storage at 22°C. $r = 0.98$; regression line $y = 3.08x + 4.74$.

TABLE IV. Effect of storage temperature on platelet viability

	Storage conditions		Platelet		
No. of observations	*Interval (h)*	*Temperature (°C)*	*Recovery (%)*	*Survival (d)*	*Viability index*
15	24	22	51±3	8.2±0.2	0.93±0.05
8	24	4	61±7	1.3±0.1	0.18±0.02*
11	72	22	40±3	7.9±0.2	0.71±0.06*
8	72	4	40±5	1.0±0.1	0.09±0.01*

Plasma volume for 24 and 72 h storage were 20 ml and 50 ml, respectively; ACD anticoagulant. Results are expressed as the mean±1 SE.

* Significantly different from unstored concentrates ($P<0.01$).

TABLE V. Effect of centrifugation on viability of stored platelets

	Centrifugation of PRP		Storage interval (h)	Platelet		
No. of observations	*Force (g)*	*Time (min)*		*Recovery (%)*	*Survival (d)*	*Viability index*
38	3000	20	0	54±2	8.2±0.2	1.00±0.05
8	3000	20	72	46±3	7.9±0.3	0.81±0.06*
16	4000	10	0	55±3	7.6±0.3	0.94±0.05
6	4000	10	72	34±2	7.2±0.3	0.55±0.05†

Unstored concentrates, ACD and CPD anticoagulant; stored concentrates, CPD. Results are expressed as the mean average±1 SE.

Significantly different from unstored concentrates: *$P<0.05$; †$P<0.01$.

Temperature

The effect of storage temperature (4°C and 22°C) was investigated with platelet concentrates prepared from ACD anticoagulated blood. Platelet recovery after 24 h at 4°C averaged 61±7%, with a survival of 1.3±0.1 d and a viability index of 0.18±0.02 (Table IV). In studies of 72 h storage at 4°C, half the concentrates were mixed during storage and half were not. No difference in viability was found (0.10±0.02 and 0.08±0.02, respectively). The pooled data gave an average recovery, survival and viability index of 40±5%, 1.0±0.1 d and 0.09±0.01, respectively (Table IV). All units stored at 4°C maintained a pH above 6.2. Even when concentrates were prepared in a plasma volume of 20 ml (average platelet concentration 5.0×10^{12}/l.), the pH never dropped below 6.0 for storage up to 96 h.

Platelets stored at 4°C for 24 and 72 h had considerably lower viability than those stored the same periods at 22°C ($P<0.01$). A comparison of platelet viability data for unstored and stored concentrates (72 h at 4°C and 22°C) is given in Fig 4.

Centrifugation

The effect of centrifugation on viability of unstored platelets has been reported (Slichter & Harker, 1976). To determine its effect on stored platelets, concentrates prepared at 3000 **g**

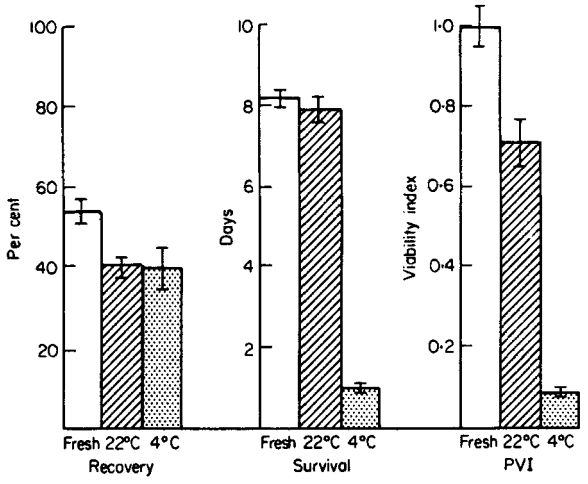

FIG 4. Platelet viability data (height of column, mean; bar, ±1 SE) for unstored ACD concentrates and those stored 72 h at 4°C or 22°C.

and 4000 *g* were measured for viability following storage for 72 h at 22°C. The viability index of the platelets centrifuged at 4000 *g* was significantly lower ($P<0.01$) than those centrifuged at 3000 *g* (Table V).

