Platelet Storage at 22°C; Metabolic, Morphologic, and Functional Studies

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Abstract Platelets stored at 22°C for transfusion purpose have been examined with metabolic, morphologic, and functional studies. Evaluations were made of platelet-rich plasma (PRP) stored for 3-4 days and platelet concentrates (PC) stored for 24 hr. During these periods, lactate accumulated continuously without significant change in platelet count, pH, or plasma glucose. Platelet glycogen fell dramatically both chemically and by electron microscopy, but adenosine triphosphate (ATP), adenosine diphosphate (ADP), and intracellular potassium did not change. After storage, the cell's capacity for glucose utilization through glycolysis, the hexose monophosphate shunt, and the tricarboxylic acid cycle appeared to be intact. Although platelet volume during storage did not change, disc to sphere transformation was observed by phase microscopy. Platelet aggregation with ADP was reduced even after 1 day of storage. After transfusion of stored platelets to thrombocytopenic recipients, recovery of platelet glycogen and capacity for aggregation occurred within 24 hr. In summary, the platelet remains surprisingly intact during the intervals studied; those defects which do develop are reversible in the circulation of a thrombocytopenic recipient if viability has been maintained. A "storage lesion" responsible for loss of viability has not been defined.

Introduction Recent studies (1, 2) have indicated that platelet viability for transfusion purposes is best maintained at 22°C rather than at 4°C. Platelets stored at the higher temperature are suitable for use for as long as 3-4 days with variations depending on whether the cells are maintained

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Abbreviations used in this paper: ACD, acid-citrate-dextrose; ADP, adenosine diphosphate; ATP, adenosine triphosphate; PC, platelet concentrates; POPOP, 1,4-bis[2-(5-phenyloxazolyl)]benzene; EPO, 2,5-diphenyloxazole; PPP, platelet-poor plasma; PRP, platelet-rich plasma.

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Over-all, our data suggest that the platelet "storage lesion" will prove to be quite different from that of the red cell. Metabolic and functional defects do develop during storage. However, these are reversible in the circulation of a thrombocytopenic recipient after transfusion suggesting that the functional integrity as well as the viability of these cells will be acceptable for the clinician.

METHODS

PRP and PC were prepared as previously described (1) except that PC were resuspended to a final volume of 25 ml rather than 4 ml (2). All PC studied were prepared from PRP which had been acidified with one volume of extra acid-citrate-dextrose (ACD) NIH Formula A for 18 volumes of PRP. During storage, the plastic bag containing PRP or PC was agitated at 20 cycles/min on an aliquot mixer and maintained in a constant temperature incubator at 22°C. For studies of rates of glucose utilization, fresh or stored PRP or PC was centrifuged at 1000 g for 20 min and resuspended to a platelet count of 500,000 per mm² in modified Krebs-Ringer buffer. Platelets in PRP and PC were counted with a Coulter Counter Model F. The method of Brecher and Cronkite (8) was used for whole blood platelet counts. In all preparations, erythrocyte and leukocyte contamination did not exceed 1/10,000 platelets. pH was measured at room temperature with a pH meter.

Glucose and lactate concentrations were measured directly on aliquots of PRP, PC, and platelet suspensions in buffer. Platelet adenosine diphosphate (ADP) and ATP, glycerol, and potassium were measured on platelet buttons prepared by centrifugation at 1400 g for 10 min at 4°C and related to platelet number. The content of platelets in the button was determined by multiplying the volume of the platelet-rich material centrifuged by the difference between the platelet counts of the platelet-rich material and the resulting, platelet-poor supernatant. Glucose was measured by the glucose oxidase method. After extraction with perchloric acid and neutralization of the extract with KOH, lactate, ADP, and ATP were measured by standard enzymatic techniques (9). In the majority of experiments platelet glycerol was determined by the anthrone reaction after digestion of the button in hot 30% KOH and precipitation of glycerol in 66% ethanol (10). In additional studies carried out to confirm results with the anthrone technique, the button was lysed by solution in distilled water, heating at 90°C for 3 min, and three cycles of freezing and thawing. Glycerol content of the tissue homogenate was then assayed by enzymatic hydrolysis with Diazyme® and subsequent measurement of glucose liberated by the glucose oxidase method (11). Platelet potassium was measured by flame photometry after lysis of the platelet button in 1 ml of distilled water followed by three cycles of freezing and thawing. Correction was made for plasma potassium trapped in the button by adding albumin to PRP before centrifugation and subsequently measuring the radioactivity of the button and supernatant platelet-poor plasma (PPP) and the potassium concentration of PPP. Potassium in trapped plasma never contributed more than 5% of the total button potassium.

