The Use of

FROZEN BLOOD

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During open heart surgery blood is required for priming the pump oxygenator and for replacing the blood lost during surgery. Depending upon the type of oxygenator used and on the degree of hemodilution employed, blood needs vary. In the past, this has meant several units of blood for each patient.

In order to prevent the decrease of the level of ionized calcium following transfusion of large amounts of acid-citrate-dextrose (ACD) anticoagulated whole blood, it was customary to provide fresh heparinized blood for patients during cardiopularmony bypass. The collection of large amounts of fresh, heparinized blood presented logistical problems especially when a patient with a rare blood type was concerned. The blood collection problems were somewhat simplified when it was recognized that recalcified, heparinized ACD blood could be used successfully even if the blood had been stored at 4° C for four days.[1 2 3]

Post-transfusion hepatitis has been a serious complication of open heart surgery. The prevalence of this condition has been reported to be between 0.7 to 18 to 51 percent, [4 5 6] the wide range probably depending upon the incidence of the carrier of hepatitis virus B in the donor population and upon the state of immunity in the recipient population. Since hemodilution was employed successfully during open heart surgery,[5 7] hepatitis could be eliminated by avoiding homologous transfusions. However, it is not possible to perform all cardiac surgery without donor blood.

It appears that frozen blood is suited for transfusion during open heart surgery since it has been observed that preservation by freezing converts blood that is Australian antigen positive to negative.[8] Moreoever, clinical reports confirm the lack of post-transfusion hepatitis following the use of frozen blood.[9] In addition, transfusion of frozen blood that contains reduced amounts of white cells and platelets will probably decrease the likelihood of sensitizing the patients to the histocompatibility antigens.

Development of cryotechnology has made the use of previously frozen erythrocytes practical in large scale. As a rule, glycerol is used as cryoprotective agent during frozen storage. Satisfactory storage can be achieved by mechanical refrigeration at -80° C if glycerol is used in high concentration (about 45%) or in liquid nitrogen at -150° C if glycerol is used in low concentration (about 18%). The removal of high concentration of glycerol can be accomplished by using glycerol-electrolyte gradient dilution and centrifugation (Cohn method[10]), by using gradient dilution with hypertonic glucose and other sugar solutions and mechanical removal of the supernatant (Huggins method[11]) or using gradient silution with hypertonic sodium chloride and centrifugation (Meryman method[12]). Various amounts of red blood cells are lost during *in vitro* processing. Depending on the method used, this can amount to 1 to 30 percent. [12 13]

The following aspects relating to the use of frozen blood given during extracorporeal circulation will be discussed:

1. The effect of free hemoglobin upon the kidney function of the recipient.
2. The viability and function of the frozen red blood cells.
3. The effect of frozen blood upon the coagulation parameters.

SUPERNATANT HEMOGLOBIN

The concentration of free hemoglobin in frozen blood far exceeds levels in liquid blood. The average concentration of supernatant hemoglobin in Huggins blood is approximately 300 mg percent,[14] whereas it is about 135 mg percent in previously frozen, ACD collected blood processed by the Meryman method.[15] Hanson et al. studied the behavior of Meryman's blood when it was circulated *in vitro* for three hours in a bubble oxygenator.[16]

If no plasma was added, hemolysis of frozen blood was excessive and debris accumulated in the pump. This is, of course, an artificial situation, since during the cardiac bypass, patient's own plasma is added to the blood even if the pump is primed with frozen red blood cells resuspended in saline. Even when previously frozen red cells were suspended in plasma and used in these *in vitro* experiments, higher supernatant hemoglobin levels were observed than when fresh blood was used. This higher supernatant hemoglobin level was related to the initially higher values. There was no evidence that destruction of the frozen cells was increased during circulation in the bubble oxygenator.

