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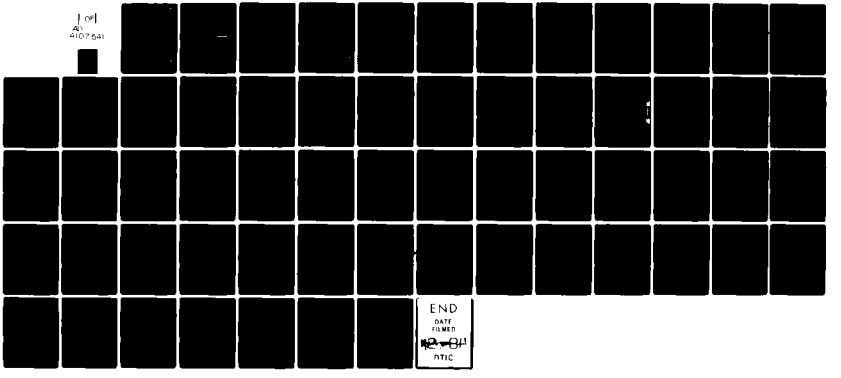

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February 1, 1976 to January 31, 1981

KINETICS OF NEUTROPHIL-RELEASING ACTIVITY OF POST-LEUKOPHERESIS PLASMA

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Humoral factors are elaborated by animal and human granulocyte donors during filtration leukapheresis. Plasma obtained from leukapheresed rats (PPP) may be used to increase granulocyte yields in donors when given prior to leukapheresis. This effect is dose and time related. The titer of neutrophil releasing activity is related to the duration of pheresis. There is a positive correlation between the donor pre- and postgranulocyte count and the titer of neutrophil releasing activity of the PPP. This activity

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in PPP is stable for up to three weeks at 4°C. The maximum granulocyte increment observed following injection of PPP occurs at three hours after injection. Intraperitoneal injection of PPP is ineffective. The standard 100% plasma eluent for removing granulocytes from the filter can be replaced by 50% plasma/saline, phosphate buffered saline enriched with bovine or human albumin, or hydroxyethyl starch. The plasma/saline eluent can be used on two successive days. Whole-body radioautography can be used to determine the viability and fate of transfused granulocytes. Pretreatment of granulocyte donors with PPP one hour before pheresis appears to be optimal for improving granulocyte yields. Kinetic studies suggested that approximately 20,000 granulocytes per mm^3 was the limit to which granulocyte mobilization in the pheripheral blood could be pushed while granulocytes were simultaneously being removed in large numbers by filtration leukapheresis. A mathematical model was constructed to show the actual number of granulocytes being mobilized during pheresis. We suggested that some procedural steps in the pheresis procedure such as recovery from anesthesia and blood cell/plastic interaction contribute to the granulocytosis in granulocyte donors, although granulocyte removal appears to provide the greatest impetus in this regard. Chemical contributions from the system, except for broken granulocytes, appear to play little or no role in this granulocytosis. The most important data to come from these studies show that in rats the yield of granulocytes obtained by filtration leukapheresis for two hours can be obtained by an intravenous injection of postpheresis plasma given one hour before initiation of the pheresis.

CONTENTS

INTRODUCTION

The studies carried out under this contract were designed to (a) confirm our previous observations that infusion of adequate amounts of plasma from leukapheresed rats (PPP) into normal rats consistently results in a marked granulocytosis in the recipient, (b) determine the optimal dosage of this plasma to improve yields of granulocytes from donor rats, (c) determine the optimal time to pretreat donor rats with PPP, (d) investigate the means by which PPP mobilizes granulocytes from body reserves, (e) study any artifactual contributions by the animal system used in these studies, and (f) use the phenomenon of the induction of granulocytosis by leukapheresis and by injection of post-leukapheresis plasma to elucidate normal granulocyte kinetics.

Details of the studies carried out between 1 February 1976 and 31 January 1980 are contained in Annual Reports No. 1, 1 March 1977; No. 2, 1 March 1978; No. 3, 31 March 1979, and No. 4, 15 March 1980. These details will be omitted from this report. The results are stated and reference to these annual reports is made. Details of the studies carried out between 1 February 1980 and 31 January 1981 are included in this report. The report is divided into two sections. Section I lists the results described in Annual Reports 1, 2, 3, and 4, with proper reference to these reports. Section II contains the details of the studies not covered earlier.

I. WORK CARRIED OUT BETWEEN 1 FEBRUARY 1976 - 31 JANUARY 1980

RESULTS

1. More than 98% of normal rats given PPP at a dosage of 1.5 ml/kg or more obtained from donors pheresed for at least 15 minutes demonstrated a significant (>4000/mm^3) increment in the granulocyte count (2).

2. Injection of plasma from rats pheresed for two hours into normal rats invariably induced a leukocytosis within 60 minutes, which was progressive for at least three hours (2).