For measurement of glucose utilization, lactate production, and evolution of 14CO2 from glucose-14C or glucose-

135A platelets in buffer (500,000 per mm²) were incubated in 5-ml aliquots in eight siliconized 25-ml flasks gassed with 5% CO2-95% O2 and sealed with rubber caps. 1 µCi glucose-14C was included in each flask. The flasks were shaken at 80 cycles/min at 37°C in a Dubnoff metabolic shaker. At the start of the incubation and at hourly intervals thereafter, the reaction in one of the flasks was stopped by inserting a needle through the rubber stopper to allow the injections of 1.0 ml of 4 N perchloric acid into the platelet suspension and 0.4 ml of hydroxide of hyamine 10-X® into a plastic cup suspended above it. The flask was then incubated for 1 hr at 22°C to allow trapping of evolved CO2. The contents of the cup were added to scintillation solution while the acid-precipitable material in the flask was washed five times with 0.5 N perchloric acid and solubilized in NCS-Solubilizer for addition to scintillation solution. The combined acid extracts were neutralized with 4 N KOH. Aliquots of the neutralized extracts were assayed enzymatically for lactate while one-third of the final extract was passed through an anion exchange column which was washed with 40 ml of distilled water. The anionic products of glucose metabolism were then eluted with 30 ml of 1 N HCl; an aliquot was added to scintillation solution. Samples were counted in Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3325) and the results corrected for quenching with an internal standard. Preliminary studies indicated that longer periods of incubation to collect CO2 further washes of the acid-precipitable material, and larger volumes of 1 N HCl in the elution step did not significantly increase the radioactive yield. The total recovery of isotope in all fractions averaged 95% and was always greater than 90%. With knowledge of the specific activity of glucose in the original platelet suspension, amoles glucose converted to CO2, anionic glucose metabolites, and acid-precipitable material were determined. Their sum was interpreted as amoles glucose utilized recognizing that an undetermined glucose metabolite might be excluded from the three fractions. The percentage of glucose utilized passing through the hexose monophosphate shunt during the 1st hour of incubation was estimated according to Wood, Katz, and Landau (12). This method of measuring glucose utilization was adapted from one originally described by Rose and O’Connell for red cells (13).

1 Squibb Radioiodinated (131I) serum albumin (human), 5 

mCi/g protein.

2 Siliclad, Clay-Adams, Parsippany, N. J.

3 New England Nuclear, Boston, Mass. Final specific activity in platelet suspensions, approximately 0.03 mCi/ 

mmole. The glucose concentration of the platelet suspensions in each study was measured so that the final specific activity was always accurately known.

4 Packard Instrument Co., Downer’s Grove, Ill.

5 Half mixture of toluene: ethanol. PPO, 5 g/liter. PO- 

POPOP, 0.3 g/liter.

6 Amersham/Searle, Des Plaines, Ill.

7 Anion exchange resin, AG 1-X8, 100–200 mesh, acetate form, Calbiochem, Los Angeles, Calif.

8 (See p. 372 for footnote 14.)

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Platelets in PRP and PC were studied in wet preparations in their own plasma by oil-phase microscopy (×1000). For electron microscopy, equal volumes of PRP and 0.1% glutaraldehyde in White's saline (14) were mixed and centrifuged at 22°C to obtain a platelet button. The button was further fixed in 5% glutaraldehyde-5% paraformaldehyde in 0.1 M cacodylate buffer at 22°C and postfixed at 4°C in 2% osmic acid in 0.2 M s-collidine buffer. The samples were then washed with 0.5% uranyl acetate in Veronal-acetate buffer, dehydrated in alcohol, embedded in Epon 812, stained with uranyl acetate and lead citrate, and examined with either a Phillips 300 or Jeolco-JEM 7 microscope.

Estimations of platelet volume were made by diluting PRP 1:2000 in Isoton. After 22°C and obtaining a size distribution plot with a Coulter Counter Model B and histogram plotter attachment. The mean window of the distribution was calculated by the method of Brecher, Jakobek, Schneiderman, Williams, and Schmidt (15). For the latter calculation, the first three windows were not included since they consisted largely of electrical "noise." Since the smallest platelets blended with this noise, the calculated mean volumes should be regarded not as absolute but as relative expressions useful for comparing fresh and stored platelets. The counter was calibrated daily with a suspension of polyvinyltoluene particles whose modal volume (3.86 μ) was similar to that of platelets. Mean platelet volume was expressed in cubic microns by comparison of the mean window of the distribution with the window in which the standard particle peaked.

