Functional Platelets After Storage *in Vitro* for 15–21 Days

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Blood platelets are notoriously difficult to preserve *in vitro* for long periods of time. Despite many efforts to solve the problem and improve conditions for storage, platelets lose their ability to respond to aggregating agents after 72–96 hours and are routinely discarded by blood banks after three days if not used for transfusion. The present study has evaluated the influence of raising the pH of the anticoagulant used to collect blood on the functional viability of platelets during storage at room temperature. Twenty-four samples of C-PRP were followed for 15 days and 12 samples for 21 days. Although platelet counts fell steadily during the 3-week storage period, a significant proportion of the cells remained viable. After 5–10 days the platelets responded as well to threshold concentrations of ADP and sodium arachidonate (SA) as on Day 0, and reactions to the same agents on Day 15 were nearly as impressive. Even on Day 21, responses to ADP and SA could still be elicited. Biochemical studies on samples stored for 15–21 days revealed normal levels of serotonin after 2 weeks and a fall of less than 30% after 3 weeks. The ability of the cells to convert ^{14}C-arachidonic acid into thromboxane B_2 was well maintained over the 3-week period. Adenine nucleotide levels fell 25% over 15 days and over 50% by 21 days, but the capacity of the cells to take up ^{14}C-adenine and convert it to AMP, ADP, and ATP was increased, and ATP/ADP ratios were not greatly different from those on Day 0. Physical changes were apparent in most platelets by Day 15. However, 5–20% of platelets in 15–21-day-old samples were discoid in shape and contained circumferential bands of microtubules and small amounts of glycogen. The findings suggest that high pH during collection of blood, preparation of C-PRP, and early phases of storage may foster long-term preservation of viable platelets *in vitro*. (Am J Pathol 1980, 101:613–634)

THROMBOCYTOPENIA is a common symptom in patients with leukemia and aplastic anemia, regularly complicates medical management of malignant disease, and seriously influences the outcome of treatment in therapeutic endeavors such as bone marrow transplantation.[1] As a result, blood banks have been faced with an ever-increasing demand for concentrates of viable platelets for transfusion.[2–3] The need has been met by centralizing resources, plateletpheresis, expanding donor pools and devoting increased time and effort to preparation of platelet concentrates.[4] A major obstacle, however, has been the limited viability of platelets when stored in the liquid state.[5–7] Platelets appear to lose their functional capability and ability to circulate after only a few days *in vitro* and are

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virtually useless to thrombocytopenic patients after transfusion. As a result, platelet concentrates which have not been transfused are routinely discarded by blood banks within 3 days after collection.[4]

Innumerable attempts have been made to improve the viability of stored platelets. The anticoagulant used to collect blood, the conditions employed to separate platelets, the procedures required to concentrate the cells, the temperature and pH at which they are maintained, the influence of agitation, the effects of plastic bag thickness, and the nature of the polymer, the importance of oxygen saturation, and other environmental factors in the maintenance of platelet numbers, biochemistry morphology, and function have been studied exhaustively.[5-10] Yet platelets stored *in vitro* under any set of conditions appear to lose their viability and functional capacity within 3–5 days.

Efforts to define the nature of the lesion in stored platelets have been under way in this laboratory for several years.[11] Recently we have found that a simple modification of the anticoagulant used to collect blood has a profound effect on the survival of platelets *in vitro*. Results of our study indicate that a significant proportion of stored platelets can retain biochemical and functional integrity for periods of 15–21 days.

Materials and Methods

General

Blood for the present study was obtained from healthy adult donors, after informed consent, who had not taken drugs of any kind for at least 2 weeks. The methods used to collect blood samples were identical to those described in many previous investigations.[12-14] After venepuncture, blood was drawn into plastic syringes and immediately transferred to plastic tubes containing citrate phosphate dextrose (CPD) in a ratio of 8.6 parts blood to 1.4 parts anticoagulant. The critical modification in our study was the adjustment of CPD anticoagulant to a pH of 9 prior to mixing with blood. Platelet-rich plasma was separated from whole blood by centrifugation at 200g for 20 minutes at room temperature. Supernatant C-PRP was collected from the several tubes and transferred to a plastic reservoir for storage. About 70 ml of C-PRP was recovered and placed in storage from the 150 ml of blood given by each donor.

Materials

The citrate phosphate dextrose (CPD) anticoagulant used in these studies consisted of 3.27 g of citric acid. H_2O, 26.3 g of sodium citrate · $2H_2O$, 25.5 g of dextrose, and 2.2 g of $NaH_2PO_4 \cdot H_2O$ per liter.[15] Fourteen ml of this anticoagulant was adjusted to pH 9 by drop-wise addition of 6N potassium hydroxide. The plastic reservoirs chosen for storing platelet-rich plasma were the receptacle portions of sterile, disposable, 0.22-μ filter systems (Falcon 7103, Oxnard, Calif). Platelets were stored at room temperature. The Reservoirs were agitated during sampling but were otherwise left undisturbed.

Procedures

Samples of the stored platelets for study were drawn from the side port of the reservoir under sterile conditions. The pH of each sample was measured with a glass electrode

(Beckman Instruments, Palo Alto, Calif), and platelet counts were determined by an electronic particle analyzing system (Coulter Model B, Hialeah, Fla). Aggregation studies were carried out on a Payton dual channel aggregometer. Maximum amplitude of the recording pen was preset with platelet-poor (PPP) and platelet-rich plasma (PRP) from the same sample.[13]

