Red Blood Cell Hemolysis During Processing

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Red blood cell (RBC) hemolysis has been reported in units of RBC for transfusion. This has significant clinical implications for transfused patients because the free hemoglobin dissociates into dimers that have to be bound to haptoglobin to be removed by the reticuloendothelial system. Once the binding capacity of haptoglobin has been exceeded, hemoglobinemia occurs. Hemolysis is caused by the breakdown of the RBC, causing release of hemoglobin and resulting in the discoloration of the plasma. Abnormal hemolysis in an individual RBC unit may be caused by several factors including inappropriate handling during processing of blood, inappropriate storage conditions, bacterial hemolysins, antibodies that cause complement lysis, defects in the RBC membrane, or an abnormality in the blood donor. The degree of hemolysis is described as the percent of free hemoglobin in relation to the total hemoglobin with appropriate correction for the hematocrit. The acceptable level of hemolysis has not been established in North America, but the value of 1% currently is used to assess biocompatibility of blood storage materials, whereas the Council of Europe has set the standard at 0.8%. This report emphasizes the need for the adequate control of the various processes that are involved in the preparation of RBCs from whole blood to minimize the occurrence of hemolysis. Careful evaluation of manufacturing processes will minimize RBC wastage caused by hemolysis.

HEMOLYSIS REPRESENTS the breakdown or disruption of the integrity of the red blood cell (RBC) membrane causing the release of hemoglobin. Hemolysis in blood products is usually manifested by the presence of free hemoglobin in the red cell suspending media, such as plasma or additive solutions. Some diseases such as hemolytic anemia or processes such as centrifugation of blood can cause premature breakdown of RBCs. Although much has been accomplished to improve RBC stability during processing, storage, and transfusion, being outside the body enhances the risk of hemolysis. Some of the factors that are believed to cause hemolysis are discussed later and include shear stress caused by high velocities and turbulence in flowing blood or during processing and contact with plastic surfaces in tubing, bags, and so on.

FACTORS ASSOCIATED WITH HEMOLYSIS DURING STORAGE

Preparative Procedures

The procedures that are used in blood banks to collect and process whole blood into different components may also cause RBC breakdown to release hemoglobin into the supernatant plasma. Examples include the delay between collection and separation, rapid anticoagulation (including mixing of anticoagulant with blood), large variation in centrifugation speeds, rapid resuspension of packed red cells in additive solutions, and variations in blood storage bag configurations or compositions.[1-9] When a full unit of blood cannot be collected from a donor, there is a risk of RBC damage because of the relatively high ratio of the anticoagulant solution to blood. In addition, high centrifugation speeds that are used to enhance maximum recovery of plasma may result in excessive RBC packing to a hematocrit of over 80%. Hard-packed RBCs in citrate phosphate dextrose anticoagulant have been shown to have reduced viability and increased hemolysis during storage.[1-9] The containers in which RBCs are stored before filtration have also been found to affect the extent of hemolysis. Some of the commonly used storage bags contain extractable and nonextractables plasticizers such as di-(2-ethylhexyl) phthalate (DEHP) and tri-(2-ethylhexyl) trimellate, respectively. DEHP has been shown to decrease the rate of hemolysis during storage.[10-16] Plastic containers that do not contain DEHP have been shown to be associated with significant increase in RBC hemolysis well above 1% at the end of 42 days of storage. For example, RBC units that are stored in polyvinylchloride (PVC) containers plasticized with butyrl-n-trihexyl-citrate (BTHC) had a mean supernatant hemoglobin concentration of 850 mg/dL with an upper limit of 1,470 mg/dL.[10] Some of

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these non-DEHP bags are used by a number of manufacturers for the licensed storage of packed RBCs.[11-16] Therefore, RBC units that are damaged during the preparative procedures and then stored in non-DEHP bags are more likely to hemolyze either during storage or further manipulation or handling. In addition to the preceding discussion, packed RBCs are also sometimes washed in saline before transfusion. This additional manipulation of the cells may also contribute to RBC damage as more of the protective plasma protein layers covering the cells are removed by washing.

Shear Stress and Mechanical Hemolysis

Hemolysis is sometimes caused by turbulent shear stress (Reynolds stress), which may occur at the following: the edges of kinked tubing, partially opened transfer tube closures, and entry ports into blood collection bags during stripping of red cells in sample tube segments into partially opened blood collection bags. Shear-induced damage to RBC may occur during the resuspension of hard-packed RBC. Shaking or agitation of the blood bag during mixing before filtration may also cause the more fragile and older RBCs to lyse. Low shear stress in the range of 1,000-1,500 dyne/cm^2, similar to that during gravity priming or filtration, may not by itself be sufficient to cause hemolysis.[17-24] However, in situations of turbulent flow in blood storage containers with abnormal surface roughness or geometric imperfection, hemolysis may occur.[21,22] For example, turbulent shear stresses created by fluid dynamic characteristics of prosthetic valve can damage RBCs, resulting in significant hemolysis in patients with valve replacements.[25] RBCs may be damaged if they are forced through leukocyte reduction filters, small bore needles, narrow openings, kinked or twisted intravenous tubing, or partially obstructed or occluded blood storage bags.[26-30] Mechanical or traumatic hemolysis is more likely to occur in undiluted RBC concentrates with high hematocrits than in whole blood or diluted RBC concentrates having lower hematocrit levels and viscosities.[29]

Bacterial Contamination

Abnormal hemolysis in an RBC unit may also be caused by bacterial contamination. Therefore, the presence of particular matter, clots, change in color (if the cells or plasma have brownish or purplish discoloration), abnormal masses in the liquid blood, opaque or muddy plasma, presence of gas or peculiar odour in the blood product should raise suspicion that the unit is contaminated.[30,31]

