Principles of Blood Separation and Component Extraction in a Disposable Continuous-Flow Single-Stage Channel


A single-stage disposable channel and seal that provides for leukocyte and granulocyte collection by continuous-flow cell separation (CFCS) has been designed by the IBM Corporation. This paper describes (1) the separation characteristics of whole blood as it responds to varying gravitational (G) forces and flow rates through the channel; (2) the mechanism by which the buffy coat accumulates and is extracted; (3) the efficiency of extraction; (4) those donor and procedural variables that contribute to the final yield; (5) posttransfusion increment response in patients; and (6) the functional integrity of the cells collected.

INCREASED emphasis on specific blood component replacement for specific physiologic deficiencies has directed attention toward the procedures used to collect components. Currently, granulocyte components are quite variable in terms of volume collected, cellular quantity, and cellular character. The variability resides in hematologic differences between donors, day-to-day variability in each donor, nonuniform response to leukocytosis-inducing agents, the efficiency of the technology used for collection, and the operator performing the procedure.

The biomedical systems group of the IBM Corporation has designed disposable blood pathways, consisting of separation channels and ceramic seals, to extract leukocytes by continuous-flow cell separation (CFCS).

The design objectives were (1) to increase the total cellular yields over those currently obtained by CFCS, (2) to minimize operator-related variability, which strongly influences total yield, and (3) to provide some standardization in collection by expanding scientific understanding of the response of whole blood to centrifugal forces in a CFCS system, and what that response realistically allows in the way of component collection.

This article describes (1) the principles of separation and the operation of the single-stage channel; (2) its performance over a range of flow rates, centrifuge speeds, procedure time, and total blood processed; (3) those donor variables that influenced the yield; (4) a stepwise forward multiple regression analysis that

Abbreviations: CFCS, continuous-flow cell separator; IFPP, interface positioning port; BCS, blood cell separator, NCI-IBM or Amino Celltrifuge; ACD, acid citrate dextrose; HES, hydroxyethyl starch; BSA, body surface area; TBV, total blood volume; ACD:WB, ACD:whole blood dilution.

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provides statistical correlation and ranking of the variables; (5) in vitro function studies of cellular components; and (6) posttransfusion recipient increment responses. Evidence for mobilization of cells from storage pools outside the circulating pool is demonstrated. An empiric formula is derived that gives mathematical expression to the relationship between donor and procedure variables that predicts, with reasonable accuracy, what the yield should be for each donor.

MATERIALS AND METHODS

Channel Description, Principles of Separation and Operation

Description

The single-stage channel consists of an extruded semirigid rectangular polyvinyl chloride plastic tube that is attached to the input and collection chamber to form a closed loop, as shown in Fig. 1. Four tubes from the face seal provide passages for whole blood, buffy coat, packed RBC, and plasma or a mixture of RBC–plasma. There is no direct communication of blood between the input port and collection chamber. Three exit ports have different radial positions within the collection chamber. The packed RBC port reaches the outermost wall of the chamber. The buffy coat and the interface positioning port (IFPP) are radially positioned to lie on the center line of the channel. They are separated from each other by a barrier that extends from the top to the bottom of the chamber. Plasma can pass around the barrier along the inner wall, and RBC flow around the barrier along the outer wall of the chamber. Downstream from the barrier, RBC and plasma are recombined and extracted by the IFPP.

Principles of Separation

The new disposable single-stage separation channel differs in two important ways from current CFCS devices. (1) The blood flow during separation is circumferential, i.e., the flow is in a circle around the axis of rotation. (2) The interface position within the channel is stabilized by the design of the collection chamber so that only minimal operator attention is required. Current CFCS are axial flow devices, i.e., blood flow during separation is parallel to the axis of rotation. The interface position and collection are under the direct control of the operator, who must vary the relative extraction flow rates of packed RBC and plasma to obtain the desired component.

Separation occurs as anticoagulated whole blood enters the channel and flows in a counterclockwise direction toward the collection chamber. Centrifugal separation, which allows leukocyte collection, can be characterized in terms of a packing factor (PF) defined as:

\[ PF = \frac{G \tau s}{d} \]  

where \( G \) = separation acceleration, \( \tau \) = time of separation, \( s \) = sedimentation rate of blood, and \( d \) = distance through which sedimentation takes place.

For the channel used in this work, the separation time, \( \tau \), is expressed as:

\[ \tau = \frac{A l}{Q} \]  

where \( A \) = channel area, \( l \) = channel length, and \( Q \) = mean flow rate through the channel.