3. The degree of leukocytosis following injection of PPP was dose- and time-dependent (2).

4. The granulocytosis produced by leukapheresis was time dependent. The mean maximum increment observed in leukapheresed rats was more than 27,000 mm^3 (2).

5. The response of rats to a standard dose of PPP obtained from rats which had undergone filtration leukapheresis for two hours is independent of the pretreatment granulocyte count (2).

6. There was no difference in the ability of rats to mobilize granulocytes on two successive days in response to treatment with postpheresis plasma (2).

7. There is a positive relacionship between the donor postpheresis granulocyte count and the ability of that plasma to induce a granulocytosis in normal rat recipients (2).

8. The neutrophil-releasing activity in postpheresis plasma is stable during storage at $4^{o}C$ for at least 21 days (3).

9. The mean maximum increment in granulocyte counts of rat recipients of PPP occurred at three hours after injection (3).

10. Intraperitoneal injection of PPP resulted in a granulocyte increment in normal rat recipients which was 29% of that seen following an equivalent dosage given intravenously (3).

11. Prewashing the nylon wool filters with saline to remove contaminating red blood cells prior to harvesting the granulocytes resulted in loss of more than half the granulocytes into the prewash (3).

12. Dilution of the plasma used to wash granulocytes from the filter 1:1 with saline does not reduce its effectiveness in eluting granulocytes from the filter (3).

13. The granulocytes obtained from rats by filtration leukapheresis were shown to be 90 to 95 percent viable as measured by incorporation of fluorescein diacetate uptake and ethidium bromide exclusion (3).

14. In preliminary studies, PPP given one hour before pheresis increased the granulocyte yields from 9.69 to 16.34 x 10^{7} cells. Pretreatment two hours before pheresis showed no greater increment (4).

15. Previous studies had focused on granulocyte increments one or more hours following injection of PPP. A later study focused on granulocyte increments during the first hour following such injection. There was a small but progressive increase in the mean granulocyte

increment of normal rats during each ten minute time period during the first hour following injection of 1.5 ml/kg of PPP (4).

16. There was a direct correlation between the donor granulocyte count following filtration leukapheresis and the granulocyte harvest from those animals (4).

17. There was a direct correlation between the prepheresis count versus the postpheresis granulocyte increment. Animals with low prepheresis granulocyte counts had significantly lower postpheresis increments than those with higher counts (4).

18. A greater mean increment of granulocyte counts was seen in smaller (younger) rats undergoing filtration leukapheresis than in larger (older) rats (4).

19. Plasma/saline eluent can be used at least twice without loss of efficiency in harvesting granulocytes from normal filters (4).

20. Phosphate buffered saline enriched with 2% bovine albumin (but not with 2% human albumin) was as effective as 50% plasma saline in eluting granulocytes from nylon filters (4).

21. Fresh or frozen guinea pig granulocytes tagged with ^{14}C-DFP were sequestered in the kidney, spleen, lung, and liver within 30 minutes of injection. Fresh cells were sequestered in decreasing concentrations in the spleen, lung, liver, and kidney with no evidence of circulation in the blood. In animals given frozen-thawed granulocytes, the highest concentration of cells was found in the lung followed by liver, spleen, and kidney. The primary differences between animals

receiving fresh or frozen cells were an apparently greater deposition of cells in the spleen and liver by fresh cells as compared with the frozen cells. There was no obvious difference in cell deposition in lungs and kidney (4).

22. Filtration leukapheresis was accomplished in the guinea pig. On the basis of these experiments, it was concluded, because of anatomical differences, that the guinea pig is inferior to the rat for studying filtration leukapheresis (4).

23. Studies involving combinations of modalities for increasing granulocyte counts in normal rats were carried out. Pheresis alone, PPP injection alone, or PPP injection followed at varying times by pheresis suggested that mobilization of greater than 20,000/mm^3 of circulating blood is rarely possible when granulocytes are being removed concomitantly by pheresis (4).

24. Study of the animal model for filtration leukapheresis showed that anesthesia, anticoagulation and blood/plastic interaction may contribute to the granulocytosis resulting from this procedure in the rat. However, removal of granulocytes appears to be the most potent stimulus for this phenomenon (5).

25. Continued deep anesthesia provided by intraperitoneal boluses of Nembutal prevented even the small but evident granulocyte increment observed in rats which were only anesthetized over a period of at least five hours (5).

26. The use of normal rat plasma as a control for experiments in which rat PPP is used contributes little, if any, to the small but

consistently seen granulocyte increment observed following its use in normal anesthetized rats (5).

27. Human plasma in doses greater than 1.0 ml/kg when injected into normal anesthetized rats stimulates granulocyte mobilization which is both dose- and time-dependent. Therefore, human plasma is not a suitable control medium for these studies in spite of its greater economy and availability (5).

28. Granulocytes can be mobilized by adrenal corticosteroids in rats as well as in human beings. However, the mobilization of rat granulocytes appears to be significantly quicker by this means than that of humans (5).

