Use of Postpheresis Plasma to Improve Granulocyte Yields for Transfusion

By Albert J. Roy, Alberts Brivkalns, and Ronald A. Yankee

Humoral factors which stimulate release of mature granulocytes from body reserves are presumed to be the mechanism through which high yields of granulocytes are obtained from donors by filtration leukopheresis. Postpheresis plasma (PPP) obtained 2 hr after leukopheresis, when infused into normal rats, induced a peak granulocytosis at 3 hr of 22,000/cu mm above controls. A substance in the nylon filters, which caused a peak granulocytosis at 4 hr of 7600/cu mm above controls, was eliminated by washing the filter with 30 volumes of saline. Injection of PPP obtained following leukopheresis with washed filters resulted in an 8000/cu mm increase in granulocytes. One milliliter of PPP given 1 hr before pheresis increased the granulocyte yield from 4.3 to 8.7 × 10^7 granulocytes in a 2-hr run.

We conclude that (1) a humoral substance elaborated by the host during filtration leukopheresis induces a granulocytosis in the donor, (2) a substance in commercial leukopaks, which can be eliminated by vigorous washing of the filters, may be responsible in part for granulocytosis observed during leukopheresis, (3) PPP may be used to increase granulocyte yields in donors undergoing leukopheresis.

FILTRATION LEUKOPHERESIS is one of the most efficient methods for obtaining granulocytes in large quantities for transfusion to septic patients with severe granulocytopenia.[1-3] The procedure utilizes sterile nylon-packed columns to which granulocytes adhere and are collected with an efficiency of over 85%. It is possible to process up to 2.5 blood volumes of donor blood and to obtain more than 30 billion granulocytes during a single 2-hr procedure.

Donors undergoing the procedure have demonstrated a transient granulocytopenia followed by a granulocytosis.[3,4] It is likely, as has been suggested, that the increased numbers of circulating cells are derived from release of bone marrow stores upon activation of humoral substances in response to the initial granulocytopenia.[4-8]

In order to facilitate the study of events related to factors associated with neutrophil release, we developed a model for filtration leukapheresis in the rat utilizing a miniaturized nylon filter system.[4,9] The procedure is analogous to the method currently used in the human in that, for equivalent duration of pheresis, the efficiency of removal of granulocytes related to the volumes of blood processed is equivalent. Our previous studies demonstrated that, in this model, granulocytosis occurs in the same manner as in the human system.[4] Transfusion

---

From the Platelet Pheresis Laboratory, The Children's Cancer Research Foundation, The Department of Pathology, Children's Hospital Medical Center and The Harvard Medical School, Boston, Mass. 02115.

Submitted May 16, 1974; accepted September 30, 1974.

Supported by Research Grants CAO-8855 and C-6526 from the National Cancer Institute and NI PRO-5526 from the Division of Research Facilities and Resources, National Institute of Health.

Address for reprint requests: Albert J. Roy, Ph.D., *Children's Cancer Research Foundation, 35 Binney Street, Boston, Mass. 02115.*

of granulocytes obtained with this system into rats rendered granulocytopenic with cyclophosphamide resulted in granulocyte increments that paralleled those observed in human studies.[3,4]

Our objectives in the present experiments were: (1) to establish the presence of a substance in postpheresis plasma capable of releasing mature granulocytes from body reserves, (2) to determine whether this factor is present as a result of interaction of the granulocytes with the nylon filter material itself or whether it represents release in response to depletion of circulating granulocytes, and (3) to determine whether the substance can be used to increase granulocyte recovery and improve yields in subsequent donors.

