In Vitro Function and Post-Transfusion Survival of Granulocytes Collected by Continuous-Flow Centrifugation and by Filtration Leukapheresis

By Jeffrey McCullough, Barbara J. Weiblen, Amos R. Deinard, James Boen, Ignacio E. Fortuny, and Paul G. Quie

The function of granulocytes collected by continuous-flow centrifugation (CFC) and by filtration leukapheresis (FL) was studied in vitro, and the post-transfusion recovery and intravascular survival of these cells was studied by autologous transfusion in normal donors. Granulocytes collected by both FL and CFC leukapheresis (CFCL) functioned normally in the quantitative nitroblue tetrazolium, oxygen consumption, and chemotaxis assays. Bacterial killing was slightly but consistently decreased in FL but not CFCL granulocytes. The post-transfusion recovery of control granulocytes collected by ordinary phlebotomy averaged 52% in eight transfusions, compared with 34% for six CFCL granulocyte concentrates and 16% for six FL concentrates. The intravascular half-times were 3.8 hr for phlebotomy and 3.0 hr for CFCL granulocytes. FL granulocytes had survival curves which were nonlinear and a single half-life could not be calculated. The average half-time 30 min after transfusion was 1.3 hr, and 3 hr after transfusion it was 2.6 hr. Granulocytes collected by FL had a mild impairment of bactericidal killing, decreased post-transfusion recovery, and altered intravascular kinetics. None of these abnormalities was found in granulocytes collected by CFCL.

With the development of the continuous-flow centrifuge and filtration leukapheresis, granulocyte transfusion has become increasingly practical and more widely used in research and in the general clinical management of infected leukopenic patients. Despite the increasing use of granulocyte transfusion, the effects of collection methods on the function and survival of these cells have not been settled. Initial studies of the function of granulocytes collected by continuous-flow centrifuge leukapheresis (CFCL) used light microscopy or trypan blue dye exclusion, and some included assessment of bactericidal and hexose monophosphate shunt activity. However, most of these studies involved granulocytes from chronic myelogenous leukemia patients, not normal donors.

The function of granulocytes collected by filtration leukapheresis (FL) remains controversial. We, and others, have found a slightly reduced bactericidal activity in normal granulocytes collected by FL. Djerassi et al. reported normal trypan blue viability and others found normal bactericidal...
and chemotactic activity, quantitative iodination, and ¹⁴C-1-glucose oxidation by FL granulocytes. Thus the present study was undertaken to define the functional capacity of normal granulocytes collected by both FL and CFCL using the same function assays in the same laboratory.

Post-transfusion granulocyte increments appear to be greater for granulocytes collected by CFCL than by FL when comparable numbers of cells are transfused.⁶⁻¹² Therefore, we have studied the post-transfusion recovery and intravascular survival of granulocytes collected by FL and CFCL from normal adults returned to those same donors as an autologous transfusion.

MATERIALS AND METHODS

Granulocytes were collected by FL and CFCL from normal, healthy adults who met all the criteria for ordinary blood donation¹³ and had given informed consent to participate. This study was approved by the University of Minnesota Committee on the Use of Human Volunteers. Peripheral venous blood samples were obtained from the donors immediately before and immediately after leukapheresis to serve as controls. An additional control was granulocytes from blood freshly collected in CPD solution from normal blood bank donors.

Continuous-Flow Centrifuge Leukapheresis

CFCL was performed as previously described.¹⁴ The donors underwent bilateral venipuncture with 16-gauge intravenous catheters and blood was pumped through the continuous-flow centrifuge (Celltrifuge, American Instrument Co., Silver Spring, Md.) at 40–60 ml/min. The centrifuge speed was 400–450 rpm, and granulocytes were collected at approximately 1 ml/min. The anticoagulant regimen consisted of 2000 units of a heparin intravenous push at the beginning of the procedure followed by the infusion of heparin 15 U/min and a 3%, citrate hydroxyethyl starch (HES) solution¹⁵ in a ratio of 1:20 with whole blood. Approximately 9000 ml of whole blood were processed during the 3½-hr leukapheresis procedure, and the donor received 4500–5000 units of heparin and 400–500 ml of citrate HES solution. The characteristics of the granulocyte concentrate obtained by this procedure and the effects of the procedure on the donor have been reported previously.¹⁴,¹⁵ An average of 1 x 10¹⁰ granulocytes was collected and served as the source of granulocytes for the in vitro function studies.

