Use of Frozen Blood

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ABSTRACT

Frozen blood is most easily and economically prepared using high glycerol (40 percent). Storage is at $-80^\circ$ in a mechanical freezer. Thawing must be rapid and washing can be carried out with low electrolyte sugar solutions and agglomeration or with high electrolyte solutions and continuous flow centrifugation.

These processed cells have many advantages not found in raw routinely stored blood. When transfused a few hours after washing, the red cells are as good as fresh red cells. The undesirable elements including hepatitis virus, leukocyte and tissue antigens, low pH citrate and plasma antibodies are effectively removed.

Frozen blood is increasing in use and the variety of clinical use is broadening to include renal dialysis, transplantation, transfusion reactions and even open heart surgery. In our experience, the use of freezing for rare blood is infrequent.

Liquid storage of blood in ACD or CPD anticoagulant has been limited to 21 days with a recognized loss of 20 to 30 percent of viable cells in that interval.

Freezing red cells for clinical use was introduced in the late 1950's to provide prolonged storage of rare blood cells. In recent years, detailed studies on frozen red cells have demonstrated numerous advantages that suggest frozen red cells are preferable to raw blood in other clinical situations as well as when rare blood is needed.

This paper will review recent observations on the methods of freezing and the effects of washing on removing undesirable elements and reducing some of the risks associated with ordinary blood transfusion.

Glycerol was chosen as the solution in which to freeze blood because it is a normal physiologic compound of human metabolism. It seems to act to maintain water and electrolyte relationships inside and outside the cell thus preventing dehydration and increased salt concentration. These properties make it an ideal molecule for preventing cell damage during freezing. Under circumstances of physiologic salt concentration, lowering the temperature of blood results in ice crystal formation which excludes ions. The ion buildup in the region of the cell produces, in effect, a relative dehydration. Water leaks preferentially from the cell resulting in cell shrinkage. At temperatures approximating $-10^\circ$ or salt concentrations about 2.8 M, dehydration is
USE OF FROZEN BLOOD

TABLE I
METHODS OF PREPARATION OF FROZEN RED CELLS

<table>
<thead>
<tr>
<th>Glycerolization</th>
<th>Freeze</th>
<th>Storage</th>
<th>Deglycerolization</th>
</tr>
</thead>
<tbody>
<tr>
<td>High glycerol</td>
<td>Slow</td>
<td>Polyethylene bags</td>
<td>Agglomeration or Continuous flow centrifugation</td>
</tr>
<tr>
<td>40%</td>
<td>1 C/min</td>
<td>Mechanical freezer $-80^\circ$</td>
<td></td>
</tr>
<tr>
<td>Low glycerol</td>
<td>Fast</td>
<td>Stainless steel container</td>
<td>Continuous flow centrifugation or Manual batch wash</td>
</tr>
<tr>
<td>20%</td>
<td>1000 C/min</td>
<td>Liquid nitrogen $-196^\circ$</td>
<td></td>
</tr>
</tbody>
</table>

severe enough to produce cell membrane damage and, eventually, hemolysis.\(^5\) Addition of glycerol to a high concentration (40 percent W/V) will prevent this sequence. Glycerolization can be accomplished in two steps, 2.2 M at equilibration and 4.5 M at equilibration, by adding 100 ml of 6.2 M glycerol with shaking to 250 ml of red cells, then adding 300 ml more glycerol with gentle mixing to complete the process.\(^6\)

The two step procedure is convenient and economically more efficient than the continuous flow centrifugation of Tullis.\(^11\) The gradual addition of glycerol is necessary because glycerol traverses the cell membrane with a half time of seconds while water traverses the membrane with a half time measured in milliseconds.\(^5\) The temporary shrinkage of the cells in glycerol is reversed as the glycerol enters the cell.

When the temperature is reduced, ice does not form in such a way as to exclude ions. Dehydration is not as severe and cryoprotection is produced.

Some methods employ a lower concentration of glycerol 2.2 M (18 percent W/V) to allow one step equilibration and easy removal by washing.\(^4,8,9\) Lower temperatures and faster freezing are necessary for preservation of red cells in reduced glycerol concentrations (table I).

However, Meryman points out that the high glycerol method provides the greatest flexibility and greatest safety. Blood can be frozen for prolonged periods at mechanical freezer temperatures ($-80^\circ$) and can be transported in dry ice. Special freezing and thawing apparatus is not needed and ordinary plastic containers can be used.\(^6\)