Oxidation of arachidonic acid by platelet cyclo-oxygenase into specific metabolites was followed by a modification [16] of the method of Hamberg and Samuelsson.[17] Five-milliliter volumes of fresh or stored platelets were mixed with 0.5 ml of 0.1 M EDTA and centrifuged at 600g for 15 minutes for the purpose of obtaining pellets. Platelet pellets were gently resuspended in 4 ml of Hanks' balanced salt solution (HBSS) without calcium or magnesium. One ml of citrate-citric acid dextrose (CCD) [16] anticoagulant was added to the suspended cells, and then each sample was sedimented again. The platelet pellet was resuspended in 1 ml of HBSS. Each sample containing 2×10^8 platelets was stirred on the platelet aggregometer at 37 C for 3 minutes with 1 μg of ^{14}C-arachidonic acid and 10 μl of 100 mM $CaCl_2$. The reaction was stopped by addition of 2 ml of ethyl acetate, and after acidification, each sample was extracted twice more with ethyl acetate. the several acetate extracts were pooled and concentrated under a stream of nitrogen before application of silica gel G thin layer plates. Chromatography of the free acids was accomplished with diethyl ether, methanol, and acetic acid (135:3:3 v/v) as the solvent system. Plates were scanned for radioactive spots with a Berthold radioactivity scanner. Areas with radioactivity were scraped from the platelet, placed in scintillation vials, and analyzed in a Beckman scintillation counter. The acounts of uncoverted ^{14}C-AA remaining and the concentration of thromboxane B_2 formed in the reaction mixtures were determined and the percentage of conversion derived by calculation.

Perchloric acid extracts of the platelet samples were analyzed spectrophotofluorometrically for endogenous serotonin content [18] and by high pressure liquid chromatography (HPLC) for adenine nucleotide profiles.[19] Nucleotide kinetics were followed by incubating 5 ml samples of platelet-rich plasma with ^{14}C-adenine (250 mCi/m mole) for 80 minutes at 47 C.[20] At the end of the incubation period an equal volume of CCD anticoagulant was added and the sample rapidly sedimented at 7000g in a microfuge for the purpose of obtaining a platelet pellet. Perchloric acid extracts were partitioned by HPLC and fractions representing peaks for AMP, ADP, and ATP were collected into scintillation vials. Radioactivity was determined in a Beckman scintillation counter. Quantitation of each nucleotide was done with a Hewlett-Packard integrator and a programmable Canon model 1614P calculator. Special computer programs were used to process data to yield micromoles of specific adenine nucleotides per 10^{11} cells, ATP/ADP ratios, and specific activities.[21]

Platelet samples were fixed in glutaraldehyde and osmic acid with or without 0.1% potassium ferrocyanide for study in the transmission electron microscope according to methods developed in this laboratory and reported previously in detail.[12-14] Ferrocyanide added to osmic acid enhances the preservation of unit membranes and selectively stains glycogen.[22] However, it has the undesirable effect of failing to preserve platelet dense bodies as well as osmic acid without ferrocyanide. Biochemical assays were carried out on at least ten separate samples of stored platelets at 15 days and eight samples maintained for 21 days. Each separate data point in the tables represented the mean of duplicate determinations on the same sample. Standard errors of the means were obtained by the Student *t* test.

Experimental Observations

The pH of the stored platelet samples was measured daily. After collection in the CPD anticoagulant adjusted to pH 9, the pH of samples of PRP, following separation from whole blood, averaged 7.68–7.8. The pH fell slowly to about 7.4 over a period of 3 days. If the pH fell below 7.3, it was raised to 7.4 by dropwise addition of 6N KOH. Spontaneous increases in the pH of stored platelets above 7.6 were modified by addition of 0.6 N hydro-

chloric acid to reduce the pH to 7.4–7.5. Platelet counts were obtained at the time of collection (Day 0) and after 5, 10, 15, and 21 days of storage. Aggregation responses were evaluated on Day 0, Day 5, Day 10, Day 15, Day 19, and Day 21. Samples were prepared for electron microscopy on Day 10, Day 15, Day 19, and Day 21. Evaluation of serotonin, adenine nucleotides, and nucleotide kinetics were performed on Days 0, 15, and 21. Twenty-four samples from different donors have been followed through 15 days of storage, and 12 have been evaluated for 3 weeks. At the onset of the study we did not anticipate that functional integrity of stored platelets would be preserved beyond 5 days. As a result, the volumes of stored platelets in the initial sets of experiments were too small for all of the required studies. As a result, all of the biochemical morphologic and physiologic studies were carried out on 12 of the 24 samples at 15 days and 8–10 of the samples preserved for 21 days.

Results

Platelet Counts

Platelet numbers in samples of C-PRP on Day 0 averaged 380,000/cumm (Table 1). After 5 days in storage the average number had fallen to 357,000/cumm, and by Day 10 the mean value was 260,000/cumm. As late as Day 15 the average number of stored platelets was still 73% of the mean value on Day 0 but had fallen to less than 50% of the original count by 21 days of storage. The fate of the platelets lost over this period of time has not been determined.

Platelet Aggregation

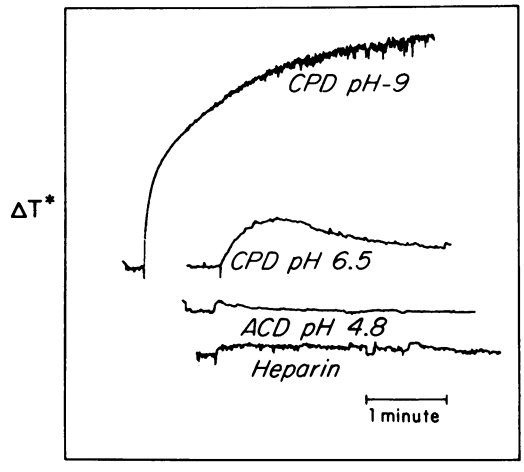
Adenosine diphosphate and sodium arachidonate were chosen as the agents to test platelet aggregation. A concentration of 3 μM ADP represents a test of platelet membrane sensitivity as well as platelet integrity.[12,19–31,23] The ability of sodium arachidonate (0.45 mM) to stimulate platelet aggregation is dependent on an intact pathway of prostaglandin synthesis.[17] Platelets after 5 days in storage responded in the same manner as platelet samples on day 0. The response of the platelets stored under the conditions described in this study was in sharp contrast to the reaction

Table 1—Platelet Counts of Stored Samples of Platelet Rich Plasma.

	Stored platelets ($\times 10^3$/mm)
Day 0	380 ± 9.4*
Day 5	357 ± 10.8
Day 10	260 ± 16.1
Day 15	276 ± 9.3
Day 21	178 ± 9.9

* Mean and standard error; n = 12.