Granulocyte collection for the autologous transfusion and intravascular survival studies was carried out exactly as described above except that the procedure was shortened to 2 hr and produced an average of 5 x 10⁹ granulocytes in a volume of 200 ml. This shortened collection was to minimize the disruption of the donor's homeostatic mechanisms and to produce a granulocyte concentrate for autologous transfusion containing a number of granulocytes similar to the average of 1.4 x 10⁹ obtained by ordinary phlebotomy of 450 ml of whole blood. The autologous transfusion studies were carried out using an average of 2 x 10⁹ granulocytes in a volume of 100 ml.

Filtration Leukapheresis

FL was performed in a manner similar to that described by Herzig et al.³ The FL system (Filtration Leukapheresis Pump and Filtration Leukapheresis Set, Fenwal Laboratories, Morton Grove, Ill.) was primed with 2 liters of heparinized saline solution (heparin 1000 U/liter); bilateral venipunctures were performed with 16-gauge catheters; and the donors were given 2000 units of a heparin intravenous push at the beginning of the procedure, followed by infusion of 4000 U/hr. Blood flow was maintained at 50–60 ml/min during the 2½-hr procedure. Approximately 9000 ml of blood were processed. Protamine sulfate, 50 mg, was administered at the end of the procedure. Granulocytes were eluted from the two filters using 1500 ml of ACD plasma–saline solution composed of 250 ml ACD-A, 220 ml fresh frozen CPD plasma, and 1000 ml of normal saline. During elution the filters were tapped firmly but not extremely hard. An average of 2 x 10¹⁰ granulocytes was collected and served as test cells for the in vitro studies.

Granulocytes for the autologous transfusion and intravascular survival studies were obtained as described above, except that the procedure was shortened to 2 hr, with processing of only approximately 7500 ml of blood. This procedure was used to minimize the disruption of the donor's
homeostasis and also to produce a granulocyte concentrate that would not have a number of
granulocytes much larger than that obtained by phlebotomy of 450 ml of whole blood. The average
number of granulocytes collected with the shortened procedure was $9 \times 10^7$ in a volume of 225
ml. The autologous transfusions were carried out using an average of $4.5 \times 10^7$ granulocytes
in a volume of 100 ml.

In some of the autologous transfusions, the post-transfusion recovery and intravascular survival
were determined on granulocytes collected by phlebotomy of 450 ml of whole blood. This study
was carried out using standard blood bank procedures. The 450 ml of blood were collected into
plastic bags (No. 4R0514 Fenwal Laboratories) containing 2250 units of heparin. Granulocytes
collected in this manner served as a control to evaluate the effects of the FL and CFCL methods
of collecting granulocytes.

Laboratory Procedures

The granulocyte intravascular survival procedure\textsuperscript{16} involved in vitro labeling of granulocytes
with disopropylphosphofluoride-\textsuperscript{32}P (DF-\textsuperscript{32}P). The granulocyte concentrates prepared either by
FL or CFCL, or the 450 ml of whole blood (phlebotomy) were labeled with 50 μCi of DF-\textsuperscript{32}P
(Amersham-Searle Corporation, Des Plaines, Ill.) by incubation at room temperature for 30 min.
Following incubation, the granulocytes were infused into the original donor over 15-30 min through
a standard blood administration set. Blood samples were obtained from the bag prior to infusion
and from the donor immediately at the end of infusion and at 30 min, 1, 2, 3, 4, 5, and 6 hr.
Portions of these whole blood samples were passed over a column of nylon fibers where granulo-
cytes were trapped. The nylon was washed free of contaminating red cells, platelets, lymphocytes,
and plasma proteins, and the residual radioactivity was measured in a liquid scintillation counter.
Monitoring of absolute granulocyte counts before and after blood flow over the nylon fibers al-
lowed calculation of the number of granulocytes trapped. Results are reported as counts per min-
ute (cpm) per 100% trapping of granulocytes per 5 ml of whole blood applied to the column. A line
of best fit for all of the points was calculated by the method of least squares. The intersection of
this line with the Y-axis at zero time was used as the value for calculation of post-transfusion
recovery.