Substitution of (2) into (1) gives:

\[ PF = \frac{G}{Q} \left( \frac{A l}{d} \right) \]  

The channel area (\( A \)), length (\( l \)), and distance through which sedimentation takes place (\( d \)) are geometrical properties of the system and are fixed. Sedimentation (\( s \)) is variable and not directly controllable. Thus, the degree of separation in the single-stage channel can be characterized by the ratio of separation acceleration, \( G \), to flow rate, \( Q \), through the channel. If this ratio is held constant, similar separation should be obtained at different \( G \) and \( Q \). In early work with this system using channels of different geometric design, acceptable yields of \( 3.0 \times 10^6 \) cells/liter of blood processed were found at a packing factor equivalent to 380 RPM/40 ml/min flow rate. The combination of centrifuge speed and
flow rate, which maintains that constant ratio, indicated that 650/50, 720/60, and 820/80 should provide comparable yields.

**Operation**

Anticoagulated whole blood is drawn into the channel by extracting priming saline through the IFPP. As the system fills with blood, separation occurs, and plasma replaces saline in the IFPP. After approximately 250 ml of blood have been processed, RBC–plasma appear in the IFPP. The downstream stabilized interface is related to the physical position of the IFPP. The location of this interface, in turn,
forces the interface on the upstream side of the barrier to take the same radial location so that WBC collection relates to channel design and not visualization of a buffy coat. As processing continues, a dense layer of packed RBC forms on the outside wall of the collect channel, and the upstream interface moves radially inward. The amount of radial offset between the upstream and downstream interface location can be controlled by the extraction rate of packed RBC through the RBC port. A leukocyte–platelet buffy coat forms on the upstream interface, and both components are extracted through the WBC collect port. Collection begins when sufficient packing of the RBC has occurred (500–1000 ml processed) to introduce RBC into the WBC collect line. Controlling the extraction rate of packed RBC controls the cellular makeup of the buffy coat collection. Simultaneous platelet collection is enhanced in this system compared to current BCS procedures, but optimal platelet collection requires higher RPM (1100–1600) than that required for optimal granulocyte collection (580–820 RPM).

Donor Selection

Donors were randomly selected from the daily list of normal donors scheduled to undergo leukapheresis procedures on the NCI-IBM 2990, Aminco Celltrifuge, and Haemonetics M30 separators. Donors were evaluated by the usual screening procedures, and consent forms were signed. Seventy-six donors were treated with etiocholanolone, 10 mg, and dexamethasone, 2.0 mg, intramuscularly. Fifty-one donors received dexamethasone, 8.0 mg, orally, 10–12 hr prior to leukapheresis. Ten donors received no prior medication.

Donor parameters studied included pre- and postprocedure blood pressure, temperature, pulse rate, CBC with differential and platelets for all donors. Some donors were studied for SMA chemistries, prothrombin time (PT), partial thromboplastin time (PTT), Lee-White clotting time, serum citrate, and ionized calcium (Ca++) levels. Cells collected were transfused to 125 patients entered on replacement protocols being carried out in the Department of Developmental Therapeutics.

Procedure

Hydroxyethyl starch, 500 ml 6%, with ACD concentrate followed by ACD-A in a 1:13 ACD:WB dilution was used for all procedures. A mean of 8.8 liters of blood was processed (range, 4.4–10); mean procedure time was 142 min (range, 78–200) with a mean flow rate of 60 ml/min (range, 40–80) at a mean RPM of 720 (range, 580–820). Donor selection for each flow rate centrifuge speed combination was based on BSA-TBV measurement. Donors <1.6 sq m were processed at 50 ml/min, 1.6–1.9 sq m at 60 ml/min, and >1.9 sq m at 80 ml/min. When insufficient bleeding rates occurred, usually related to some limitation in venous access, processing was carried out at 40 ml/min.

Collection Bag Data

Samples were collected for determination of leukocyte, platelet, and RBC concentration. Collection bag weights are converted to milliliter volume, and total yields are expressed as leukocytes × 10^9, and platelets and RBC × 10^12 cells.

Statistical Methods

Statistical analysis of the data was performed by the Department of Biostatistics, using a Control Data Corporation Cyber 172 computer and Statistical Package for the Social Sciences program. The Student’s t test was used for comparison of means between two groups. For comparison of means between more than two groups, the Scheffe test was applied. This method allows examination of all possible linear combinations of group means, not just pair-wise comparisons.