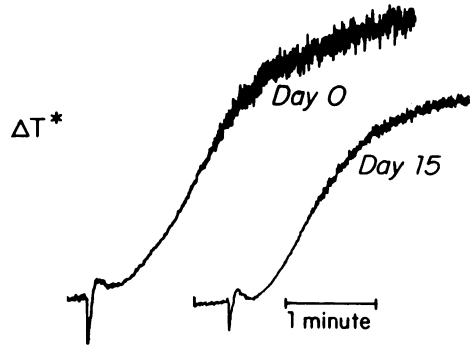
Average counts fell less than 10% by Day 5 but had decreased almost 30% by Day 15 and by nearly half on Day 21.



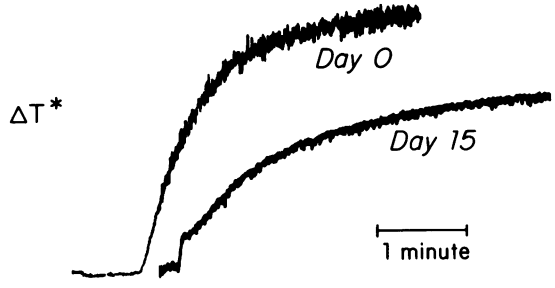
TEXT-FIGURE 1—Influence of short-term storage on the response of platelets prepared from blood collected in different anticoagulants and maintained at room temperature for 5 days. Platelets from blood collected in CPD anticoagulant at pH 9 responded vigorously to 3 μM (not shown), while none of the other anticoagulated samples responded to this concentration of nucleotide. At ten times the threshold concentration (30 μM), platelets from blood obtained in CPD at pH 6.5 showed a single wave of response, which reversed completely. Platelets from blood collected in heparin or ACD at pH 4.8 revealed no response to stimulation by ADP, while platelets from blood anticoagulated by CPD, pH 9, developed irreversible aggregation. *Change in light transmission.

of platelets prepared from blood collected in CPD anticoagulant at pH 6.5, ACD at pH 4.8, heparin, or EDTA and preserved under identical conditions,[11] even when the different preparations were exposed to 30 μM ADP (Text-figure 1).

Samples stirred with ADP and sodium arachidonate after 10 days in storage were nearly as responsive as platelets evaluated on Day 0 and Day 5. Fifteen days after storage *in vitro* platelet samples aggregated reasonably well when stimulated with ADP and nearly as well as platelet samples on Day 0 to sodium arachidonate (Text-figures 2 and 3). At 19 days the response to sodium arachidonate was intact and could be completely blocked by prior exposure of the samples to aspirin (1×10^{-4}M) (Text-figure 4). Even after 21 days a response to ADP and sodium arachidonate



TEXT-FIGURE 2—Influence of storage on the response of platelets to arachidonic acid (0.45 mM). Both aliquots are from the same sample of C-PRP. The 15-day-old sample responded as rapidly as fresh C-PRP when stirred with arachidonic acid, and the rate of aggregation slopes are nearly superimposable. Fifteen-day-old platelets reach the maximum aggregation plateau more quickly probably because the cell number is reduced.[39] *Change in light transmission.

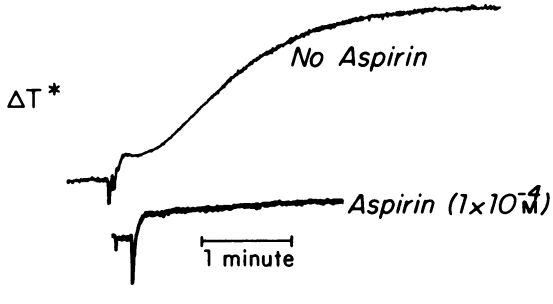


TEXT-FIGURE 3—Influence of storage on the response of C-PRP to ADP (3 μM). The threshold concentration of ADP generates a rapid response in 15-day-old platelets, but the slope of the tracing is decreased, compared with the sample of C-PRP on day 0, and the rate of aggregation begins to level off more quickly. *Change in light transmission.

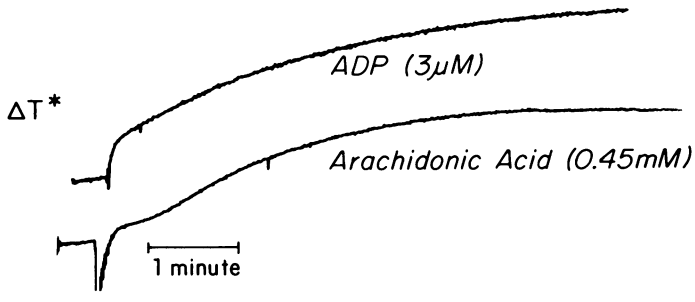
could still be elicited (Text-figure 5). However, the slow rate of aggregate formation and the tendency to level off before tracings had reached maximal excursion of the recording pen suggested that the number of healthy platelets available for aggregation had decreased substantially. Yet an increase in the concentration of aggregating agents caused an increased level of response by the 21-day-old samples (Text-figure 6).

Biochemical Evaluation

After 15 days in storage the platelet samples had lost less than 10% of the endogenous levels of serotonin recorded on Day 0 and still retained slightly over 50% of the Day 0 concentration after 3 weeks in storage (Table 2). Adenine nucleotides were more severely affected by storage than levels of serotonin (Table 3). Total adenine nucleotides were reduced by 30% of Day 0 values in 15-day-old platelet samples and by over 55% in platelet samples kept for twenty-one days. However, the distribution of AMP, ADP and ATP in platelets was similar to Day 0 values in stored samples (Table 3). As a result the ATP/ADP ratios at 15 days were the same as on Day 0, and had fallen to a negligible extent by Day 21 due to greater losses in ATP levels. In addition, the uptake of ^{14}C-adenine was increased in 15- and 21-day-old platelets, compared with samples on Day 0, and incorporation of the label into AMP, ADP, and ATP took place as



TEXT-FIGURE 4—Influence of aspirin (1×10^{-4} M) on the response of stored platelets to stimulation by arachidonic acid (0.45 mM). The aliquot incubated with aspirin shows no response to arachidonic acid. However, the untreated sample stored for 19 days aggregates when stirred with arachidonic acid nearly as well as platelets on Day 0 and Day 15. *Change in light transmission.

