Blood Cell Separation in the Dog by Continuous Flow Centrifugation

By Dean Buckner, Robert Eisel and Seymour Perry

There is evidence that homologous granulocytes are beneficial in the treatment of bacterial infection when supplied to a granulocytopenic recipient. Control studies have not been done but Freireich et al. and Schwarzenberg et al. have demonstrated lysis of fever, and clinical improvement in granulocytopenic patients given granulocytes from donors with chronic myelocytic leukemia (CML). Cross circulation studies in animals and man suggest that therapeutic benefit is derived from the transfer of large quantities of leukocytes. It has been estimated that $1 \times 10^{11}$ CML granulocytes per m$^2$ of body surface area are needed for an effective therapeutic transfusion. Unfortunately, it is impractical to attempt to obtain this number of normal granulocytes routinely by present technic since more than 40 units of normal blood would be required. However, this estimate may be too high and data from cross circulation studies indicate that fewer normal leukocytes may be effective.

Current methods for platelet procurement are also difficult and laborious although adequate quantities may be obtained for therapeutic purposes. Finally, the procurement of large numbers of lymphocytes is desirable for experimental and perhaps therapeutic purposes.

The National Cancer Institute and the International Business Machines Corporation initiated a joint project in 1964 in an attempt to develop a machine to separate the various cellular components of the blood on a continuous in vivo basis and to return plasma and red blood cells to the donor.

In the studies reported initially, the separation of leukocytes from bank blood was moderately efficient with yields of 30–60 percent at flow rates up to 100 ml./min. However, attempts at continuous flow separation in patients with leukemia and lymphoma were unsuccessful with leukocyte yields of less than 20 percent. Subsequently, there have been many changes in the blood cell separator with an increase in efficiency and reliability.

The present studies in dogs were initiated in an attempt to evaluate blood cell separation by the present NCI-IBM Blood Cell Separator and to determine the effects of the centrifugation procedure on the donor and on the viability of processed blood components.
The purpose of this report is to present the results of continuous flow cell separation in dogs utilizing the NCI-IBM Blood Cell Separator.

**Materials and Methods**

**Preparation of Donor**

*Shunts.* Indwelling silastic-teflon arterio-venous shunts* were placed between the carotid artery and jugular vein of mongrel dogs, weighing between 10 and 24 kg., as previously

*Extra Corporeal and Medical Specialties Co., Inc., Medford, New Jersey, or Swedish Freezer Corporation, Seattle, Washington.*
BLOOD CELL SEPARATION

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ACKED RED BLOOD CELLS
BLOOD BUFFY COAT PLASMA

Fig. 2.—Diagrammatic cross section of Centrifuge Bowl.

described. Most studies were performed on unanesthetized dogs 1–4 days following surgery.

Vein to vein. Teflon cannulae* were inserted into a jugular vein and a forelimb vein of
anesthetized dogs and tubing connections were made in a similar manner as for dogs with
indwelling shunts.

Anticoagulation. The donor dog was pretreated with 5,000 units of heparin i.v. followed
by an infusion of heparin into the arterial line at a rate sufficient to maintain anticoagulation
(approximately 3,000 units/hr.). Cells were collected in 5 ml. ACD (NIH formula A).

Description of the NCI-IBM Blood Cell Separator (Fig. 1)

Two prototype machines have been developed, one for use in patients on a vein to vein
basis† and the other, used in the present studies, for processing bank blood and for animal
use. The centrifuge for patient use is basically the same as that described below with the
addition of a buffer bag and multiple safety features.

Components. The essential components of the blood cell separator are: (1) the centrifuge
bowl; (2) the face seal; and (3) the pumps and tubing connections.

Centrifuge bowl. The bowl consists of three separate parts made of polycarbonate
(Fig. 2): (1) a transparent top containing the entrance port for whole blood and the exit ports
for separated components; (2) an inner core which is solid except for the entrance passage
for whole blood through its center and (3) an outer shell. The effective radius is 2.5 inches
and the depth is 4 inches with the distance between the inner core and the sides and bottom
of the shell being 0.040 inches. The bowl rests in an enclosed fly wheel which is driven by a
D.C. motor capable of producing 350 g. (2200 r.p.m.) of centrifugal force at 2.5 inches
radius.

Seal. The most important part of the machine is the face seal (Fig. 3) through which
whole blood and separated components must pass. It is composed of two matching parts:
(1) an upper stainless steel portion which is stationary and (2) a lower portion composed of
synthetic plastic† or ceramic which rotates with the centrifuge bowl. The upper portion

*Becton, Dickinson and Company, Rutherford, New Jersey.
†Rulon, Dixon Corporation, Briston, Rhode Island.
contains the tubing connections for all seven of the input and output ports: (1) whole blood, (2) buffy coat, (3) plasma, (4) packed red blood cells, (5) seal lubrication, (6) outer ring irrigation entrance and (7) outer ring irrigation exit. The seal is designed to keep components separate as they enter and exit through the rotating face seal, and this is accomplished by concentric matching channels in each half of the seal for each blood component. These channels are separated by concentric lands containing small grooves into which saline is forced under pressure (10-12 psi) at a flow rate of 20 ml/hr. The flow of saline from these grooves into the channels effectively prevents the migration of cells from the channels to the contacting surfaces of the seal. The outer groove is irrigated with saline which cools the seal and presents a barrier to the entrance of air. The grooves and lands in the bottom half appear to be unnecessary and in recent studies a flat ceramic seal has been used successfully.

