Shake, rattle, and roll . . . preventing platelets from turning into Golden Oldies

Platelets (PLTs) for transfusion have been stored at room temperature with constant agitation following the seminal work of Murphy and Gardner1 that demonstrated improved in vivo recovery compared to storage at refrigerator temperatures. The early recognition of the requirement for adequate O2 to maintain aerobic metabolism and the effect of the storage bag material gas permeability led to the development of a series of storage containers with O2 and CO2 permeabilities that allow an adequate supply of oxygen into the bag to support oxidative metabolism and necessary CO2 egress, thus preventing rapid pH decline in the bag during extended storage.2

Although the best in vitro storage condition to hold PLTs before transfusion appears to be with continuous agitation, there are important practical aspects that must be considered in the supply line from donor to patient. For example, Blood Systems, Inc. (Scottsdale, AZ), estimates that 8.5 percent of their PLT inventories are transported overnight (Greg Moase, personal communication, August 6, 2008). An estimated 16 to 20 percent of American Red Cross (ARC) PLTs are transported between blood center regions (Richard J. Benjamin, MD, PhD, personal communication, September 4, 2008). One can imagine that an important fraction of the ARC PLT inventory may be spending a night in a box. AABB Standards permit PLTs to be without agitation for a maximum of 24 hours, although not specified as either a contiguous interval or a cumulative time. There is the requisite movement of PLTs from the point of collection to the point of testing and labeling, electrical interruptions, and agitator mechanical failures that lead to periods of static rest for the PLT, in addition to shipping logistics which may result in cumulative time without agitation exceeding 24 hours.

What are the effects on the PLTs when off of the agitator? What are the limitations of and when should we have concern over the agitator being off too long or the PLTs being in transit too long? Are the effects cumulative for a given product? When do these valuable blood components turn into “golden oldies”?7

Several recent studies examine these questions. Consistent observations among all of these studies are that during static storage of the PLT (whether during shipping, packaged and held in the lab, or simply with the agitator turned off) the mean rate of glycolysis is increased, the pH declines at an accelerated rate compared to continuously mixed controls in spite of adequate oxygen availability within the storage bag, and the chance of reaching unacceptably low pH by the end of extended storage is higher when agitation has been interrupted.3 Under actual shipping, BEST Collaborative investigators observed that the glycolytic rate was restored toward the baseline or control values upon resumption of the proper agitation,4 confirming the simulated shipping observations of Hunter and colleagues.5 The accumulated lactate is not diminished after replacement on the agitator; however, the passive buffering action of the bag mitigates the accumulated acid by the escape of CO2. Would there be an accumulated effect for PLTs subjected to several cycles of static storage? Building on their earlier work,6 Wagner and colleagues7 have addressed this question directly in the first of two articles in this issue of TRANSFUSION as they compare static storage for 24 and 30 hours either as a continuous interval or broken into three distinct periods. Their data suggest that the intermittent agitation does mitigate the cumulative effect of static storage. These data also show the effects from time without agitation and differences between apheresis devices.

In related studies, van der Meer and colleagues8,9 have examined static PLT storage in the presence of PLT additive solutions (PAS) for hold times from 16 hours to 4 days for PLTs prepared from whole blood. While used in Europe and elsewhere for years, there has been an increased US interest in PAS as a means to reduce the plasma dose as a transfusion-related acute lung injury preventive action. PAS has the metabolic advantage of providing alternative energy sources, such as acetate and, in some cases, an added buffer to help maintain pH. In the studies by van der Meer and coworkers with PAS, he also observed increased glycolysis and lactic acid production during periods of static hold. As expected, the number of PLTs in the bag and the time period of hold were important factors in determining the overall effect. Controls in these studies were not in plasma, preventing an estimation of PAS effects. In their second paper appearing in the issue, Wagner and colleagues have directly evaluated the potential effect of an experimental PAS in a well-designed in vitro study with PLTs prepared from whole blood undergoing simulated shipping for 30 hours. In this study, PAS did not prevent but
certainly mitigated the effect of static hold of the PLT—especially evident after 7 days of storage.\textsuperscript{10}

What may be happening in the PLT bag that causes this increase in glycolysis? Are we seeing a Pasteur effect in spite of high oxygen concentrations? What can be done to provide adequate protection of the PLT quality in light of current practices of transporting PLTs?

The mechanism(s) leading to increased glycolysis and pH decline during static storage of PLT have not been specifically described. When PLT bag agitation is stopped, or when the bag is so full so as to prevent mixing wave motions on the reciprocating agitator, PLTs are no longer suspended in the storage medium and fall to the bottom of the bag forming a layer of PLTs. To my knowledge, the thickness of this PLT layer has not been measured, but a layer of approximately 90 \( \mu \text{m} \) might be expected in a typical apheresis PLT bag. Oxygen must reach the PLTs in this biomass through diffusion. The specific diffusion rate of oxygen through this PLT layer has not been determined, but may be less than half of that in serum at \( 20^\circ\text{C} \).\textsuperscript{11} The specific oxygen concentration in the PLT mass will be a function of the diffusion rate, oxidative consumption rate, and the oxygen concentration in the supernatant storage media. We might expect the concentration of oxygen to be reduced in this layer based on work in other tissues.\textsuperscript{12-14} Gnaiger and coworkers\textsuperscript{15} have shown in closed-chamber respirometry that oxidative metabolism slows by approximately 10 percent as oxygen tension is lowered from 150 to 7.5 mmHg (20 to 1 kPa), well above the level of the Pasteur effect (pO\textsubscript{2} approx. 2 mmHg) when there is a major shift from oxidative phosphorylation to anaerobic glycolysis. Therefore, we could expect an upregulation of glycolysis in the PLT biomass to meet a constant ATP demand within the PLTs. This potential mechanism may be worth further investigation.

Like many studies related to PLT function, all of the studies addressing shipping and interrupted agitation of PLTs cited here have only evaluated in vitro characteristics of the PLT. Is this adequate to address a clinical question of how well shipped PLTs will perform in the patient? Early experiments by Murphy\textsuperscript{16} have clearly shown that the efficacy of PLT transfusions, as measured by in vivo radiolabeled recovery, is severely compromised when the PLT pH measured at \( 22^\circ\text{C} \) (pH\textsubscript{22°C}) is less than 6.2 for PLTs stored in plasma. The other in vitro characteristics reported in these studies are useful in describing the PLT, but have not been consistently shown to predict in vivo performance. Therefore, an opportunity remains to extend some of these observations to in vivo studies to strengthen our knowledge base, especially in the context of PLT storage in PAS.

Finally, in a world of current Good Manufacturing Practices and Food and Drug Administration regulation, it is tempting to latch onto studies such as these to set rules or specifications; for example, PLTs may be off of the agitator for no longer than “\( X \)” consecutive hours. However, the authors have shown that this is a complex metabolic system sensitive to not only time off of the agitator but also the collection method, storage container, the number of PLTs in the bag, intervening agitation between static periods, storage media, and perhaps donor variation. Even the seemingly simple variable of the number of PLTs in the bag is subject to very large variation between counting devices. To fully and adequately define this acceptable operational space for a specification seems a daunting task that would demand many resources from manufacturers, blood centers, and regulators. Would a better approach be monitoring of the pH as indicated for special cases (e.g., 26 hr of shipping), intermittently, or even continuously? This outcome would automatically account for all of the other variable inputs into the system that affect pH and perhaps be a better use of our resources to detect and possibly avoid the Golden Oldie PLT.

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