MATERIALS AND METHODS

Pheresis Procedure

Adult Sprague-Dawley rats weighing 350–380 g were anesthetized by intraperitoneal injection of pentobarbital-sodium, 60 mg/kg. A midline incision was made in the ventral aspect of the neck between the chin and the manubrium of the sternum. The left common carotid artery was isolated and cannulated using a length of sterile PE-50 Intramedic (Clay-Adams, Parsippany, N. J.) tubing which had been filled with saline and clamped. The left external jugular vein was similarly isolated and cannulated. The animal was anticoagulated by injection of 400 U (0.4 ml) of heparin-sodium (Liquamin Sodium "10", Organon, Inc., West Orange, N.J.) via the jugular vein cannula. The cannulae were connected to each end of a miniaturized leukopak filter constructed of Tygon (Norton Plastics and Synthetics Div., Akron, Ohio) tubing with an outside diameter of 1 cm, a length of 8.5 cm, and filled with 2.20 g of nylon wool (Fenwal Laboratories, Morton Grove, Ill.), as described previously.[4] After priming the filter with 4 ml of normal heparinized rat blood, the clamps were removed, and the blood was allowed to circulate through the filter for 2 hr. Blood flow, determined by insertion of a bubble flow meter into the system, averaged 0.85 ml/min. Total leukocyte counts and differentials were performed by standard techniques on blood obtained by incision of the tail vein. In selected animals, counts were performed before the start of the pheresis and at 30-min intervals until termination of the procedure.

Infusion of Postpheresis Plasma Into Normal Animals

Following a 2-hr pheresis, the filter was disconnected, and the heparinized animal was exsanguinated by insertion of a needle into the abdominal aorta. The blood was centrifuged at 1500 *g* for 25 min at 4°C in an International model PR-3 refrigerated centrifuge, and the supernatant plasma was separated using a sterile Pasteur pipette. One milliliter of plasma was then injected into the femoral vein of a normal rat which had been anesthetized as described above. Total white blood cell counts and differentials were obtained before injection and at 30-min intervals thereafter, as appropriate for each study.

In Vitro Filtration Studies

In order to determine the effect of the interaction of blood with the filter material, either 30 ml of saline or whole blood obtained from normal Sprague-Dawley rats were gravity filtered three times through a miniature filter prepared as above. Following filtration of the whole blood, the plasma was separated, and 1 ml was injected intravenously into normal rats. The filtered saline was similarly injected in 1-ml amounts into normal rats without further processing. White blood cell counts and differentials were obtained on the recipient rats before injection and at 30-min intervals thereafter.

These experiments were repeated using filters which had previously been flushed with 300 ml of normal saline. In all experiments, appropriate controls using nonfiltered saline or plasma were studied concomitantly.

Elution of Granulocytes From the Filters

Granulocytes were eluted from the nylon filters by flushing with a 50-ml solution containing 50% rat plasma and 50% normal saline adjusted to pH 6.5 with ACD-A [2.2% sodium citrate

(hydrous), 0.8% citric acid (hydrous), 2.45% dextrose (hydrous)]. This solution was forced through the filter at the rate of 10 ml/min using a 25-ml syringe, during which time the filter was gently tapped as described previously.[2]

Determination of Effect of Postpheresis Plasma on Granulocyte Yields

Fresh plasma obtained following a 2-hr pheresis was injected into the femoral veins of a separate group of anesthetized rats in which the filter cannulae were already in place. One hour after receiving 1 ml of postpheresis plasma, recipient animals underwent pheresis for 2 hr. The filters were then detached and the granulocytes eluted. Control rats were given 1 ml of plasma obtained from nonpheresed rats 1 hr prior to undergoing pheresis.

RESULTS

Effect of Pheresis on the Granulocyte Count of Donor Rats

During filtration leukopheresis with washed filters, granulocytosis occurred shortly after initiation of the procedure, with a peak at 210 min (Fig. 1). The mean increment in granulocytes at that time was 8900/cu mm (range, 0–19,439). This was a 1.2-fold increase above the prepheresis level. In a series of rats treated similarly but using unwashed filters, a peak granulocytosis occurred at 150 min following initiation of the procedure. The mean granulocyte increment seen at that time was 17,600/cu mm (range, 2676–38,251), a 2.0-fold increase. The maximum mean increment observed in sham-pheresed rats was less than 3000/cu mm. The difference between the two peak increments obtained with washed and unwashed filters is large but the p value is greater than 0.10. The differences between the granulocyte increments observed in the sham-operated rats and both the pheresed groups were significant after the first 90 min (Fig. 1).