VIABILITY AND FUNCTION OF FRESH AND STORED POOLED RANDOM-DONOR PLATELETS TRANSFUSED INTO THROMBOCYTOPENIC PATIENTS

Platelet viability. Transfusions of fresh and stored platelets were given to 12 selected thrombocytopenic patients for measurements of platelet viability, function or both. The platelet concentrates were prepared from ABO compatible random blood bank donors unselected for ASA ingestion unless otherwise specified. Twenty-four of the transfused concentrates had been stored at 22°C for 72 h; they contained an average of $0.63\times10^{11}\pm0.04$ platelets before storage and $0.59\times10^{11}\pm0.04$ platelets after storage. The eight concentrates stored for 72 h at 4°C had an average platelet content of $0.78\times10^{11}\pm0.10$ before and $0.72\times10^{11}\pm0.10$ after storage. Since the loss during storage was only 6% at 22°C and 8% at 4°C, no artifact of recovery could be attributed to cell lysis during storage. Similarly, radiolabelled concentrates stored at either 4°C or 22°C gave the same post-infusion platelet recoveries using either the radioactive standard prepared before or after platelet storage.

Results obtained using electronic particle counting and ^{51}Cr labelling techniques were compared. In transfusions of unstored pooled concentrates to seven thrombocytopenic patients, platelet recovery and survival averaged $62\pm6\%$ and 2.6 ± 0.6 d using the particle counter. By radiolabelling techniques, recovery and survival were $59\pm6\%$ and 2.9 ± 0.8 d, respectively. The difference is not significant ($P>0.5$). When recovery and survival were measured following three transfusions of platelets stored for 72 h at 22°C, again the difference between these techniques were not significant ($P>0.5$): by particle counting, $60\pm11\%$ and 3.6 ± 1.3 d; by ^{51}Cr techniques; $65\pm20\%$ and 4.0 ± 1.3 d. Similarly, after three transfusions

of concentrates stored for 72 h at 4°C, the recoveries were 51 ± 25% and 42% ± 22% with survivals of 1.4 ± 0.6 d and 1.7 ± 0.2 d, respectively ($P > 0.5$). In subsequent measurements of viability, the two methods are used interchangeably.

Table VI summarizes the data on viability of pooled random-donor platelets infused into the six thrombocytopenic patients in whom these measurements were made. Unstored platelet concentrates were given to each recipient and all patients demonstrated a sustained rise in the platelet count indicating that they were not refractory to platelet transfusions. Patient 1 (Table VI) alternately received 4°C stored, 22°C stored and unstored pooled platelet transfusions. In patients 2–6 (Table VI), pooled platelet concentrates which had been stored at 4°C or 22°C were infused alternately, followed by a last transfusion of unstored pooled platelet concentrates to ensure that they had not become immunized by the previous transfusions. The control values for recovery and survival of unstored platelets in these six aplastic patients averaged 53 ± 3% and 4.0 ± 0.5 d, respectively. These results demonstrate the same recovery but reduced survival compared with the reinfusion of autologous platelets into normal subjects (Tables I and VI). There was no difference in survival times of unstored and stored (22°C) platelets in thrombocytopenic patients; 4.0 ± 0.5 d and 4.1 ± 0.6 d, respectively. Recovery of 22°C, 72 h stored platelets was the same as in the normal autologous control studies. Platelets stored at 4°C generally gave comparable platelet recovery and survival results in patients as in the normal autologous controls (Table VI); except that platelets stored at 4°C for 24 h gave a recovery of 41 ± 7% in patients compared to 61 ± 7% in normal autologous controls. Thus, platelet viability measurements using normal autologous controls proved a useful technique in evaluating the effect of platelet transfusions in thrombocytopenic patients.

Platelet function. Platelet count and post-transfusion bleeding time were measured in seven aplastic thrombocytopenic patients following 20 transfusions of unstored platelets. In all cases the pre-transfusion bleeding time was greater than 60 min and the platelet count less than 10×10^9/l. For 16 of the 20 transfusions an immediate correction of the bleeding time occurred corresponding to the rise in platelet count (Fig 5). Of the four transfusions giving a delayed response, two showed normal function at 2½ and 18 h, and the other two showed improved function at 17 and 24 h, becoming normal at 40 h post-transfusion. In the majority of cases there was a direct correlation between platelet count and bleeding time at about 90 min post-transfusion ($r = 0.94$). When platelet counts fell below 20×10^9/l., the bleeding time increased beyond 30 min. The slope of the correlation line (Fig 5) fits that seen in a large group of untransfused patients with thrombocytopenia secondary to marrow failure (Harker & Slichter, 1972b).

