Use of an in vitro biotinylation technique for determination of posttransfusion survival of fresh and stored autologous red blood cells in Thoroughbreds

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Objective—To evaluate N-hydroxysuccinimide (NHS)-biotin labeling of equine RBCs and determine posttransfusion survival of autologous equine RBCs stored in citrate phosphate dextrose adenine-1 (CPDA-1) for 0, 1, 14, and 28 days.

Animals—13 healthy adult Thoroughbreds.

Procedures—Serial dilutions of biotin and streptavidin-phycoerythrin (PE) were evaluated in vitro in blood collected from 3 horses. One horse was used to determine RBC distribution and recovery. Twelve horses were allocated to 4 groups for in vivo experiments in which blood was collected into CPDA-1. Blood was labeled with biotin and reinfused or stored at 4°C for 1, 14, or 28 days prior to labeling with NHS-biotin and reinfusion. Posttransfusion blood samples were collected 15 minutes and 1, 2, 3, 5, 7, 14, 21, 28, and 35 days after reinfusion. Biotin-labeled RBCs were detected via flow cytometry by use of streptavidin-PE. Posttransfusion lifespan of RBCs and RBC half-life were determined.

Results—Optimal biotin concentration was 0.04 pg of biotin/RBC, and the optimal streptavidin-PE ratio was 1.2 µg of streptavidin-PE/1X10^6 RBCs. Posttransfusion lifespan of autologous RBCs was 99, 89, 66, and 59 days after storage for 0, 1, 14, and 28 days, respectively. Storage did not result in significant alterations in RBC lifespan. Mean posttransfusion RBC half-life was 50, 45, 33, and 29 days for 0, 1, 14, and 28 days of storage, respectively.

Conclusions and Clinical Relevance—Biotin can be used to label equine RBCs for RBC survival studies. Posttransfusion survival of equine autologous RBCs was greater than previously reported. (Am J Vet Res 2010;71:960–966)

Preoperative blood donation is routinely performed in horses as part of a presurgical autologous blood transfusion protocol used before elective surgical procedures, such as sinus surgery. Traditional homologous donor transfusions are expensive and time-consuming procedures, and they are associated with numerous risks, which include disease transmission and immune-mediated reactions. Autologous blood transfusion may result in a longer lifespan of transfused RBCs and may minimize the risk of transfusion reactions. In addition, autologous blood transfusion is considered important because routine RBC compatibility tests, including crossmatching and blood typing, can fail to detect minor incompatibilities between a donor and recipient and often do not accurately predict the survival of the transfused RBCs.

For preoperative autologous blood transfusion in humans, posttransfusion lifespan of the RBCs and the effects of storage solutions on survival of RBCs are used to determine the duration for storage of RBCs prior to transfusion and to calculate transfusion frequency. Knowledge of these factors is important for establishing appropriate transfusion protocols. Little is known about the posttransfusion survival of RBCs in horses or the effects of RBC storage solutions on posttransfusion survival of RBCs in horses. In 1 study, the half-life of transfused autologous RBCs in 4 foals ranged from 7 to 18 days, with a mean of 12 days. In that study, the
Horses received transfusions between 2 and 5 days of age. The cause of the variable RBC half-life was unknown. In contrast to humans, horses do not have a distinct fetal hemoglobin; therefore, increased postnatal turnover of RBCs can presumably be ruled out as a reason for the observed variability. Experiments in neonates may underestimate the actual posttransfusion survival of RBCs because of ongoing erythropoiesis and growth of neonates, with a commensurate increase in blood volume during the time at which survival of RBCs is evaluated. In another study, the half-life of transfused autologous RBCs in 4 ponies was estimated by use of radioactive RBC labels to be approximately 14 days. Evaluation of posttransfusion survival of RBCs has suggested that use of RBC-radiolabel techniques underestimates survival of RBCs in humans by as much as 50%. Furthermore, it has been reported that chromium radiolabels elute from equine RBCs much faster than they do from RBCs of other species. Therefore, studies conducted to evaluate posttransfusion lifespan of equine RBCs by use of chromium as an RBC radiolabel may have underestimated survival of RBCs.