Granulocytes collected by FL and CFCL were studied for in vitro function using the bactericidal
assay, the quantitative nitroblue tetrazolium (NBT) test, the oxygen consumption assay, and the
chemotaxis assay.

The bactericidal assay\textsuperscript{17} consisted essentially of incubating $1 \times 10^6$ granulocytes with $5 \times
10^8$ Staphylococcus aureus, strain 502-A, in the lag phase of growth. Fresh frozen human serum
was used as a standard source of opsonin. The phagocytic mixtures were incubated with end-over-
end rotation at 10 rpm in plastic tubes to insure maximum contact between bacteria and granulo-
cytes. Viable bacteria were determined immediately after bacteria were added to the phagocytic
mixture and during incubation by removing aliquots from the incubation mixture with a calibrated
loop. Controls consisted of tubes with granulocytes and opsonin without bacteria, bacteria and
opsonin without granulocytes, and tubes incubated without rotation to determine extracellular
bactericidal activity.

The quantitative nitroblue tetrazolium test\textsuperscript{18} consisted essentially of incubating $2.5 \times 10^6$
granulocytes in a phosphate-buffered saline, pH 7.4, with 0.05 ml of dialyzed 0.8-μ latex particles,
0.1 ml of 0.1 M KCN, and 0.1% NBT at 37°C for 30 min. After extraction with ethyl acetate,
the optical density of the NBT was read spectrophotometrically. Results are reported as the differ-
ence in optical density between phagocytic mixtures with and without latex particles added.

In the oxygen consumption assay\textsuperscript{19} granulocytes were adjusted to a concentration of $3 \times 10^6/$
ml in phosphate-buffered saline, pH 7.4. Oxygen consumption was measured using a YSI model
53 Biological Oxygen Monitor. A 3-ml suspension of granulocytes was equilibrated with air for
5 min at 37°C. Oxygen consumption was recorded for 10 min, then 0.15 ml of dialyzed 0.8-μ
latex particles was added and the oxygen consumption was recorded for an additional 10 min. The
rate of oxygen consumption for the resting or nonstimulated cells was calculated from the slope
of the first 10-min record. The oxygen consumption during phagocytosis was determined from the
initial 2-min period following addition of the latex particles. The oxygen consumption value was
recorded as microliters of oxygen utilized per $9 \times 10^6$ granulocytes per hour. Results are re-
ported as the difference in oxygen consumption between resting and phagocytic granulocytes.
Average ± SD.

tGranulocyte concentrate bacterial killing < donor before (p < 0.05).

Table 1. Morphological Appearance of Leukocytes Collected by CFCL and FL

<table>
<thead>
<tr>
<th></th>
<th>CFCL(%)</th>
<th>FL(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>63</td>
<td>61</td>
</tr>
<tr>
<td>Increased vacuolization</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>Severely damaged</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>26</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Granulocyte chemotaxis was studied using a modification of the method of Boyden as previously described. A standardized granulocyte suspension consisting of $8 \times 10^5$ granulocytes suspended in Medium 199 (Microbiological Associates, Bethesda, Md.) was utilized. The chemotactic activity of granulocytes was tested using Escherichia coli bacterial factor. Chemotaxis chambers were incubated for 3 hr at 37°C, the filter removed, stained with hematoxylin, and examined for the number of cells that had passed from one side of the filter to the other. All tests were run in triplicate, and the results were reported as the number of granulocytes per 10 high power fields which migrated through the filter.

RESULTS

Granulocytes collected by CFCL appeared morphologically normal by light microscopy. Approximately 3% of the granulocytes had increased vacuolization, and an additional 8% of the cells were severely damaged (Table 1). In contrast, 23% of FL granulocytes had increased vacuolization, and 13% were severely damaged.