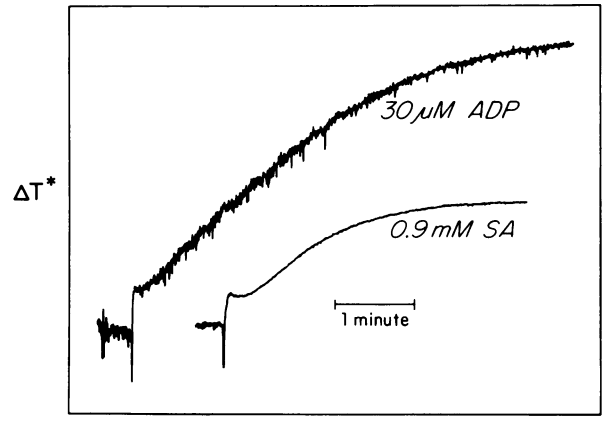


TEXT-FIGURE 5—Aggregation of platelets stirred with threshold concentrations of ADP (e μM) and arachidonic acid (0.45 mM) after storage *in vitro* for 21 days. Slopes of the tracings are decreased, and the plateaus of maximal aggregation are reached quickly. The responses are similar to those obtained from samples of fresh C-PRP in which the platelet count has been reduced to about 50,000/cumm. *Changes in light transmission.

rapidly as the control C-PRP (Table 4). Thus, the mechanisms necessary to take up adenine and the enzymes required to convert the substrate into nucleotides were clearly intact.[20] The ability of stored cells to convert ^{14}C-arachidonic acid into thromboxane B_2 was well maintained in samples stored for 15 and 21 days (Table 5). After 2 weeks stored platelets converted 90% of the labeled substrate into metabolites, and 70% of the arachidonate was transformed even after platelets had been kept *in vitro* for 3 weeks.

Ultrastructure

Platelets stored for 15 to 21 days under these conditions developed many physical changes. Most of the cells had become irregular, with multiple pseudopods, after 5–10 days in storage, although most of their ultrastructural features were well preserved (Figure 1). However, the general



TEXT-FIGURE 6—Response of platelets stored for 21 days to increased concentrations of aggregating agents. A tenfold increase in the threshold concentration of ADP to 30 μM produced more rapid and complete aggregation than the lower concentration. Doubling the concentration of sodium arachidonate to 0.9 μM had a slight but definite effect on the response of 3-week-old platelets.

Table 2—Levels of Serotonin in Stored Human Platelets

	Serotinin (ng/10^9 cells)
Day 0 (n = 10)	717 ± 43*
Day 15 (n = 10)	658 ± 45
Day 21 (n = 8)	374 ± 42

* Mean and standard error.

Endogenous serotonin levels fell less than 10% by Day 15 but had decreased nearly 50% after storage for 3 weeks.

appearance of morphologic integrity was in sharp contrast to the virtual destruction observed previously in samples of platelets from blood collected in CPD at pH 6.5, ACD at pH 4.8, EDTA, or heparin and maintained at either 22 C or 4 C.[11] About 15% of cells appeared swollen and devoid of internal contents after Day 15. An occasional microaggregate was observed, but in general they were uncommon, even in samples stored up to 21 days under these conditions. Membrane systems were distorted in some platelets (40%) 15 days after storage (Figure 2), but in most (60%), the surface-connected open canalicular system and the dense tubular system were intact, and their interaction resulted in normal membrane complexes (Figure 3). Glycogen could be identified with ease in 15-day-old platelets (Figure 2), and some was evident in platelets at 21 days after storage. About 10–20% of the platelets in 15-day-old samples were discoid in configuration and possessed circumferential bands of microtubules (Figures 2, 4, and 5). Approximately 5–10% of the cells stored for 21 days were also discoid, with circumferential bundles of microtubules (Figures 8 and

Table 3—Levels of Adenine Nucleotides in Stored Human Platelets

	Nucleotides (μmoles/10^{11} cells)				
	AMP	ADP	ATP	Total	ATP/ADP
Day 0 (n = 10)	0.82 ± 0.5*	3.68 ± .19	4.48 ± .18	8.99 ± .37	1.24 ± .06
Day 15 (n = 10)	0.63 ± .04	2.51 ± .25	3.11 ± .24	6.25 ± .51	1.24 ± .07
Day 21 (n = 8)	0.48 ± .04	1.71 ± .20	1.74 ± .21	3.93 ± .42	1.02 ± .04

* Mean and standard error.

Total adenine nucleotides decreased to 70% of control values by Day 15 and were reduced to under 45% of the Day 0 levels after 3 weeks in storage. However, the distribution of AMP, ADP, and ATP remained relatively unchanged during storage, so that the ATP/ADP ratio on Day 15 was similar to the value on Day 0. The slight decrease in the ATP/ADP ratio on Day 21 was due to the relatively greater loss of ATP during storage for 3 weeks.

Table 4—Incorporation and Conversion of ^{14}C-Adenine by Stored Human Platelets

	CPM × 10^3/nmoles*		
Day 0 (n = 10)	24.7 ± 2.1*	21.72 ± 2.2	23.4 ± 2.7
Day 15 (n = 10)	33.1 ± 6.3	35.40 ± 2.5	54.7 ± 4.4
Day 21 (n = 8)	36.0 ± 2.4	41.80 ± 3.7	55.2 ± 4.2

* Mean and standard error.

After 2 and 3 weeks in storage, platelets took up greater quantities of ^{14}C-adenine than the cells on Day 0 and converted larger amounts into AMP, ADP, and ATP.

