

## Transient Neutropenia Induced by Transfusion of Blood Exposed to Nylon Fiber Filters

By Charles A. Schiffer, Joseph Aisner, and Peter H. Wiernik

During the course of granulocyte collection by continuous-flow filtration leukopheresis, an abrupt fall in neutrophil count was noted (mean decrease 77%, range 64%–95%). Neutropenia occurred within 5 min of return of blood exposed to the nylon fiber filters and lasted less than 30 min. Saline exposed to the fibers, withdrawal and reinfusion of whole blood, and heparin did not cause neutropenia. Heparinized blood passed by gravity through isolated filters and reinfused immediately also induced neutropenia (mean decrease 64%  $\pm$  8%, range 11%–19%). Blood anticoagulated with ACD (de-

crease 19.5%  $\pm$  6%, range 6%–56%), heparinized plasma ( $N = 10$ , decrease 15%  $\pm$  3%, range 3%–29%) and platelet-rich plasma exposed to the filters failed to produce neutropenia. 91%  $\pm$  2% of the neutrophils adhered to the fibers using heparinized blood as compared to 21%  $\pm$  5% using ACD ( $p < 0.001$ ). All donors were asymptomatic during the infusions. These results suggest that during neutrophil adherence a substance is released which produces profound, transient neutropenia perhaps by inducing margination of cells.

**T**RANSIENT NEUTROPENIA has been described after intravenous administration of endotoxin,<sup>1</sup> dextran,<sup>2</sup> histamine,<sup>3</sup> iron oxide,<sup>4</sup> and leukocyte lysates.<sup>5</sup> More recently, profound transient neutropenia has been noted to be an almost uniform occurrence in patients undergoing hemodialysis.<sup>6–9</sup> A dramatic fall in neutrophil count occurs within the first few minutes of dialysis with a return to normal in less than 1 hr. Subsequently, a reactive leukocytosis with an increase in the number of band forms often occurs, and labeling studies with DF<sup>32</sup>P suggest that this “rebound” is due to egress of cells from the bone marrow pool.<sup>10</sup>

The stimulus responsible for this phenomenon has not been isolated, but certain possible factors have been eliminated. Although large numbers of granulocytes adhere to the dialysis cellophane membranes and are lost to the patient, the absolute number lost cannot account for the fall in white count.<sup>11</sup> Simple withdrawal and reinfusion of blood does not appreciably affect the neutrophil count.<sup>9</sup> Nor will administration of heparin,<sup>6</sup> exposure of blood to intravenous tubing,<sup>6</sup> use of a peristaltic pump, or infusion of saline or albumin exposed to dialysis coils cause neutropenia.<sup>9</sup> Whole blood exposed to dialysis membranes in a plastic blood bag will cause neutropenia upon reinfusion, however, and indeed has been suggested as a means of measuring granulocyte reserve.<sup>9</sup>

Continuous-flow filtration leukopheresis (CFFL) is a method of harvesting granulocytes which selectively adhere when heparinized blood is passed through

---

*From the Section of Medical Oncology, NCI-Baltimore Cancer Research Center, Baltimore, Md. 21201.*

*Submitted April 5, 1974; accepted July 15, 1974.*

*Address for reprint requests: Dr. Charles A. Schiffer, Baltimore Cancer Research Center, 9th floor, 22 South Greene Street, Baltimore, Md. 21201.*

© 1975 by Grune & Stratton, Inc.

nylon fiber filters.<sup>12,13</sup> During the course of CFFL, we have observed transient neutropenia similar in timing and magnitude to that seen during hemodialysis. The most obvious feature common to both systems is the occurrence of granulocyte adherence. It is possible that neutrophils elaborate a substance during the process of adhesion to surfaces and/or each other which can produce temporary redistribution of cells when transfused. The following investigation was undertaken to test this hypothesis.

#### MATERIALS AND METHODS

CFFL was performed using techniques previously described.<sup>12,13</sup> In this closed system, heparinized blood is pumped through two nylon fiber filters (Leukopak) (Fenwal, Morton Grove, Ill.) drawing blood from one arm and returning it to the other. Twenty-five hundred units of heparin are administered intravenously at the beginning of the procedure. Prior to receiving blood which has been exposed to the fibers, approximately 200 ml of heparinized saline, used to fill and irrigate the tubing and filters, is returned to the donor. In this study samples were taken proximal to the filters at 5-min intervals beginning when blood exposed to the fibers was first returned to the donor.

