Increased Efficiency of Leukocyte Collection by the Addition of Hydroxyethyl Starch to the Continuous Flow Centrifuge

By John Milton Mishler, Daniel C. Hadlock, Ignacio E. Fortuny, Robert W. Nicora, and Jeffrey J. McCullough

A total of 67 leukaphereses were performed with the continuous-flow centrifuge (CFC) on 27 healthy donors for the purpose of obtaining increased yields of granulocytes for infusion into septic patients with acute leukemia accompanied by severe granulocytopenia. The addition of hydroxyethyl starch (HES) to the input line of the CFC significantly ($p < 0.005$) increased the total number of leukocytes and/or granulocytes collected per donation. A mean yield of 9.72×10^9 and 4.65×10^9 total granulocytes were collected by the HES-treated and control-group donors, respectively. The efficiency of cell collection as evidenced by the total number of leukocytes and/or granulocytes harvested per liter of processed blood was also significantly ($p < 0.005$) improved by the addition of HES to the continuous-flow centrifuge. Significant alterations in hematologic parameters were not experienced by HES-treated donors undergoing initial and multiple procedures. Pre- and postdonation leukocyte and platelet counts, hemoglobin, prothrombin time, partial thromboplastin time, and leukocyte differential counts were no different whether or not HES was employed for granulocyte collection. The results of the present study demonstrate that increased yields of granulocytes can be harvested by the addition of HES to the continuous-flow centrifuge and made available for supportive therapy to patients experiencing granulocytopenia induced by malignant disease or its treatment.

BACTERIAL AND FUNGAL infections are the major contributors of morbidity and mortality in patients with acute leukemia.[1,2] The malignant process, radiation therapy, and antineoplastic agents contribute to the occurrence of granulocytopenia in these patients through bone marrow depression, thus rendering them susceptible to an increased incidence of infection and death as the result of severe and overwhelming septicemia, rather than as the result of the primary disease.

Consequently, many attempts have been undertaken to supplement granulocytopenic patients by administering leukocyte transfusions. The administration of granulocyte transfusions from donors with chronic myelocytic leukemia (CML) to septic patients with acute leukemia has been reported.[3-5] The use of

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From the University of Minnesota Hospitals, Minneapolis, Minn. 55455 and the Medical Affairs Department, McGaw Laboratories, Division of American Hospital Supply Corporation, Glendale, Calif. 91201.

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Address for reprint requests: John Milton Mishler, M.Sc., McGaw Laboratories, 1015 Grandview Avenue, Glendale, Calif. 91201.

CML leukocytes, however, has several disadvantages: the number of donors is limited, and transfusion of neoplastic stem cells occasionally results in transient engraftments and adverse reactions in the recipient.[6]

In the past, attempts at harvesting leukocytes from normal donors have been limited by the low number of cells collected.[8,9] However, improved techniques have been devised based on clinical observations that increased white cell collections can be achieved from normal donors possessing high leukocyte counts and/or an elevated blood sedimentation rate. These techniques utilize the mechanism of peripheral blood granulocytosis produced by steroid treatment and/or the erythrocyte sedimentation properties of high-molecular-weight polymers. It is now possible for normal donors to serve as the significant source of leukocytes for transfusion to granulocytopenic patients with acute leukemia.[7,10-17]

The present study demonstrates that increased yields of granulocytes can be collected from normal donors by the addition of hydroxyethyl starch (HES)* to the input line of the continuous-flow centrifuge (CFC).†

METHODS AND MATERIALS

Patients

Candidates for leukocyte-replacement therapy were septic patients hospitalized with acute leukemia or lymphoreticular malignancies, accompanied by severe leukopenia (< 1500 WBC/cu mm). The leukopenia experienced clinically was the result of daunorubicin as the method of inducing remission in acute leukemia. Supportive therapy during the period of infection was augmented by the use of reverse protective isolation. Pretransfusion testing of patients included ABO and Rh typing, red cell and leukocyte antibody screening, complete blood count (CBC), and HL-A typing when possible.