In Vitro Cell Function Studies

Granulocytes

The test method selected for granulocyte phagocytic function was a quantitative measurement of formazan produced by conversion of nitro-blue tetrazolium (NBT) dye. A modification of the method of Baehner and Nathan was standardized relative to polymorphonuclear cell (PMN) concentration (1.6 × 10^6 PMN), NBT concentration (0.5%), incubation time, latex particle size (0.8 μl), and concentration (1:9 dilution). Spectrophotometric measurements at 510 nm were performed on a
flow-through Gilford spectrophotometer, and results were expressed as micrograms formazan/PMN concentration/NBT concentration/reaction time at 10-min intervals from 5 to 60 min.

Granulocyte morphology was examined and graded for cytoplasmic integrity and nuclear configuration on 1000 cells in a Wright-stained preparation of collected cells.

Lymphocytes

Specimens were examined by the trypan blue exclusion test to determine percent intact surface membranes of viable cells, which excluded dye, and nonviable cells, which took up the dye.

The rate of DNA synthesis, as measured by pulse labeling with thymidine(TdR)-3H on serial days of lymphocyte culture, was carried out according to the method of Thurman et al. Incorporation of 3H-TdR was measured on days 2 through 7 and expressed as cpm/1.5 x 10³ lymphocytes.

Red Cells

Aliquots of donor blood pre- and postprocedure were exposed to decreasing concentrations of saline, from 0.85% to 0.30%. Hemoglobin freed from lysed RBC was measured on a Gilford flow-through spectrophotometer. Results are given as percent hemolysis/NaCl concentration.

Plasma hemoglobin by benzidine reagents was carried out for 18 procedures. Cyanmethemoglobin method was used on donor blood specimens pre- and postprocedure when benzidine reagents became unavailable.

RESULTS

Performance

The mean total cellular yields, standard deviation, and ranges are shown in Table 1. Mean collect volume was 170 ml (range, 70–306) with mean collect bag cellular concentration of 202 × 10⁹ WBC/µl (range, 70–379), 693 × 10⁵ platelets/µl (range 130–2260), and 556 × 10⁶ RBC/µl, which represented a mean hematocrit (Hct) of 4.4%.

Procedure Variables

Flow Rate and RPM

The four centrifuge speeds and flow rates were determined relative to separation characteristics already described. While the equations predicted comparable yields, experiments documented that the WBC yields increased with centrifuge speed at 820 RPM, as shown in Fig. 2. Although the correlation was low (r = .243), the differences in yield were statistically significant (p = 0.003). While total WBC yield increased, a subtle shift in differential was noted, in that the percent PMN dropped from 80% to 85%, seen at 580–720 RPM, to 70%–75% at 820 RPM. These data support blood separation principles in that optimal PMN collection requires low G forces. Donor precounts, bag collection volume, and RBC contamination

<table>
<thead>
<tr>
<th>Total (n = 130)</th>
<th>Cell Yield ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC x 10⁹</td>
<td>33.0 ± 11.6</td>
<td>13.7–63.6</td>
</tr>
<tr>
<td>PMN x 10⁹</td>
<td>26.7 ± 10.2</td>
<td>9.0–55.4</td>
</tr>
<tr>
<td>Lymph x 10⁹</td>
<td>4.4 ± 2.3</td>
<td>0.85–13.7</td>
</tr>
<tr>
<td>Mono x 10⁹</td>
<td>2.0 ± 2.0</td>
<td>0.0–8.8</td>
</tr>
<tr>
<td>Plate x 10¹¹</td>
<td>1.1 ± 0.6</td>
<td>0.2–3.3</td>
</tr>
<tr>
<td>RBC x 10¹¹</td>
<td>1.1 ± 0.5</td>
<td>0.1–2.1</td>
</tr>
</tbody>
</table>
were comparable so that changes in yield related to centrifuge speed were not influenced by these variables. Increasing flow rates reduces donor time required to collect WBC–PMN. Variability in flow rates represents first efforts to individualize procedures for each donor and avoids inappropriate calcium depletion, which is influenced by donor blood volume and citrate infusion rates (Hester JP, unpublished data).