Results of the aforementioned studies have been used to support the proposition that autologous (and allogeneic) RBC transfusions in horses are of questionable benefit because transfused RBCs appear to have a significantly shorter lifespan, compared with that for nontransfused RBCs. In comparison to other species, the lifespan of RBCs in cats is 76 days (as determined by 32P-disopropyl fluorophosphate and 14C-glycine evaluations) and the half-life of fresh, transfused autologous feline RBCs is 38 days (50% of the lifespan of nontransfused feline RBCs). Although cats have a shorter RBC lifespan than do horses, and given the reported posttransfusion RBC half-life in cats, the half-life of fresh transfused autologous RBCs in horses would be in the range of 70 to 80 days (50% of the lifespan of nontransfused equine RBCs), given an RBC lifespan of 140 to 160 days for nontransfused equine RBCs. The shortened lifespan of transfused autologous equine RBCs has been attributed to small amounts of naturally occurring alloantibodies not detected with routine in vitro cross-matching procedures. However, this explanation does not adequately explain the reason that investigators in other studies have found a decreased lifespan for transfused autologous equine RBCs.

Radioactive isotopes have traditionally been used to label RBCs for survival studies. More recently, the vitamin biotin has been used as a simple, nontoxic, safe alternative to the use of radioisotopes for labeling of RBCs. Biotin is stable on RBCs in vivo. This stability is important because serum contains biotinidase, which is an enzyme capable of cleaving biotin from certain substrates that could lead to elution of biotin from RBC surfaces in vivo. The use of biotin facilitates the study of large animals without the need for special containment facilities for radioactive waste and minimizes elution of radioactive labels from RBCs. The high binding constant between biotin and avidin allows biotin-labeled RBCs to be diluted substantially in vitro or in vivo and still be accurately quantified by incubating blood with streptavidin tagged with a fluorochrome. Biotinylation has successfully been used to label RBCs in mice, rabbits, dogs, baboons, and humans to evaluate both RBC circulating volume and posttransfusion survival of RBCs.

Storage of blood products also can alter posttransfusion survival of RBCs. Effects of storage of blood products on survival of RBCs have been evaluated for a number of animal species, although there has been minimal evaluation for equine RBCs. When RBCs are stored in an anticoagulant preservative solution, viability and function of RBCs decrease over time, and the RBCs progressively lose their ability to survive in vivo. These storage lesions include membrane alterations of RBCs and loss of energy stores and metabolites, including ATP and 2,3-diphosphoglycerate, from RBCs that can lead to a decreased lifespan of transfused RBCs. After relatively brief periods of storage, most RBCs will survive in vivo, but a small subset of RBCs will be destroyed within the first 24 hours after transfusion. In 1 report, CPDA-1 was identified as the best storage medium to minimize storage lesions of equine RBCs; however, that study did not include in vivo experiments.

The objectives of the study reported here were to evaluate use of a nonradioactive-labeling technique (biotin) in equine RBCs and to compare posttransfusion half-life and lifespan of autologous RBCs stored for various amounts of time in accordance with current blood-banking protocols. We hypothesized that the posttransfusion survival of autologous RBCs in adult horses would be greater than that previously reported and would be inversely related to duration of RBC storage.

Materials and Methods

Horses—Thirteen healthy adult (5- to 15-year-old) Thoroughbreds were used in the study. Horses were judged to be healthy on the basis of results of physical examination, a CBC, and serum biochemical analysis. Blood collected from 3 horses was used in the in vitro evaluation of RBC labeling, 1 horse was used in an in vivo evaluation of distribution and recovery of labeled RBCs, and 12 horses were used in the in vivo evaluation of the effects of duration of storage on posttransfusion survival of RBCs and RBC half-life. Those horses were allocated into 4 groups (3 horses/group; groups A through D, respectively) by randomly drawing horse names out of a box, with the first 3 names drawn comprising group A. This process was continued until all groups were filled. All horses were housed at the University of California-Davis Center for Equine Health, and all procedures and animal care were performed in accordance with a protocol approved by an institutional animal care and use committee.