Selection of Donors

Normal healthy donors were initially selected from the donor files of the University of Minnesota Blood Bank or from the immediate family of the patient. Consent forms were obtained from donors participating in the program. Final donor acceptability was determined by a thorough medical history, complete blood count, platelet count, serum calcium, red cell and leukocyte compatibility testing, and coagulation screening prior to their entry into the study.[18] Donors with a history of heart disease or presenting hematologic abnormalities were excluded from participation in the program. Laboratory data obtained prior to and immediately following the completion of each procedure included the monitoring of vital signs, total leukocyte and platelet counts, leukocyte differential counts, hemoglobin, hematocrit, prothrombin time, partial thromboplastin time, and serum calcium.

Leukocyte Procurement (Vein-to-Vein Technique)

The procedure used in our laboratory for granulocyte collection with the CFC has been previously described.[18] Donors in the experimental (HES) and control groups were anticoagulated with a combined total of 4000–5000 U of sodium heparin and 300–450 ml of acid citrate dextrose-A USP (ACD-A). Each donor initially received 2000 U of sodium heparin intravenously at the beginning of the procedure, followed by the addition of the remaining heparin (15 U/min) and ACD-A solution (1 ml ACD-A to 20 ml whole blood), directly to the input line of the CFC. The initial ratio of anticoagulant to whole blood in both donor groups was employed to insure a

*Volex 6% hetastarch (6% hydroxyethyl starch) in 0.9% sodium chloride injection, McGaw Laboratories, Glendale, Calif.

†Celltrifuge, American Instrument Company, Silver Spring, Md.

constant level of anticoagulation during the entire donation period. Hydroxyethyl starch was added directly to the input line of the CFC in the ratio of 1 ml HES to 14–16 ml of whole blood. An average of 400 ml of HES was used during each leukapheresis. Hydroxyethyl starch was selected for use in these studies because it possesses erythrocyte sedimentation properties similar to dextran,[19,20] but has been shown to be nonantigenic.[21–24] The speed of rotation for the centrifuge bowl was usually 450 rpm, and the flow rate varied between 40 and 70 cc/min. The mean duration of the leukocyte collection period was 210 min, the range being 180–270 min.

In Vitro Studies of Granulocyte Function

Granulocytes collected using the CFC with and without the addition of HES were tested for in vitro activity using the bactericidal assay and quantitative nitroblue tetrazolium techniques.[25] Granulocytes collected directly from the donor immediately before and immediately after leukapheresis were included with each assay.

*Hepatic Function Determinations**

In order to ascertain the effect of multiple infusions of hydroxyethyl starch on hepatic function prior to the start of the leukapheresis program, 18 normal healthy male volunteers each received three infusions of 500 ml of 6% HES in 0.9% sodium chloride, within a 96-hr period. Each 500-ml unit of HES was administered in 60 min, 48 hr after the last infusion. Each subject was evaluated clinically by monitoring vital signs prior to the first infusion and 1 hr following the administration of the 500-ml unit of HES. Alkaline phosphatase, SGPT, and serum bilirubin (total, direct, and indirect) were determined within a 1-wk period prior to the first infusion, and 24 and 96 hr following the third and final infusion.

RESULTS

The Collection of Leukocytes

The results of 67 leukaphereses performed on 27 normal donors are presented in Table 1. A mean yield of 15.10×10^9 and 9.49×10^9 total leukocytes were harvested from the HES-treated and control-group donors, respectively. The difference in yield was subjected to t test analysis and found significant to $p < 0.005$. A mean yield of 9.72×10^9 total granulocytes were collected from HES-treated donors; in comparison, 4.65×10^9 total granulocytes were harvested from control-group donors. The difference in yield was again significant by t test analysis to $p < 0.005$. The percentage of granulocytes collected per total

Table 1. The Results of Leukocyte Procurement With the Continuous Flow Centrifuge

	Number of Procedures	Total Leukocytes Collected ($\times 10^9$)	Total Granulocytes Collected ($\times 10^9$)	Per cent Granulocytes Collected/Total Leukocyte Collection
(1) Hydroxyethyl Starch	50			
Mean		15.10	9.72	65
Range		(5.0–25.0)	(2.2–16.8)	(35–99)
(2) Control	27			
Mean		9.49	4.65*	46
Range		(3.9–19.6)	(1.6–13.7)	(27–70)
Level of Significance		1 > 2 ($p < 0.005$)	1 > 2 ($p < 0.005$)	1 > 2 ($p < 0.005$)

*Data computed on 24 procedures.