Intrinsic RBC Membrane Defects and Deformability

The ability of the RBC to deform is a very important requirement for these cells to negotiate narrow capillaries in vivo. However, a decrease in deformability or membrane defect may play a significant role in the spontaneous or storage-induced hemolysis.[32-44] Examples of conditions in which a decrease in deformability plays a role in hemolysis include hereditary spherocytosis and the closely related elliptocytosis.[45,46] RBCs from blood donors with glucose-6-phosphate dehydrogenase deficiency, sickle cell anemia, sickle trait, or other forms of hemoglobinopathies possess abnormal membrane defects that may result in hemolysis.[36,37-40] RBCs from uremic and diabetic patients have poor deformability, rendering them more susceptible to mechanical damage.[47-49] RBC deformability is also affected by internal viscosity as in sickle cell disease and hemoglobin C disease and to a limited extent by properties of the membrane as in thalassemia.[37-40]

Temperature

The temperature of the blood and component during storage, at filtration, during filtration, or processing is a very important factor in hemolysis. The temperature greatly affects membrane deformability[50-56] and, therefore, the stability of the membrane during processing. RBCs can be lysed by accidental freezing, if, for example, the blood is stored in a refrigerator in which the temperature is not properly controlled or placed in a freezer without a cryoprotective agent. RBCs are damaged if warmed to a temperature of 40°C.[54] Therefore, excessive heat from a heat sealer that is used to make sample tube segments may result in thermal damage. However, the thermo-sensitivity or temperature at which RBCs are damaged may be significantly reduced in some individuals.[56] Such thermally damaged RBCs may be broken down during processing, centrifugation, and separation of the blood units into different components. In addition, extreme cold conditions or placement of the blood bags at temperature below 1°C may result in hemolysis either during filtration with leukocyte reduction filters or during mixing and

processing of the blood units before filtration. Blood can also be frozen inadvertently during shipping. Blood components must be transported in a manner that will ensure maintenance of temperature of 1°C to 10°C.[57] Therefore, the presence of an abnormal level of free hemoglobin in the supernatant plasma from the donor blood may occur as a result of damage by improper temperature during shipping, storage, or mishandling at the time of blood donation.

Osmotic (Hypotonic and Hypertonic) and pH Changes

Sudden exposure of RBCs to hypotonic or hypertonic solutions, to extremes of pH changes, to anticoagulants, and to additive solutions in the blood storage bags may result in either damage or lysis of the more fragile populations of RBCs.[58-62] The threshold at which RBCs are damaged when suspended in hypotonic or hypertonic solutions may be lowered by changes in temperature of the suspending media.[58,59]

Blood Age and Storage Duration

Blood donations for transfusion are routinely stored for 35 to 42 days, depending on the composition of the anticoagulant and preservative solutions. Previous reports on the effects of blood storage have shown significant alteration in RBC membrane integrity and flow properties and significant increase in the levels of free hemoglobin.[63-65] Studies conducted by various investigators to quantify the levels of free-plasma hemoglobin in packed blood cells during storage showed significant increase in free hemoglobin.[63-65] A typical 2-day-old unit of unfiltered packed RBC in Adsol® additive solution (Baxter Health Care Corporation, Fenwal Division, Deerfield, IL) has a free plasma hemoglobin concentration of 17.4 mg/dL (range, 3.7 to 45.5 mg/dL). These levels appear as straw-colored plasma. At 26 days of storage, an unfiltered unit of packed RBC has a plasma hemoglobin level of 90.2 mg/dL (range, 46.5 to 151.5 mg/dL). Finally at 40 days of storage, the typical plasma concentration for unfiltered RBCs is about 193.0 mg/dL (range, 49.0 to 413.9 mg/dL). These levels of hemoglobin at day 40 of storage appear straw colored to slightly red at the low end and visibly red at the upper end of the concentration range (Fig 1). Hogman et al[10] reported that the type of storage containers used can significantly affect RBC hemolysis during storage. They showed that unfiltered RBC units that are stored for up to 42 days in PVC containers plasticized with BTHC had mean supernatant hemoglobin of 830 mg/dL (2.5% hemolysis) with an upper limit of 1470 mg/dL (4.5% hemolysis).[10]

Presence of Leukocytes

The presence of leukocytes in unfiltered RBC units may also contribute significantly to the increase in hemolysis during storage.[66-69] During storage, leukocytes break down and release a number of chemicals and enzymes such as hydrogen peroxide and proteases. Proteases released by leukocytes during storage have been reported to cause RBC lysis during storage and are detrimental to their metabolism and viability.[66,68] These detrimental effects of leukocytes can be reduced or abolished by removal of leukocytes with leukocyte-reduction filters.[68]

Drug-Induced Hemolysis

Certain drugs when taken in high concentrations by blood donors before donation may cause RBC lysis through osmotic, oxidative, or immune-mediated mechanisms. Examples of such drugs are penicillin, vitamin C, quinidine, and alpha methyldopa. Blood donors using these types of drugs are not excluded from donating blood. Therefore, it might be useful to review donor history in cases of red cell hemolysis. Many drugs can cause lysis of red cells in a patient with glucose-6-phosphate-dehydrogenase (G6PD) deficiency and even in normal healthy blood donors if administered in higher than normal doses.[70-78]

Irradiation of Packed RBCs

Irradiation of whole blood and cellular components is currently the only accepted methodology to prevent transfusion-associated graft-versus-host disease (TA-GVHD).[79] However, several investigators have reported significant changes in RBC membrane integrity after gamma irradiation as shown by increase in osmotic fragility, cell lysis, potassium leakage, and reduction in 24-hour, in vivo survival.[80,81] Whole blood and RBC units that are gamma irradiated are more likely to lyse and release hemoglobin either during filtration or during the preparative procedures to obtain the RBC