Evaluation of RBC biotinylation technique in vitro—Blood (50 mL) was collected from a jugular vein of each of 3 horses of similar age (range, 8.9 to 13 years old). The blood was mixed with CPDA-1 (1 mL of CPDA-1:9 mL of blood). Aliquots of blood were removed and centrifuged (1,000 × g) at 22°C for 10 minutes. Plasma and the buffy coat were removed and discarded. The RBCs then were washed 3 times with PBS solution.

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Biotin was prepared in accordance with the manufacturer’s instructions. Briefly, NHS-biotin4 was dissolved in a DMSO solution4 to achieve a concentration of 100 mg/mL. The biotin solution was then diluted to a concentration of 20 mg/mL by use of another DMSO solution. This biotin solution was then sterilized by filtering through 0.2-µm-pore nylon syringe filters. Finally, the solution was diluted with 6 volumes of saline (0.9% NaCl) solution.4

Biotin labeling of RBCs was performed as described for other species.15,18–20 The optimum biotin concentration that saturated RBCs was determined. Equine RBCs (7 × 10^6 RBCs suspended in 10, 20, 30, 40, and 45 µL of saline solution) were labeled with various concentrations of NHS-biotin (0, 0.04, 0.06, 0.10, 0.50, 1.00, 2.00, 3.00, 4.00, and 5.00 pg of biotin/RBC). The RBC-biotin mixture was incubated at 22°C for 30 minutes and then washed twice with PBS solution. To determine optimal saturating concentration of the secondary label, aliquots of RBCs from biotinylated samples were incubated at 22°C for 30 minutes with serial dilutions of streptavidin-PE (0, 0.05, 0.1, 0.15, 0.2, 0.5, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 µg of streptavidin-PE/100 µL of a solution containing 1 × 10^6 RBCs). The RBCs then were washed twice with PBS solution. Samples were measured on a fluorescence-activated cell sorter.1 Fluorescence-activated cell sorter voltages were set with the first samples evaluated on the instrument. At least 100,000 cells/sample were acquired to determine the number and percentage of biotin-labeled RBCs in each sample. Data were analyzed by use of a software program.3 Unlabeled RBCs, RBCs labeled with biotin with no streptavidin-PE, and RBCs labeled with streptavidin-PE with no biotin were used as negative control samples to set cell population gates and establish background fluorescence.

Dilutions of biotin-labeled RBCs were mixed with unlabeled RBCs to determine whether excess amounts of biotin in the plasma (biotin not bound to leukocytes, platelets, RBCs, or plasma proteins) would bind with the unlabeled RBCs. Briefly, an established number of biotin-labeled RBCs in a known volume of plasma in which there was an excess of biotin were mixed with an established number of unlabeled RBCs in a known volume of plasma. Biotin-labeled RBCs were further labeled with fluorescent streptavidin-PE. Samples were evaluated via flow cytometry.5 The number of fluorescent events was calculated and compared with the number of labeled RBCs in the mixture. The use of fluorescence-activated cell sorter analysis of biotin-labeled RBCs stained with fluorescent streptavidin-PE allowed us to analyze only RBCs and to exclude other labeled cells.