In vivo function of platelets stored at 22°C was investigated in nine transfusions into four aplastic thrombocytopenic patients. Two of the pooled concentrates had been stored for 24 h and seven for 72 h. For four of the nine transfusions, correction of the bleeding time was delayed from 1 to 2½ h (Fig 6a). However, in all cases platelets were functional by 2½ h post-transfusion and remained functional.

Two patients were infused with platelets stored at 4°C (three stored for 24 h and five for 72 h). There was no measurable improvement in bleeding time following two of the transfusions (Fig 6b). Despite the maintenance of circulating platelets at levels above 20 ± 10^9/l., bleeding time prolonged to more than 30 min within 2½ h of transfusion in the remaining six transfusions. Only a single measurement showed any function after 2½ h.

TABLE VI. Platelet viability in thrombocytopenic patients

	Unstored			*Stored at 22°C for 72 h*			*Stored at 4°C*			
		Platelet			*Platelet*		*Transfusions*		*Platelet*	
Patient	*No. of transfusions*	*Recovery (%)*	*Survival (d)*	*No. of transfusions*	*Recovery (%)*	*Survival (d)*	*No.*	*Storage time (h)*	*Recovery (%)*	*Survival (d)*
1	11	50±6	2.9±0.4	5	37±3	3.4±0.6	1	24	40	0.5
							5	72	40±7	1.4±0.2
2	1	58	5.2	1	45	5.2				
3	1	41	4.0	1	39	3.3				
4	1	78	2.5	1	67	2.7	1	24	54	1.1
							1	72	35	1.3
5	1	38	4.1				1	24	29	1.1
6	1	53	5.1	1	22	6.0				
Total patients	16	53±3	4.0±0.5	9	42±7	4.1±0.6	3	24	41±7	0.9±0.2
							6	72	40±6	1.4±0.1
Normal controls*	38	54±2	8.2±0.2	11	40±3	7.9±0.2	8	24	61±7	1.3±0.1
							8	72	40±5	1.0±0.1

Results are expressed as the mean ± 1 SE.

* Autologous transfusions.

Effect of ASA. To assess the effect of ASA on platelet function, platelets were collected by plateletpheresis from six normal donors who had not ingested ASA in the preceding 7 d. Later, platelets from these same donors were collected after a regimen of 1.2 g of ASA twice daily for 3 d prior to and on the morning of plateletpheresis. Platelet function was measured following transfusion of unstored ASA and non-ASA platelets into two aplastic thrombocytopenic patients (Figs 7a and 7b). While the non-ASA platelets corrected the bleeding time appropriately, ASA platelets showed a 7 h delay in correction in one patient (Fig 7a) and an 18 h delay in the other (Fig 7b). Survival of unstored ASA and non-ASA platelets was comparable.

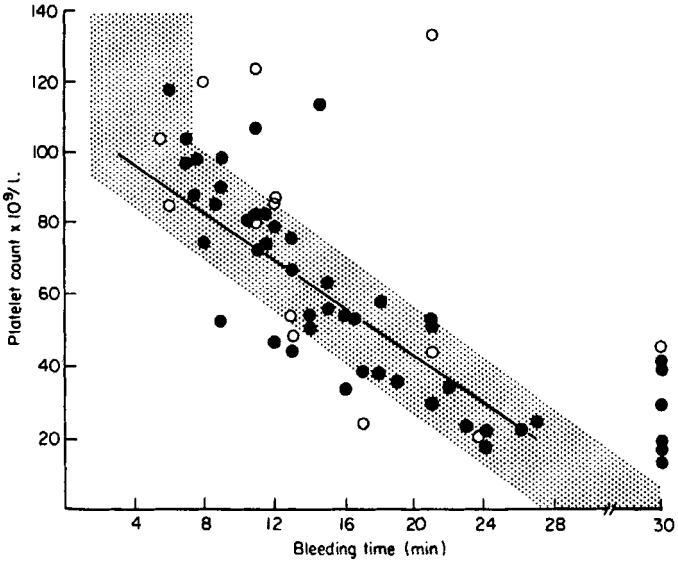

FIG 5. Bleeding times and platelet counts in seven aplastic thrombocytopenic patients following 20 transfusions of unstored platelets. Pre-transfusion bleeding times were greater than 60 min and platelet counts less than 10×10^9/l. in all cases. ○, Measurement between 5 min and 2½ h post-transfusion; ●, after 2½ h. Shaded area represents 95% confidence limits of relationship between bleeding time and platelet count in untransfused aplastic thrombocytopenic patients (Harker & Slichter, 1972b); regression line for fresh transfused platelets is not statistically different ($y = -3.37x + 110.05$).