**Pumps and tubing connections.** Peristaltic roller pumps are used to pump plasma and red cells back to the donor dog, buffy coat cells into a collection bag, anticoagulant into the arterial input line and saline into the seal for lubrication. They are mounted on the cabinet top to the rear of the centrifuge bowl with their controls and flow rate meters located on the front (Fig. 1). These pumps can be modified, with plastic adapters, to accommodate tubing from .010 to .125 inches I.D., permitting flow rates from 0.1 ml/min. to greater than 100 ml/min.

Tubing connections are fabricated by hand from polyvinyl chloride tubing, nylon fittings, 3-way stopcocks, solution infusion sets and polyethylene tubing.

The bowl and tubing parts can be sterilized with ethylene oxide, but sterilization was not performed for these studies.

**Operation and Function of the NCI-IBM Blood Cell Separator**

The centrifuge bowl and tubing are primed with heparinized saline (5 units/ml.) and all air is removed. The arterial portion of the A-V shunt, or a venous cannula in a vein to vein procedure, is connected to the input line and whole blood enters the machine via a stainless steel fitting on the upper half of the face seal (Fig. 2 and 3). The blood then passes through...
the seal and downward through the center of the solid inner core. The flow path is from the center across the bottom, up the sides of the bowl and into the flared portion at the top. The process of separation occurs during the upward flow of blood with red blood cells collecting in the outer portion of the flared area, plasma in the central area, and buffy coat cells at the plasma-red cell interface.

There are three collecting ports in the transparent cover: one at the outer wall which continuously collects from the bottom of the packed red cell layer, one at the inner wall which collects from the top of the plasma layer and an intermediate port for buffy coat collections at the plasma-red cell interface. Separated components exit through the rotating face seal and the rate of removal is controlled by individual peristaltic pumps. Buffy coat cells can be removed continuously or allowed to accumulate in the bowl and removed intermittently. Red blood cells and plasma are recombined after passing through the pumps and the resulting buffy coat poor blood is returned to the donor.

The total volume of the bowl and tubing is 220 ml., and by flushing the system with saline at the end of a centrifugation, all but 20–25 ml. of packed red blood cells can be returned to the donor with insignificant loss of plasma.

**METHODS**

White blood cell counts were performed in duplicate with an electronic counter. Samples were diluted with saline and red blood cells were lysed with saponin. All counts below 1,000 per mm. were repeated in duplicate by the chamber technic. White blood cell differential counts were done utilizing standard technics. Polymorphonuclear leukocytes, bands, basophils and eosinophils were recorded as granulocytes; lymphocytes, mononuclear cells and atypical forms were recorded as mononuclears. Packed cell volumes were determined in duplicate utilizing a microcapillary centrifuge. Platelet counts were done in duplicate by phase microscopy. Plasma hemoglobin levels were measured by the benzidine method on plasma from arterial blood prior to a centrifugation and then repeated on plasma from the plasma line at the end of the procedure. Aliquots of arterial blood for baseline studies were collected in siliconized tubes and centrifuged at room temperature.

Granulocyte kinetic studies with tritiated diisopropylfluorophosphate ($^3$H-DFP). Buffy coat collections were incubated with 100 μCi of $^3$H-DFP for 45 minutes and transfused into normal dogs over a 5–20 minute period. One infusion of labeled autologous cells was performed. White blood cells were isolated and the radioactivity determined in a liquid scintillation spectrometer by a method previously reported from this laboratory. The fraction of transfused cells appearing in the circulating leukocyte pool was determined by the method of Mauer et al. and the sample with the highest post transfusion specific activity (counts per $10^8$ granulocytes) was plotted as 100 percent.

$^{51}$Cr red blood cell survival. Blood samples, anticoagulated with heparin (5-10 units/mL), were obtained from the packed red blood cell line at the termination of a centrifugation for red blood cell survival studies. Ten ml. aliquots were incubated at room temperature for 30 minutes with 25 μCi. $^{51}$Cr. Ascorbic acid, 100 mg., was then added and 15 minutes later the labeled blood was reinfused. Five ml. whole blood samples were drawn at 15 minutes and at appropriate intervals thereafter and counted simultaneously at the termination of the study in a gamma spectrometer. A control red blood cell survival was also performed in a dog whose blood was not centrifuged.

Buffy coat infusions into dogs with bone marrow aplasia. Bone marrow aplasia was produced in six dogs by a single intravenous dose of 75 mg./kg. of cyclophosphamide. These dogs then received a total of 21 separate buffy coat infusions between day

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* Coulter Electronics, Hialeah, Florida.
† International Equipment Company, Needham Heights, Massachusetts.
¶ Cytoxan, Mead Johnson Laboratories, Evansville, Indiana.
and 11 following the administration of cyclophosphamide. Donor and recipient pairs were matched for canine red cell antigen A.6