Evaluation of RBC recovery and distribution in vivo—To determine whether a measurable percentage of RBCs could reliably be recovered after labeling of a standard transfusion volume of blood, 4 L of blood was collected to permit labeling of 1-in-1,000 equine RBCs with biotin; this ratio has been successfully achieved in other species by use of biotin as an RBC label. Blood from 1 adult Thoroughbred was collected into CPDA-1. The blood was labeled with biotin (0.04 pg of biotin/RBC as determined on the basis of an RBC count of the sample) and reinfused within 2 hours after collection (time of reinfusion was designated as time 0). Blood samples (10 mL) were collected via jugular venipuncture at 3, 6, 12, 18, 24, and 36 hours and 2, 3, 4, 5, 6, and 7 days. Blood samples were collected into evacuated tubes6 that contained acid citrate-dextrose. An aspirate was obtained concurrently from the spleen at 3 hours and 3 days. Red blood cells from both sites were isolated, labeled with fluorescent streptavidin-PE (1.2 µg of streptavidin-PE/1 × 10^6 RBCs), and analyzed by use of flow cytometry to determine the percentage of RBC recovery from both sites. All samples were evaluated in triplicate. Coefficients of variation were determined for all time points.

Determination of RBC survival in vivo—for blood collection, each horse was restrained in a standing position in stocks and sedated with xylazine hydrochloride (0.2 to 1.1 mg/kg, IV) or detomidine (10 µg/kg, IV). The hair over a jugular vein was clipped, and the area was prepared by use of chlorhexidine and alcohol in accordance with standard aseptic protocols. The area was infiltrated with anesthetic (2 mL of a 2% lidocaine solution). A 10-gauge, 3-inch (3.4 × 76-mm) catheter was inserted into the jugular vein, and 4 L of blood was collected into commercially available 1-L blood bags6 that contained CPDA-1 as an anticoagulant-preservative solution. Blood was collected by gravity flow. The appropriate volume of biotin-DMSO-saline solution (biotin concentration, 0.04 pg of biotin/RBC as determined on the basis of an RBC count of the sample) was injected into each bag, and the bags were slowly rotated (4 revolutions/min) on an orbital mixer for 30 minutes at 22°C.

Blood was collected from horses in group A, biotinylated, and reinfused into those same horses within 2 hours after collection (ie, no storage). Blood was collected from horses in group B, stored at 4°C for 24 hours, biotinylated, and then reinfused into those same horses (ie, storage for 1 day). Blood was collected from horses in group C, stored at 4°C for 14 days, biotinylated, and then reinfused into those same horses (ie, storage for 14 days). Blood was collected from horses in group D, stored at 4°C for 28 days, biotinylated, and then reinfused into those same horses (ie, storage for 28 days). In accordance with a standard blood bank protocol,26 the blood was maintained in a blood bank refrigerator in which the temperature was maintained between 1° and 4°C. Blood bags were inverted and mixed once daily. Blood was warmed to 22°C prior to labeling with biotin.

Determination of posttransfusion survival of RBCs—Blood samples (10 mL) were collected from a jugular vein of all horses in the 4 groups by use of evacuated tubes6 that contained acid citrate-dextrose. Samples were collected 15 minutes and 1, 2, 3, 5, 7, 14, 21, 28, and 35 days after transfusion of biotinylated RBCs (end of transfusion was designated as time 0). Blood was diluted to 1 × 10^6 RBCs/mL in saline solution with 0.37% dextrose; aliquots of each sample were prepared in triplicate. Then, 1 × 10^6 RBCs were labeled with 1.2 µg of streptavidin-PE diluted with saline solution with 0.37% dextrose. The RBCs were incubated in the dark
for 30 minutes at 22°C. The RBCs then were washed with 1 mL of saline solution with 0.37% dextrose, pelleted by centrifugation at 1,000 × g for 1 minute, and resuspended in 850 µL of saline solution with 0.37% dextrose. The percentage of cells with positive results for biotin was determined by use of flow cytometry, and results from triplicate samples were used to calculate mean, SD, and SEM.