9). Randomly dispersed microtubules were apparent in most of the 15-day-old irregular platelets and in some of the 21-day-old cells. Dense bodies were present in usual numbers in 5- and 10-day-old samples but appeared decreased after 15 and 21 days in storage, though they could be identified (Figures 1 and 4). Granules were also present in platelets stored for 15 or 21 days (Figures 2–5, 8, 9, and 10). In most cells they resembled the granules in platelets on Day 0, but in 10% or more of stored platelets the α granules had fused into giant organelles.[24] Mitochondria generally were morphologically normal, even in 3-week-old stored platelets (Figures 8 and 9).

Platelet aggregates formed in response to stimulation by ADP or sodium arachidonate at 15 and 21 days after storage were morphologically indistinguishable from aggregates from samples exposed to these agents on Day 0 (Figures 6, 7, 10, and 11).

Table 5—Incorporation and Conversion of ^{14}C-Arachidonic Acid by Stored Human Platelets

	CPM × 10^3		
	Unconverted AA	Thromboxane B_2	% Conversion
Day 0 (n = 10)	78.8 ± 4.2*	41.7 ± 2.9	33.8 ± 1.0
Day 15 (n = 10)	78.3 ± 2.9	34.0 ± 1.3	30.4 ± 0.8
Day 21 (n = 8)	90.2 ± 2.6	27.4 ± 1.9	23.7 ± 1.6

* Mean and standard error.

Washed and resuspended samples of stored platelets were able to convert ^{14}C-arachidonic acid to thromboxane B_2 nearly as well as fresh platelets. After 15 days in storage the cells lost less than 10% of this activity and were still 70% as efficient as platelets on Day 0 after 3 weeks in storage.

Discussion

The rationale for the present study evolved from several lines of evidence. Ultrastructural studies of platelets prepared from blood collected in several different anticoagulants buffered to pH 6.5 or lower and stored for intervals at 4 C or 22 C revealed a pattern of early transformation in the midst of severe damage which resembled the response of fresh platelets to aggregating agents.[25] The finding suggested that a compromise in the ability of stored platelets to keep calcium out of the cytoplasm resulted in an influx of the cation, a triggering of the contractile mechanism, and disappearance of microtubules. Since the choice of anticoagulants and storage conditions in that study fostered the development of low pH, it seemed worthwhile to evaluate the influence of high pH on platelet preservation.

A second line of evidence favoring exploration of the influence of high pH on storage resulted from investigations into the effects of pH on inhibition of platelet function mediated by prostacyclin (PGI_2).[26] PGI_2 is the major metabolite formed from arachidonic acid during prostaglandin synthesis by blood vessels.[24] The compound is a powerful vasodilator and a potent inhibitor of platelet function *in vivo* and *in vitro*.[27-29] During the course of our investigation it was found that the inhibitory effect of PGI_2 on platelet function was prolonged by the maintaining of the pH of the sample at 7.8–8.0.[26] After 24–48 hours, when the pH was reduced to cause hydrolysis of PGI_2, the platelets responded in a normal manner to aggregating agents. These findings suggested that PGI_2 might be a useful agent for preserving the functional activity of stored platelets, but preliminary studies did not appear to support that viewpoint. Platelet samples maintained at pH 8 with or without added PGI_2 lost their ability to respond to aggregating agents in 3–5 days.

Though the findings in regard to hoped-for beneficial effects of PGI_2 were disappointing, we did note that platelet function was preserved in control samples. Blood for these had been collected in the citrate-citric acid dextrose (CCD) anticoagulant used routinely in our laboratory,[12-14] except that the pH of the CCD had been raised to 9 by dropwise addition of 6 N KOH. After separation of platelet-rich plasma and placement in plastic tubes for storage at room temperature, no attempt was made to maintain the pH at 8. Instead the pH was allowed to fall spontaneously over a few days. PGI_2 was not included, as it would surely have been degraded as the pH fell toward neutral values.[30,31] The pH declined to a range of 7.4 to 7.6 over the period of 5 days. At this time platelet function was the same as it had been on Day 0, in contrast to the samples maintained at pH 8, which no longer responded to aggregating agents.

On the basis of these findings the present investigation was initiated. We chose to use the standard blood bank formula for CPD anticoagulant,[15] rather than CCD, so that the experiment would have direct relevance to platelet concentrate preparation and storage. The reservoir selected for storage was chosen because removal of the top half permitted some air exchange across the surface of the 0.22-μ filter without bacterial contamination. The side ports permitted introduction of the sample and periodic sampling under sterile conditions. It seemed to be a reasonable substitute for the plastic tubes used in our preliminary studies, although the primary reason for selecting the particular system was its availability in the laboratory.

The aggregation response of platelets after prolonged storage was excellent. After 5–10 days the platelets responded to ADP and sodium arachidonate nearly as well as they had on Day 0. At 15 days they responded less well, but the aggregation patterns, particularly after stirring with sodium arachidonate, were very impressive. After 21 days in storage the aggregation response was clearly modified. However, the fact that threshold concentrations of both ADP and sodium arachidonate could stimulate irreversible aggregation indicated that a small but significant population of platelets was physically and functionally intact after 3 weeks in storage.

The functional nature of the response is emphasized by the aspirin study carried out after 19 days. Incubation with aspirin completely blocked the response to sodium arachidonate, while a companion sample without aspirin responded nearly as well as platelets on Day 0. Arachidonic acid is the essential substrate for platelet cyclo-oxygenase, which converts the fatty acid into endoperoxides and thromboxanes.[17] The labile products produced are responsible for the aggregation caused by sodium arachidonate. The ability of arachidonate to stimulate aggregation of 15–21-day-old platelets indicates that platelet cyclo-oxygenase remains intact, can still be acetylated by aspirin,[32] and can generate products capable of aggregating platelets, that the cells remain sensitive to the stimulating influence of these compounds, and that stored platelets can consume oxygen on stimulation.[33]

Morphologic studies also indicated that a significant number of platelets remained physically as well as functionally intact for 15–21 days. By 15 days most platelets had undergone some degree of shape change, many had developed internal alterations in membrane systems and granules,[24] and a few resembled empty spherical bags. However, the majority appeared physically intact, and 10–20% retained their discoid form. Circumferential bundles of microtubules supported the lentiform shape of these platelets, and randomly dispersed microtubules were evident in platelets

whose discoid shape was not preserved. In samples stored 21 days, only a few of the irregular platelets contained microtubules. However, 5–10% of the cells were discoid, and their shape was supported by circumferential bands of microtubules just under the cell wall.