**RBC Contamination**

There was a negative correlation between RBC contamination and yield. There was a trend toward higher bag Hct (mean 4.8%) and lower mean yields, $24 \times 10^6$ WBC and $20 \times 10^9$ PMN, in donors with precounts $< 10,000/\mu l$, compared to a mean Hct of 4.0% and yields of $40 \times 10^6$ WBC and $32 \times 10^9$ PMN in donors with precounts $> 12,000/\mu l$. The differences in bag Hct were not statistically significant. The highly significant differences in total yields (Scheffe) related to differences in donor precount. However, no gain in yield can be expected by collecting deep within the packed red cell layer.

**Bag Volume**

In current technology, a wide range of collect volumes is obtained. Neither a specific collect volume nor a range of volumes has been identified that could be correlated with optimal yields. Volumes collected during the earlier part of the research period were based on a mean volume of 169 ml obtained in 980 BCS procedures that indicated a low but positive correlation between collect volume and yield. Research on this channel has indicated that as a single variable there is positive correlation ($r = .37$) between collect volume and yield that is significant ($p = 0.0001$), but volume contribution to yield diminishes beyond 300 ml.

**Donor Variables**

The concentration of WBC and PMN in the peripheral blood of the donor is highly correlated ($r = .607; p = 0.001$) with yield. Figure 3 shows this relationship.
Unstimulated donors have the lowest WBC-PMN concentrations and total yields. Etiocholanolone–dexamethasone-treated donors had the highest precounts and total yields. Values for dexamethasone-treated donors are intermediate. All three groups differed significantly (Scheffe, \( p = 0.01 \)) in precounts and yield, and the shift to granulocytosis by pretreatment of the donor is also reflected in the granulocyte fraction of the total yield from treated donors.

Yields obtained in the single-stage channel exceed our mean performance with the reusable bowls (NCI-IBM:Aminco) in which a mean of \( 18.5 \times 10^6 \) WBC and \( 13.8 \times 10^6 \) PMN were obtained in 980 procedures. Three-hundred and fifty concurrent BCS procedures were analyzed to match both donor precount and bag volumes. At each level of donor precount and volume collected, the performance exceeds that of the reusable bowl, as seen in Fig. 4.

Multiple Variable Stepwise Forward Regression Analysis

The statistical method by which single variables contributing to a dependent variable (yield) can be ranked statistically in order of their relationship to each
other and to the dependent variable is a forward stepwise multiple variable regression analysis. Nine variables contributing to PMN yield were analyzed by this method and are summarized in Table 2. The analysis indicates that donor precount \((p < 0.0001)\), centrifuge speed \((p = 0.004)\), and donor hemoglobin (Hb) \((p = 0.025)\) are statistically the most significant variables in PMN collection.

Differences in flow rate, RBC content, procedure time, recirculation, blood processed, and bag volume did not attain statistical significance in this multivariable ranking. Donor Hb and donor precount are linked and relate primarily to the fact that male donors had higher hemoglobin values and etiocholanolone–dexamethasone induced a greater leukocytosis in males than females. Whether these two variables contribute to the yield dependently or independently is not known at this time.

**Efficiency of Extraction**

Previous estimates of efficiency of leukocyte extraction have been based on multiplying the arithmetic mean of the precount and the postcount by the total blood processed and expressing the extraction efficiency as a ratio of actual cell yield to total cells passing through the system. Although there was an overall 33% drop in donor WBC counts, the arithmetic mean does not accurately reflect the extraction efficiency. In this study, assessment of the extraction efficiency of the leukocyte channel was carried out by serially measuring the WBC present in blood entering and leaving the channel. WBC counts obtained preprocedure, at 3.0, 6.0, and 9.0 liters of blood processed, and postprocedure revealed that a linear fall in

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**Table 2. Regression Analysis for Variables Contributing to Granulocyte Yield**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Multiple (r)</th>
<th>Simple (r)</th>
<th>(r^2) Square</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precount</td>
<td>.853</td>
<td>.853</td>
<td>.728</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>RPM</td>
<td>.911</td>
<td>358</td>
<td>.831</td>
<td>.004</td>
</tr>
<tr>
<td>Donor Hb</td>
<td>.935</td>
<td>.696</td>
<td>.875</td>
<td>.025</td>
</tr>
<tr>
<td>Flow</td>
<td>.943</td>
<td>.059</td>
<td>.889</td>
<td>NS</td>
</tr>
<tr>
<td>Bag RBC</td>
<td>.947</td>
<td>-.227</td>
<td>.897</td>
<td>NS</td>
</tr>
<tr>
<td>Procedure time</td>
<td>.949</td>
<td>-.299</td>
<td>.902</td>
<td>NS</td>
</tr>
<tr>
<td>Recirculation</td>
<td>.951</td>
<td>.122</td>
<td>.905</td>
<td>NS</td>
</tr>
<tr>
<td>Blood processed</td>
<td>.953</td>
<td>-.002</td>
<td>.908</td>
<td>NS</td>
</tr>
<tr>
<td>Bag volume</td>
<td>.956</td>
<td>.321</td>
<td>.915</td>
<td>NS</td>
</tr>
</tbody>
</table>