**Statistical analysis**—Data were analyzed with 2 statistical programs. The measured percentage of labeled RBCs at 15 minutes was used as the baseline value and assigned a value of 100%. For example, if biotinylation was 5% at 15 minutes and 4% at 12 hours, then the percentage biotinylation decreased from 100% to 80%. The posttransfusion survival curve of labeled RBCs was determined by dividing the percentage of biotin-labeled cells in circulation 15 minutes after transfusion (baseline value) by the percentage of labeled cells at each time point thereafter. Biotinylation data from all horses in each of the 4 storage groups were evaluated for differences among groups via a nonparametric ANOVA with a Dunn multiple comparison post hoc test. Best-fit linear regression lines were forced through the point of x = 0, y = 100% biotinylation for each storage group. Slopes of regression lines for each of the 4 groups were compared via an ANCOVA. The x-intercept values at 0% biotinylation (equivalent to RBC lifespan) and 50% biotinylation (equivalent to RBC half-life) were calculated by use of linear regression from data for individual horses; values then were evaluated for differences among groups via a nonparametric ANOVA with a Dunn multiple comparison post hoc test. For all statistical tests, the value of α was set at P ≤ 0.05.

**Results**

**Validation of RBC labeling in vitro**—The optimal biotin concentration determined was 0.04 pg of biotin/RBC, and the optimal streptavidin-PE ratio determined was 1.2 µg of streptavidin-PE/1 × 10⁷ RBCs (data not shown). Biotin concentrations of 0.04 pg of biotin/RBC resulted in consistent, complete labeling of RBCs, as determined by results for labeled blood serially diluted with unlabeled blood. Higher biotin concentrations resulted in excessive label reacting with admixed unlabeled RBCs. There was a clear distinction between labeled and unlabeled cells for flow cytometric evaluation (> 50-fold increase in mean channel fluorescence with labeling). The biotin label was stable over 5 days in vitro and over 35 days in vivo, with only a mild shift to the left for fluorescence intensity (toward lesser intensity on the log₁₀ scale) observed over time. This provided evidence that the number of biotin labels per RBC was relatively stable. No new discrete populations of biotinylated RBCs appeared at any time point, which suggested that the biotin label was neither recycled nor exchanged at a level detectable by use of flow cytometry.

**RBC recovery and distribution in vivo**—Biotin labeling of 4 L of blood resulted in labeling of 3.9% of recovered RBCs at 3 hours (baseline value). Biotinylation of labeled RBCs was stable (range, 3.9% to 4.7%) from 3 hours to 7 days after transfusion. No significant differences were detected between the percentage of labeled cells at various time points. There was no significant difference between the percentage of labeled RBCs in blood samples obtained from the jugular vein, compared with results for the aspirates obtained from

![Figure 1](image1.png)

**Figure 1**—Mean ± SEM values for biotinylated RBC count (expressed as the percentage of the baseline biotinylated RBC count) over time for each of 4 blood storage groups (n = 3 horses/group). Time of reinfusion was designated as time 0, and the baseline value was the value at 15 minutes, which was assigned a value of 100%. Values did not differ significantly (P > 0.05) among group means and between paired means. Groups were as follows: group A (no storage [diamonds]), group B (storage for 1 day [circles]), group C (storage for 14 days [triangles]), and group D (storage for 28 days [squares]).

![Figure 2](image2.png)

**Figure 2**—Scatterplot of biotinylated RBC values at 24 hours after transfusion for each of the 4 blood storage groups (n = 3 horses/group). Blood (4 L) was collected from each horse and reinfluenced (no storage [black diamonds]) or stored for 1 day (black circles), 14 days (gray triangles), or 28 days (gray squares) before biotinylation and transfusion. Blood samples (10 mL) were collected from each horse at various time points after transfusion and evaluated via flow cytometry to determine the percentage of biotinylated RBCs. Biotinylated RBC values are expressed as the percentage of biotinylated RBCs at 15 minutes after transfusion (ie, baseline). The dotted horizontal line represents the minimum acceptable survival of RBCs at 24 hours after transfusion for human RBCs. Values did not differ significantly (P > 0.05) among group means and between paired means.
the spleen. The coefficient of variation ranged from 2% to 11% within replicate samples. Substantial splenic sequestration of labeled RBCs was not observed.