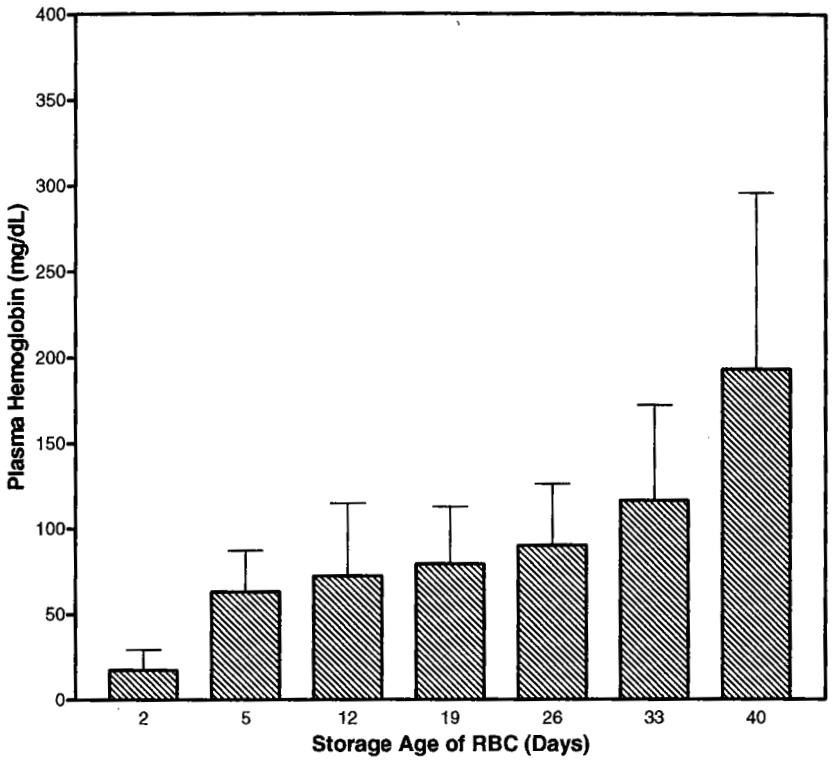

Fig 1. Levels of free hemoglobin in RBC concentrates in AS-1 additive solution at different storage durations. Each data point represents the mean ± SD of 6 to 14 samples.

component. Other forms of irradiation such as the use of ultraviolet light (UV-A, UV-B, and UV-C) that are used for pathogen inactivation in blood and blood components may also cause extensive damage to RBCs.[82,83]

Complement Activation and Platelet Activation

There are reports in the literature that the activation of platelets or leukocytes may lead to the release of chemicals that may damage RBCs to make them susceptible to lysis during storage or further manipulation or handling. In addition, activation of complement proteins in the blood may also result in significant RBC damage.[84-87]

Biological Variations and Hemolytic Tendencies

There are also some situations in which the cause of hemolysis could not be ascribed to any of the factors described previously. There is recognition that blood samples from different normal healthy blood donors can show different hemolytic tendencies.[88] Hemolysis rates also vary with the time at which the blood is collected from the donor. For example, blood samples that are withdrawn from donors after meals are known to be more susceptible to hemolysis than samples taken after a fast.[88]

EFFECTS OF FILTRATION ON RBC HEMOLYSIS

Leukocyte Reduction With Filters

The preceding discussions show that there are several factors that may cause RBCs to lyse that are unrelated to filtration. Published data[89,90] and results from our studies[91] show that Pall leukocyte reduction filters when used according to the manufacturer's instructions do not cause RBC hemolysis above the most stringent regulatory standard of 0.8% hemolysis.[92] On the contrary, the presence of leukocytes in unfiltered red cell units has been suggested to contribute significantly to an increase in RBC hemolysis during storage.[66-69] During storage, leukocyte breakdown is associated with the release of a number of chemicals and enzymes such as hydrogen peroxide and proteases.[93-95] Proteases that are released by leukocytes during storage have been reported to cause red cell lysis

during storage.[93-95] Reports from several investigators indicate that the prestorage reduction of leukocytes in RBC concentrates significantly improves the storage characteristics including significant reduction in the hemolysis when compared with control unfiltered RBC units.[96-98] In a comprehensive crossover study, 4 clinical research centers investigated the effects of filtration on hemolysis.[91] For the study, 40 volunteers donated blood on 2 different occasions. The units were processed after 6 to 8 hours at either room temperature or 4°C. The results shown in figure 2 show significant ($P < .0001$) reduction in storage hemolysis of the Pall Leukotrap RC filtered RBC units when compared with control unfiltered units at the end of a 42-day storage period. Similar studies have been performed by other investigators with whole blood and packed RBC units.[96-98] The results from these studies confirmed our observation of significant reduction in hemolysis of filtered units compared with control unfiltered blood.

Filtration of RBC at Different Storage Ages

A series of experiments were also performed to determine the effects of filtration on hemolysis associated with the duration of RBC storage. In these experiments, different ages of RBCs in AS-1 additive solutions with a mean hematocrit of 46% were filtered through Pall BPF4® leukocyte-reduction filter (Pall Corporation, East Hills, NY) according to manufacturer's instructions for use. Sampling was done aseptically through one of the inlet ports on the storage bags. The results showed that during storage hemolysis rates increased steadily with time in both filtered and unfiltered units in agreement with previous reports (Fig 3). Although the levels of free hemoglobin in filtered units were slightly higher than in 2-day-old unfiltered RBC units (Fig 4), the hemolysis levels were still well below 1% or 0.8% levels for USA-licensed additive solutions and European guidelines, respectively.[92,99] Figures 3 and 4 show the levels of hemolysis in packed RBC units in AS-1 additive solutions at different ages in unfiltered and Pall BPF4 filtered units. The data in figure 3 show that leukocyte filtration of RBC concentrates protects the cells from the leukocyte-induced hemolysis and maintain the levels of hemolysis below 1% during storage. A recent study by Gammon et al[90] confirmed these results and indicated that the lev-