Fig. 5. Cell depletion during leukocyte collection single-stage channel.
donor WBC does not occur during leukapheresis, as shown in Fig. 5. There is a 28% reduction in donor WBC during the first 3.0 liters processed, but further decrease of only 4% is seen between 3.0 and 6.0 liters, and 7% between 6.0 and 9.0 liters. Instantaneous measurements of leukocyte counts taken on the input and return lines, however, demonstrated that 60%–65% of cells entering the channel were being extracted through the collection port during the infusion of HES. This extraction efficiency dropped to about 50%–55% when NIH ACD-A was infused following completion of HES. This suggests that mobilization from storage pools outside the circulating pool was occurring. Further evidence for this can be seen by comparing the number of cells cleared from the circulating pool (donor pre–post WBC x TBV) to that which was collected.

**Predictive Equation for Yield**

The regression analysis indicates that with the single-stage channel, we could account for 91.5% of the yield by the variables studied, and additional procedural variables are not likely to significantly affect the yield. It further implies that separation, extraction, and collection parameters are sufficiently well characterized in this system to allow predictability of yield.

Granulocyte yield appears to be most significantly related to donor count. The number of cells available to be collected is a function of donor precount and total blood volume. An empiric equation was derived to give mathematical expression to these relationships, which predicts with reasonable accuracy what PMN yields ($Y_p$) should be for a given donor. This represents a log ratio of what was collected relative to what was available to be collected. The value, 8.2, is a constant ($k$) derived from least-squares fit to the data. The equation is:

$$Y_p = 2C_p V_B \ln \left[ 1 + \frac{8.2 \ V_c}{C_p V_B} \right] \quad (1)$$


![Fig. 6. Correlation of observed yields to predicted yields.](image)
Table 3. Posttransfusion Corrected Granulocyte Increments × 10^9/μl

<table>
<thead>
<tr>
<th>HLA</th>
<th>ABO</th>
<th>Single-Stage</th>
<th>BCS</th>
<th>Incompatible</th>
<th>Single-Stage</th>
<th>BCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compatible</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t - 0</td>
<td></td>
<td>.813</td>
<td>.624</td>
<td></td>
<td>.288</td>
<td></td>
</tr>
<tr>
<td>t - 1 hr</td>
<td></td>
<td>.803</td>
<td>.652</td>
<td></td>
<td>.408</td>
<td></td>
</tr>
<tr>
<td>Incompatible</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t - 0</td>
<td></td>
<td>.830</td>
<td>.476</td>
<td></td>
<td>.556</td>
<td>.150</td>
</tr>
<tr>
<td>t - 1 hr</td>
<td></td>
<td>.736</td>
<td>.464</td>
<td></td>
<td>.550</td>
<td>.212</td>
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</table>

Figure 6 shows the correlation between our observed yields and the predicted yields. It indicates that most of the observed yields lie very close to the predicted yield. Less than 10% of the observed yields lie outside the lower standard deviation and can be accounted for by procedural problems or nonuniform bag sampling with resultant lower WBC concentrations.

Recipient Data

Recipients were acute leukemia patients receiving induction chemotherapy. All had previously been transfused extensively with red cells and platelet components and were receiving therapeutic or prophylactic PMN replacement. Alloimmunization could have been expected, but was not documented. The mean absolute PMN increment immediately after transfusions was 1050 PMN/μl (range, 0–5000 PMN/μl). Increments corrected for donor body surface area and total cells transfused and arranged according to HLA and ABO compatibility are shown in Table 3. These are compared to those obtained in an analysis of 980 therapeutic replacement transfusions, obtained with the reusable bowl in which a regression analysis of 8 variables revealed ABO (p = 0.005) and HLA (p = 0.02) made the most statistically significant contribution to posttransfusion increments.