**Posttransfusion survival of RBCs**—The baseline biotinylation percentage did not differ significantly among storage groups (range, 4% to 6%). The posttransfusion survival curves for groups A through D were plotted (Figure 1). The RBC survival kinetics of all 4 groups was similar on the graphs, and no significant differences were detected among groups. The mean survival of labeled RBCs at 24 hours after transfusion was 91%, 83%, 78%, and 73% for groups A through D (ie, 0, 1, 14, and 28 days of RBC storage), respectively (Figure 2). Storage time did not significantly alter survival of RBCs at 24 hours after transfusion. Mean half-life of transfused RBCs (which was defined as the time at which half of the transfused cells remained in the circulation [ie, 50% of baseline biotinylated]) was 50, 45, 33, and 29 days for 0, 1, 14, and 28 days of RBC storage, respectively (Figure 3). No significant difference in RBC half-life was observed, as determined by comparison of the slopes of regression lines for groups A through D. Comparison between 0 and 28 days of storage yielded a value that was not significant (P = 0.057). The posttransfusion lifespan of autologous RBCs was 99, 89, 66, and 59 days after 0, 1, 14, and 28 days of storage, respectively. Duration of RBC storage did not alter RBC lifespan in vivo because no significant differences were observed between x intercepts, as determined by comparison via regression analysis.

**Discussion**

Blood transfusion is an important component of medical and surgical care of equine patients. However, there is a paucity of information on the effects of RBC storage before transfusion and the overall survival of transfused RBCs. The determination of autologous posttransfusion survival of RBCs in horses will enable clinicians to better plan anticipated transfusion treatments for their equine patients.

Other studies conducted to evaluate posttransfusion survival of autologous equine RBCs have several limitations. First, radioactive RBC labels may underestimate posttransfusion lifespan of RBCs. Second, low numbers of animals were used, and they were not representative (neonatal foals and ponies) for extrapolation of results to adult Thoroughbreds. Third, those studies were primarily focused on the posttransfusion survival of cross-transfused or allogeneic RBCs. Finally, it is unclear whether RBCs were maintained in strict accordance with blood bank guidelines that would reflect the current standard of care for veterinary blood banks.

The results of our study support the contention that biotin is an effective RBC label for evaluation of transfusion in horses; that 4 L of blood can be removed, biotinylated, reinfused, and recovered; and that labeled RBCs can be reliably enumerated via flow cytometry. Biotin is a safe, stable product that is easy to measure. We did notice some loss of labeling intensity as indicated by a shift of the mean fluorescence histogram to the left during flow cytometric assessment. Although it has been reported that biotin is relatively stable as an RBC marker for at least 50 days, there may be a loss of RBC labeling intensity over time. We speculated that this loss could have been attributable to the effects of biotinidase. Given that horses also have the protein sequence coding for biotinidase (GenBank accession No. XM 001494901.1), this may be a possible explanation for our findings. In addition, it has been reported that the anticoagulant and pH of the medium in which the RBCs are labeled affect in vivo elution of the label from the RBCs, which may result in reduced posttransfusion survival. However, investigators in that study did not evaluate the effects of CPDA-1 (the anticoagulant solution used in the study reported here). Additionally, we did not measure pH of the anticoagulant after RBC biotinylation or before reinfusion into the study subjects, so effects of pH on RBC lifespan in the present study are not known. As a result, we propose that although use of biotin labeling may also underrepresent the posttransfusion lifespan of autologous RBCs, we believe that it represents a closer approximation of posttransfusion RBC lifespan in horses than that provided by use of radioactive RBC labels.