ASA and non-ASA platelets prepared in a similar fashion from another group of six normal donors were stored for 72 h at 22°C and infused into the same two aplastic patients. The non-ASA platelets functioned appropriately, while ASA platelets were markedly dysfunctional during the first 4–6 h post-transfusion (Figs 7c and 7d). Two additional studies (not shown) in which non-ASA random donor platelets stored for 72 h at 22°C were infused into these patients showed immediate normal function.

All six transfusions of non-ASA platelets showed immediate normal function. In the previous studies in which platelets were prepared from donors unscreened for ASA ingestion, initial dysfunction was seen in four of 20 (20%) transfusions of unstored platelets and in four of nine (44%) transfusions of stored platelets.

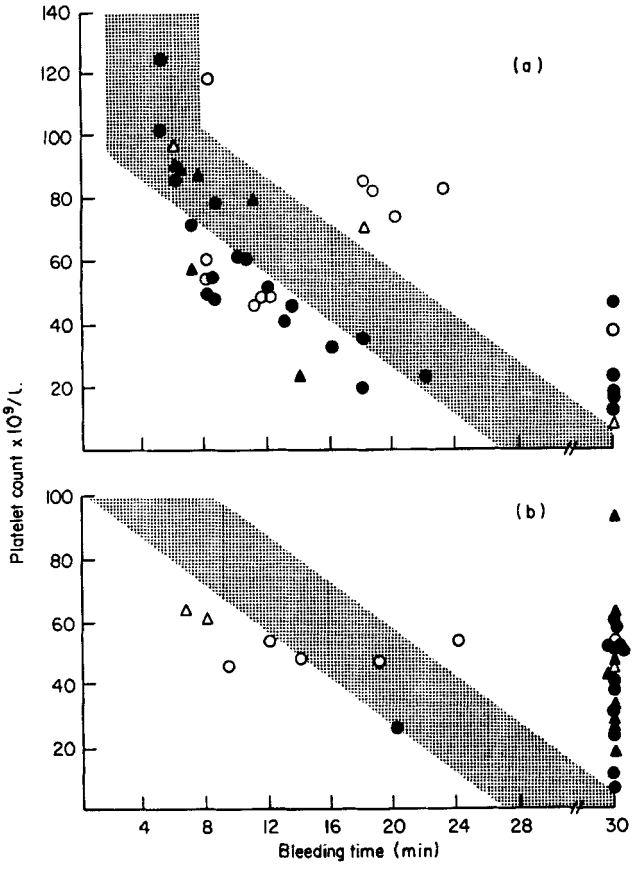

FIG 6. Bleeding times and platelet counts in aplastic thrombocytopenic patients following transfusion of platelets stored at 22°C (a) and 4°C (b). Pre-transfusion bleeding times greater than 60 min and platelet counts less than 10×10^9/l. in all cases. (a) 22°C: data on four patients following nine transfusions (two stored for 24 h and seven stored for 72 h). (b) 4°C: data on two patients after eight transfusions (three stored for 24 h and five stored for 72 h). Platelets are functional in every instance within 2½ h post-infusion of 22°C stored platelets and remain functional (a) while only a single measurement showed any function after 2½ h post-transfusion of 4°C stored platelets (b). Triangles = 24 h storage; circles = 72 h storage. Open symbols = measurement between 5 min and 2½ h post-transfusion; closed symbols = after 2½ h. Shaded area represents 95% confidence limits of relationship between bleeding time and platelet count in untransfused aplastic thrombocytopenic patients (Harker & Slichter, 1972b).

Bacterial Monitoring

No evidence of bacterial growth was found in the 310 concentrates monitored for 3 weeks following 72 h storage at 22°C. The Gram stain was negative in all instances.

DISCUSSION

To be of use in a transfusion service, platelet concentrates must be able to withstand storage with minimal loss of viability or function. Six critical variables related to optimum platelet storage have been identified.