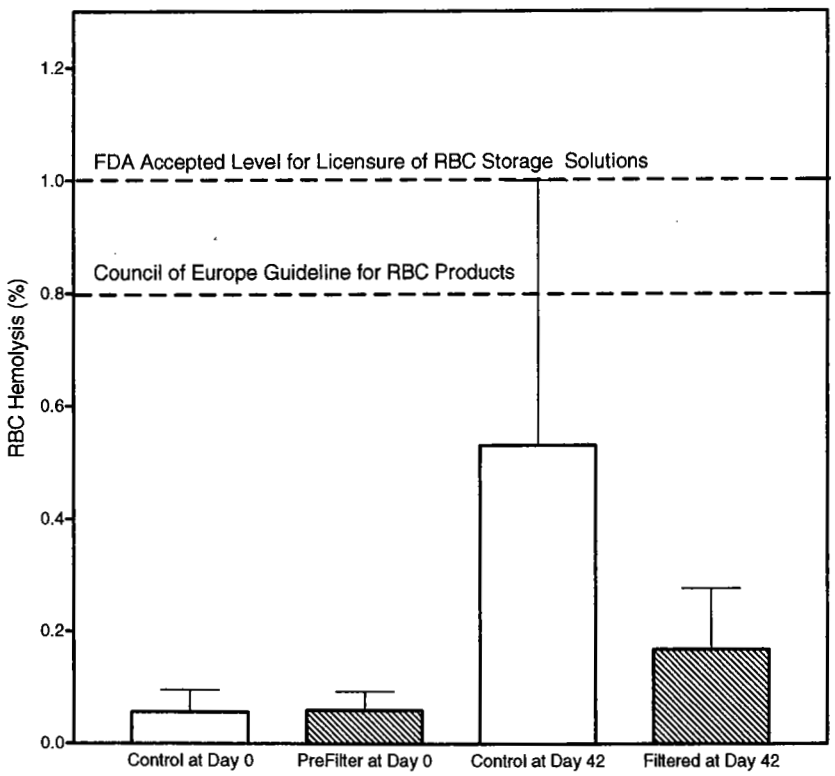

Fig 2. Effects of prestorage removal of leukocytes on RBC hemolysis. Leukocytes were removed from the RBC concentrates on day 0 and then stored for 42 days in AS-2 additive solution at 4°C. Each data point represents the mean ± SD of 41 samples.

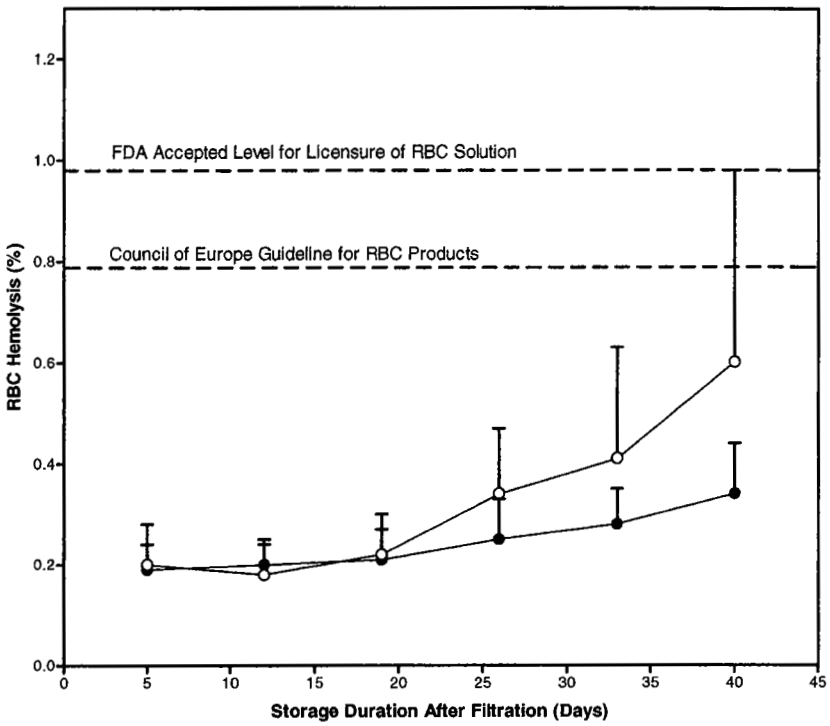

Fig 3. RBC hemolysis during extended storage period. RBC concentrates in AS-1 additive solution were filtered on day 5 and then stored at 4°C for an additional 35 days. Each data point represents the mean ± SD of 6 samples. (●, filtered unit; ○, control unfiltered RBC.)