On the basis of our hypothesis that posttransfusion survival of equine RBCs is greater than previously reported, we anticipated the half-life of nonstored transfused autologous RBCs to be in the range of 70 to 80 days. However, our observed results identified the half-life of nonstored RBCs was 50 days (62% to 71% of our anticipated value). This discrepancy between the predicted and observed half-life may have been the re-
sult of 1 or more factors. First, although the nonstored RBCs were only exposed to the CPDA-1 anticoagulant-preservation solution for a brief period (< 2 hours), this may have been a sufficient amount of time for a storage lesion to develop. However, investigators in another study conducted to evaluate 4 blood storage methods for autologous equine RBCs stored in CPDA-1 did not detect substantial differences in the development of RBC biochemical storage lesions in horses, compared with those in humans and dogs. We believed blood bags that contained CPDA-1 were the best method for preserving the ATP concentration in equine RBCs, and a significant correlation has been reported between the amount of ATP and the RBC viability in humans. However, RBCs of domestic animals may react differently to storage in CPDA-1 than do RBCs of humans. Rat RBCs stored in CPDA-1 for 1 week had a decrease in deformability, similar to that observed in human RBCs after 4 weeks of storage.

One explanation for our observations may have been the development of storage lesions not detected by routine measurement. Variables (including electrolytes, ATP, 2,3-diphosphoglycerate, pH, lactate, glucose, hemolysis, ammonia, and PCV) are used to assess the effects of routine storage solutions on RBC viability. Other than gross observation of RBC morphology, more sensitive evaluations of membrane structure and function are not routinely conducted. An RBC membrane lesion could have developed such that the antigenic structure of the RBC surface was altered or a membrane transport lesion developed. Alterations in band 3 that lead to a disruption of the interaction of membrane bilayer proteins as well as oxygen-dependent metabolic modulation (also related to modification of band 3) have been described. Such alterations could lead to premature RBC removal or sequestration by the spleen. Future studies conducted to evaluate this possibility in equine RBCs may be beneficial.

We chose the 15-minute postinfusion time point to represent 100% of infused biotinylated RBCs because this was deemed a sufficient interval to allow for biotinylated RBCs to mix in the circulation. The choice of this time point assumed that the rate of RBC destruction was no greater during the first 15 minutes than it was subsequently. There is substantial evidence that when the overall posttransfusion survival of RBCs is poor, the rate of RBC destruction within the mixing period is higher than it is subsequently. However, it was concluded in 1 study that if the survival of RBCs at 24 hours after transfusion is > 70% (as was detected in our study), then the error in detecting the 100% value by extrapolation of observations between 5 and 15 minutes will be small.

Although the RBC lifespan for each of the 4 experimental groups differed numerically, no significant differences were observed in the slope of the lines among the 4 storage times. Comparison between 0 and 28 days of storage yielded a value that was not significant (P = 0.057). However, given a larger sample size, we believe RBCs stored for 4 weeks would likely have had a significantly shorter in vivo survival time because human RBCs have a storage-related decrease in posttransfusion survival of RBCs. In the study reported here, survival of RBCs at 24 hours after transfusion was acceptable for all storage groups. Poststorage transfused RBC survival has traditionally been defined as the percentage of transfused RBCs in the circulation 24 hours after the transfusion, as determined by use of radioactive chromate labeling, on the basis that the surviving RBCs then appear to have a typical lifespan. In general, stored RBCs are deemed appropriate for transfusion in humans only when at least 70% remain in the circulation 24 hours after transfusion. Given this criterion, analysis of the results of our study suggests that autologous equine RBCs stored in blood bags containing CPDA-1 for up to 4 weeks are acceptable for transfusion. Our findings support our hypothesis that posttransfusion survival of autologous RBCs in horses is greater than values reported previously. Furthermore, autologous RBCs stored in accordance with routine blood bank conditions appear to have a similar, although slightly decreased, posttransfusion half-life and lifespan, compared with those of other companion animal species. Studies conducted to evaluate storage-associated RBC membrane lesions may be beneficial in helping to examine this discrepancy.

References