Bactericidal Activity

The activity of eight granulocyte concentrates collected by CFCL was normal (Table 2). Eighty-seven per cent of the bacteria in the assay system were killed during the 2-hr incubation, whereas in 12 concentrates collected by FL an average of 68% of the bacteria in the assay were killed compared with 78%-82% for the FL controls. This 10%-12% decrease in bacterial killing was consistent. Bacterial killing by FL granulocytes was statistically significantly less than the controls included with that assay ($p < 0.05$). The impaired killing was evident throughout the 2-hr assay. By 30 min only 32% of the bacteria remained in the assays using CFCL granulocytes, whereas 54% of the bacteria remained when FL granulocytes were used.

Harris et al. have suggested that differences in elution techniques could account for the differences in function of granulocytes collected by FL. Our elu-

Table 2. Bactericidal Activity of Granulocytes Collected by CFCL and FL

<table>
<thead>
<tr>
<th></th>
<th>CFCL Granulocytes</th>
<th>FL Granulocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Tested</td>
<td>Bacteria Killed at 2 hr (%)</td>
</tr>
<tr>
<td>Granulocyte concentrates</td>
<td>8</td>
<td>$87 \pm 5^*$</td>
</tr>
<tr>
<td>Donor before</td>
<td>8</td>
<td>$87 \pm 7$</td>
</tr>
<tr>
<td>Donor after</td>
<td>8</td>
<td>$85 \pm 6$</td>
</tr>
<tr>
<td>Fresh CPD control</td>
<td>6</td>
<td>$86 \pm 3$</td>
</tr>
</tbody>
</table>

$^*_{Average \pm SD.}$

$^{f}_{Granulocyte concentrate bacterial killing < donor before (p < 0.05)}.$
Table 3. Bactericidal Activity and Morphology of Granulocytes From Three Different Elution Fractions of the Same Filter Compared With Granulocytes Obtained Simultaneously From a Second Filter From the Same Donor

<table>
<thead>
<tr>
<th>Granulocyte Suspension</th>
<th>Granulocytes Eluted From Each Filter (%)</th>
<th>Bacteria Killed at 120 min (%)</th>
<th>Morphologically Normal Granulocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filter 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First 150 ml</td>
<td>52</td>
<td>80</td>
<td>83</td>
</tr>
<tr>
<td>Second 150 ml</td>
<td>31</td>
<td>65</td>
<td>63</td>
</tr>
<tr>
<td>Third 150 ml</td>
<td>17</td>
<td>55</td>
<td>61</td>
</tr>
<tr>
<td>Filter 2—500 ml</td>
<td>100</td>
<td>83</td>
<td>75</td>
</tr>
<tr>
<td>Donor before leukapheresis</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Normal control</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Elution technique was similar to that of Herzig et al. and used a solution of saline, ACD, and citrated plasma. To evaluate elution techniques, we also used the procedure suggested by Harris et al. in which the eluting solution was composed entirely of citrated plasma. In addition, we collected elution fractions as follows: elution from one of the pair of filters was collected in three parts each of approximately 150 ml each, and the elution from the second filter (500 ml) was collected together. A bactericidal assay was performed on each of these four granulocyte suspensions. Bacterial killing by control granulocytes and by the donors’ own granulocytes before FL was 91% and 92%, respectively (Table 3). The granulocytes eluted from filter two killed 83% of the bacteria and granulocytes in the first elution fraction from filter one killed 80% of the bacteria (Table 3). Granulocytes in the later elution fractions of filter one had more severe impairment of bacterial killing (Table 3) which was even more marked when the bactericidal assay was carried out for 180 min (Fig. 1). Morphologically, the granulocytes in the second and third elution fractions of filter one were more abnormal (Table 3). In the first elution fraction 83% of the granulocytes were normal, whereas only 61% and 63% were normal in the second and

![Fig. 1. Bactericidal activity of granulocytes from three different elution fractions of the same filter compared with granulocytes obtained simultaneously from a second filter from the same donor.](image)
third fractions. These findings suggested that granulocytes which were more loosely bound to the filters or possibly adherent to other granulocytes and not to the filters sustained little or no damage, whereas the most adherent granulocytes and the last to come off the filters were the most severely damaged.

Chemotactic Response

Chemotactic response to E. coli bacterial factor was studied in 23 granulocyte concentrates collected by CFCL and in 10 concentrates collected by FL. Both CFCL and FL granulocytes had normal chemotactic responses (Table 4). The chemotactic response of granulocytes collected by FL was severely impaired in the standard assay system using Medium 199. However, when fresh frozen heparinized plasma was used as the suspending medium for granulocytes, chemotaxis of FL cells was normal as shown in Table 4.