*Study conducted by Hill Top Research, Inc., Cincinnati, Ohio, under the direct supervision of Thomas G. Brown, M.D.

leukocytes harvested was shown to increase significantly with the addition of hydroxyethyl starch to the CFC. Sixty-five per cent of the total leukocytes harvested in the HES-treated donors were granulocytes, in comparison to leukocyte collection in the control-group donors, in which only 46% of the harvested cells were granulocytes.

The Efficiency of Leukocyte Collection

The efficiency of collection results are presented in Table 2. The addition of HES significantly increased the total number of leukocytes and in particular granulocytes harvested per liter of blood processed by the CFC. This increased efficiency of collection in the HES-treated donors was also evidenced by the percentage of circulating leukocytes and/or granulocytes harvested in each donation period. The use of HES resulted in a twofold increase in the total percentage of circulating granulocytes collected per procedure.

Previous studies[26,27] have shown that a leukocyte gradient can be established in the buffy coat and the first nine layers (millimeters) of interfacing erythrocytes by subjecting the blood sample to a gravitational force ranging from 17 to 360 *g* (500–2250 rpm). The percentage distribution of the different forms of leukocytes was shown in these cited studies to vary from each layer of buffy coat and packed red cells. In the buffy coat and the first layer of erythrocytes, 95% and 85%, respectively, of the leukocytes were lymphocytes. The leukocytes comprising the last erythrocyte layer were 80% granulocytes. It is suggested by the present study that the addition of HES with increased red cell sedimentation properties improves the separation of granulocytes from red cells in these lower layers; thus, granulocyte collection efficiency is improved.

Hematologic Studies

The addition of hydroxyethyl starch to the CFC did not significantly alter the hemoglobin, platelet count, and prothrombin time in donors undergoing initial donations (Table 3). Leukocyte and platelet counts performed immediately following the initial procedure decreased from predonation levels in both experimental and control group donors, with a significant reduction ($p < 0.005$) in peripheral leukocytes occurring in the HES-treated donors. Donors undergoing multiple procedures with HES (Table 4) experienced no serious alterations in leukocyte or platelet counts, as evidenced by minimal decreases and/or increases in these parameters, following a series of three or more procedures. Hemoglobin levels decreased following leukapheresis but usually returned to predonation levels within a 24-hr observation period for the first few consecutive procedures. Thereafter the hemoglobin in all donors began to decrease. This gradual decrease in hemoglobin levels following repeated donations was also observed in control donors experiencing multiple leukaphereses. The partial thromboplastin time was altered immediately following the donation, and this elongation was thought to reflect the presence of residual heparin and ACD-A in the donor blood sample, along with the dilutional effects of the HES. Follow-ups in these donors were unremarkable.

Table 2. Efficiency of Collection Using the Continuous-Flow Centrifuge

	Number of Procedures	Volume of Processed Blood (ml)	Total Leukocytes Collected/Liter of Processed Blood ($\times 10^9$)	Total Granulocytes Collected/Liter of Processed Blood ($\times 10^9$)	Per cent Leukocytes Collected/Total Circulating WBC	Per cent Granulocytes Collected/Total Circulating Granulocytes
(1) Hydroxyethyl Starch	50					
Mean		7929	1.92	1.25	31	32*
Range		(6000–10,500)	(0.74–3.47)	(0.33–2.33)	(12–47)	(8–54)
(2) Control	26					
Mean		7921	1.23	0.61†	18	13‡
Range		(6300–12,000)	(0.53–2.97)	(0.19–2.08)	(8–32)	(4–30)
Level of Significance			1 > 2 ($p < 0.005$)	1 > 2 ($p < 0.005$)	1 > 2 ($p < 0.005$)	1 > 2 ($p < 0.005$)

*Mean computed on 42 procedures.
†Mean computed on 23 procedures.
‡Mean computed on 21 procedures.