After preliminary results using CFFL were obtained, a simplified system was devised which allowed repeated study of the phenomenon under varying conditions. Five hundred milliliters of whole blood were removed and collected in plastic blood bags containing either heparin (2200 U) (Fenwal JHI) or acid-citrate-dextrose (ACD) (Fenwal PA 220) as anticoagulant. This blood was then immediately passed by gravity through a single nylon fiber filter and reinfused. Blood samples were obtained prestudy, preinfusion, and every 1-2 min during infusion from a site in the opposite arm. Samples were also obtained from the bag and the filtrate to determine the per cent adherence.

Heparinized cell-free plasma, obtained by centrifugation of whole blood at 5000 g at 25°C was studied in a similar fashion except that incubation of plasma and fibers were allowed to take place for at least 5 min before reinfusion. Infusion time averaged 9-10 min for the whole blood and 6-7 min for the plasma. In other experiments, heparinized whole blood was passed through filters into a collection bag and separated into plasma and red blood cells by centrifugation. This was carried out at room temperature (approximately 25°C) and at 37°C on different occasions. The plasma and red cells were then directly infused in sequence and blood counts obtained. In all the studies the filters were washed with 500 ml of normal saline before blood or plasma were passed through, in order to evacuate trapped air.

White blood cell counts were performed on a Coulter Model F Counter (Coulter Electronics, Hialeah, Fla.) and differentials performed on Wright-stained slides using standard methods.

The following definitions were used:

1. Neutrophil adherence (%):

$$100 \frac{\text{bag neutrophil count} - \text{filtrate neutrophil count}}{\text{Bag neutrophil count}}$$

2. Neutrophil count decrease (%):

$$\frac{\text{Preinfusion neutrophil count} - \text{lowest neutrophil count postinfusion}}{\text{Preinfusion neutrophil count}}$$

The degree of platelet adherence during passage through the filters was also measured in some of the studies with whole blood. Platelet counts were performed on a Coulter Model S Counter. In addition, to test the possible contribution of platelet adherence, platelet-rich plasma (PRP), which is neutrophil poor, was prepared by plateletpheresis technique<sup>14</sup> from single units of heparinized blood. Autologous PRP was then infused through a filter, and serial neutrophil counts obtained.

Normal volunteers and patients with a variety of tumors with normal neutrophil counts were studied. No patient was receiving chemotherapy at the time of study. The procedure was explained in detail to all potential participants and consent obtained.

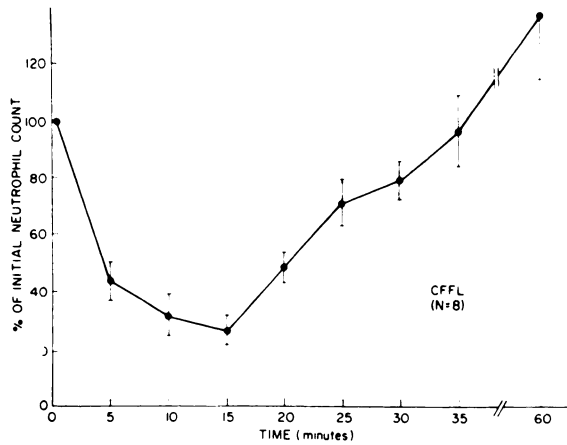


Fig. 1. Pattern of fall in neutrophil count in eight donors during continuous-flow filtration leukopheresis. Brackets indicate  $\pm 1$  SE.

RESULTS

Eight normal donors undergoing CFFL were studied. Neither the administration of heparin nor the return of saline exposed to the fibers produced a change in white blood cell count in these donors. All eight donors developed neutropenia detectable in the first sample obtained after receiving blood exposed to the fibers. Average maximal neutrophil count decrease was 77% (range 64%–95%) with a nadir of 76/ $\mu$ l in one individual. The count sequence in these donors is demonstrated in Fig. 1. Neutrophil counts returned to normal within 25–30 min, and no change in the lymphocyte count was noted. All donors were asymptomatic during this period of time and remained so throughout the procedure. Administration of 7.5 mg of dexamethasone intravenously 1 hr before CFFL did not block the fall in neutrophil count in one donor.

Profound transient neutropenia also occurred using the simplified system and heparinized whole blood (Table 1). In some patients the fall in count began within 1–2 min of return of blood exposed to the fibers (Fig. 2). Counts re-

Table 1. Neutrophil Adherence and Count Decrease

	Maximal Neutrophil Count Decrease (%)	Neutrophil Adherence (%)
Heparinized blood (N = 10)	64 $\pm$ 8 (11–91) range	91 $\pm$ 2 (76–99)
ACD blood (N = 8)	20 $\pm$ 6 (6–56)	21 $\pm$ 5 (5–40)
Heparinized plasma (N = 10)	15 $\pm$ 3 (3–29)	—
CFFL (N = 8)	77 $\pm$ 4.5 (64–95)	—

Values are expressed as means  $\pm$  SE.  
NS, not significant.