NBT Activity

Ten granulocyte concentrates collected by CFCL and eight collected by FL were assayed for NBT activity. The activity of both CFCL and FL granulocytes in the quantitative NBT assay was similar to the controls and was normal for this assay procedure (Table 5). The absolute values differed slightly for CFCL compared with FL cells because these studies were carried out at different times using different batches of NBT and other reagents.

Oxygen Consumption

Oxygen uptake in response to phagocytosis of latex particles was studied in 15 granulocyte concentrates collected by CFCL and in 13 collected by FL. Oxygen consumption of granulocytes collected by FL and CFCL was similar to their respective controls (Table 6). Although the oxygen uptake for CFCL and

<table>
<thead>
<tr>
<th>Table 4. Chemotactic Response of Granulocytes Collected by CFCL and FL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFCL Granulocytes</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>No. Tested</td>
</tr>
<tr>
<td>Granulocyte concentrates</td>
</tr>
<tr>
<td>Donor before</td>
</tr>
<tr>
<td>Donor after</td>
</tr>
<tr>
<td>Fresh CPD control</td>
</tr>
</tbody>
</table>

*HPF, High power field.
†Average ± SD.

<table>
<thead>
<tr>
<th>Table 5. Nitroblue Tetrazolium Activity of Granulocytes Collected by CFCL and FL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFCL Granulocytes</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>No. Tested</td>
</tr>
<tr>
<td>Granulocyte concentrates</td>
</tr>
<tr>
<td>Donor before</td>
</tr>
<tr>
<td>Donor after</td>
</tr>
<tr>
<td>Fresh CPD control</td>
</tr>
</tbody>
</table>

*Average ± SD.
Granulocyte Collection

Table 6. Oxygen Consumption Assay Activity of Granulocytes Collected by CFCL and FL

<table>
<thead>
<tr>
<th></th>
<th>CFCL Granulocytes</th>
<th>FL Granulocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Tested</td>
<td>μl/O2*</td>
</tr>
<tr>
<td>Granulocyte concentrates</td>
<td>15</td>
<td>12.64 ± 4.55 †</td>
</tr>
<tr>
<td>Donor before</td>
<td>14</td>
<td>9.60 ± 3.14</td>
</tr>
<tr>
<td>Donor after</td>
<td>15</td>
<td>10.41 ± 3.97</td>
</tr>
<tr>
<td>Fresh CPD control</td>
<td>7</td>
<td>11.47 ± 3.04</td>
</tr>
</tbody>
</table>

*Microliters of oxygen per 9 x 10⁶ granulocytes/hr.
†Average ± SD.

FL granulocytes was higher than the corresponding control, these values were not significantly different (p > 0.05).

Granulocytes used in the bactericidal, NBT, and oxygen consumption assays were washed and resuspended in balanced salt solutions or buffered saline. It is possible that damaged cells were lost in this washing process. Thus the abnormalities found in granulocytes collected by FL may be less severe than actually occurs in mixtures of these cells used for transfusion.

Post-Transfusion Recovery

Eight different donors underwent granulocyte collection by conventional phlebotomy and received a transfusion of their own radiolabeled granulocytes. The average recovery immediately following transfusion was 52% with a range of 25%–78% (Table 7). The average recovery of granulocytes collected by CFCL from six different donors was 34% (range 14%–68%), which was slightly less than for the phlebotomy-collected granulocytes. However, there was not a statistically significant difference between these two means. Only 16% (range 9%–21%) of the FL granulocytes were recovered immediately following transfusion. Post-transfusion recovery of granulocytes collected by FL was significantly decreased compared with the phlebotomy (p < 0.05) and CFCL (p < 0.05) cells.