Table 3. Comparative Data on Donors Experiencing Their Initial Procedure

	Number of Donors	Leukocyte Counts (10^3/cu mm)	Platelet Counts (10^4/cu mm)	Hemoglobin (g/100 ml)	Prothrombin Time (sec)	Differential (%)				
						Neutro.	Lympho.	Mono.	Eosino.	Baso.
Hydroxyethyl Starch	15									
Pre		6.1	23.6	14.8	10.6*	62	32	3	2	1
Post		5.1	20.6	13.2	11.5*	66	31	1	3	0
Control	12									
Pre		7.3	33.0	14.8	11.1*	67	28	2	2	0
Post		7.1	29.4	13.5	11.7†	68	29	1	2	1

Pre, mean preprocedure value; post, mean value determined immediately following completion of the procedure.
*Calculated from data on nine donors.
†Calculated from data on four donors.

Table 4. Hematologic Parameters in Normal Donors Undergoing Multiple Leukapheresis With Hydroxyethyl Starch

Donor	Date of Donation	Leukocyte Counts (10^3/cu mm)	Platelet Counts (10^4/cu mm)	Hemoglobin (g/100 ml)	Act. PTT (sec)	Differential (%)				
						Neutro.	Lympho.	Mono.	Eosino.	Baso.
B.A.	(HES) 3-27-73									
	pre	7.3	26.2	14.7	34.3	79	16	3	1	1
	post*	5.3	21.8	12.7	54.7	71	25	2	1	1
	(Control) 3-28-73									
	pre	7.7	25.4	14.4	†	75	17	4	4	0
	post	6.9	22.8	12.7	†	63	36	1	0	0
	(HES) 3-29-73									
	pre	7.0	25.2	13.6	†	68	20	8	3	1
	post	5.8	18.0	11.0	51.0	71	27	0	0	2
	(HES) 4-20-73									
	pre	8.1	28.2	11.5	†	†	†	†	†	†
	post	6.7	24.6	9.1	58.5	†	†	†	†	†
M.M.	(Control) 4-10-73									
	pre	8.2	29.2	17.3	†	63	36	0	1	0
	post	7.4	12.1	15.9	†	78	20	1	1	0
	(HES) 4-11-73									
	pre	7.5	29.0	16.5	†	†	†	†	†	†
	post	5.5	16.6	14.2	45.9	55	39	†	†	†
	(Control) 4-12-73									
	pre	6.3	26.4	15.5	†	60	37	1	1	1
	post	4.9	21.0	13.5	40.5	73	24	0	2	1
	(HES) 4-13-73									
	pre	6.6	23.4	14.3	†	73	22	†	†	†
	post	5.8	22.4	13.1	39.6	68	29	†	†	†
	(Control) 4-17-73									
	pre	5.8	23.0	13.9	†	†	†	†	†	†
	post	5.4	†	12.8	39.0	†	†	†	†	†
	(HES) 4-18-73									
	pre	5.9	29.2	13.5	†	†	†	†	†	†
	post	4.8	24.4	11.6	42.0	†	†	†	†	†
	(Control) 4-19-73									
	pre	6.0	19.0	12.3	†	64	28	5	0	1
	post	4.8	21.4	11.3	41.5	62	35	1	2	0
R.S.	(HES) 7-10-73									
	pre	6.0	†	†	†	46	45	2	6	1
	post	5.2	25.0	13.5	50.3	66	31	0	2	1
	(HES) 7-12-73									
	pre	4.9	†	†	†	45	39	8	1	1
	post	4.4	27.4	12.7	†	64	30	2	3	1
	(HES) 7-13-73									
	pre	5.3	†	†	†	58	33	3	6	0
	post	4.7	21.6	12.6	†	53	40	5	2	0
	(HES) 7-17-73									
	pre	7.0	†	†	†	57	38	3	1	1
	post	5.1	24.0	12.7	41.5	45	45	6	3	1
E.P.	(Control) 6-19-73									
	pre	8.3	27.6	16.5	30.9	80	16	0	2	2
	post	9.1	27.0	†	36.7	76	21	0	1	2
	(HES) 6-26-73									
	pre	8.0	18.4	14.5	35.0	77	21	1	1	0
	post	10.0	18.8	13.7	44.5	75	21	1	3	0