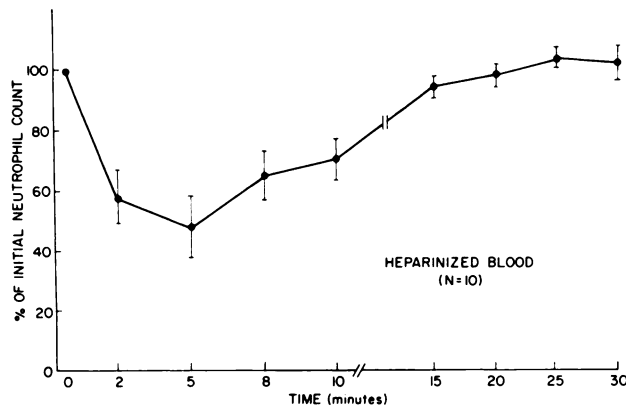


Fig. 2. Pattern of fall in neutrophil count in ten donors utilizing heparinized blood and the simplified system. Abrupt falls in neutrophil count occurred within 2 min of transfusion of blood exposed to the nylon fiber filter. Brackets indicate  $\pm 1$  SE.

turned to normal within 20 min in all but two patients and in one patient returned to normal while the infusion was still in progress.

As shown in Table 1, whole blood anticoagulated with ACD and heparinized plasma did not produce significant count changes. The differences between heparinized blood and these two groups are highly significant ( $p < 0.001$ ) (Student's *t* test). There is no significant difference between the ACD and heparinized plasma groups. As expected, the addition of ACD, a calcium chelating agent, tended to prevent adherence by granulocytes, and the difference in adherence between the ACD and heparin groups is again highly statistically significant ( $p < 0.001$ ). Because granulocyte adherence varied over a rather narrow range in the heparin group, it was not possible to correlate per cent adherence and per cent neutrophil count decrease. Absolute lymphocyte counts did not change appreciably during any of the maneuvers.

The neutrophil count did not change in three donors who received filtered heparinized platelet-rich plasma. In another subject infusion of filtered platelet-poor heparinized blood produced a fall in neutrophil count of 37%, demonstrating that neutropenia can be induced in the absence of platelets. A moderate degree of platelet trapping occurred when heparinized blood was passed through the filters (mean platelet loss, 45%, range 4%–77%,  $N = 5$ ). When fibers were examined using light microscopy, amorphous clumps of platelets were seen adhering to the fibers and each other. There was essentially no platelet trapping with ACD blood (loss, 6%, range 0%–16%,  $N = 4$ ).

Plasma prepared at 37°C from heparinized blood passed through nylon fibers induced a mean count decrease of 31% in three studies (range 19%–45%). Infusion of plasma similarly prepared at 25°C ( $N = 3$ , mean count decrease 5%, range 0%–14%) failed to produce significant changes in neutrophil count. All subjects were asymptomatic during all phases of the study.

An increase in neutrophil count was noted in some subjects in the 30-min period postinfusion. There was no significant difference between the ACD group (mean increase 18%), the heparin group (mean increase 13%), and the heparinized plasma group (mean increase 11%). In addition, there was no correlation between the degree of neutropenia produced and subsequent leukocytosis.

## DISCUSSION

These results suggest that the adherence of granulocytes to nylon fibers is associated with the production of transient neutropenia upon reinfusion. Heparin, plastic tubing, peristaltic pumping of blood, and mere "exposure" of plasma, platelet-rich plasma, or ACD whole blood to the fibers did not produce neutropenia. The rapidity of the fall, the absence of symptoms in the donors, and previous experience with other experimental models strongly suggest that margination rather than destruction of cells takes place.<sup>15</sup> The phenomenon seems limited to neutrophils and reverses quickly, despite continuation of the procedure and presumed continued release of the stimulus.

During recovery from neutropenia, rebound leucocytosis and an increase in the number of band forms occurred during CFFL (Fig. 1) but was not a consistent feature in subjects studied with the simplified system (Fig. 2). It is possible, however, that if the studies had been extended for a longer period of time, further increase may have been detected.

The chemical mediator inducing neutropenia has never been isolated in any of the previous studies, although the possible role of histamine release has been suggested.<sup>3</sup> Recent studies have demonstrated that leukocyte and platelet lysates are capable of inducing histamine release both *in vitro* and *in vivo*.<sup>16,17</sup> Histamine can induce transient neutropenia probably by producing margination, at least when injected in doses causing symptomatology in the recipient.<sup>3</sup> Experiments in our laboratory have demonstrated that morphologic changes occur in granulocytes which have adhered to fibers<sup>18</sup> as well as release of small amounts of the lysosomal enzyme, muramidase.<sup>19</sup> It is therefore conceivable that during the process of adherence an unidentified and perhaps labile substance is released into the plasma which is capable of producing histamine release in the recipient, which in turn induces neutropenia.