Intravascular Survival

Granulocytes collected by conventional phlebotomy had a survival which followed a straight-line disappearance (Fig. 2) and appeared to be of a single component when plotted using the log 10 of the number of cpm. The average intravascular half-time for these cells was 3.8 hr (Table 7). Six granulocyte concentrates collected by CFCL also had a single component disappearance which

Table 7. Per Cent of Granulocytes Recovered and Their Intravascular Survival Following Autologous Transfusion of DF³²P-Labeled Cells

<table>
<thead>
<tr>
<th>Collection Method</th>
<th>No. of Transfusions</th>
<th>Recovery (%) Mean</th>
<th>Range</th>
<th>Half-Life (hr) Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phlebotomy</td>
<td>8</td>
<td>52</td>
<td>25–78</td>
<td>3.8</td>
<td>2.7–4.8</td>
</tr>
<tr>
<td>CFCL</td>
<td>6</td>
<td>34</td>
<td>14–68</td>
<td>3.0</td>
<td>2.0–4.6</td>
</tr>
<tr>
<td>FL</td>
<td>6</td>
<td>16</td>
<td>9–21</td>
<td>1.3*</td>
<td>0.9–1.8</td>
</tr>
</tbody>
</table>

*Half-life at 30 min.
†Half-life at 3 hr.
followed a straight line (Fig. 2). The average intravascular half-time of these cells was 3.0 hr (Table 7), which was not significantly shorter \((p > 0.05)\) than the half-time of granulocytes collected by phlebotomy.

Five of six granulocyte concentrates collected by FL had observed survival curves which were nonlinear and were approximated by a parabolic curve fitted by the method of least squares (Fig. 2). Because of the continuously changing slope of the parabolic curves a single half-life could not be calculated. Therefore, 30 min and 3 hr were arbitrarily chosen as points of interest for determination of half-times during the 6-hr period of study. The average half-time 30 min after transfusion was 1.3 hr and at 3 hr was 2.6 hr (Table 7). Both of these half-times were significantly less \((p < 0.05)\) than the half-times for granulocytes collected by phlebotomy, and the half-time at 30 min was significantly less \((p < 0.05)\) than the half-time of cells collected by CFCL.

**DISCUSSION**

Granulocyte transfusion is gaining widespread use in the management of infections in granulocytopenic patients. However, the effects of different collection techniques on the function of granulocytes has not been established. Alterations of function caused by collection techniques should be identified so that techniques can be improved if indicated. Also, alterations of function should be recognized and taken into consideration in planning and analyzing clinical studies of granulocyte transfusion.

Previous studies have shown that granulocytes collected by CFCL from patients with CML are normal in vitro when studied by light microscopy, trypan blue dye exclusion, and bactericidal and hexose monophosphate shunt activity.\(^1,2\) The data presented here, in combination with our previous report,\(^4\) establish that granulocytes collected from normal donors by CFCL show very slight morphological changes but have normal in vitro function. Hexose monophosphate shunt and chemotactic activity are normal, as is overall bacterial killing. The normal function of these granulocytes also suggests that the con-
centrations of hydroxyethyl starch used do not interfere with granulocyte function. Although we have not studied other concentrations of HES, these results confirm our previous observations.\textsuperscript{15}

Post-transfusion recovery of 52\% of the granulocytes collected by phlebotomy is similar to the ideal maximum of 50\%, allowing for margination of half of the transfused cells.\textsuperscript{21} The intravascular half-time of 3.8 hr for these granulocytes is somewhat shorter than reported by others. However, we have observed intravascular survivals of this duration in previous studies of other normal individuals,\textsuperscript{16} and similar values have been found by other investigators who considered them normal.\textsuperscript{22,23} Kauder et al.\textsuperscript{23} observed a range of 3.5-6.0 hr intravascular half-life in five normal autologous transfusions.

The 34\% recovery of CFCL granulocytes is slightly but not significantly ($p > 0.05$) less than the recovery of cells collected by phlebotomy and is further evidence that these cells are relatively unaffected by the collection process. The straight-line single-component disappearance pattern of granulocytes collected by CFCL indicates that there is a single population of cells and that these cells leave the circulation randomly in a manner similar to cells collected by phlebotomy.\textsuperscript{24} In addition, the straight-line single-component survival pattern suggests that if there is some minor cell damage in the process of collection damaged cells are removed almost instantaneously, accounting for the decreased recovery but apparent random removal of cells during the subsequent 6 hr. Thus it appears that granulocytes collected by CFCL function normally in vitro and almost normally during the intravascular component of their life span following transfusion.