(Continued)

Table 4. Hematologic Parameters in Normal Donors Undergoing Multiple Leukaphereses with Hydroxyethyl Starch (Contd)

Donor	Date of Donation	Leukocyte Counts (10^3/cu mm)	Platelet Counts (10^4/cu mm)	Hemoglobin (g/100 ml)	Act. PTT (sec)	Differential (%) Neutro.	Lympho.	Mono.	Eosino.	Baso.
	(HES) 6-27-73									
	pre	8.2	†	†	35.1	78	20	0	1	1
	post	6.8	†	†	41.5	70	28	0	2	0
	(HES) 6-28-73									
	pre	5.9	24.4	13.2	†	78	12	4	5	1
	post	5.2	18.8	10.8	†	68	27	3	2	1
	(HES) 6-29-73									
	pre	5.4	†	†	†	77	16	5	1	1
	post	4.5	22.4	9.9	69.2‡	59	29	7	3	2
	(HES) 7-2-73									
	pre	6.6	†	11.9	†	74	15	7	4	0
	post	5.5	25.2	9.5	64.3‡	65	29	6	0	0
	(HES) 7-5-73									
	pre	9.4	†	11.6	†	74	15	6	4	1
	post	5.1	24.9	9.4	96.2‡	73	19	4	2	2
L.S.	(HES) 7-23-73									
	pre	5.2	†	†	†	62	29	8	1	0
	post	4.1	16.0	13.5	53.1	66	29	4	0	0
	(HES) 7-24-73									
	pre	4.8	†	†	†	50	40	8	2	0
	post	5.6	15.2	13.7	72.6	71	19	7	1	1
	(HES) 7-25-73									
	pre	5.1	†	†	†	62	34	3	1	0
	post	4.9	20.2	12.2	60.6	62	30	5	3	0
	(HES) 7-26-73									
	pre	4.4	†	†	†	59	38	3	1	0
	post	4.2	17.2	12.0	64.3	58	41	0	1	0
E.H.	(Control) 1-30-73									
	pre	8.6	45.8	17.3	36.0	62	34	1	1	2
	post	7.2	32.6	15.6	53.3	59	35	1	4	1
	(Control) 1-31-73									
	pre	8.9	35.0	16.2	†	75	24	0	1	0
	post	8.3	42.0	15.5	49.1	70	30	0	0	0
	(HES) 2-1-73									
	pre	7.5	30.2	16.3	†	65	26	2	4	3
	post	5.5	29.8	14.0	48.2	54	42	0	4	0
	(HES) 2-2-73									
	pre	6.8	38.8	15.1	†	59	36	0	2	3
	post	5.3	31.0	12.6	52.9	†	†	†	†	†
	(HES) 2-8-73									
	pre	7.9	55.8	14.7	†	54	45	0	0	1
	post	5.2	33.8	12.8	†	37	58	0	3	2
	(HES) 2-9-73									
	pre	6.7	44.4	13.2	†	73	23	3	0	1
	post	4.9	40.0	11.8	61.3	50	42	5	2	1
C.R.	(Control) 6-4-73									
	pre	6.6	33.2	15.1	†	60	28	10	2	0
	post	7.7	44.0	14.7	47.1	60	38	2	0	0
	(HES) 6-5-73									
	pre	5.3	26.4	14.5	†	53	45	1	1	0
	post	5.0	24.0	13.2	51.8	72	18	6	4	0

(*Continued*)

Table 4. Hematologic Parameters in Normal Donors Undergoing Multiple Leukaphereses with Hydroxyethyl Starch (Contd)