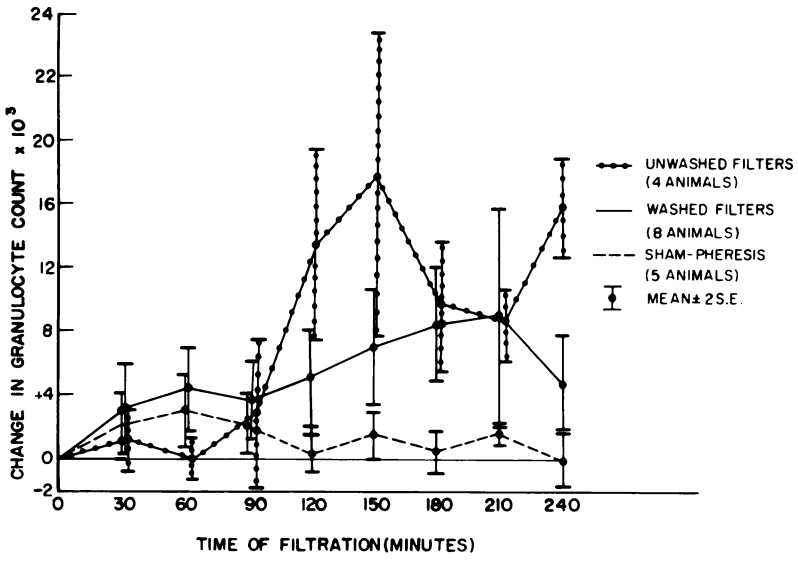

Fig. 1. Effect of filtration leukopheresis on the granulocyte count of normal rats.

In Vitro Filtration Studies

Injection into normal animals of 1 ml of normal saline which had been passed three times through unwashed filters resulted in a progressive increase in recipient granulocyte counts which persisted for at least 4 hr (Fig. 2). The mean granulocyte increment at 4 hr was 7600/cu mm, or a 3.8-fold increase above initial counts. Saline passed three times through washed filters resulted in an initial granulocytopenia followed by a slight but not significant granulocytosis. Injection of 1 ml of fresh nonfiltered saline resulted in a mean granulocytosis which did not exceed 1000/cu mm during the entire 4-hr period of observation. Injection of normal rat plasma separated from blood which had been passed three times through washed filters resulted in little or no increase in the granulocyte counts of the recipient rats (Fig. 3). When such plasma was passed through unwashed filters, however, a progressive increase in the recipients' granulocyte counts was observed, with a resulting mean increment of 16,700/cu mm (range, 8561–24,230) at 4 hr, representing a 3.6-fold increase over pretreatment values. The differences between washed and unwashed filters are significant ($p < 0.005$) by analysis of variance.

Effect of Postpheresis Plasma

Injection into normal rats of 1 ml of plasma obtained following leukopheresis with washed filters resulted in a 2.9-fold increase above initial granulocyte levels, with a peak granulocyte increment of 7755/cu mm (range, 3854–22,902) 3 hr after transfusion (Fig. 4). Injection of plasma obtained using unwashed filters resulted in a 5.5-fold increase, with a peak increment of 22,298/cu mm (range, 5680–40,310) 3 hr after transfusion. An increment of 800/cu mm (range, 156–4986) was observed with injection of normal plasma. Peak activity with this nonpheresed plasma occurred at 60 min after injection. Complete differentials were determined on three animals showing the greatest increase in neutro-

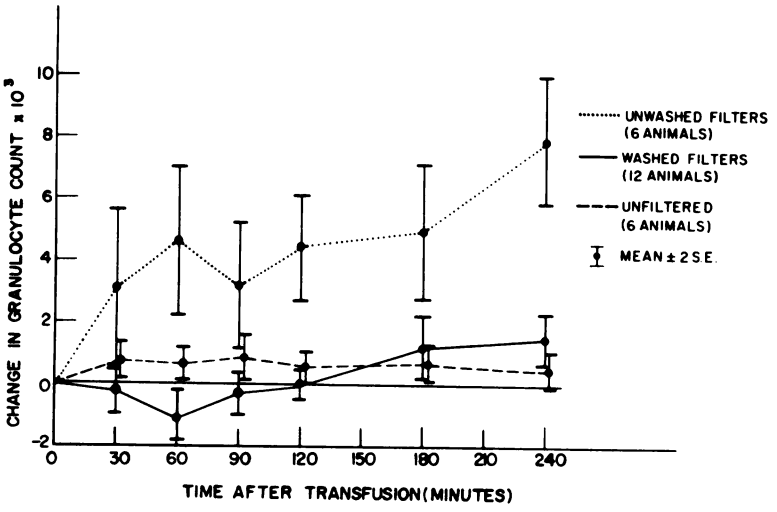

Fig. 2. Change of granulocyte count of normal rats given filtered saline.