Prolonged continuous centrifugation. Nineteen experiments of 12 hours duration were carried out to determine the effects of the centrifugation procedure or an equivalent period of heparin infusion on circulating platelet levels. Shunts were placed in these dogs 1–3 days prior to study. Heparin (5,000 units) was given i.v. prior to the study and was infused into the arterial line at rates of 80–508 units per kg. per hour during the study in all animals. Five groups of dogs were studied. Group A (5 dogs): 220 ml. of whole blood was collected in 30 ml. of ACD (NIH formula A) from the arterial cannula with simultaneous infusion of 220 ml. of saline into the venous cannula. Heparin was infused for 12 hours through the arterial shunt but the dogs were not attached to the centrifuge. The blood was reinfused at the end of the 12-hour period. Group B (4 dogs): Dogs were connected to the centrifuge as before and blood was pumped through the centrifuge system, without application of centrifugal force during the 12-hour period of study. Group C (3 dogs): These animals were connected to the centrifuge as in Group B but blood was centrifuged for 12 hours at low speed (250–500 r.p.m., 5–20 g.) anduffy coat was returned to the donor continuously via a white blood cell line to venous return line connection. Group D (4 dogs): These animals were similar to Group C except that blood was centrifuged at a higher centrifugal force (1100–1700 r.p.m., 80–200 g). Group E (3 dogs): Studies in this group were similar to those in Group D except thatuffy coat was removed.

Phagocytosis. Phagocytosis was assessed by incubating buffy coat cells processed by the NCI-IBM blood cell separator with heat-killed staphylococci. Three drops of the bacterial suspension (6 × 10^8 particles per ml.) were added to tubes containing 10 drops each of buffy coat cells and heparinized peripheral blood cells. The white blood cell concentration of the buffy coat preparation was adjusted to that of the peripheral blood control by the addition of autologous plasma. The control and test cells were incubated simultaneously at 37 C. for 15 minutes and then smears were made on clean glass slides. After staining with Wright's, one hundred consecutive granulocytes (excluding eosinophils and basophils) were counted and the percentage containing staphylococci determined.

Lymphocyte culture with phytohemagglutinin. Lymphocytes from peripheral blood and from buffy coat collections were obtained and incubated for three days with phytohemagglutinin.13,14 One thousand cells were counted at the end of incubation and the percentage of small lymphocytes, medium lymphocytes, transformed lymphocytes, mitoses and dead cells determined.

Calculations

Dog blood volume was estimated to be 80 ml./kg.15 The quantity of circulating leukocytes and platelets was determined by multiplying the estimated blood volume by the number of cells per ml. The number of cells collected or infused was calculated by multiplying counts per ml. of the sample by the volume (by weight). The number of blood volumes centrifuged was ascertained by dividing the volume of blood centrifuged by the estimated blood volume of the donor. The number of blood volumes cleared of leukocytes or platelets was determined by dividing the estimated cells in circulation at T. by the quantity of cells collected. The percent yield of cells was calculated by dividing the number of cells that entered the centrifuge by the number collected. The number of cells entering the centrifuge was estimated by multiplying the mean arterial counts per ml. by the volume of blood centrifuged. Post transfusion increment was determined by subtracting the

*Canine red cell antisera prepared by Dr. Raymond D. Zinn, Division of Research Services NIH, and typing was performed by Miss Coralyn Colladay, Experimental Surgery Section, NIH.
pre-transfusion count from the post-transfusion count (per mm.³). The percent recovery of transfused cells was determined by multiplying the post-transfusion increment per ml. by the estimated blood volume and dividing by number of cells infused.

RESULTS

One hundred fifty-five separate centrifugations were performed utilizing 75 dogs. The results of 83 separate centrifugations are considered in detail in this report. The first 40 centrifugations and 12 vein to vein procedures are omitted as they were developmental studies and data were often incomplete. Fifteen studies were performed for purposes unrelated to cell collection and five later studies were excluded because of technical problems that occurred during centrifugation. Fifty of the reported centrifugations were performed in order to collect large quantities of leukocytes and platelets in a small volume and with minimal red blood cell contamination. Ten centrifugations were performed in an attempt to procure large quantities of lymphocytes free of other cellular components. Nine centrifugations were performed to collect granulocytes with a low concentration of platelets by leaving platelets suspended in the plasma. Fourteen prolonged centrifugations were performed to study platelet viability.

Effects of the Procedure on Donor Animals

Most donor dogs tolerated the procedure very well and had no major changes in heart rate, respiration or temperature. Shivering was occasionally seen and could be prevented by warming the returning blood.

Seventy of the 75 dogs used in these studies recovered and most have subsequently been used in other experiments. Five dogs died following the centrifugation procedure: one each of infection, hemorrhage, carotid artery thrombosis, pulmonary edema and unknown cause.

Results of Cell Separation and Collection

Plasma separation. The separation of red blood cells from plasma in this system was extremely efficient, occurring in some dogs at 250 r.p.m. (5 g.) and in most dogs at 600 r.p.m. (25 g.) at flow rates of 40–50 ml./min.

Hematocrit determinations on samples from the packed red blood cell line during centrifugation at 800-1200 r.p.m. (40–100 g.) averaged 71 volumes percent (61–85) as compared to 36 (29–49) volumes percent for arterial blood. A few red blood cells were present in the plasma when centrifugations were performed at low speed, i.e., under 600 r.p.m. Clean plasma separation was accomplished at plasma flow rates of up to 100 ml./min. (total flow of 200 ml./min.) by increasing the g. force up to 350 (2200 r.p.m.).