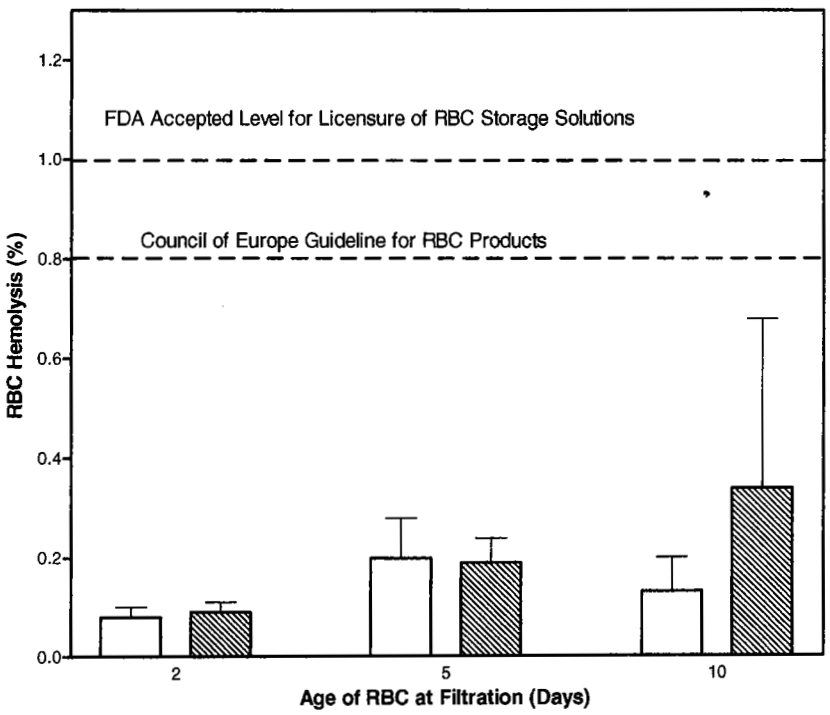

Fig 4. Effects of RBC age on hemolysis. RBC in AS-2 additive solutions at different storage ages were filtered at room temperature with Pall BPF4 leukocyte-reduction filters. Each data point represents the mean ± SD of 6 samples. (□, control unfiltered sample; ▨, filtered units.)

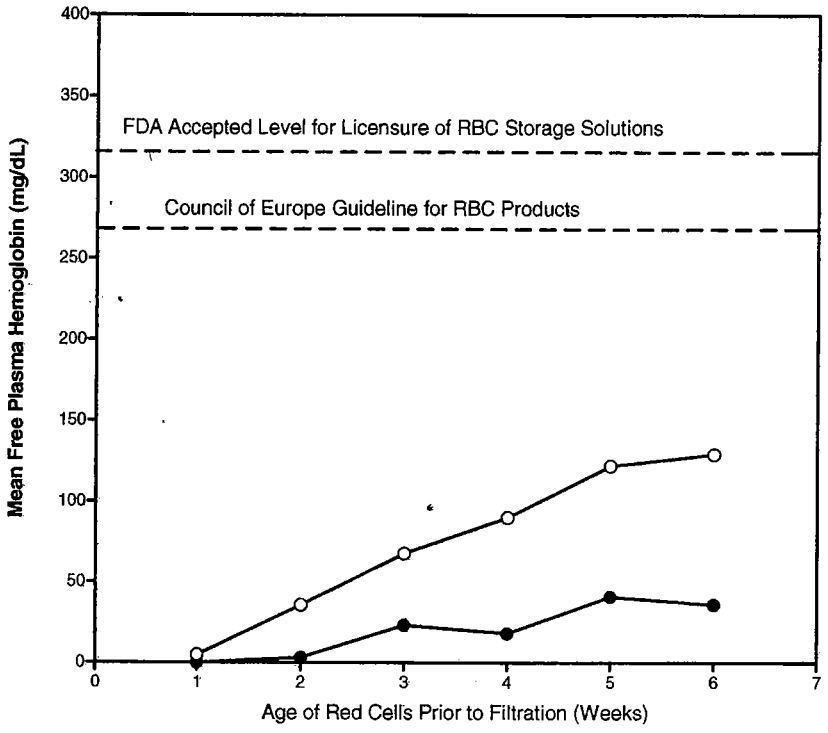

Fig 5. Effects of RBC age and filtration on hemolysis. At each of the 6 weeks of the shelf life of an RBC concentrate, 10 units were filtered with a Pall BPF4™ or a Purecell RCQ™ (Pall Corporation, East Hills, NY) leukocyte reduction filter at room temperature. Each data point represents the mean post-filtration plasma hemoglobin minus the prefiltration plasma hemoglobin concentration in RBC units that were filtered after 1 to 6 weeks of storage.[90] (○, Pall BPF4™; ●, Pall RCQ™.)

els of hemolysis in filtered units are significantly below the most stringent regulatory guidelines (Fig 5).

REGULATORY GUIDELINES AND CLINICAL IMPLICATIONS OF HEMOLYSIS

Acceptable levels of plasma-free hemoglobin have not been established in the United States except for deglycerolized RBCs.[99] Grossly visible pink discoloration of plasma or red cell suspending medium occurs with free hemoglobin levels as low as 25 mg/dL (approximately equivalent to 0.09% hemolysis at 45% hematocrit and 16 g/dL of total hemoglobin in RBCs).[100] When the hemoglobin concentration is 100 mg/dL, the suspension is clearly red in color. Figure 7 shows the color of the supernatant fluid at different levels of hemolysis and free hemoglobin concentrations for RBCs in plasma. Hemolysis is a very important parameter for assessing the quality of stored RBCs. Free hemoglobin in the body dissociates into α dimers, which have to be bound to haptoglobin to be removed by the reticuloendothelial system. The normal haptoglobin level in adult human is 30 to 200 mg/dL.[101] Each molecule of haptoglobin, a dimeric glycoprotein, can bind 2 hemoglobin dimers or approximately 1 gram per liter of plasma. Thus, in an adult, about 3 grams of free hemoglobin (or approximately 10 units of RBCs each with 0.5% hemolysis) can be transfused to a patient without the occurrence of hemoglobimuria. Hemoglobin does not characteristically appear in the urine until plasma levels exceed 100 mg/dL (1 g/L)[100] In practice, tolerance is greater because of the rapid formation and the metabolic turnover of haptoglobin. The US Food and Drug Administration (FDA) has not established an official guideline for acceptable level of hemolysis in blood products for transfusion. However, the FDA has recommended a maximum of 1% hemolysis for deglycerolized RBCs and has approved and licensed additive solutions for long-term storage of packed RBC units, with less than 1% hemolysis at the end of the storage period.[99] In contrast to the FDA, the official guideline in Europe for hemolysis in RBC products for transfusion is 0.8%.[92]