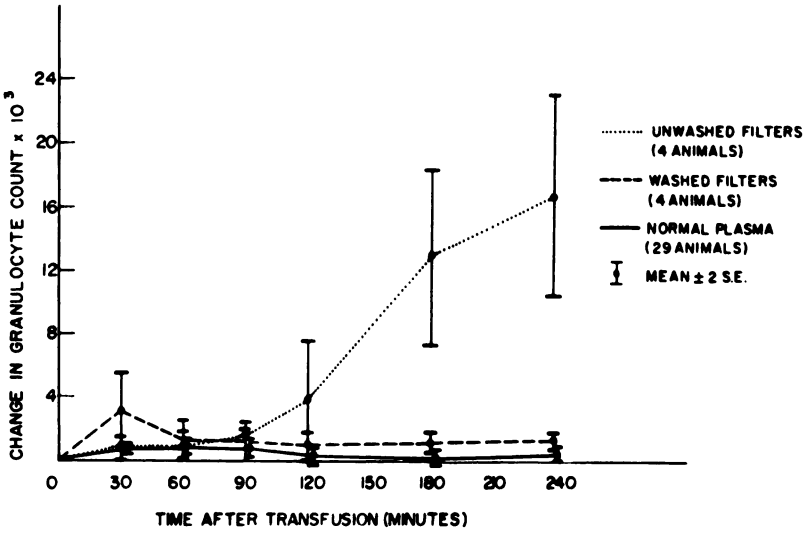

Fig. 3. Effect of plasma from blood filtered in vitro on the granulocyte count of normal rats.

phil count following infusion of PPP obtained with washed filters. Both segmented and nonsegmented neutrophils were increased at each time period. The proportional increase in segmented cells was far greater than that of nonsegmented neutrophils (Fig. 5). A transient lymphocytosis which occurred with normal plasma (29 animals), PPP from washed filters (16 animals), and PPP from unwashed filters (ten animals) was not significantly different from that seen in 11 noninjected control animals (Fig. 6). There was no quantitative change in the other blood elements.

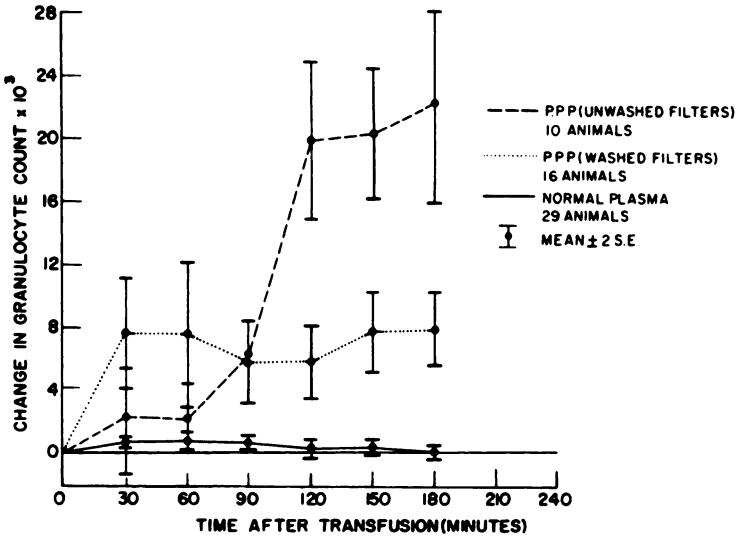

Fig. 4. Effect of postpheresis plasma on the granulocyte count of normal rats.