Buffy coat collection. In 50 buffy coat collections (Table 1), an attempt was made to collect large quantities of granulocytes, lymphocytes and platelets in a small volume with as little red blood cell contamination as possible. A large proportion of the centrifuged granulocytes was present in the upper portion of the packed red blood cell layer. It was not possible to collect large quantities of granulocytes without also collecting red blood cells.
Table 1.—Buffy Coat Collections—50 Centrifugations

<table>
<thead>
<tr>
<th></th>
<th>White Blood Cells</th>
<th>Granulocytes</th>
<th>Mononuclear Cells</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total collected $\times 10^9$</td>
<td>24.0</td>
<td>17.0</td>
<td>7.0</td>
<td>197.0</td>
</tr>
<tr>
<td>Bag counts $\times 10^3$ mm.³</td>
<td>223.1</td>
<td>71.0%</td>
<td>29.0%</td>
<td>1,850.0</td>
</tr>
<tr>
<td>and differential</td>
<td>(6.9-55.1)</td>
<td>(4.3-38.6)</td>
<td>(7.7-18.7)</td>
<td>(50.7-810.0)</td>
</tr>
<tr>
<td>Yield %</td>
<td>34.0</td>
<td>28.0</td>
<td>66.0</td>
<td>36.0</td>
</tr>
<tr>
<td>No. B. V. cleared‡</td>
<td>1.0</td>
<td>.8</td>
<td>1.8</td>
<td>.9</td>
</tr>
<tr>
<td>Collection volume (ml.)</td>
<td>117</td>
<td>(35.0-310.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit of collection (Vol. %)</td>
<td>14.0</td>
<td>(3.0-33.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time of centrifugation (min.)</td>
<td>146</td>
<td>(75-240)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R.P.M.</td>
<td></td>
<td></td>
<td></td>
<td>(800-1500)</td>
</tr>
<tr>
<td>Force (g.)</td>
<td></td>
<td></td>
<td></td>
<td>(40-160)</td>
</tr>
<tr>
<td>Flow rate (ml./min.)</td>
<td></td>
<td></td>
<td></td>
<td>40 (30-70)</td>
</tr>
<tr>
<td>Total volume centrifuged (L.)</td>
<td>8.3</td>
<td>(3.0-13.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. B. V. centrifuged</td>
<td></td>
<td></td>
<td></td>
<td>4.8 (2.0-15.8)</td>
</tr>
</tbody>
</table>

*Mean.
†Brackets indicate range.
‡Fraction of original quantity of cells in circulating pool collected.

Table 2.—Comparison of Low and High Speed Centrifugation for the Collection of Buffy Coat

<table>
<thead>
<tr>
<th>R.P.M.</th>
<th>500-600</th>
<th>1000-1200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collection vol. (ml.)</td>
<td>134 (88-164)†</td>
<td>83 (62-122)</td>
</tr>
<tr>
<td>Hematocrit (Vol. %)</td>
<td>26 (16-40)</td>
<td>18 (9-28)</td>
</tr>
<tr>
<td>WBC ($\times 10^9$ per mm.³)</td>
<td>119 (72.0-193.0)</td>
<td>303.0 (183.0-414.0)</td>
</tr>
<tr>
<td>Platelets ($\times 10^9$ per mm.³)</td>
<td>551 (128.0-1,600.0)</td>
<td>1,800.0 (917.0-3,800.0)</td>
</tr>
<tr>
<td>Leukocytes collected ($\times 10^9$)</td>
<td>15.6 (6.4-28.4)</td>
<td>25.3 (15.0-40.9)</td>
</tr>
<tr>
<td>Granulocytes collected ($\times 10^9$)</td>
<td>13.0 (5.1-22.7)</td>
<td>19.6 (11.5-34.4)</td>
</tr>
<tr>
<td>Lymphocytes collected ($\times 10^9$)</td>
<td>2.6 (0.6-7.0)</td>
<td>5.7 (3.3-7.0)</td>
</tr>
<tr>
<td>Platelets collected ($\times 10^9$)</td>
<td>67.2 (13.5-191.4)</td>
<td>143.5 (64.4-288.8)</td>
</tr>
<tr>
<td>Yields %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>39.2 (17.0-57.7)</td>
<td>39.2 (24.5-49.6)</td>
</tr>
<tr>
<td>Platelets</td>
<td>5.4 (1.5-10.8)</td>
<td>44.0 (27.0-76.4)</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>40.2 (17.8-49.6)</td>
<td>35.2 (19.7-48.4)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>36.7 (11.0-89.0)</td>
<td>69.0 (46.2-100.0)</td>
</tr>
</tbody>
</table>

*Mean.
†Brackets indicate range.

Buffy coat collections at lower g. forces (25 g.) were carried out in three dogs for two hours per day on three successive days. In these studies, it was attempted to keep platelets suspended in the plasma while collecting leukocytes. At this speed, some red cells remained in the plasma. Plasma platelet counts averaged 140 percent (83-200), and plasma white blood cells (predominantly lymphocytes) averaged 29 percent (0-75) of simultaneous arterial counts. Table 2 and Figure 4 show the results of these nine centrifugations as compared to nine centrifugations carried out in the same manner in three dogs.
Fig. 4—Percent change in peripheral platelet level at low g. (●—●) as compared to high g. centrifugation (○—○) with the collection of buffy coat. Shaded areas represent centrifugation periods.

at increased g. force, i.e., 1000–1200 r.p.m. (70–100 g.). At this speed, the plasma is virtually cell free.