Donor Data

Donor hematologic data pre- and postprocedures are shown in Table 4. The 19% drop in Hb is comparable to that with our current BCS procedures and reflects dilution with HES:ACD and sampling for multiple studies. The 30%–33% reduction in leukocyte and platelet counts exceeds that for current BCS and reflects the greater efficiency of the disposible blood pathway in extracting cellular compo-

| Table 4. Donor Data for Leukocyte Collection Pre- and Postprocedure |
|------------------------|------------|----------------|---------------|-------------|---------------|
| Value                  | Pre ± SD   | Post ± SD      | Percent Change| p Value‡    |
| Hb (g/100 ml)          | 14.3 ± 1.7 | 11.7 ± 1.6     | 19%           | 0.0001      |
| WBC (x 10^9/μl)        | 11.5 ± 3.4 | 8.0 ± 2.0      | 31%           | 0.0001      |
| PMN (x 10^9/μl)        | 9.4 ± 2.9  | 6.6 ± 2.0      | 30%           | 0.0001      |
| Lymph (x 10^9/μl)      | 1.6 ± 1.0  | 1.0 ± 0.5      | 38%           | 0.038       |
| Mono (x 10^9/μl)       | 0.3 ± 0.4  | 0.2 ± 0.2      | 34%           | NS          |
| Platelets (x 10^9/μl)  | 277 ± 64   | 215 ± 52       | 33%           | 0.0001      |
| Blood volume           | 4.6 ± 0.6  | —              | —             | —           |

‡Two-tailed t test.
ments. Prothrombin time, partial thromboplastin time, and Lee-White clotting times were normal, confirming that anticoagulation was limited to extracorporeal blood. SMA screening studies disclosed no significant abnormalities. No temperature elevation, shaking, chills, or hypotension occurred in any donor. Headaches and peripheral edema, related to volume expansion by HES:ACD, were noted in a pattern similar to that with BCS-M30 procedures performed in our laboratory.

In Vitro Function

Granulocytes

Phagocytic function is intact, as shown in Fig. 7. Micrograms of formazan produced by nonstimulated (resting) PMN and duplicate cultures stimulated (active) by latex particles for cells collected by our current BCS reusable bowl, the research prototype (Apheresor), and the 2997 are compared. There were no differences among the three groups. Nonstimulated cells produced approximately 4 \( \mu g \) formazan after a 30-min incubation period. This is the same value in 23 studies of peripheral blood granulocytes from normal blood. A rise to 16–19 \( \mu g \) formazan is seen in all three groups when granulocytes are stimulated by latex beads.

Normal PMN morphology, as related to membrane integrity and nuclear structure, was noted on Wright-stained smears of collected cells. A small percentage (0%–3%) of smudge cells can be seen on slides of cells collected by any of these systems currently in use.

Lymphocytes

Figure 8 shows the mean values (± standard deviation) of \(^{3}H\)-TdR incorporation of control and mitogen-stimulated (PHA and SLO) lymphocytes from etiocholanolone and/or dexamethasone-treated donors. Each donor served as his own control, as lymphocytes were isolated from peripheral blood prior to the procedure, and a sample was obtained from the bag collected.
**Erythrocytes**

Standard erythrocyte osmotic fragility curves were constructed and demonstrated no significant differences in donor peripheral blood, pre- and postprocedures, for the IBM 2997 procedures \((n = 76)\) compared to Aminco \((n = 36)\) or Haemonetics M30 \((n = 8)\) procedures. Plasma hemoglobin determinations by the cyanmethemoglobin method were unchanged after the procedure \((1.195 \text{ mg/100 ml pre and } 1.172 \text{ mg/100 ml post})\), indicating no injury occurred to donor blood in the seal or outflow parts of the 2997.
DISCUSSION

NCI-IBM blood cell separator was developed in the 1960s to provide an effective, reliable, closed centrifuge system in which a mixture of blood and anticoagulant could undergo uninterrupted processing while collection of components was in progress.7,8

The first trials, reported by Freireich et al.9 in 1965, produced good in vitro recovery of leukocytes. The in vivo separation initially involved chronic leukemia patients, and leukocyte yields were primarily lymphocytes.

Two factors subsequently made a significant contribution to increasing granulocyte yields in a CFCS system. One was donor pretreatment to induce leukocytosis/granulocytosis; the other was the addition of the red cell sedimenting agent hydroxyethyl starch.