Donor	Date of Donation	Leukocyte Counts (10^3/cu mm)	Platelet Counts (10^4/cu mm)	Hemoglobin (g/100 ml)	Act. PTT (sec)	Differential (%)				
						Neutro.	Lympho.	Mono.	Eosino.	Baso.
	(Control) 6-6-73									
	pre	4.6	†	14.0	†	59	34	4	2	1
	post	4.8	†	12.9	44.6	58	40	1	1	0
	(HES) 6-7-73									
	pre	†	†	†	†	†	†	†	†	†
	post	4.0	19.4	11.9	47.4	50	47	1	1	1
	(HES) 6-19-73									
	pre	5.2	28.2	13.2	†	59	30	9	2	0
	post	4.6	31.0	11.7	†	57	40	0	2	1
	(HES) 6-20-73									
	pre	4.2	32.6	12.7	†	66	29	3	2	0
	post	4.4	28.8	12.2	†	53	41	4	2	0
	(HES) 6-21-73									
	pre	3.6	28.0	12.3	†	52	35	9	4	0
	post	4.0	27.0	10.4	†	55	35	9	2	0

*Post, value obtained immediately following termination of procedure.

†Data not recorded.

‡Donor E.P. had shunt implanted, received a mean of 6225 U of heparin per procedure.

Granulocyte Function

Granulocytes collected with or without HES displayed no difference in bactericidal activity (Table 5). The average per cent of bacteria killed by granulocytes collected by both methods was 87%, and this did not change significantly when the granulocytes were stored for 18 hr at 4°C.

Hepatic Function Studies

The results of hepatic function studies conducted on 18 healthy male volunteers are displayed in Table 6. Significant differences were not observed between pre- and postinfusion results for any of the hepatic function determinations. There were no clinical laboratory alterations witnessed in this study which were considered to be related to the hydroxyethyl starch infusions. Serum bilirubin, including direct, indirect, and total, SGPT, and alkaline phosphatase levels were within normal limits for each volunteer on every examination day. Volunteers tolerated the infusions well, with no significant alterations occurring in vital signs or clinical condition. Adverse effects associated with the multiple

Table 5. Effects of HES on the Bactericidal Capacity and NBT Activity of Granulocytes Collected Using the CFC

	Number of Concentrates Studied	Average Per cent of *S. Aureus* Killed During 120-min Incubation	Number of Concentrates Studied	Average ΔOD in Quantitative NBT Assay
Immediately after collection without HES	5	87	4	0.108
Immediately after collection with HES	4	87	6	0.092

Table 6. Hepatic Function Determinations in Normal Volunteers Following Infusion of 3 × 500-ml U of Hydroxyethyl Starch within a 96-hr Period

	Number of Samples	Sampling Period (hr)		
		Preinfusion*	24†	96†
Serum glutamic-pyruvate transaminase (SGPT)	18			
Mean (U/ml)		15.0	15.3	13.9
SD		9.3	4.9	6.0
Range		(5.0–35.0)	(9.0–26.0)	(6.0–30.0)
Alkaline phosphatase	18			
Mean (U)		34.4	24.1	25.2
SD		10.9	5.3	5.7
Range		(17.0–58.0)	(13.7–34.0)	(16.0–34.0)
Total bilirubin	18			
Mean (mg/100 ml)		0.38	0.48	0.44
SD		0.16	0.19	0.12
Range		(0.16–0.66)	(0.30–0.84)	(0.34–0.66)
Direct bilirubin	18			
Mean (mg/100 ml)		0.10	0.10	0.13
SD		0.05	0.05	0.04
Range		(0.00–0.25)	(0.00–0.25)	(0.00–0.17)
Indirect bilirubin	18			
Mean (mg/100 ml)		0.28	0.35	0.32
SD		0.14	0.10	0.12
Range		(0.08–0.56)	(0.20–0.59)	(0.17–0.57)

*Preinfusion level (control).

†Samples drawn 24 and 96 hr following third (final) infusion of hydroxyethyl starch.

infusions of HES were not observed. The results demonstrate that hydroxyethyl starch was not shown to be associated with alterations in hepatic function, even 24 hr after a series of three 500-ml infusions.