In order to test this hypothesis, two donors were studied after administration of antihistamines. Diphenhydramine 50 mg was administered orally the night before and again 1–2 hr before a reinfusion of filtered heparinized blood. Falling counts of 24% and 20% were noted, suggesting a preventive effect of the antihistamine. Premedication with antihistamine did not, however, prevent the occurrence of significant neutropenia in one CFFL donor. A further point against this proposed sequence is that the maximal fall in neutrophil count was only 66% in the study by Bierman et al. in which large doses of histamine were administered.<sup>3</sup> Serial measurement of blood histamine levels could help to clarify this issue.

The model for inducing neutropenia described in this report is simple and safe, and involves minimal discomfort to the donor. It allows repeated study of normal individuals and may provide a new tool for the study of granulocyte kinetics and autoregulation.<sup>20</sup>

## ACKNOWLEDGMENT

The authors wish to express their appreciation to Ms. Theda Strohecker, Ms. Susan Betts, and Ms. Valerie Coppola for their technical assistance, and to Ms. Nancy Cusack for the preparation of the manuscript.

## REFERENCES

1. Quesenberry P, Morley A, Stohlman F, Rickard K, Howard D, Smith M: Effect of endotoxin on granulopoiesis and colony-stimulating factor. *N Engl J Med* 286:227, 1972
2. Leusen IR, Essex HE: Leukopenia and changes in differential leucocyte counts produced in rabbits by dextran and acacia. *Am J Physiol* 172:231, 1953
3. Bierman HR, Kelly KH, Petrakis NL, Cordes F, Foster M, Lose EA: The effect of intravenous histamine administration on the level of the white blood count in the peripheral blood. *Blood* 6:926, 1951
4. White LP, Bierman HR, Kelly KH, Byron RL: The immediate hematologic effects of intravenous saccharated iron oxide. *Blood* 7:897, 1952
5. Weisberger AS, Heinle RW, Hannah R: Transfusion of leukocytes and products of disintegrated leukocytes. *Proc Soc Exp Biol Med* 70:749, 1949
6. Kaplow LS, Goffinet JA: Profound neutropenia during the early phase of hemodialysis. *JAMA* 203:133, 1968
7. Papadimitriou M, Baker LRI, Seitanidis B, Sevitt LH, Kulatilake AE: White blood count in patients on regular haemodialysis. *Br Med J* 4:67, 1969
8. Smith EKM, Jobbins K: Observations on neutropenia associated with haemodialysis. *Br Med J* 4:70, 1969
9. Jensen DP, Brubaker LH, Nolph KD, Johnson CA, Nothum RJ: Hemodialysis coil-induced transient neutropenia and overshoot neutrophilia in normal man. *Blood* 41:399, 1973
10. Brubaker LH, Nolph KD: Mechanisms of recovery from neutropenia induced by hemodialysis. *Blood* 38:623, 1971
11. Buscarini L, Bassi F: Leucocyte loss in haemodialysis. *Acta Haematol* 48:278, 1972
12. Herzig GP, Root RK, Graw RG Jr: Granulocyte collection by continuous flow filtration leukopheresis. *Blood* 39:554, 1972
13. Buchholz DH, Schiffer CA, Wiernik PH: Description and evaluation of a low cost system for filtration leukopheresis. *Transfusion* 13:354, 1973
14. Schiffer CA, Buchholz DH, Wiernik PH: Intensive multi-unit platelet-pheresis of normal donors. *Transfusion* 14:388, 1974
15. Toren M, Goffinet JA, Kaplow LS: Pulmonary bed sequestration of neutrophils during hemodialysis. *Blood* 36:337, 1970
16. Kelly MT, Brashear RE, Martin RR, White A: Histamine release in the dog after leukocyte lysate injection. *Infect Immunol* 4:228, 1971
17. Kelly MT, Martin RR, White A: Mediators of histamine release from human platelets, lymphocytes, and granulocytes. *J Clin Invest* 50:1044, 1971
18. Aisner J, Sanel FT: Ultrastructural and functional studies of granulocytes obtained by filtration leukopheresis. *Proc Am Assoc Cancer Res* 15:69, 1974
19. Schiffer CA: unpublished data
20. McGarry MP, Mirand EA, Murphy GP: Colony-stimulating activity. Association with effect of hemodialysis on neutrophil granulocytes. *NY State J Med* 73:1864, 1973