Platelet aggregation was measured at 37°C by the turbidometric procedure of Born and Cross (16) using an aggregometer.

RESULTS

Metabolic events during storage. Neither PRP stored for 4 days nor PC stored for 1 day showed significant change in platelet count or pH. PRP and PC platelet counts were 150,000–300,000 per mm³ and 1,500,000–3,000,000 per mm³ respectively. The ranges of pH were 6.8–7.0 for PRP and 6.3–6.7 for PC. When PC were stored for longer periods, unpredictable and occasionally extreme reductions in pH occurred (2) making metabolic evaluation beyond 24 hr difficult. Plasma glucose, initially 20–30 mmole/liter, did not fall more than 10% during PRP storage for 4 days or PC storage for 1 day. Lactate production occurred continuously as time passed (see Fig. 1). Fresh platelet glycogen was 0.57 ± 0.03 μmoles/10⁹ platelets (10 studies) by the anthrone method (10). In four studies, the glycogen content of platelets in fresh PC was found not to differ significantly from that of platelets in the parent PRP. This value is comparable to the results of Scott (17) and Karpatkin (18) who reported 0.56 μmoles/10⁹ platelets and 0.43 μmoles/10⁹ platelets respectively, using the same method. Fig. 2 shows the decline of platelet glycogen with storage, greater in PC than PRP. By the diazyme method, platelet glycogen in fresh PC was 0.41 ± 0.03 μmoles/10⁹ platelets (6 studies) and 0.05 ± 0.02 μmoles/10⁹ platelets after 24 hr of storage confirming the profound decline observed with the anthrone method.

ATP and ADP levels of platelets in fresh PRP were 37 ± 2 μmoles/10⁹ platelets (17 studies) and 25 ± 2 μmoles/10⁹ platelets (7 studies). These values are essentially identical with those reported by Karpatkin (18) but one-half to two-thirds of those reported by others (19, 20). After PRP storage for 3 days, ATP and ADP levels were 35 ± 2 μmoles/10⁹ platelets and 25 ± 2 μmoles/10⁹ platelets respectively; neither of these values represents a statistically significant change. In four samples of PC stored for 24 hr, ATP levels did not change significantly. Significant decline in platelet potassium was not detected during 3 days of PRP storage. Fresh platelets contained 0.90 ± 0.05 μEq/10⁹ platelets (10 studies) compared to 0.95 ± 0.03 μEq/10⁹ platelets 3 days later. Our value for fresh platelets is about 15% higher than values recently reported (21–23).

The intermediates of glycolysis, the hexose monophosphate shunt, and the tricarboxylic acid cycle should be recovered in the anionic fraction. It was shown, for example, that citrate-C¹⁴ was recovered quantitatively in this fraction. Glycogen, protein, and lipid are recovered in the acid precipitate. It was shown using the anthrone method (10) that 87% of platelet glycogen was recovered in the precipitate. Amino acid recovery is incomplete. Using C-labeled compounds, we found that alanine is excluded from the three fractions while aspartate and glutamate are recovered quantitatively in the anionic fraction.

Coulter Diagnost Inc., Hialeah, Fla.

An aperture tube with a 50 μ opening was used. Machine setting were: 1/amplification = 1 1/aperture current = 1, matching switch = H16, gain control = 75.

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Metabolic capacity after storage. When fresh or stored platelets were incubated in modified Krebs-Ringer buffer for 4 hr, glucose utilization and lactate production were linear with time from the 1st to the 4th hour (Fig. 3) while the small amount of glucose-$^{14}$C converted to CO$_2$ increased exponentially. Of the glucose-$^{14}$C which had been utilized at the end of the 4 hr incubation, 1–2% was found in CO$_2$ and 1–2% was found in the acid precipitate. During the 1st hour of incubation, approximately 3% of labeled carbon utilized from glucose-$^{14}$C was converted to CO$_2$ and 1–2% was found in the acid precipitate. During the 1st hour of incubation, approximately 1–2% of the glucose-$^{14}$C utilized was found in CO$_2$. The percent of glucose utilized via the pentose cycle in four experiments was 1.2 ±0.1%. When NaCN at final concentration, $10^{-4}$ mole/liter, was added after 1 hr of incubation (Fig. 3), production of labeled CO$_2$ from glucose-$^{14}$C was completely inhibited and glucose utilization and lactate production increased fivefold. A similar increase in glycolytic rate could be achieved during incubations under 5% CO$_2$–95% N$_2$ although the technique was more cumbersome and complete suppression of labeled CO$_2$ production could not be achieved reproducibly.