Although, there are no extensive clinical trials on the toxicity of free hemoglobin solutions in human beings, autologous hemolyzed blood has been infused into humans for various experimental investigations. Spector and Crosby[102] infused normal human volunteers with hemolyzed blood to

induce hemoglobinemia. The volunteers were found to be asymptomatic after 540 mg/dL bolus injection of free hemoglobin followed by a 5-hour maintenance infusion of 240 mg/dL. Furthermore, the induction of moderate hemoglobinemia in normal subjects did not result in the development of disseminated intravascular coagulation.[102] Various other studies have reported higher levels of plasma hemoglobin levels in transfused blood. Aaron et al[103] reported transfusion of unwashed salvaged blood with a mean free-hemoglobin level and plasma hemoglobin levels of 1000 ± 625 mg/dL (about 3% hemolysis) in patients undergoing arthroscopic surgery. Their study confirms that such levels of plasma hemoglobin are tolerated without clinical sequelae.[103] This observation is true for normovolumic subjects and may not be true for other types of patients (ie, those with hypovolemia).

INVESTIGATION OF HEMOLYSIS

Hemolysis is usually recognized by free hemoglobin in the RBC-suspending media. The presence of pink discoloration in the suspending media either in the prefiltration or post-filtration RBC unit or blood should prompt an immediate investigation for the factors that mediate the break down of the cells. All the various stages in the manufacturing of the leukocyte-reduced RBC products are outlined in figure 6, which may be useful in the investigation to identify the source of RBC damage. The first test is to ensure that the donor RBCs have not been damaged before filtration by any one of the factors discussed in the preceding section. For example, exposure to extremes of heat or cold, obtaining blood under excessive pressure through a too-small needle, or contact with incompatible blood containers may cause RBC damage and release of hemoglobin before subsequent filtration with a leukocyte-reduction filter or processing by using centrifugation procedures. Visual examination of the supernatant of the blood remaining in the blood bag or in the administration tubing against a white background may reveal the presence of pink discoloration as result of RBC lysis or damage. An RBC unit that had been inadvertently lysed by exposure to excessive cold has a purple appearance that could serve as a warning that hemolysis has occurred.[87,88] Although visual observation is adequate for identification of hemolyzed units at plasma hemoglobin level of 25 mg/dL when pink discoloration is apparent to the unaided eye, the level of free hemoglobin must be accurately measured with one of the methods described later for proper quality control and for establishing rejection criteria for RBC units for transfusion purposes. Different levels of supernatant hemoglobin in plasma are shown in figure 7 for comparison. Note that at 40 mg/dL, which is still well below 1%, the plasma appears visibly red in color.

Investigating post-filtration (leukocyte reduction) hemolysis requires detailed inquiry into the circumstances surrounding the donation of the blood, preparation of the RBCs, and the filtration process. Some examples of determinants of hemolysis associated with filtration that one must look for include the following:

1. Forcing the RBCs or whole blood through the filter to achieve adequate priming of the filter material before filtration
2. Stripping of the administration tubing to prepare sample tube segments for analysis
3. Trapped air pockets either in the filter or foam generated during the mixing of the blood unit
4. Extremes of temperature for filtration
5. Deviation from manufacturer's instruction for use of the leukocyte reduction or filtration system
6. Irradiation of RBC units prior to filtration

However, to determine whether the levels of hemolysis in the units is above 1%, it is suggested that appropriate analytical methods be used to quantify the levels of hemoglobin in the units. This will avoid unnecessary rejection of units that are within the acceptable level that is recognized by regulatory agencies for the licensure of additive solutions for the long-term storage of RBCs.

GUIDELINES FOR PREVENTION OF HEMOLYSIS DURING FILTRATION

The following proposed guidelines are based on the available literatures and may help reduce the potential for hemolysis during leukocyte filtration or blood processing. Note that many of the findings relate to generalized standard or "good" blood management techniques.

1. Follow manufacturer's instructions for use of leukocyte filtration devices. Adequate training of laboratory technologists is important. Those processing blood units must be properly trained and regularly monitored