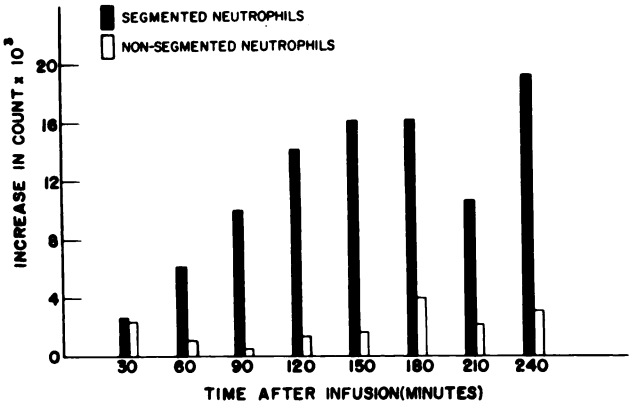

Fig. 5. Increments of segmented and nonsegmented neutrophils following infusion.

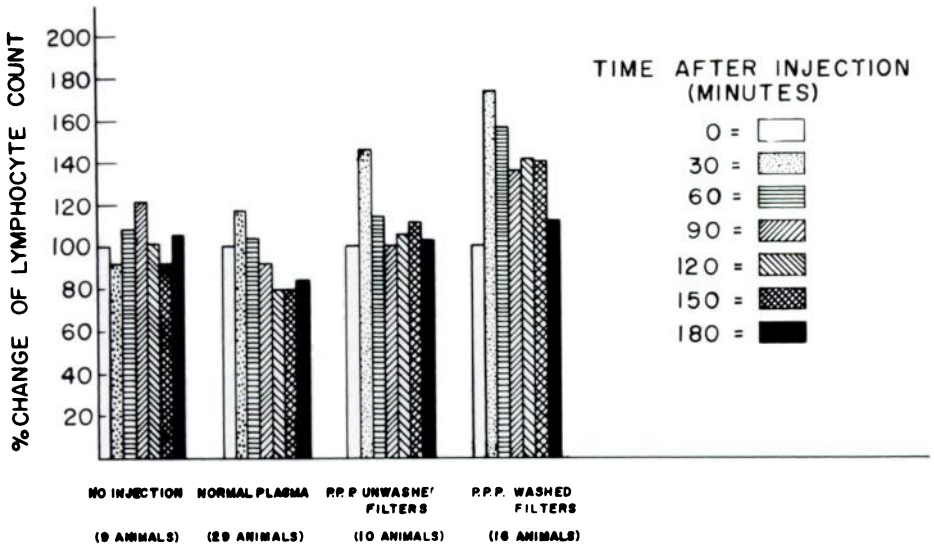

Fig. 6. Change in lymphocyte counts of rats given postpheresis plasma.

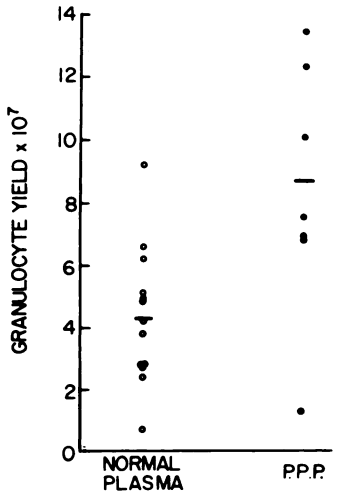

Fig. 7. Yield of granulocytes from leukopheresed rats pretreated with postpheresis plasma.

Pretreatment With Postpheresis Plasma

Pretreatment of seven normal rats 1 hr prior to leukopheresis with 1 ml of plasma obtained from rats following a 2-hr filtration leukopheresis with washed filters resulted in an average yield of 8.7×10^7 granulocytes. This compares favorably with the average yield of 4.3×10^7 granulocytes from 14 rats receiving 1 ml of normal plasma given 1 hr prior to pheresis (Fig. 7). This difference is statistically significant ($p < 0.005$ by analysis of variance).

DISCUSSION

Observations by others have clearly demonstrated the appearance of a leukocytosis-promoting substance in blood following rapid induction of leukopenia by a variety of means, including drugs,[10,11] peritoneal lavage,[6] x-irradiation,[12] endotoxin,[13] and typhoid vaccine.[6] This substance, which appears to rapidly mobilize mature granulocytes from body reserves, has been referred to as "leukocytosis-inducing factor" (LIF) by Gordon[6] and "neutrophil-releasing factor" (NRF) by Rothstein.[8] Plasma obtained from individuals rendered granulocytopenic by the above means characteristically induces granulocytosis within a few hours following injection.[11] The rapidity with which the granulocytosis occurs and the maturity of the cells suggest that this represents a mobilization of marrow stores rather than maturation of granulocyte precursors.