Lymphocyte collection. In these ten collections (Table 3), the object was to collect lymphocytes with little or no red blood cell contamination and with minimal granulocytes. The only major variable in these studies compared to the buffy coat collections was that cells were collected from the top of the buffy coat layer. Accordingly, there were fewer red blood cells and granulocytes, but platelet contamination was unavoidable.

Although there was no systematic attempt to collect platelet-rich plasma, the results of low g. centrifugation indicate that this is feasible.

Effects of the Centrifugation Procedure on Processed Cells

Red Blood Cells

Hemolysis. Gross hemolysis was rarely detected in the separated plasma during 3–4 hours of centrifugation and when present could usually be accounted for by malfunction of the face seal. The number of these episodes
Table 3.—Lymphocyte Collections—10 Centrifugations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min.)</td>
<td>*140 (60-180)†</td>
</tr>
<tr>
<td>R.P.M.</td>
<td>(800-1200)</td>
</tr>
<tr>
<td>Force (g.)</td>
<td>(40-105)</td>
</tr>
<tr>
<td>Flow rate (ml/min.)</td>
<td>(30-60)</td>
</tr>
<tr>
<td>Total volume centrifuged (L.)</td>
<td>5.9 (2.4-10.8)</td>
</tr>
<tr>
<td>No. B. V. centrifuged</td>
<td>3.9 (2.2-7.7)</td>
</tr>
<tr>
<td>Collection volume (ml.)</td>
<td>112 (35-310)</td>
</tr>
<tr>
<td>Hematocrit (Vol. %)</td>
<td>5.0 (1.0-15.0)</td>
</tr>
<tr>
<td>WBC ($\times 10^3$ per mm$^3$)</td>
<td>104.1 (40.6-152.0)</td>
</tr>
<tr>
<td>Platelets ($\times 10^3$ per mm$^3$)</td>
<td>2,329 (1,280-4,450.0)</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>71.0 (57.0-100.0)</td>
</tr>
<tr>
<td>Granulocytes (%)</td>
<td>29.0 (0.0-43.0)</td>
</tr>
<tr>
<td>Lymphocytes collected ($\times 10^6$)</td>
<td>7.4 (3.4-18.9)</td>
</tr>
<tr>
<td>Lymphocyte yield (%)</td>
<td>48.2 (13.9-100.0)</td>
</tr>
<tr>
<td>No. B. V. lymphocytes cleared</td>
<td>2.0 (0.6-6.1)</td>
</tr>
</tbody>
</table>

*Mean.
†Brackets indicate range.

Fig. 5.—Autologous $^{51}$Cr red blood cell survivals in four dogs. Curves A, B, and C (●●●●) represent disappearance of centrifuged red blood cells and D (● - - - ●) is a control.

decreased with increasing experience in operating the centrifuge and were rare in the later studies. Plasma hemoglobin determinations were performed on samples obtained from 14 separate centrifugations of 2–4 hours duration at 800–1400 r.p.m. (40–150 g.). Control pre-centrifugation arterial samples collected in siliconized tubes and centrifuged at room temperature had an
Table 4.—Pre- and Post-Centrifugation Peripheral Blood Counts of Donor Animals in 50 Studies Where Buffy Coat Collections Were Made

<table>
<thead>
<tr>
<th></th>
<th>Pre $\times 10^3$ mm.$^3$</th>
<th>Post $\times 10^3$ mm.$^3$</th>
<th>Decline $\times 10^3$ mm.$^3$</th>
<th>Per cent Decline</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>22.5</td>
<td>9.6</td>
<td>12.9</td>
<td>57.3</td>
</tr>
<tr>
<td></td>
<td>(9.3–33.6)</td>
<td>(2.5–19.8)</td>
<td>(0.4–29.6)</td>
<td>(3.0–88.0)</td>
</tr>
<tr>
<td>Platelets</td>
<td>209.1</td>
<td>81.5</td>
<td>127.6</td>
<td>61.0</td>
</tr>
<tr>
<td></td>
<td>(24.0–420.0)</td>
<td>(12.5–340.0)</td>
<td>(11.5–310.0)</td>
<td>(32.8–90.7)</td>
</tr>
</tbody>
</table>

*Mean.
†Brackets indicate range.

Table 5.—Summary of 21 Buffy Coat Infusions into Dogs with Cyclophosphamide Induced Marrow Aplasia

<table>
<thead>
<tr>
<th>Infused cells $\times 10^8$</th>
<th>Granulocytes $\times 10^4$</th>
<th>Mononuclear Cells $\times 10^3$</th>
<th>Platelets $\times 10^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>*21.0</td>
<td>15.0</td>
<td>6.0</td>
<td>170.0</td>
</tr>
<tr>
<td>(6.0–39.0)</td>
<td>(5.1–32.0)</td>
<td>(1.0–11.0)</td>
<td>(33.7–386.0)</td>
</tr>
<tr>
<td>Increment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.1</td>
<td>2.7</td>
<td>0.4</td>
<td>79.0</td>
</tr>
<tr>
<td>(0.2–8.0)</td>
<td>(0.2–8.0)</td>
<td>(0–8)</td>
<td>(0–197.0)</td>
</tr>
<tr>
<td>Percent recovery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.0</td>
<td>24.0</td>
<td>5.6</td>
<td>50.0</td>
</tr>
<tr>
<td>(2.0–34.4)</td>
<td>(3.0–54.0)</td>
<td>(0–19.0)</td>
<td>(0–90.0)</td>
</tr>
</tbody>
</table>

*Mean.
†Brackets values indicate range.

average plasma hemoglobin level of 8.9 mg. percent (5.3–16.7) while plasma line samples taken at the termination of study and not further processed had a mean value of 3.1 mg. percent (0.0–9.0).