When glycolytic rate was measured after PRP storage for 72 hr (Table I), glucose utilization and lactate production were approximately twice the control. With one exception (study 2), incorporation of label into CO$_2$ and the acid precipitate was increased after storage as well. In one study, incubations with glucose-$^{14}$C were carried out simultaneously and the percent glucose utilized through the hexose monophosphate shunt was found to be 1.0% both before and after storage. In the presence of $10^{-4}$ M NaCN, glycolytic rate increased beyond the level achieved before storage. A similar main-

![Figure 2](image-url) Platelet glycogen during storage. As measured by the anthrone method, there was a profound decline in platelet glycogen with storage, greater in PC than PRP. Numbers in parentheses refer to number of studies.

![Figure 3](image-url) Metabolic events during incubation of fresh platelets in modified Krebs-Ringer buffer: representative experiment. Glucose utilization and lactate production proceeded at a linear rate after the 1st hour and were stimulated fivefold in the present of $10^{-4}$ M NaCN. 2% of glucose-$^{14}$C utilized was completely oxidized to CO$_2$ after 4 hr. No labeled CO$_2$ was produced in the presence of cyanide.

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microscopy. During the first 48 hr of PRP storage, the majority of cells retain their normal disc configuration (1). From 48 to 96 hr, the cells tend to become spherical and develop both multiple short, pointed projections and one or two long, dendritic projections. Beyond 96 hr, clumping is frequent and the majority of cells are spheres, many of which demonstrate eccentricity of the granulomere with veil-like projections of cytoplasm arising from the cell surface (24). Although these morphologic changes occurred, platelet volume as assessed by the Coulter Counter was unchanged during 96 hr of PRP storage. In eight studies, the mean volume of fresh platelets was 5.4 ±0.2 μ; 96 hr later, mean volume for these same eight samples was 5.5 ±0.2 μ. The control value is somewhat lower than 7.1 μ reported by Bull and Zucker (25) and 7.5 μ by Karpatkin (18).

In thin sections viewed by electron microscopy, fields of glycogen particles (26) were markedly depleted after 48 hr of PRP storage and entirely absent after 24 hr of PC storage. Otherwise, we could detect no definite, consistent ultrastructural change even after 96 hr of PRP storage; specifically, mitochondria, granules, and the circumferential band of microtubules (26) seemed well preserved.

**Aggregation studies.** Platelets in fresh ACD PRP demonstrate a primary wave of aggregation in the presence of ADP at concentrations as low as 1–2 μmoles/liter. The so-called "secondary wave" (27) was not demonstrated in ACD PRP as it has been in PRP from blood anticoagulated with sodium citrate. After 24 hr of storage, the aggregation response was usually clearly reduced; it was frequently absent at 2 μmoles/liter and sometimes at concentrations as high as 10 μmoles/liter (Fig. 4). The addition of 1/4 volume of fresh PPP to stored PRP did not improve the response, and conversely PPP obtained by centrifugation of stored PRP did not impair the aggregation of fresh PRP. These studies tend to exclude the loss of a necessary plasma cofactor or the evolution of a plasma inhibitor as explanations for the decrease in aggregation. It is more likely that a change in the platelet itself takes place.

**Studies of stored platelets after in vivo circulation.** In a patient, M.C., with aplastic anemia and platelet count of 6000 per mm³, platelets concentrated from 20 U of PRP stored for 24 hr were infused. The platelet count rose to 120,000 per mm³ (calculated recovery, 50%). The stored platelets before infusion were nearly unreactive.
even at 10 μM ADP (Fig. 4). However, at 8 and 24 hr after transfusion, platelets obtained from the patient’s circulation aggregated with a response similar to that of fresh, normal platelets.

On three occasions, patients A.C. and K.C., with aplastic anemia and platelet counts less than 10,000 per mm³, were transfused with PC stored for 24 hr so that their platelet counts were raised to 80,000–140,000 per mm³; calculated in vivo recoveries were 35–55%. Serial samples were then obtained and platelets were harvested for measurement of glycogen. As shown in Fig. 5, platelet glycogen rose within 3–6 hr and was normal at 24 hr. Similarly, whereas glycogen was morphologically absent in electron micrographic preparations of stored PC, it was plentiful in platelets obtained from the circulation 24 hr after transfusion.