Microtubules are labile structure [34,35] and are known to be particularly sensitive to calcium concentrations.[36] Their presence in significant numbers in platelets after storage for 15–21 days suggests that the conditions developed in this study foster preservation of mechanisms that keep calcium out of the platelet cytoplasm.

A relationship between the ability of platelets to assemble microtubules and the preservation of function during storage has been pointed out by McGill.[37,38] He demonstrated that platelets stored at 4 C and warmed to 37 C every 12 hours retained the ability to assemble microtubules for up to 96 hours. However, by the fourth day only a few microtubules were observed in temperature cycled platelets,[37,38] and none were organized into circumferential bands supporting platelet discoid shape. Thus, as for preservation of microtubule numbers, organization into circumferential bundles, and association with platelet discoid form, the conditions designed in the present study appear to have advantages over temperature cycling in microtubular preservation. Their persistence for 15–21 days may be related to the continuing sensitivity of the stored platelets to aggregating agents.

Biochemical studies supported the morphologic and physiologic results. Endogenous serotonin levels were well preserved for 15 days and fell less than 50% by Day 21. Adenine nucleotides were more labile, but the ability of the cells to incorporate ^{14}C-adenine into AMP, ADP, and ATP after 15–21 days in storage indicates that the enzymes essential for nucleotide kinetics are well preserved.[20] The ability of the stored cells to synthesize prostaglandin products was maintained over the 3-week period of study. Clearly the new conditions seem capable of fostering the survival of several of the most labile enzyme systems in the platelet.

In conclusion, the present study has demonstrated that increasing the pH of CPD anticoagulant used to collect blood can influence the functional viability of stored platelets. Conditions employed in the study were not optimal, according to currently recommended blood banking procedures.[4] However, the results indicate that a significant population of morphologically and biochemically intact platelets that respond well to aggregating agents tolerate storage for 15–21 days at room temperature. Efforts to evaluate the influence of high pH CPD on the viability of platelet concentrates prepared in the blood bank are currently in progress.

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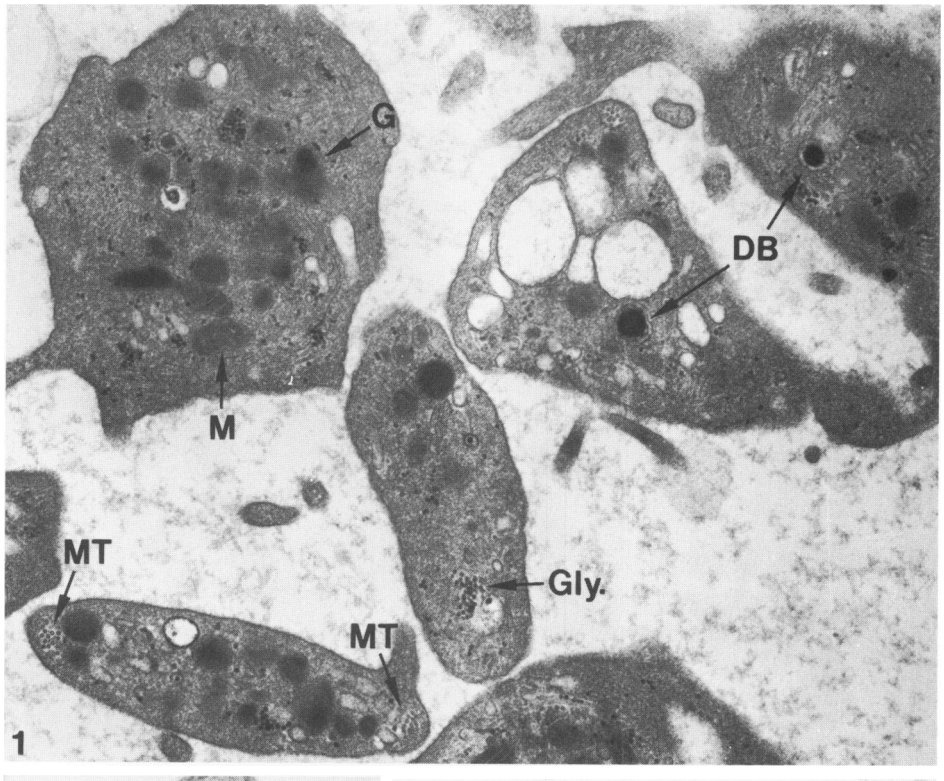
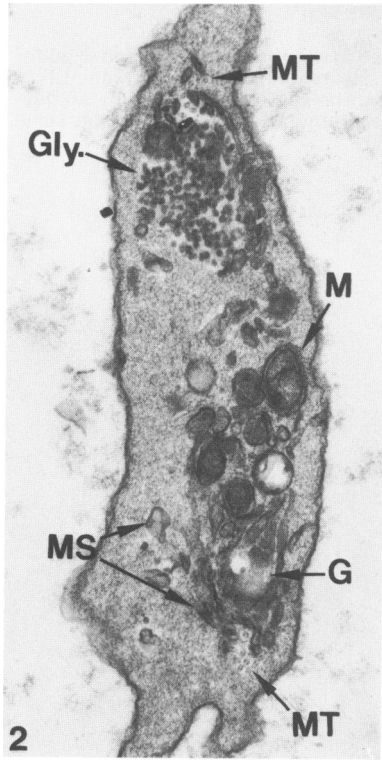
[*Illustrations follow*]