The only disadvantage in this system is the cost and inconvenience of removing the glycerol by washing prior to transfusion. The Meryman wash technique, primarily reviewed here, consists of adding hypertonic salt (12 percent NaCl) to shrink the cells and initiate the removal of glycerol from the cells. Subsequent washes with two liters of 1.6 percent sodium chloride and one liter of 0.8 percent sodium chloride and 0.2 percent glucose are carried out by continuous flow centrifugation in a Latham-ADL bowl driven by an International PR-2 centrifuge. The procedure takes one hour and the cost of supplies is $18. The other washing procedures to remove high concentration glycerol include Huggins’ cyto-agglomerator, which employs an initial mixing with 500 ml of 50 percent glucose to remove glycerol and two subsequent washes in 2 liters of 8 percent glucose and 1 percent fructose.\(^2,3\) The cells are recovered by sedimentation and disaggregated with 250 ml of isotonic saline. The advantages of the Huggins system is that eight units can be processed simultaneously by one technician so mass production of washed units is possible for military or routine hospital use. The expense of supplies is higher than that of the Meryman technique, approximating $24 per unit. One scientific observation which is of concern is that cells exposed to salt-free sugar solu-
TABLE II

DETECTION OF HAA AND OTHER VIRUSES IN ARTIFICIALLY CONTAMINATED BLOOD AFTER WASHING

<table>
<thead>
<tr>
<th>Agent</th>
<th>Assay</th>
<th>Raw Blood</th>
<th>Final Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAA</td>
<td>CF</td>
<td>8–32*</td>
<td>-2*</td>
</tr>
<tr>
<td>HAA</td>
<td>RIA</td>
<td>pos</td>
<td>neg</td>
</tr>
<tr>
<td>Poliovirus</td>
<td>Plaque assay</td>
<td>2 X 10^4</td>
<td>20–260</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>Tissue culture</td>
<td>1 X 10^4</td>
<td>0</td>
</tr>
</tbody>
</table>

* Reciprocal titer.

(tions in this technique tend to leak potassium and the interval between thawing and transfusion must be kept short (6 to 24 hours) or excessive loss of cells occurs in vivo after transfusion. Another consideration is the length of frozen storage time which is shorter for cells frozen in low glycerol solutions.

Another wash procedure involves continuous flow with electrolyte solutions and utilizes a Sorvall centrifuge with a special harness (Elutramatic). This method allows washing of two units simultaneously in a cone-shaped bag. It is suggested that a more thorough wash may occur in such an apparatus, but this remains to be confirmed. The interesting feature of the Elutramatic is that it has been studied from the point of fine particle washout.

Blood deliberately contaminated with hepatitis associated antigen (HAA) polio virus or cytomegalic inclusion virus was washed with 2,000 ml 5 percent D/S by continuous flow. HAA and cytomegalovirus were undetectable and polio virus was reduced 10,000 times in concentration after this type of washing (table II).

The possibility that the risk of post-transfusion hepatitis could be reduced by processing raw blood through glycerolization, freezing and deglycerolization has intrigued a number of investigators. Tullis reported as early as 1970 that frozen, deglycerolized red cells resuspended in albumin did not transmit hepatitis when compared to red cells suspended in frozen plasma. Huggins in Massachusetts and Sumida in Japan have indicated marked reduction in hepatitis risk with frozen blood. Elimination of this risk alone would almost pay for the increased expense of frozen blood in lives saved, morbidity and hospitalization avoided, and costs of health care and loss from work considered.

Another advantage of frozen blood is the removal of leukocytes during the process of freezing and deglycerolization. It has been found in our laboratory, as in others, that there is a marked reduction in leukocytes in frozen blood. Patients receiving frozen

TABLE III

FINAL LEUKOCYTE CONCENTRATION (LEUKOCYTE PER MM^2 OF FINAL PRODUCT)

<table>
<thead>
<tr>
<th>N</th>
<th>X</th>
<th>S.D.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPRC</td>
<td>51</td>
<td>4,074</td>
<td>±3,123</td>
</tr>
<tr>
<td>FRC</td>
<td>72</td>
<td>291</td>
<td>±261</td>
</tr>
<tr>
<td>GdGRC</td>
<td>7</td>
<td>815</td>
<td>±304</td>
</tr>
<tr>
<td>SWRC</td>
<td>6</td>
<td>2,749</td>
<td>±921</td>
</tr>
</tbody>
</table>

LPRC—leukocyte poor red cells (inverted spin); FRC—frozen red cells; GdGRC—glycerolized-deglycerolized red cells—not frozen; and SWRC—saline washed red cells—not glycerolized.

cells are exposed to one tenth the number of leukocytes as those receiving traditional leukocyte poor preparations (table III). This reduction may be particularly important to the renal transplant patient since such patients are frequently transfused during dialysis and, if they develop leukocyte antibodies, they are likely to have hyperacute rejection of a transplanted kidney incompatible with the tissue antibodies.

Schechter and MacFarlane have demonstrated an immunologic response in patients receiving blood transfusion. Atypical lymphocytes and increased uptake of tritiated thymidine occurred between seven and 10 days after transfusion in those patients receiving fresh blood. Lower response was found in those patients receiving stored blood; no response was observed in those patients receiving frozen cells. These observations make it clear that routine processing of blood through the freezing deglycerolization wash technique may be particularly useful in open heart surgery. The length of time 2, 3 DPG remains high during post-thaw storage is short, however, and more work must be done in this area.

References