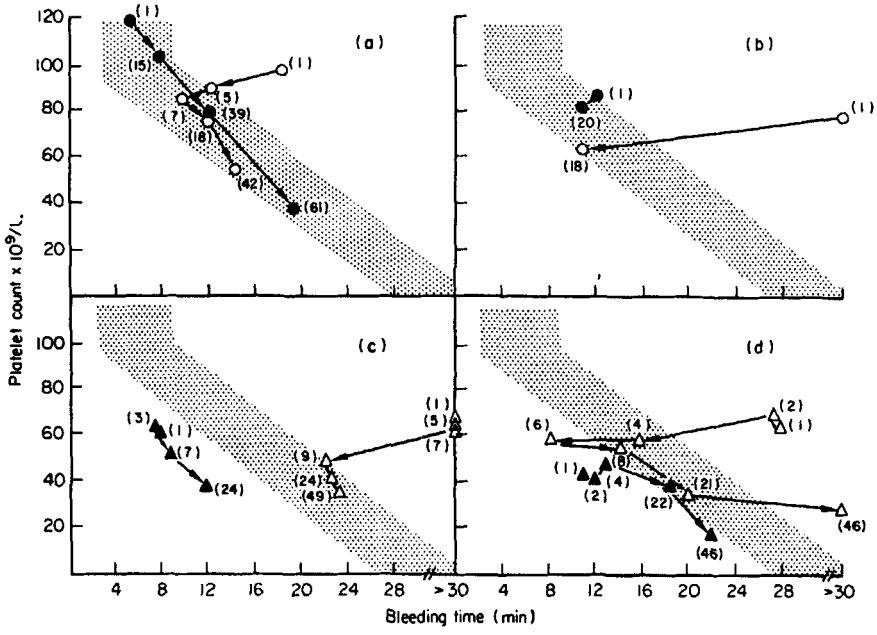

FIG 7. Effect of ASA ingestion on bleeding time correction. (a) and (b), Bleeding time in two thrombocytopenic patients after transfusion of unstored ASA (○) and non-ASA (●) platelets prepared from the same six donors. (c) and (d), Bleeding times in the same two thrombocytopenic patients following transfusion of stored (72 h at 22°C) ASA (△) and non-ASA (▲) platelets prepared from the same six donors. Numbers in parentheses are post-transfusion times in hours.

Anticoagulant. A positive correlation was found between initial pH of the PRP and post-storage platelet viability. Thus platelet concentrates prepared from CPD anticoagulated blood (pH 7.2) appear to have slightly greater post-storage viability than those from ACD-anticoagulated blood (pH 7.0) (Table III and Figs 2 and 3). When ACD-acidified PRP is used (pH 6.5), platelet viability is significantly reduced after storage. Even though ACD-acidified units can be resuspended immediately after centrifugation avoiding the 1½ h delay required with nonacidified ACD and CPD units, we feel this time-saving technique is not justified because of the reduced viability.

Centrifugation. It has been demonstrated (Slichter & Harker, 1976) that viability of unstored platelets prepared at forces greater than 3000 *g* is significantly impaired. When platelets prepared in this manner are stored for 72 h, viability is further reduced (Table V). Thus the optimum speed for removing platelets from PRP is 3000 *g*.

Plastic bag. The type of bag used has a significant effect on viability of stored platelets (Table I). Only two commercially available materials, Fenwal PL-146 and McGaw bags and one investigational plastic (Cutter CL-2383), proved satisfactory in tests after storage for 24 and 72 h. Viability of platelets stored in Cutter PR2370A and Fenwal PL-130 bags was significantly impaired. The results obtained with Fenwal bags are in accord with previous work (Murphy *et al*, 1970). While the mechanism of impairment is unknown, factors in bag construction such as internal surface, plastic composition, thickness or leachables may be

implicated. It has been reported that the plasticizer DEHP is toxic to blood cells (Contreras *et al*, 1974). However, platelet viability and function are well maintained despite high levels of DEHP in the suspending plasma (Contreras *et al*, 1974).

Temperature. After storage at 4°C for 24 and 72 h, platelet viability dropped to 18% and 9% of that of unstored controls, respectively. These results are in agreement with the findings of others (Levin & Freireich, 1964; Murphy & Gardner, 1969; Murphy *et al*, 1970; Becker *et al*, 1973). In contrast, platelets stored at 22°C for 24 and 72 h maintained viability of 97% and 81% that of unstored controls, respectively. Other investigators (Murphy *et al*, 1970) reported that after 72 h storage at 22°C, platelet viability dropped to 65% of that of unstored controls. While he attributed the loss in viability to platelet ageing *in vitro*, we believe it was probably due to suboptimal storage conditions.