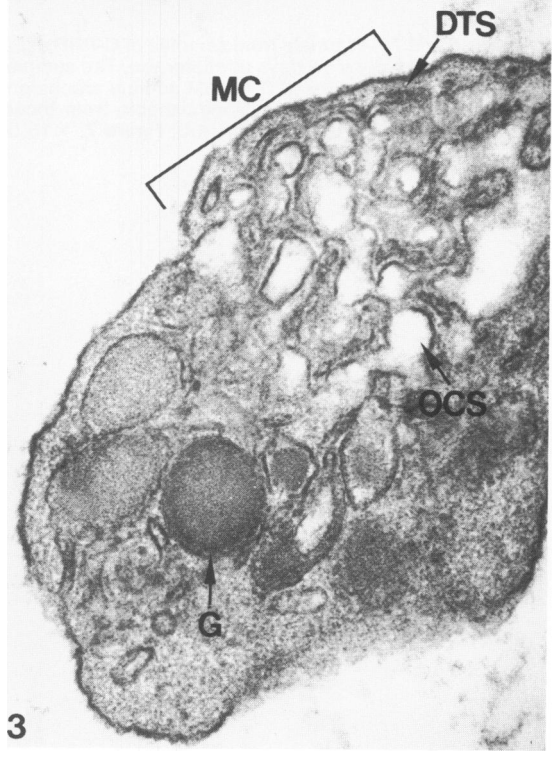
Figure 1—Platelets prepared from blood collected in CPD anticoagulant at pH 9 and stored at room temperature for 10 days at room temperature. Although most of the cells are irregular, about 20% have a discoid shape. Microtubules (*MT*) are present in all of the cells, either as a circumferential band or randomly dispersed in the cytoplasm. Granules (*G*), dense bodies (*DB*), mitochondria (*M*) and glycogen (*Gly*) deposits appear unchanged from samples fixed for study on Day 0. Vacuoles are evident in some of the irregular cells. (×23,000)

Figure 2—Discoid platelet from a sample of C-PRP stored for 15 days. A circumferential band of microtubules (*MT*) supports the discoid shape of the cell. Granules (*G*), mitochondria (*M*) and elements of the membrane systems (*MS*) are preserved in the 2-week-old cell. A large aggregate of glycogen particles is also present after the long period of *in vitro* storage.

Figure 3—Platelets from blood collected in CPD at pH 9 and maintained at room temperature for 15 days. The open canalicular system (*OCS*) of clear channels and the dense tubular system (*DTS*) are preserved in the 2-week-old cells. In the example they are interwoven to form a membrane complex (*MC*). Granules (*G*) have a normal appearance. (×60,000)

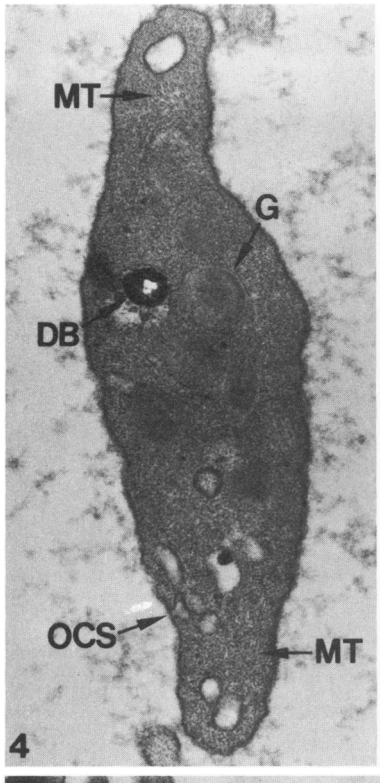

G
DB
M
MT
Gly.
MT
1

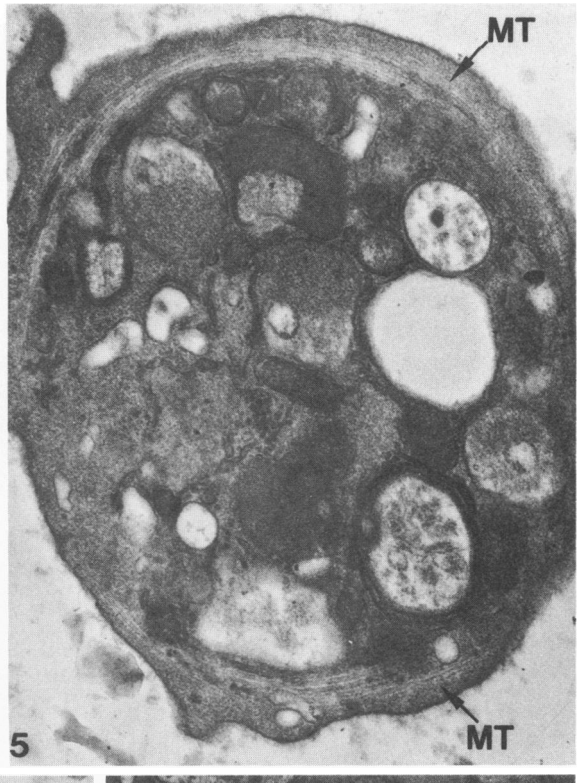

MT
Gly.
M
MS
G
MT
2

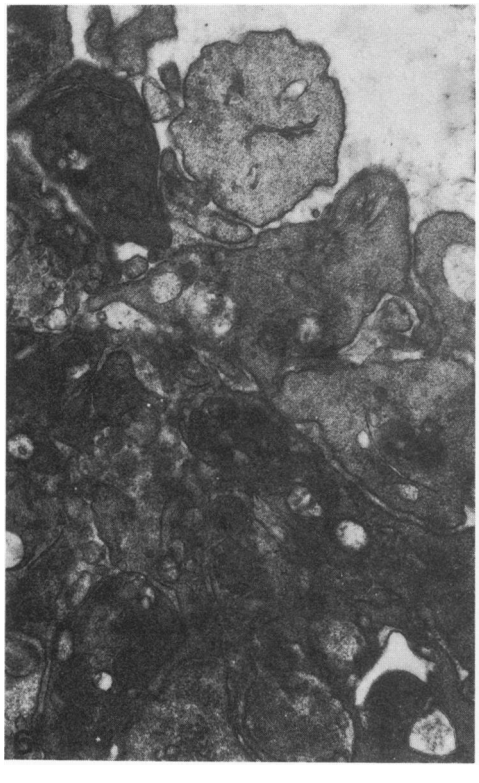

DTS
MC
OCS
G
3

Figures 4 and 5—Discoid platelets 15 days after storage at room temperature. Smooth surface contours are apparent on the cross-sectional platelet in **Figure 4.** Its discoid shape is supported by a circumferential band of microtubules (*MT*). A channel of the open canalicular system (*OCS*) is continuous with the cell surface. Granules and dense bodies are well preserved in the compact cytoplasm. The cell in **Figure 5,** sectioned in the horizontal plane, has a nearly complete bundle of circumferential microtubules (*MT*). Granules in the cytoplasm reveal various degrees of swelling. (**Figure 4,** ×30,000; **Figure 5,** ×36,000)