Our previous observations have demonstrated a nadir of granulocytopenia within 15–30 min following initiation of the pheresis.[4] This was followed by a granulocytosis of greater than 30% above prepheresis values occurring at 45 min.

Rothstein has demonstrated the appearance of a diffusible granulocytopoietic stimulator (DGS), as well as a colony-stimulating factor (CSF), in mice following injection of endotoxin.[14] DGS was clearly distinguishable from NRF and CSF by the appearance of peak activity at 60 hr after injection of endotoxin. This contrasts with peak CSF levels which were observed within 12 hr. Shadduck and Nagabhushanam demonstrated peak CSF concentrations within 2 hr after injection of antineutrophil serum.[15] Boggs has shown[10] that the increment of circulating granulocytes observed following injection of plasma containing neutrophil-releasing factor in dogs is derived primarily from the bone marrow, with little or no contribution from the marginating granulocyte pool. The material in our studies has similar properties, as evidenced by appearance of a slightly less mature population of circulating cells. Release of marginating cells would not be expected to alter the differential. Unfortunately, to our knowledge there has been no comparative study of the effect of CSF and NRF on the granulocyte count to determine difinitively whether, in fact, these are two separate factors.

It is clear that the granulocytosis observed in our studies was due in part to a substance in the filter which could be removed by vigorous washing of the filter with normal saline. Removal of the substance, however, did not eliminate the granulocytosis in donors undergoing leukapheresis nor the ability of PPP to increase granulocyte counts when given to normal rats. The eluted material was not endotoxin, as demonstrated by negative results in the limulus assay, which was kindly performed by Dr. John Das using the method of Levin and Bang.[16]

We believe that the substance is derived from the nylon filter material rather than from the plastic tubing. Polyvinyl chloride, which contains leachable plasticizers, and polyethylene, which does not, were both used to contain the nylon wool. The ability of saline passed through filters fashioned from either of these materials was equivalent in producing granulocytosis in the recipients.[17]

Herzig et al.[3] observed a prompt granulocytosis in three of four human recipients of autologous postpheresis plasma. Granulocytosis was not observed, however, on the one occasion in which they reinfused a single unit of fresh autologous plasma that had been passed through an unwashed filter. They suggested that the granulocytosis-promoting effect of filtration leukopheresis resulted from interaction of the granulocytes directly with the nylon fibers. In contrast, our studies did not demonstrate granulocytosis following infusion of plasma from blood filtered in vitro using washed filters. This suggests that production of NRF is of endogenous origin and not derived from interaction of the granulocytes with the nylon. We suggest that both an exogenous factor in unwashed filters and this endogenous factor triggered by the initial granulocytopenia results in the granulocytosis observed during filtration leukopheresis.

In agreement with studies by Boggs et al.,[10] we observed no significant lymphocytosis-promoting activity in postpheresis plasma. Although a slight increase in the lymphocyte count was seen following infusion of PPP, the magnitude of the increment did not differ significantly from that observed in nontreated control animals. We assume that the observed lymphocytosis resulted from manipulation of the animals. This contrasts with results reported by Gordon et al.,[6] in which a significant lymphocytosis occurred in rats given plasma from animals which had been leukopheresed by peritoneal lavage after instillation of saline.

The consistent granulocytosis in donors undergoing filtration leukopheresis appears to be responsible for the greater yields of granulocytes obtained by this method as compared with continuous-flow centrifugation. In the latter procedure, granulocytosis has not been consistently observed during pheresis carried out over comparable time periods.[3] The granulocyte turnover rate in a normal adult has been shown by Athens et al. to be of the order of 1×10^{11} per day or 4.2×10^{9} per hr.[18] With filtration leukopheresis, it becomes possible to remove more than 15×10^{9} cells per hr, a rate which is approximately four times the normal granulocyte turnover rate. Granulocyte harvest with the Celltrifuge approximates 1.25×10^{9} granulocytes per hr, a number which is considerably less than the normal turnover rate. The differences in rate of removal of granulocytes from the circulation probably account for the differences in the effects of the two techniques on the donor granulocyte count. The more rapid removal of granulocytes by filtration leukopheresis is thus more likely to induce activation of humoral regulatory mechanisms.