The in vitro function of granulocytes collected by FL was normal, except for a slight but consistent and significant decrease in bacterial killing. Assays of hexose monophosphate shunt activity were normal, suggesting that membrane damage may occur when the granulocytes adhere to the nylon fibers during the collection process. This thesis was supported by the observation that granulocytes in the later elution fractions, which were probably more adherent to the nylon fibers, showed more severe defects in morphology and bacterial killing.

Granulocytes collected by FL have more morphological abnormalities than CFCL granulocytes. However, chemotactic response to bacterial factors remains normal despite impaired bacterial killing and the morphological alterations. Fehr et al.\textsuperscript{25} also have reported that granulocytes exposed to nylon fibers have a normal chemotactic response to bacterial factors. They report that granulocytes collected by FL undergo complement-mediated degranulation and have an impaired chemotactic response to activated complement components.

The average post-transfusion recovery of FL granulocytes in this study was 16\%, which was quite similar to the 10\% reported by de Fliedner et al.\textsuperscript{10} in two patients. The low post-transfusion recovery of FL granulocytes suggested that a significant proportion of these cells underwent some alteration during the collection process which resulted in almost immediate clearance following transfusion. De Fliedner et al.\textsuperscript{10} have shown that granulocytes in the first elution fraction from FL collections were morphologically normal, whereas cells from later elution fractions were morphologically abnormal. Our studies supported
this observation in that there were more morphological abnormalities and bacterial killing was impaired when the last elution fraction was compared with the first granulocytes eluted from the filters. Differences in function did not appear to be due to elution techniques, since these studies were carried out using the elution technique of Harris et al. Thus, the usual elution techniques in FL granulocyte collection appeared to produce a granulocyte concentrate containing mixtures of normal and abnormal cells.

If the post-transfusion recovery of granulocytes is plotted on a linear scale, the expected exponential decrease is seen with cells collected by phlebotomy and CFCL (Fig. 3). Granulocytes collected by FL appear to have more of a two-component survival. The first component lasts approximately 1 hr, and during that time the cells have a half-life of approximately 1.3 hr. This time is somewhat longer than reported by Graw et al. but very similar to the 1.25 hr reported by Herzig et al. However, when these results are plotted on a log scale, the intravascular survival of FL granulocytes best fits a parabolic curve, rather than two different straight lines reported by others. We have been unable to identify a single point in these survival plots where the lines clearly change slope to separate the two components. A two-component linear survival plot implies two distinct but homogenous populations of cells. However, a parabolic curve seems more realistic, since it is likely that the FL granulocyte concentrate contains cells with a spectrum of alterations ranging from none to severe.

We did not administer epinephrine to any of the donors in this study in order to determine if the noncirculating granulocytes collected by FL could be released from the marginal granulocyte pool. Thus the fate of these cells remains unknown. However, the intravascular survival studies were carried out for 6 hr, and no increases in radioactivity that would have suggested release of margi-nated cells were observed during that time.

Data presented here indicate that granulocytes collected by CFCL have normal in vitro function and a slight but insignificant reduction in post-transfusion recovery and intravascular survival. Clinical studies indicate that transfusions
of granulocytes collected by CFCL are valuable in the treatment of gram-negative sepsis. Granulocytes collected by FL function normally in vitro, except for a slight reduction in bactericidal activity. These cells have a markedly reduced post-transfusion recovery and a very short intravascular half-life, and they have been shown by Fehr et al.25 to be coated with complement components. Reactions are more common following transfusion of granulocytes collected by FL than by CFCL.6 Whether or not all of these abnormalities interfere with the clinical effectiveness of granulocytes collected by FL remains to be established. Two reports67 indicate that FL granulocytes are clinically helpful. FL clearly produces many more granulocytes for transfusion than CFCL techniques. It may be that alterations in FL granulocytes are sufficiently mild that they are compensated for by the much larger number of cells available for transfusion. It seems clear, however, that these cells are not normal. Further studies are required to elucidate the lesion in granulocytes collected by FL and the fate in vivo of the transfused cells which are not recovered in the circulation.

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In vitro function and post-transfusion survival of granulocytes collected by continuous-flow centrifugation and by filtration leukapheresis

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