DISCUSSION

During the past two decades, the development and continued improvement of collection techniques with the continuous-flow centrifuge have resulted in the increasing application of leukocyte transfusions for supportive therapy in acute leukemia patients. The employment of techniques utilizing steroid treatment and/or the erythrocyte sedimentation properties of high-molecular-weight polymers has led to the use of normal donors for leukocyte collection purposes.

The treatment of normal donors with etiocholanolone was initially shown by Graw et al.[10] and McCredie et al.[12] to significantly increase the yield of collected granulocytes over previously reported values. Recently, dexamethasone,[7,13,16,17] prednisone,[15] and prednisolone[28] have been used successfully to increase the efficiency of granulocyte harvesting with the continuous-flow centrifuge. The employment of hydroxyethyl starch in combination with steroid treatment has further extended the efficiency of granulocyte collection.[11,13,14,16,17] The employment of steroids in these studies was normally well tolerated by donors, except in those instances where administration of etiocholanolone and dexamethasone was associated with mild influenza-like symptoms,[11,29] chills, and fever.[13,17] A number of investigators[10,30] have questioned the use of hydroxyethyl starch in

the leukapheresis procedure because of the necessity for repeated injections in donors experiencing multiple donations. In the present series of experiments and in other previously reported leukapheresis studies[11,13,17,29] employing HES, there were no adverse reactions associated with the administration of this drug following single or multiple injections. Hydroxyethyl starch has also been employed in the management of chronic myelocytic leukemia (CML) patients with no reported reactions occurring as the result of the drug.[31,32] A number of CML patients have been given over 75 U (500 ml) of HES and have not presented signs of toxicity.[33] The results of the present study demonstrate that increased yields of granulocytes can be harvested by the addition of HES to the continuous-flow centrifuge and made available for supportive therapy to patients experiencing granulocytopenia induced by malignant disease or its treatment. The employment of HES in granulocyte collection does not have a detrimental effect on the hematologic changes which occur in donors undergoing leukapheresis, and granulocytes collected using HES do not demonstrate any impairment in in vitro function.

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REFERENCES

1. Levine AS, Graw RG Jr, Young RC: Management of infections in patients with leukemia and lymphoma: Current concepts and experimental approaches. Semin Hematol 9: 141, 1972

2. Hersh EM, Bodey GP, Nies BA, Freireich EJ: Causes of death in acute leukemia. JAMA 193:99, 1965

3. Freireich EJ, Levin RH, Whang J: The function and fate of transfused leukocytes from donors with chronic myelocytic leukemia to leukopenic recipients. Ann NY Acad Sci 113:1081, 1969

4. Morse EE, Freireich EJ, Carbone PP, Bronson W, Frei E: The transfusion of leukocytes from donors with chronic myelocytic leukemia to patients with leukopenia. Transfusion 6:183, 1966

5. Schwarzenberg L, Mathe G, Grouchy P: White blood cell transfusions. Isr J Med Sci 1:925, 1965

6. Mathe G, Amiel JL, Schwarzenberg L: Bone Marrow Transplantation and Leukocyte Transfusions (ed 1). Springfield, Charles C Thomas, 1971

7. Koza I, Holland JF, Cohen G: Histocompatible leukocyte transfusions during granulocytopenia. Neoplasma 18:185, 1971

8. Judson GT, Buckner ED, Eisel R, Greenough WB, Kellogg RM, Perry S: Closed continuous flow centrifuge. Nature (Lond) 217:816, 1968

9. Lani K, Pfisterer H, Ruppert W, Bolland H, Stich W: Studies of the separation of granulocytes for transfusion from normal persons. Presented at the Thirteenth International Congress of Hematology, Munich, Germany, August 1970

10. Graw RG Jr, Herzig GP, Eisel RJ, Perry S: Leukocyte and platelet collection from normal donors with the continuous flow blood cell separator. Transfusion 11:94, 1971

11. McCredie KB, Freireich EJ, Hester JP, Vallejos CS: Increased granulocyte collection using the blood cell separator and the addition of etiocholanolone and hydroxyethyl starch. Transfusion (in press).