In the 12-hour centrifugation studies, gross hemolysis in the plasma line was noted after approximately 9–10 hours of centrifugation at 1400 and 1700 r.p.m. (150–200 g.) at which time approximately 20 donor blood volumes had been processed. However, at speeds of 250–1100 r.p.m. (5–100 g.) visible increases of hemoglobin in the plasma line were not detected during 12 hours of centrifugation.

Survival. $^{51}$Cr red blood cell survivals were performed on cells obtained from the packed red blood cell line at the termination of three separate centrifugations of three hours duration. During these three centrifugations, 9.0–12.6 liters of blood were processed representing 6.5–9.0 donor blood volumes. The results of these three studies and one control study are shown in Figure 5.

Leukocytes

Changes in white blood cell count. Table 4 shows the changes in peripheral white blood cell counts in donor dogs before and after centrifugation in 50 studies (Table 1) where buffy coat collections were made. Leukocyte counts returned to pre-centrifugation levels within four hours after cessation of the study.

Buffy coat infusions into leukopenic dogs. The results of 21 buffy coat infusions into dogs with cyclophosphamide-induced marrow aplasia are shown in Table 5. The pre-transfusion, 1–4 hour post-transfusion and 24-hour post-
transfusion white blood cell counts were 0.3 (0–1.0), 3.2 (0.7–8.5) and 0.4 (0.05–1.9) $\times 10^9$ per mm.$^3$, respectively. Figure 6 shows the white blood cell counts following five separate infusions where sufficient samples were taken to define the rate of disappearance. Granulocytes were primarily responsible for the increment since recovery of lymphocytes in these studies was low with rapid disappearance from the recipient’s circulation. The survival of dogs given an LD 100 dose of cyclophosphamide and treated with buffy coat cells is the subject of further investigation. However, three of the six dogs given buffy coat infusions survived while nine control dogs died.

Recovery and survival. Seven buffy coat collections were labeled with $^3$H-DFP and infused into homologous recipient dogs. One collection was labeled and reinfused into the donor. The mean number of granulocytes (excluding basophils and eosinophils) infused was 6.8 (1.0–14.2) $\times 10^9$. Figure 7 shows seven of the eight granulocyte disappearance curves. In one study, there was no radioactivity detected in the peripheral blood samples. In five of the studies, the mean percent recovery of granulocytes was 34.8 (21.7–62.1). The T-$\frac{1}{2}$ disappearance of three of the seven curves was between four and six hours.

Fig. 6.—White blood cell counts following transfusion of buffy coat cells into leukopenic dogs with cyclophosphamide-induced marrow aplasia.
and the remaining four curves were too complex for the determination of a single T-1/2.

Phagocytosis. The ability of collected granulocytes to ingest heat-killed staphylococci was tested in ten paired studies. The mean percentage of granulocytes demonstrating phagocytosis was 96 (88–100) for cells processed by the NCI-IBM Blood Cell Separator as compared to 94 (75–100) for control samples.
Table 7.—Characteristics of 19 Separate 12-Hour Experiments to Determine The Effects of Centrifugation on Platelets

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*Mean and 95 percent confidence limits for values obtained during and one hour following centrifugation.
†Mean.
‡Brackets indicate range.
Fig. 8.—Percent change in peripheral platelet levels during and following nineteen 12-hour centrifugations and/or heparin infusions. Group A (●—●—●): Phlebotomy of 220 ml. followed by 12-hour heparin infusion with reinfusion of blood. Group B (● — ●): Blood pumped through bowl and tubing without centrifugation. Group C (●—●—●): Centrifugation at 250–500 r.p.m. with continuous return of buffy coat to donor. Group D (●—●): Centrifugation at 1100–1700 r.p.m. with continuous return of buffy coat to donor. Group E (●—●): Centrifugation at 1100–1800 r.p.m. with collection and removal of buffy coat.

Lymphocytes

Table 6 presents the results of 12 three-day cultures with phytohemagglutinin. The mean and 70 percent confidence limits of differences in number of mitoses and transformed cells between peripheral blood and buffy coat samples were 2.1 ± 2.3 percent.

Platelets

Changes in platelet count. Table 4 shows the changes in peripheral platelet counts before and after centrifugation in 50 studies where buffy coat collections were made. Platelet counts did not return to pre-centrifugation levels for 2–3 days after removal of significant numbers of platelets.