Figures 6 and 7—Platelets from samples of C-PRP exposed to aggregating agents and fixed for electron microscopy 15 days after storage. The sample in **Figure 6** was aggregated with ADP (3 μM) and the cells in 7 with 0.45 mM sodium arachidonate. The aggregates formed by 15-day-old platelets are virtually indistinguishable from those prepared after exposure to the same agents on Day 0. (**Figure 6** ×17,000; **Figure 7,** ×15,000)


MT
G
DB
OCS
MT
4

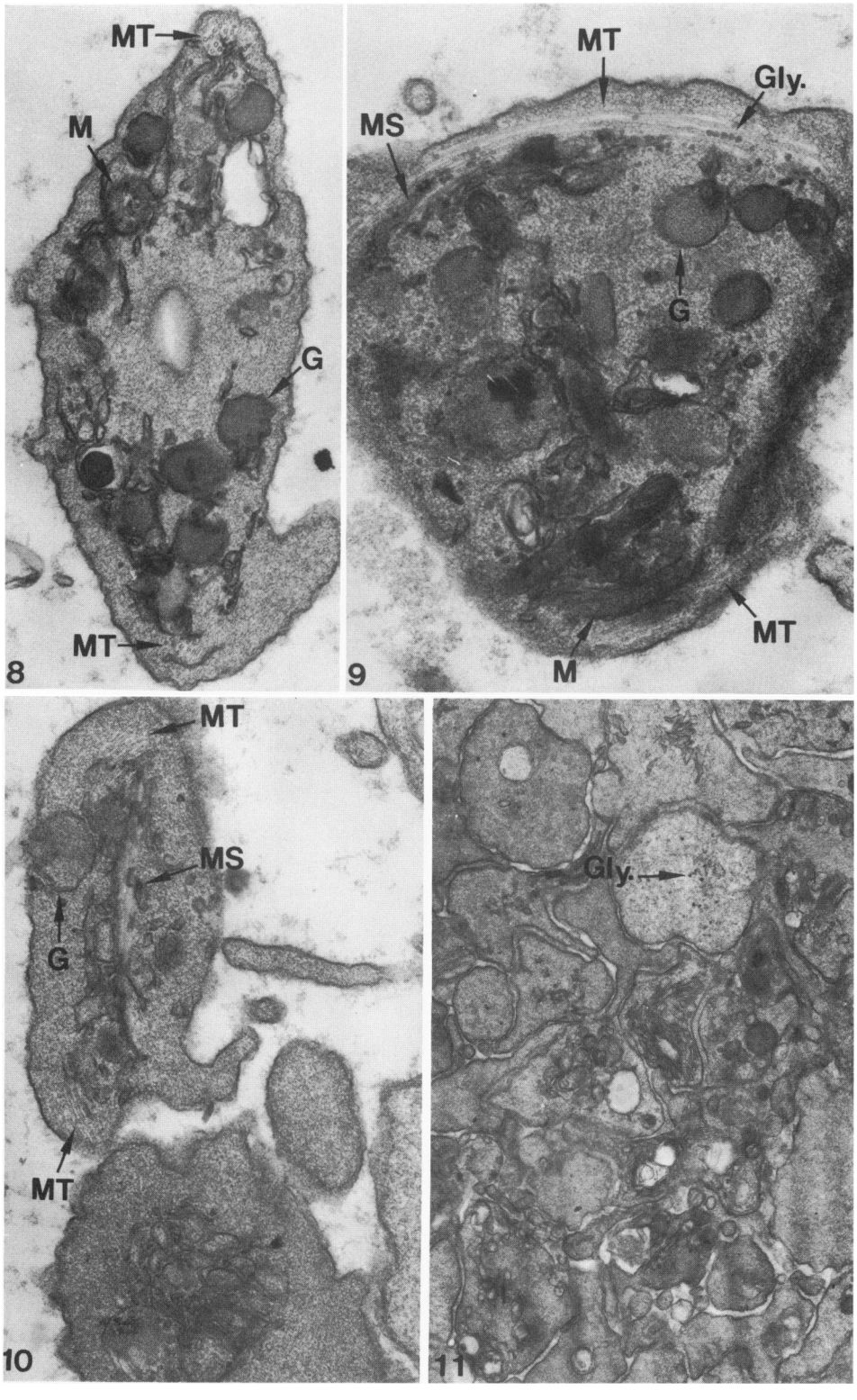

MT
MT
5


6


7

Figures 8 and 9—Discoid platelets from samples of C-PRP maintained in storage at room temperature for 21 days. Circumferential bands of microtubules (*MT*), granules (*G*), and mitochondria are reasonably well preserved in the 3-week-old platelets. A few glycogen particles (*Gly*) are apparent in the cell in **Figure 9. Figure 8,** ×30,000; **Figure 9,** ×36,000)

Figures 10 and 11—Platelet samples exposed to aggregating agents after storage *in vitro* for 21 days. Microtubules (*MT*), granules (*G*), and elements of the membrane systems (*MS*) are evident in a platelet associated with cells aggregated by 3 μM ADP in 10. A few glycogen particles (*Gly*) are apparent in the platelets aggregated by 0.45 mM sodium arachidonate in D. The aggregates are essentially identical to those formed in response to the same aggregating agents in samples of C-PRP on Day 0. **Figure 10,** ×36,000; **Figure 11,** ×16,000)


MT
M
G
MT
8
MT
Gly.
MS
G
MT
M
9
MT
MS
G
MT
10
Gly.
11

[*End of Article*]