Aggregation and adhesion of platelets *in vitro* are greatly impaired at 22°C but well preserved at 4°C (Shively *et al*, 1970; Murphy & Gardner, 1971; Kattlove *et al*, 1972). Conversely, viability and function of platelets *in vivo* are well preserved at 22°C but greatly impaired at 4°C. As a result, *in vitro* tests do not predict viability and function of platelets *in vivo*.

Platelet concentration (pH). In a plasma volume of 20 ml, pH was found to drop below 6.0 after 72 h storage, seriously impairing platelet viability. Since pH is inversely related to platelet concentration (Murphy & Gardner, 1975), a pH greater than 6.0 can be maintained by decreasing the platelet concentration to less than 1.7×10^{12}/l. (Fig 1). This requires dilution of the concentrate in 70 ml plasma. The reduced viability of stored platelets reported by others (Murphy *et al*, 1970; Becker *et al*, 1973) was probably due to the low pH which accompanies a platelet concentration greater than 1.7×10^{12}/l.

Mixing. As previously suggested (Murphy & Gardner, 1969), constant gentle mixing is essential to maintain viability of platelets stored at 22°C (Table I). The recent report (Vallejos *et al*, 1973) of poor clinical response to platelets stored at 22°C may be related to the lack of continuous mixing.

Clinical Effectiveness

Since bleeding time is a quantitative measure of platelet plug formation (Harker & Slichter, 1972b), it is an appropriate means of evaluating the haemostatic effectiveness of transfused platelets. When platelets were transfused to unimmunized aplastic thrombocytopenic patients, unstored concentrates were found to correct bleeding time predictably (Fig 5). Similarly, platelets stored for 24 and 72 h at 22°C were effective in reducing bleeding time in these patients (Fig 6a). Platelet concentrates stored at 4°C had markedly impaired function (Fig 6b). The difference between our findings and those of others (Becker *et al*, 1973; Vallejos *et al*, 1973) is probably related to variation in experimental design. For example, in a recent study (Becker *et al*, 1973), bleeding time was only measured 1 h after transfusion, so that subsequent dysfunction was not detected. Furthermore, part of the defective function reported for platelets stored at 22°C probably reflected loss of viability due to inadequate plasma volume and low pH. In that study, platelet counts corresponding to the bleeding times were not recorded, so that viability could not be assessed directly.

Carefully controlled comparisons of viability and function of stored and unstored platelets after transfusions to aplastic thrombocytopenic patients showed that platelets stored at 22°C were clearly superior to those stored at 4°C (Table VI, Fig 6).

Effect of ASA

Bleeding time correction is sometimes delayed for several hours in patients receiving either unstored platelets or platelets stored for 72 h at 22°C (Figs 5 and 6a). We suggest that this transient impairment of platelet function may be related to ASA ingestion by donors. In tests of patients receiving platelets from donors who had ingested 1.2 g aspirin for 3 d before plateletpheresis, initial dysfunction was followed by full correction within 4–9 h (Fig 7). Platelets collected from unaspirinized donors had immediate normal function. These data suggest that ASA-induced platelet dysfunction is reversible *in vivo*, contrary to a previous report (Weiss *et al*, 1968). Since the impairment is transient, screening of donors for ASA ingestion is probably unnecessary.

Bacterial Contamination

Our studies as well as those of others (Katz & Tilton, 1970; Goddard *et al*, 1973) indicate that bacterial contamination in concentrates stored 72 h at 22°C is not a problem. While a 2.4% incidence of contamination has been reported for pooled concentrates (Bucholz *et al*, 1973), the most likely source of bacterial entry is the pooling procedure itself. In one study (Cunningham & Cash, 1973), 6.3% of single units were found to be contaminated but the number of organisms was small; no clinically apparent sepsis occurred after transfusion of 1800 concentrates, possibly because of a bactericidal effect during storage (Myhre *et al*, 1974). While there is a risk of contamination, the data suggest that it is of little practical consequence. Use of aseptic technique, a closed bag system, and periodic bacteria monitoring of platelet concentrates after storage appear to provide adequate quality control.

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The studies in human subjects were approved by the Human Investigation Committee of the University of Washington School of Medicine and included fully informed consent.

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