Table 7 and Figure 8 present the conditions and results of 19 12-hour...
experiments carried out to ascertain the effects of the centrifugation procedure on platelets. One dog was excluded from group C because of seal malfunction during the experiment with resultant hemolysis, thrombocytopenia and leukopenia. The post-centrifugation platelet curve in group E represents the platelet recovery of a single dog as one dog of the group died on day 4 and appropriate platelet counts were not obtained for the other dog. The difference between the mean percent platelet decrease during the 12-hour experiments where no g. force was exerted (group B) and heparin infusion (group A) was significant (p < .05, p > .01). The difference between the mean percent platelet decrease during centrifugation at high g. (group D) as compared to low g. (group C) was significant (p < .01).

Survival. The results of 21 infusions of buffy coat containing platelets into dogs with cyclophosphamide-induced marrow aplasia are shown in Table 5. The pre-transfusion, 1-4 hour post-transfusion and 24-hour post-transfusion platelet counts were 124.0 (35.0–427.0), 214.0 (53.5–505.0), and 120.0 (36.5–427.0) × 10^9 per mm.³, respectively. Survival of transfused platelets could not be estimated as endogenous platelets were decreasing at an unknown rate.

**Discussion**

The NCI-IBM Cell Separator is the first centrifuge to accomplish separation and collection of cellular components from blood on a continuous flow basis without requiring changes in gravitational field or interruption in blood flow. Other centrifuges exist, notably the modification of the Cohn fractionator,¹ which separate and allow collection of various cellular and non-cellular components from blood but these utilize batch processing technics. The NCI-IBM blood cell separator allows the preferential collection or return to the donor of packed red blood cells, platelet-rich or platelet-poor plasma, buffy coat, buffy coat with a preponderance of either lymphocytes or granulocytes or buffy coat with a high or low platelet concentration.

The unique engineering feature of the NCI-IBM blood cell separator is the rotating face seal through which whole blood and separated components pass without apparent damage. An essential feature of this seal is the saline lubrication system which prevents the intrusion of cells between the contacting surfaces. Hemolysis of red blood cells and platelet and leukocyte destruction was a major problem prior to the introduction of the saline lubrication system. Some hemolysis probably occurs, but this is relatively insignificant as indicated by plasma hemoglobin levels, ⁵¹Cr red blood cell survivals, and observations made during prolonged centrifugation. Granulocytes appear to be relatively undamaged as determined by phagocytic ability, ³H-DFP labeling studies, and transfusion into granulocytopenic dogs. Lymphocytes undergo transformation and mitosis in response to phytohemagglutinin. Gross platelet clumping invariably occurs when platelets are collected in buffy coat preparations and this can usually be reversed by resuspension in ACD (NIH formula A). Transfusion of these platelets into cyclophosphamide treated but not severely thrombocytopenic dogs results in an increment with a variable recovery rate in most recipients. The survival of platelets could not be accurately estimated
in these animals since endogenous platelets were decreasing at an unknown rate.

Prolonged (12 hour) experiments were performed to evaluate the effect on circulating platelet levels of heparin infusion (Group A), pumping blood through the system without the application of centrifugal force (group B), low (group C) and high (group D) speed centrifugation, and centrifugation at high speed with the continuous collection of buffy coat (group E) (Table 7, Figure 8). Sampling constituted the only external loss of platelets in the first four groups. These studies show that platelet levels are decreased by pumping blood through the system and that further decrements occur when high speed separation is performed. The comparison of low versus high speed separation indicates that platelet levels are maintained better when separation is carried out at low centrifugal forces. Decreases in platelet levels are most likely the result of both mechanical and chemical factors. Mechanical aggregation of platelets during centrifugation probably causes the release of adenosine di-phosphate (ADP) with subsequent aggregation on a chemical basis. The release of ADP by damaged erythrocytes and leukocytes is also a possibility and may also play a role in these experiments.

Heparin was used in these studies because of convenience but its effects on platelets, as compared to other anticoagulants, remains to be explored. It has been shown that ACD is a better inhibitor of ADP clumping of platelets than heparin in vitro; in vivo studies in dogs have demonstrated decreased platelet survival with high concentrations of heparin but increased survival at low concentrations.

Some speculation on the potential usefulness of the NCI-IBM Cell Separator in human cell collection is warranted. The morbidity and mortality in these studies in dogs can probably be attributed to the use of non-sterile, non-pyrogen free tubing and bowls, bilateral carotid shunts, and to the production of severe thrombocytopenia in anticoagulated animals. Leukopenia of more than a transient nature was not produced. The principal potential dangers include pyrogenic reactions, bacterial contamination and the effects of anticoagulation and thrombocytopenia. The first two, with proper precautions, have not been encountered in preliminary human studies. Anticoagulation in vivo is always a risk and the ideal anticoagulant in this system has not yet been identified. Theoretically, regional anticoagulation with neutralization prior to return of the blood might be preferable. Thrombocytopenia can probably be prevented in adult human donors during the collection of granulocytes by centrifugation at low g. forces (Figure 4 and Table 4) but collection of lymphocytes without the removal of large quantities of platelets may be difficult.

Based on the separation and collection efficiency in dogs (approximately .15 x 10^11 granulocytes in 2 hrs.) and the assumption that 1 x 10^11 granulocytes mm. are needed for a therapeutic transfusion, it is apparent that a 6-7 hour period of centrifugation would be necessary to collect enough cells for a single transfusion into a small recipient. However, as previously noted, this estimate of the granulocyte requirement for therapeutic transfusion is probably too high so that shorter collection periods may be adequate.
There are many other potential uses of the NCI-IBM Blood Cell Separator other than cell collection, some of which have been evaluated. This aspect will be described in more detail elsewhere but a few of the possible uses in man include plasma exchange and dialysis, precipitation and removal of cryoglobulins in a two-stage system and infusion of cytotoxic agents which rapidly bind to albumin.