There has been quite a lot of concern about the effect of transfused free hemoglobin upon the kidney function of the recipient. It appears that free hemoglobin does not harm healthy kidneys but could be deleterious if recipient's renal function is impaired. Valeri et al. investigated the contribution of frozen red blood cell transfusions on the supernatant hemoglobin concentration during and after cardiac bypass as well as the effect of the free hemoglobin on the kidney function of the patient.[17]

They administered an average of 4.4 units of Huggins frozen red blood cells during extracorporeal circulation. Each unit contained an average of 838 mg of free hemoglobin resulting in a total quantity of 3.7 g. Hb. During the bypass period, the supernatant hemoglobin averaged 278 mg percent when frozen cells were used and 113 mg percent when only ACD blood was used. Regardless of the higher concentration of supernatant hemoglobin concentration after transfusion of frozen cells, the renal function of the patients was not deteriorated significantly when compared to that of patients who received only ACD blood. The renal function was evaluated by measuring BUN and creatinine during the immediate and laste post-operative periods.

SURVIVAL OF RED CELLS

In order to obtain benefit from red cell transfusions, it is important that preserved cells have satisfactory survival *in vitro*. Red cell survival during open heart surgery is rather difficult to assess. We have developed a method to measure the survival of a red cell population by comparing it to the survival of freshly collected cells which were administered simultaneously.[18] Results of studies using this methodology indicated that the survival of red cells stored for either two or seven days did not differ significantly.

However, the survival of red cells stored for fourteen days was significantly lower than that of red cells stored for two days. The difference in the survival characteristics

was related to the degree of preservation injury that occurred during refrigerated storage. Red cells preserved by Meryman's freezing technic have approximately 85 - 90 percent survival *in vivo,* [12] a figure that is similar to that of red cells stored for one week or less.

OXYGEN CARRYING CAPACITY OF RED CELLS

The capacity to transport oxygen to the tissues is the vital function of red cells, characterized *in vitro* by oxygen dissociation curve. During the past years, the relationship between the oxygen dissociation curve (P_{50}, partial pressure of oxygen at which 50% of the red cell hemoglobin is saturated) and 2, 3 diphosphoglycerate level has been appreciated.[19] Decreased levels of 2,3 DPG are associated with decreased values of P_{50} indicating increased affinity of the red cells to oxygen.

During storage at 4° C, the 2,3 DPG level of ACD collected erythrocytes declines approximately 50% within three days, whereas the 2,3 DPG level of CPD collected erythrocytes declines about 50% within six days[20] indicating that stored blood does not deliver oxygen efficiently immediately following transfusion. On the other hand, increased levels of 2,3 DPG facilitate the delivery of oxygen to the tissues. Meryman studied the effect of freezing on the 2,3 DPG content of red cells. He observed the mean value of 2,3 DPG to be 8.93/mol/g Hb before freezing and it was 8.65/mol/g Hb twenty-four hours following deglyceraolization,[12] whereas the level of 2,3 DPG in fresh erythrocytes was approximately 12/mol/g Hb. Meryman's data indicate only a minor decline in 2,3 DPG content following deglycerolization. Satisfactory levels of 2,3 DPG might be of critical importance for tissue oxygenation during open heart surgery.

COAGULATION

Cardiac surgery usually causes reduction in levels of coagulation Factors V and VIII as well as in thrombocyte count.[3] Reduction in the platelet count occurs presumably because they are consumed in the irregular surfaces in the pump.[21] Though serious bleeding disorders following heart surgery are rare, recent reports have indicated that disseminated intravascular coagulation occurred in 10 of 592 cases.[22]

Since frozen blood does not contain any coagulation factors, it is necessary to use fresh frozen plasma and cryoprecipitate for replacement therapy of Factors V and VIII if that might become necessary.

I have been requested to offer some opinions regarding the very interesting fact that since frozen blood was used during extracorporeal circulation in St. Elizabeth's Hospital, Boston, Massachusetts, decline in the platelet count did not occur as regularly as it did when fresh blood was used.[23] Since platelets tend to clump in whole blood that is stored for more than one day at 4° C, those platelets might initiate aggregation and consumption of even normal platelets in the pump.

It has been observed that the magnitude of the drop in the platelet count following cardiac surgery was related to the amount of blood given, especially if the blood was stored longer than 24 hours at 4° C.[24] When frozen blood is used in extracorporeal circulation, no "damaged" platelets are introduced due to the presence of negligible amounts of platelets in this product. It is possible that transfusion of frozen blood might minimize the formation of platelet deposits during cardiac surgery.

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