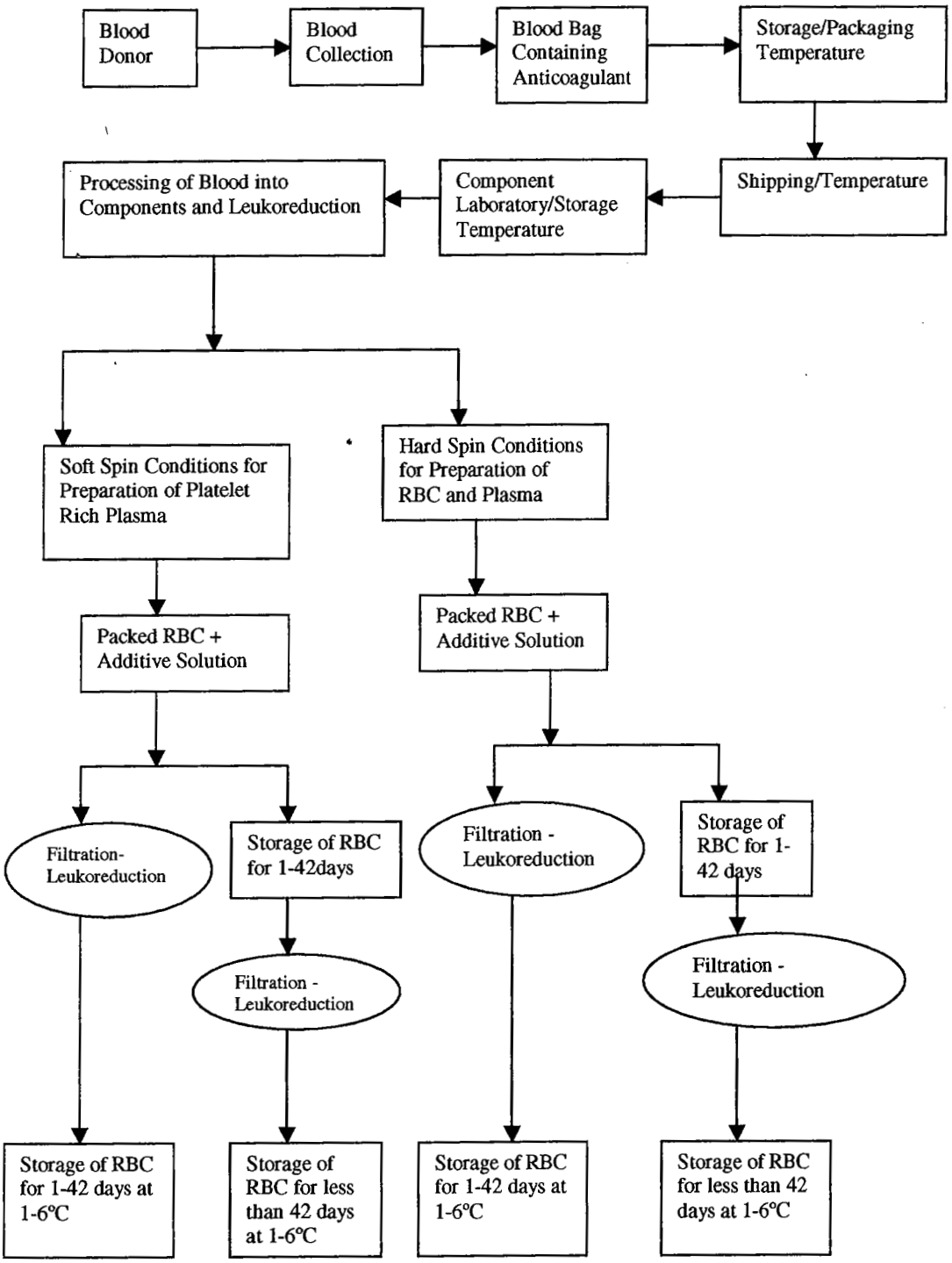

Fig 6. Flow diagram of the various stages in the production of leukocyte-reduced RBC units. Evaluation of the processes at the different stages will help to identify the source of any RBC damage and allow the appropriate corrective action to be implemented.

to ensure that manufacturers instructions for use of RBC processing equipment are followed.

2. Whole blood must be anticoagulated with the recommended volume of anticoagulant. Avoid under filling or over filling the primary blood collection bag with whole blood. RBC units must be stored in FDA-

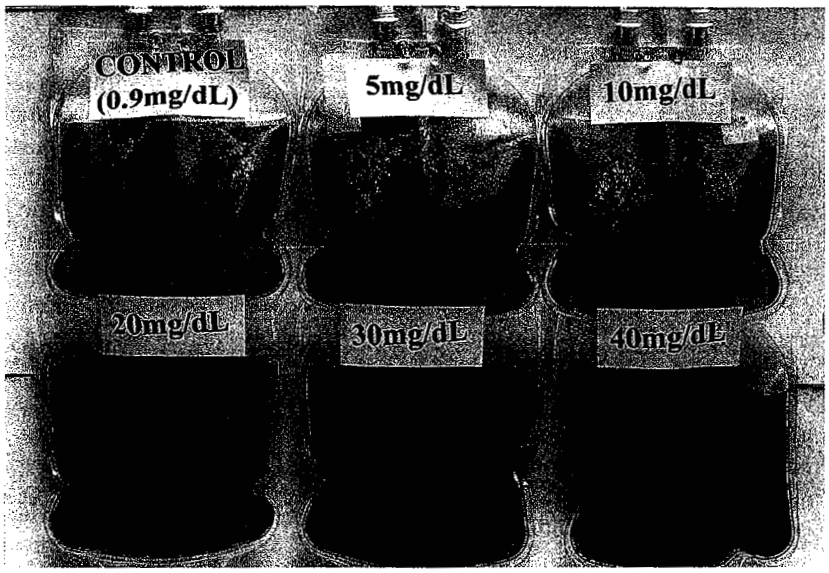

Fig 7. Different concentrations of free hemoglobin in supernatant plasma. At 40 mg/dL, which is still below the 1% hemolysis level, the discoloration of the supernatant plasma is very visible.

approved additive solutions at the appropriate composition of chemical components, osmolality, and pH.

3. Before the filtration of an RBC unit, check the supernatant for free hemoglobin against a color comparator. If the reading on the color comparator is 25 mg/dL or greater, extreme care must be used in processing the unit. Avoid repetitive shaking, squeezing, or agitation of blood storage bags containing RBC units in additive solutions or whole blood.
4. Ensure that all the tubing attachments between the blood bag and the filter are fully extended. Do not filter blood or red cell units if the tubing or connections between the filter and the blood bag are kinked or partially occluded.
5. Do not filter blood against partially opened transfer leg closure or partially clamped tubing between filter and RBC bag.
6. Occasionally check the osmolality and pH of RBC storage solutions to ensure that these parameters have not changed either during storage of the blood collection set or during shipping.
7. RBC units or whole blood that are older than 1 day old must be handled carefully during processing. For example 2 end-over-end rotations of the bag containing 7- to 42-day-old RBC units is adequate to ensure adequate mixing before filtration.
8. Do not store RBC units close to the "air vent" in the cold room where the temperature may be lower than the recommended temperature of 1° to 6°C.
9. RBC or whole blood units must be transported in an appropriate container that maintains the temperatures recommended by the prevailing standard.
10. Avoid the trapping of air bubbles into the filtration system during the leukocyte reduction process.
11. Avoid the use of force to push the RBC or whole blood unit through the leukocyte reduction filter during the initial priming. Use gravity force to prime the filter, unless it is not recommended by the manufacturer of the leukocyte reduction system.
12. Avoid the use of high centrifugation speeds (greater than 5000 g for 5 minutes) to prepare RBC concentrates from whole blood. Resuspension of tightly packed RBCs in additive solutions must be done very carefully to avoid damage.
13. Filter the RBC unit with leukocyte reduction filter before gamma irradiation.
14. Avoid transferring RBC units into blood bags that may contain hypo- or hypertonic fluid, which may have occurred as a result of a process that was used to sterilize the blood collection bags.
15. The blood unit must be fully anticoagulated before processing. Avoid the use of blood

units with visible clots as a result of inadequate anticoagulation.