**Summary**

A closed continuous flow centrifuge was used to separate and collect large quantities of buffy coat cells from the dog. One hundred fifty-five separate centrifugations of 2–12 hours duration were performed. Up to 61.0 liters of blood, representing 2.2–52 donor blood volumes, were processed. Buffy coat cells with a preponderance of granulocytes or lymphocytes, were collected while plasma and red blood cells were returned to the donor without change in flow or gravitational field. The mean total number of leukocytes, granulocytes, mononuclear cells and platelets removed was 24.0, 17.0, 7.0 and 197.0 × 10^6, respectively, which was 34.0, 28.0, 66.0 and 36.0 percent, respectively, of each cell population entering the centrifuge.

The effect of centrifugation on blood components was evaluated. Granulocytes from buffy coat collections exhibited normal phagocytic ability in vitro. The transfusion of large quantities of granulocytes (15.0 × 10^9) into leukopenic dogs produced an increment in peripheral granulocyte count (2.7 × 10^9 per mm^3). Granulocytes labeled with ^3H-DFP were also infused with a recovery of 38.6 percent at one hour and a T-1/2 of 4–6 hours. Machine-separated lymphocytes responded normally to phytohemagglutinin. Prolonged passage of blood through the pumps, tubing and bowl without centrifugation resulted in a decrease in circulating platelet levels (21 percent). A greater decrease in platelets occurred with centrifugation at high g-forces (49 percent). Hemolysis of red blood cells was not a serious problem.

**Summario in Interlingua**

Un apparato claudite a centrifugation de typo fluxo continue eseva usate pro separar e collectionar grande quantitates de cellulas a coagulo blanc ab le can. Esseva effectuate 155 separate centrifugationes de durationes in cata-un de inter due e dece-duo horas. Esseva usate un quantitate total de sanguine in le ordine de 61.0 litros, i.e., 2,2 vices le volumine normal del donationes de sanguine ab 52 donatores. Le cellulas a coagulo blanc, con un preponderantia de granulocytos o lymphocytos, esesva collectionate, durante que le plasma e le erythrocytos esesva retornate al donatores sin alteracion del caracteristicas de fluxo o del campo gravitational. Le total numero medie de leucocytos, granulocytos, cellulas mononucleari, e plachettas removite eseva 24.0, 17.0, 7.0, e 197.0 × 10^9, respectivemente, lo que representava, respectivemente, 34,0, 28,0, 66,0, e 36,0 pro cento del diverse populationes cellular que entrava ad in le apparato de centrifugation.

Le effecto del centrifugation del componentes de sanguine eseva evaluatate. Le granulocytos in le collectiones de cellulas a coagulo blanc exhibiva un normal capacitute phagocytic in vitro. Le infusion de grande quantitates de granulocytos (< 15,0 × 10^9) ad in canes leucopenic resultava in un augmento del peripheral numeration de granulocytos (2,7 × 10^9 per mm^3). Esseva etiam infusionate granulocytos marcate con DFP a tritium, resultante in un recuperation de 38,6 pro cento post un hora e un tempore de valor medie de inter quatro e sex horas. Mechanicamente separate lymphocytos respondeva normalmente.
a phytohemagglutinin. Le prolongate passage de sanguine a transverso le pumpas, le tubage, e le tank resultava, in le absentia de centrifugation, in un declino (per 21 pro cento) in le nivellos del plachettas circulante. Un plus marcate declino in ille nivellos occurreva in le centrifugation a alte valores de g (i.e., 49 pro cento). Le hemolyse de erythrocytos non representava un serie problema.

ACKNOWLEDGMENTS

The authors are indebted to Dr. William B. Greenough, III, for his direct assistance throughout these studies; to Dr. Jacqueline Whang-Peng and Mrs. Turid Knutsen for performing the lymphocyte transformation studies; and to Mr. George Judson and Dr. Robert Kellogg and the staff of the Bio-Engineering Systems Department, IBM, Endicott, New York, for engineering and technical assistance.

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NEWS AND VIEWS

SYMPOSIUM ON GNOTOBIOLOGY

A symposium on Gnotobiology: Experimental and Clinical Aspects will be held in Buffalo, New York, June 10-11, 1968. Co-sponsored by the Roswell Park Memorial Institute and the Department of Biochemical Pharmacology, School of Pharmacy, State University of New York at Buffalo, the symposium is under the auspices of the Association for Gnotobiotics, Inc.

The symposium has been divided into the following sessions: Leukemia in Germ-Free Rodents; Disease Resistance of Germ-Free Animals; Experimental Biology; Physiology; Nutrition; Immunology; Technology; Carcinogenesis; Patient Care in Sterile Environments.

Deadline for advanced registration and hotel reservations is May 15, 1968.

Further information and forms may be obtained from Dr. E. A. Mirand, Professor and Assistant Director, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, New York 14203. Telephone (Area code 716) 886-2700, ext. 253.
Blood Cell Separation in the Dog by Continuous Flow Centrifugation

DEAN BUCKNER, ROBERT EISEL and SEYMOUR PERRY