Vogel et al.,10 in a study of leukocyte kinetics, reported leukocytosis and granulocytosis in normal donors approximately 1.2 hr following an intramuscular injection of etiocholanolone, a naturally occurring steroid precursor. McCredie and Freireich11 applied this observation in 1970 to pretreatment of donors undergoing leukapheresis. PMN collection was modestly improved over untreated controls. In 1971, Graw et al.12 confirmed that total leukocyte yields could be enhanced by medicating donors with etiocholanolone or steroids.

In 1974, McCredie et al.13 and Mischler et al.14 both reported the contribution of HES, an amylopectin derivative with plasma-expanding and RBC-sedimenting properties.15 Both groups of investigators demonstrated a statistically significant increase in PMN collected by the addition of this agent to the input line.

The application of continuous-flow cell separation has broad application in addition to granulocyte collection for management of infected neutropenic patients. Mononuclear collection (lymphocyte, monocyte, stem cell), cellular depletion for patients with leukemic leukocytosis or thrombocytosis, and plasma exchange have all evolved through the technology for centrifugation of whole blood. The donor, or patient, becomes part of a "closed loop" circuit with the separator, wherein his total blood volume becomes available for processing, extracting, exchanging, or replacing specific cellular constituents or the acellular fraction.

In order to maximize the usefulness of continuous-flow cell separation, it is necessary to understand the response of whole blood to centrifugal forces as it passes through the channel, as well as the donor or patient role in the processing. The response in this disposable single-stage channel for granulocyte collection can be summarized as follows.

At low G forces, with sedimenting agents, granulocytes may be effectively collected. Because of size and density, the majority of platelets are left free in the plasma. This allows optimal granulocyte collection and adequate, but less than optimal, platelet yields.

As G forces are increased, granulocytes are replaced by mononuclear cells in the leukocyte-rich interface, and a larger number of platelets are brought down out of the plasma to the interface and are extracted with the buffy coat. This increases platelet yield, but sacrifices PMN yield.

The interface positioning port provides a semiautomatic interface control for PMN collection. The barrier allows accumulation of the buffy coat and provides superior extraction to that obtained by BCS.
The donor contribution is a biologic variable that can, to some extent, be manipulated. Both donor stimulation and HES were utilized in this study. The data reveal unambiguously the role of donor pretreatment in PMN collection by CFCS.

Mobilization of cells from storage pools outside the circulating pools appears to make a significant contribution to the final yield. Mobilization, as a result of leukocyte depletion by leukapheresis, was suggested by Bierman et al. in 1961. Although large numbers of leukocytes \((5-172 \times 10^9)\) were removed on the ADL–Cohn fractionator, the peripheral blood leukocyte level was maintained. Leukocyte collection in the disposable channel suggests that the same phenomenon is being observed. Whether the mechanism of mobilization is depletion of circulating cells or persistent influence of medication is not known.

Leukocyte yields cannot be standardized in terms of collecting a fixed quantity of cells in each procedure. The empiric equation derived in this study attempts to relate mathematically relationships between biologic variables (donor count and donor blood volume) and procedural variables (volume collected) that contributed to yield. The data indicate those circumstances in which yields \(\geq 40 \times 10^9\) WBC can be achieved—that is, collect volumes in the order of 250 ml and donor counts \(\geq 16,000/\mu l\).

Finally, there is clinical importance in the capability to deliver larger cell doses for replacement purposes. Outside the problems inherent in immunologic destruction of transfused PMN, the short half-life and inadequate cell doses delivered by transfusion to date have left patients exposed to 18 hr of inadequate circulating PMN. Applebaum et al.\(^{17}\) have reported the critical importance of cell dose in survival of neutropenic dogs with septicemia. Vogler et al.\(^{18}\) have reported a clinical study in which survival was linked with higher increments. Increasing the cellular yield from donors, coupled with a more physiologically appropriate peripheral blood PMN concentration, should allow more accurate characterization of the dose–response relationship necessary for successful replacement in neutropenic patients.

CONCLUSION

This second-generation CFCS blood pathway has expanded our scientific understanding of the characteristics of whole blood separation in response to centrifugal forces. It allows some standardization of procedure by identifying a range of flow rates, centrifuge speed, procedure time, bag volume, and interface positioning that will optimize component collection. It allows us to understand the effect of donor biologic variability on component collection, and it permits a mathematical expression of these relationships that offers guidelines for realistic component collection.

REFERENCES

Principles of blood separation and component extraction in a disposable continuous-flow single-stage channel

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