**DISCUSSION**

The studies described supplement the data already available concerning the viability of platelets stored at 22°C with information relevant to their metabolic, morphologic, and functional integrity. On the whole, contrary to traditional concepts, the platelet appears to be a relatively steady cell if it is not exposed to cold temperatures. The cell’s ability to metabolize glucose to lactate and CO₂ either via the hexose monophosphate shunt or the tricarboxylic acid cycle appears intact throughout the storage intervals studied. Similarly, the capacity to maintain a high intracellular potassium concentration is unaltered. The characteristic discoid configuration is maintained for 48 hr with a transition to spheres occurring after that point. In spite of this shape change, there is no alteration in cell volume for 96 hr.

ATP levels do not fall significantly during 72 hr of storage of PRP. After 72 hr, viability as judged by in vivo survival studies is reduced without exception (1). Therefore, it appears unlikely that loss of ATP is responsible for the decreased viability after storage as is usually the case for the red cell. These results must be interpreted with some caution since we have measured total platelet ATP. It is now clear that platelet nucleotides are present in at least two and perhaps more pools (28). It is possible that one pool of platelet ATP, quantitatively small but functionally or anatomically strategic is reduced, resulting in loss of viability. More detailed studies in this regard are planned.

We have observed a consistent fall in platelet glycogen, most striking during PC storage (Fig. 2). Again we consider it unlikely that loss of viability results from loss of glycogen stores because there is no significant difference in viability between PRP and PC stored for 24 hr (2); yet the difference in glycogen levels is dramatic. At this time, therefore, we are not able to suggest the nature of the “storage lesion.” In all in vitro systems described to date (17, 29), platelet glycogen is

![Figure 4](image-url)

**Figure 4** In vivo recovery of platelet aggregation with ADP. An aplastic patient, M.C., received 20 U of platelets stored for 24 hr as PRP. Before and after storage, 1 ml aliquots were removed from each unit; aggregation studies were carried out on the combined aliquots. Aggregation of stored platelets (B) was markedly reduced compared to fresh (A). Platelets obtained from the circulation of M.C. 8 hr after transfusion (C) had recovered an ability to aggregate similar to that of fresh platelets. The reactivity of the patient’s own platelets before transfusion could not be measured because of her extremely low platelet count, 6000 per mm³. Platelet counts in the three PRP specimens were: A, 256,000 per mm³; B, 219,000 per mm³; C, 120,000 per mm³. If the difference in OD between PRP and PRP is taken as 100%, the ΔOD for each specimen is: A, 48%; B, 8%; and C, 50%.

![Figure 5](image-url)

**Figure 5** In vivo recovery of platelet glycogen. Platelets stored for 24 hr as PC had very low levels of glycogen (mean, 0.1 μmoles/10⁹ platelets). After transfusion to aplastic patients, glycogen levels in platelets harvested from the recipient’s circulation rose to near the normal range in the first 6 hr and into or above the normal range at 24 hr. For all the points recorded, the platelet count had been raised to such an extent by transfusion that the recipient’s own platelets contributed less than 10% to the total number of circulating platelets. This excludes the possibility that the return of platelet glycogen to normal levels resulted from admixture with the recipient’s platelets.
depleted as time passes. Karpatkin, Charmatz, and Langer (29) have also observed that glycogen levels fall more rapidly when platelets are concentrated; no explanation for this phenomenon is apparent. The cell possesses the capacity to maintain ATP levels, to utilize extracellular glucose, and to increase glucose utilization dramatically when stimulated as shown by the incubations in the presence of NaCN. Furthermore, glucose is abundant in ACD plasma and increased at least fourfold over physiologic concentrations. In these circumstances, the observed glycogenolysis is paradoxical and remains unexplained.

Clinically, the critical question to ask is whether the glycogen-depleted platelet might be viable but functionally inadequate after transfusion in the thrombocytopenic patient. The in vivo resynthesis (Fig. 5) of platelet glycogen is reassuring in this regard. Similarly, aggregation with ADP is defective in the stored platelet, but recovery can be demonstrated for the platelets viable after transfusion to a thrombocytopenic recipient (Fig. 4). There is a clear similarity between these studies to those (30, 31) for stored red cells which show a fall in 2,3-diphosphoglycerate and an increase in oxygen affinity to hemoglobin during storage and a reversal of these phenomena in vivo after transfusion. They all underscore the basic principle that neither the viability nor the functional capacity of a transfusion product can be predicted with confidence by its performance in an in vitro study before transfusion. At this time, it appears that once viability has been established, only bleeding time measurements and careful clinical evaluation of recipients will demonstrate the efficacy of transfusions of stored platelets.

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