Corticosteriods and etiocholanolone have been used to improve harvesting of granulocytes by continuous-flow centrifugation.[19] Both, however, have undesirable side effects.[20,21] The use of postpheresis plasma would appear to be a more physiologic approach to improving the method of filtration leukopheresis. Leukapheresis in the rats pretreated with postpheresis plasma resulted in a

100% or greater increase in granulocyte yields. Isolation of the factor responsible may thus offer a potentially useful approach to increasing the yield of granulocytes from normal human donors.

REFERENCES

1. Djerassi I, Kim J, Mitrakul C, Suvansri U, Ciesielka W: Filtration leukopheresis for separation and concentration of transfusable amounts of normal human granulocytes. J Med (Basel) 1:358, 1970

2. Djerassi I, Kim J, Suvansri U, Mitrakul C, Ciesielka W: Continuous flow filtration leukopheresis. Transfusion 12:75, 1972

3. Herzig G, Root R, Graw R Jr: Granulocyte collection by continous flow filtration leukapheresis. Blood 39:554, 1972

4. Djerassi I, Roy A, Franklin A, Brivkalns A: Filtration leukopheresis in the rat. Exp Hematol (in press)

5. Morley A, Stohlman F Jr: Studies on the regulation of granulopoiesis. I. The response to neutropenia. Blood 35:312, 1970

6. Gordon A, Handler E, Siegel C, Dornfest B, LoBue J: Plasma factors influencing leukocyte release in rats. Ann NY Acad Sci 133: 766, 1964

7. Gordon A, Neri R, Siegel C, Dornfest B, Handler E, LoBue J, Eisler M: Evidence for a circulating leukocytosis-inducing factor (LIF). Acta Haematol 23:323, 1960

8. Rothstein G, Hugl E, Athens J: The relation of diffusible granulocytopoietic stimulator (DGS) to neutropenia and neutrophil releasing factor (NRF). Blood 38:820, 1971

9. Roy A, Djerassi I, Franklin A, Brivkalns A: Animal model for the study of filtration leukopheresis. Fed Proc 32:871, 1973

10. Boggs D, Cartwright G, Wintrobe M: Neutrophilia inducing activity in plasma of dogs recovering from drug induced myelotoxicity. Am J Physiol 211:51, 1966

11. Marsh J, Levitt M: Neutrophilia inducing activity in plasma of neutropenic human beings. Blood 37:647, 1971

12. Sodicoff M, Birnhammer R: Leukocytosis inducing factor in the blood of x-irradiated rats. Radiat Res 33:82, 1966

13. Rothstein G, Hugl E, Bishop C, Athens J, Ashenbrucker H: Stimulation of granulocytopoiesis by a diffusible factor in vivo. J Clin Invest 50:2004, 1971

14. Rothstein G, Hugl E, Chervenick P, Athens J, Macfarlane J: Humoral stimulators of granulocyte production. Blood 41:73, 1973

15. Shadduck R, Nagabhushanam N: Granulocyte colony stimulating factor. I. Response to acute granulocytopenia. Blood 38: 559, 1971

16. Levin J, Bang F: Clottable protein in limulus: its localization and kinetics of its coagulation by endotoxin. Thromb Diath Haemorrh 19:186, 1968

17. Roy A: Unpublished observations

18. Athens J, Haab O, Raab S, Mauer A, Ashenbrucker H, Cartwright G, Wintrobe M: Leukokinetic studies. IV. The total blood circulating and marginating granulocyte pools and the granulocyte turnover rate in normal subjects. J Clin Invest 40:989, 1961

19. McCredie K, Freireich E: Increased granulocyte collection from normal donors with increased granulocyte recovery following transfusions, Proc Am Assoc Cancer Res 12:58, 1971

20. Davidsohn I, Henry J (eds): Clinical Diagnosis by Laboratory Methods (ed 14). Philadelphia, Saunders 1969, p 610

21. Boggs D: Transfusion of neutrophils as prevention or treatment of infection in patients with neutropenia. N Engl J Med 290: 1055, 1974