METHODS FOR THE DETERMINATION OF PLASMA HEMOGLOBIN

Determination of free hemoglobin is of great value in the assessment of the extent of hemolysis in different leukocyte reduced and nonleukocyte-reduced blood and blood components. In most blood banks and hospitals, visual inspection of the sample tube segments that are attached to the blood bags is used as a quick and easy method to detect hemolysis in the blood units. However, such visual inspection methods are inaccurate measures of hemolysis and different results are obtained between the tube segments and the blood samples in the blood bags.[104,105] In addition to differences in the ratio of plastic surface to blood volume between the blood bag and the attached tube segments, it should be noted that the plastic used for the construction of blood storage bags may differ from those used for tubing. Thus, surface contact and the passage of gases needed for proper respiration of RBCs may be different in the tubing and the storage bags.

Quantitative Methods

The best method of assessing the levels of hemolysis in blood products is to use a validated quantitative assay. Different methods have been developed for this purpose and most procedures are based on the characteristic absorption spectra of hemoglobin.[106-116] These include spectrophotometry at discrete wavelengths,[107-109] spectral wavelength scan analysis,[110] and derivative spectrophotometry.[111,112] Plasma hemoglobin assays can classified into[1] direct optical techniques in which quantitation is based on oxyhemoglobin's absorbance peaks at 415, 541, or 576 nm. The direct spectrophotometric scanning of plasma samples is strongly subject to interference by background levels of elevated of bilirubin, plasma proteins, albumin, lipids, and other absorbing pigments. The other quantitative methods are chemical techniques in which all forms of hemoglobin (except sulhemoglobin) form a colored reaction product, cyanmethemoglobin, when mixed with chemicals such as potassium ferricyanide or tetramethylbenzidine. The International Committee for Standardisation in Haematology has recommended the cyanmethemoglobin method as the standard reference method for whole blood.[117] Although used by few organizations, the direct optical spectrophometric methods are safer, easier, and more precise and accurate than the chemical addition methods used to measure plasma hemoglobin concentration. At Pall Corporation, we use the Cripps spectrophotometric method in which oxyhemoglobin in undiluted plasma sample is quantified by using a 3 wavelength (560, 576, and 592 nm) Allen baseline correction method.[114] In this method, the partial absorbance of oxyhemoglobin at 576 nm is calculated relative to linear baseline absorbance at 560 and 592 nm. The advantage of the technique is that substances that interfere with hemoglobin assay, such as bilirubin, are corrected for using absorbance values at wavelengths on both sides of the peak.

Calculation of Percent Hemolysis

The concentration of free hemoglobin depends on the number of disintegrated RBCs and the volume of fluid. The same percentage hemolysis may thus give a 4- to 6-fold higher concentration of hemoglobin in a red cell concentrate than in whole blood.[117] Therefore, the degree of hemolysis is often described as the percent of free hemoglobin in relation to the total. Note that it is essential to correct for the hematocrit to avoid overestimation of the percent hemolysis in a product. The formula for calculating the percent hemolysis is described below with appropriate examples given in Table 1.

$$\text{Percent Hemolysis (\%)} = \frac{(100 - \text{Hematocrit}) \times \text{Free Hemoglobin in Plasma or Suspending Medium}}{\text{Total Hemoglobin}}$$

CONCLUSIONS

RBCs outside the body are challenged by processing and storage conditions. With no leukocyte reduction filter in use at anytime in their storage life, hemolysis still occurs as units age. Clinically, transfusion of 42-day-old RBC units with or without filtration has been in use for decades without any clinical adverse effects. This report shows that there are various nonfiltration-related factors that may contribute to the occurrence of hemolysis in blood units or packed RBCs. To prevent the occurrence of hemolysis, these factors must be considered carefully before the processing of blood with leukocyte reduction filters.

Table 1. Examples of Calculation of Percent Hemolysis in Samples With Different Hematocrits

Examples	Hematocrit (%)	Total Hemoglobin (mg/dL)	Free Hemoglobin in Plasma (mg/dL)	Hemolysis Not Corrected for Hematocrit (%)	Hemolysis Corrected for Hematocrit (%)
1	30	12,000	120	1.0	0.70
2	40	16,000	120	0.75	0.45
3	50	20,000	120	0.60	0.30
4	60	24,000	120	0.50	0.20
5	70	28,000	120	0.43	0.13

The highest level of free hemoglobin in either filtered or nonfiltered RBCs at the end of 42-day storage is less than 0.8% hemolysis, which is the most stringent guideline available.[92] At free-plasma hemoglobin levels of 0.8% and 1.0% hemolysis corresponding to 233 and 291 mg/dL, respectively, (calculation based on the assumption that whole blood has 45% hematocrit and total hemoglobin of 16 g/dL) the supernatant plasma is very red in color and can easily be detected by the naked eye. Although the supernatant is very red, the 1% level of free-plasma hemoglobin is acceptable and the unit can be transfused without clinical sequelae.

Many publications[15,16,64,80,89,90] using leukocyte-reduction filters show that the levels of free-plasma hemoglobin in filtered blood products are well below the most stringent regulatory standard of 0.8% hemolysis.

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