12. McCredie KB, Freireich EJ: The use of etiocholanolone to increase collection of granulocytes with the IBM blood cell separator. J Clin Invest 49:63a, 1970

13. Mishler JM, Higby DJ, Rhomberg W, Cohen E, Nicora RW, Holland JF: Hydroxyethyl starch and dexamethasone as an adjunct to leukocyte separation with the IBM blood cell separator. Transfusion (in press)

14. McCredie KB, Freireich EJ: Increased granulocyte collection from normal donors with increased granulocyte recovery following

transfusion. Proc Am Assoc Cancer Res 12:58, 1970

15. Nusbacher J, MacPherson JL, Manejias RE, Bennett JM: Leukapheresis: The effect of a single high oral dose of prednisone on granulocyte mobilization, yield, and function. Presented at the Twenty-sixth Annual Meeting of the AABB, Miami, Florida, November 1973

16. Higby DJ, Mishler JM, Cohen E, Rhomberg W, Nicora RW, Holland JF: Increased elevation of peripheral leukocyte counts by infusion of histocompatible granulocytes. Vox Sang (in press)

17. Higby DJ, Mishler JM, Rhomberg W, Nicora RW, Holland JF: The effect of a single or double dose of dexamethasone on granulocyte collection with the continuous flow centrifuge Vox Sang (in press)

18. McCullough JJ, Fortuny IE: Laboratory evaluation of normal donors undergoing leukapheresis on the continuous flow centrifuge. Transfusion 13:394, 1973

19. Gregersen MI, Chien S, Usami S: Studies on the effects of hydroxyethyl starch and other plasma expanders on the viscosity of human blood at low shear rates. Third Conference on Artificial Colloidal Agents, NAS-NRC, Washington, D.C., 1965, p 29

20. Lewis JH, Szeto ILF, Bayer WL, Takaori M, Safar P: Severe hemodilution with hydroxyethyl starch and dextran. Arch Surg 93:941, 1966

21. Brickman RD, Murray GF, Thompson WL, Ballinger WF: The antigenicity of hydroxyethyl starch in humans: Studies in seven normal volunteers. JAMA 198:139, 1966

22. Maurer PH, Berandinelli B: Immunological studies with hydroxyethyl starch (HES): A proposed plasma expander. Transfusion 8:265, 1968

23. Walton RP, Hauck AL, Herman EH: Structural specificity of dextran in producing anaphylactoid reaction in rats. Proc Soc Exp Biol Med 121:272, 1966

24. Maurer PH: Immunogenicity studies with hydroxyethyl starch. Third Conference on Artificial Colloidal Agents, NAS-NRC, Washington, D.C., 1965, p 26

25. McCullough JJ, Carter SJ, Qdie PG: Effects of anticoagulants and storage on granulocyte function in bank blood. Blood 43: 204, 1974

26. Lani K, Pfisterer H, Ruppelt W, Bolland H, Stich W: Studies on the separation of granulocytes for transfusion from normal persons by the IBM cell separator. Blut 22:35, 1970

27. Lani K, Pfisterer H, Ruppelt W, Bolland H, Stich W: Leuko- and thrombophoresis in normal subjects with the NCI-IBM blood cell separator. Klin Wochenschr 49:327, 1971

28. Pfisterer H, Ruppert W: High effective technique for procurement of viable granulocytes with the IBM blood cell separator. Presented at the Twenty-fifth Annual Meeting of the American Association of Blood Banks, Washington, D.C., August 1972

29. McCredie KB: Granulocyte transfusion becomes clinically useful. JAMA 217:1176, 1971

30. Clift RA, Buckner CD, Williams BM, Hickman RO, Thomas ED: Improved granulocyte procurement with the continuous flow centrifuge. Transfusion 13:276, 1973

31. McCredie KB, Vallejos CS, Freireich EJ: The management of chronic myelocyte leukemia (CML) by leukapheresis. J Clin Invest 51:64a, 1972

32. Vallejos CS, McCredie KB, Freireich EJ: Improved leukocyte collections with hydroxyethyl starch (HES) from patients with chronic myelocytic leukemia (CML). Clin Res 20:503, 1972

33. McCredie KB: unpublished data