29. There is a direct correlation between rat body weight (age?) and granulocyte count, i.e., the lighter animals, in general, demonstrate lower granulocyte counts (5).

30. The increment in granulocyte counts in leukapheresed rats shows a positive correlation with the weight of the animals. In general, the greater the weight, the greater is the observed granulocyte increment (5).

31. A mathematical model for measuring the actual degree of granulocyte mobilization in the presence of granulocyte removal by pheresis was constructed. This allows for more precise measurements of granulocytes within the granulocyte compartments of the body (5).

32. In collaboration with Dr. Frederick Rice of American University, "leukogenenol" measurements were made in our leukapheresed rats. On

the basis of these studies, it was concluded that the "neutrophil releasing factor" described by us as the operating hormonal mechanism for rapid mobilization of granulocytes in animals rendered leukopenic by filtration leukapheresis is probably not "leukogenenol" (5).

II. DETAILS OF WORK CARRIED OUT

BETWEEN 1 FEBRUARY 1980 AND 31 JANUARY 1981

A. INTRODUCTION

A primary goal of this contract was to determine whether or not the use of autologous plasma from animal donors who have undergone filtration leukapheresis can be used to increase granulocyte yields if given prior to a subsequent pheresis. It has already been demonstrated by other groups that it is possible to improve granulocyte yields in humans by stimulation of the donor granulocyte count with the use of potent hormones (generally adrenocorticosteroids) or red cell sedimenting agents given before granulopheresis (6-9). In fact, with most systems, it is the only means by which granulocytes in sufficient number to obtain a clinical effect can be harvested. The use of such hormones in normal donors is not without risk. Because of the lack of a safer method for mobilizing granulocytes in donors and because of the serious nature of granulocytopenia in patients with a deficiency in their immune response, this risk has been accepted in the absence of a safer method, and the long-term effects of hydroxyethyl starch are still being questioned. Ideally, a normal physiological mechanism for stimulation of the donor granulocyte count would be preferred.

We have shown previously in rats (2,4,10) that it is possible to stimulate the donor granulocyte count and to improve granulocyte yields by taking advantage of normal humoral homeostatic mechanisms.

Collection of rat granulocytes by filtration leukapheresis results in such efficient and rapid removal of granulocytes from the circulating pool that granulopoietic mechanisms are stimulated to mobilize granulocytes from body reserve stores (2). We have hypothesized that these cells are released not only from the peripheral marginating pool but also from the bone marrow reserves. Our calculations suggest that on the basis of available evidence the numbers of granulocytes which can be mobilized are too great to have come only from the marginating pool, which is considered to be approximately equal to the circulating pool (11).

The studies carried out under the present contract have been designed to elucidate the nature of the mechanisms for mobilizing granulocytes under the impetus of rapid depletion of the circulating pool and to provide some understanding of the kinetics of this mobilization.

Prior to designing clinical studies to determine the possibility of using the phenomenon of granulocyte mobilization to increase granulocyte yields in human donors, we have been making every effort to determine the kinetics of granulocyte mobilization under these very special conditions. We have determined that within certain circumscribed contexts, the animal model for filtration leukapheresis developed by us is analogous to the human system. There are certain logistic problems concerning the optimal use of time and materials for making the best use of this animal model for the kind of definitive investigations we have been carrying out. These investigations have

attempted to suggest the optimal approach to clinical studies designed to provide the greatest granulocyte yields with the system of filtration leukapheresis. In this regard, we have investigated the effect of the characteristics of the test animal, the rat, on the results obtained. We have tested factors such as depth of anesthesia, anticoagulant, and the interaction of the circulating blood and the plastic conduits of the filtration system per se on granulocyte mobilization.

We have retested control materials (homologous plasma) and potential control materials (heterologous plasma) so that any contribution they may make to the results can be assessed and taken into account when granulocyte increments are calculated.

We have investigated whether the rat model reacts to a drug in the same way as do human beings as an additional check on the similarity of the rat to the human being in this regard.

A further study of the kinetics of granulocyte mobilization and the degree to which such mobilization occurs during filtration leukapheresis has been carried out using a mathematical model which is descriptive of actual mobilization of cells and takes into account not only the cells in the circulating blood but also those removed during the filtering process. This study provides basic information on granulocyte kinetics.

Finally, in collaboration with Dr. Frederick Rice at American University, Washington, D.C., and with Dr. Harvey Bank at the Medical University of South Carolina, Charleston, South Carolina, attempts have been made to characterize by chemical and physical means the humoral

factor(s) in normal plasma and plasma from donors who have undergone filtration leukapheresis.

B. METHODOLOGY

1. Perturbations of Granulocyte Counts Induced by Chemical and Physiological Events During Filtration Leukapheresis

During filtration leukapheresis, a factor(s) is produced, released, or extracted into rat blood which causes a transient granulocytosis in pheresed animals and in recipients of homologous plasma from these animals. To identify some of the factors which may contribute to this granulocytosis, a number of chemical agents potentially extracted or produced by the procedure were tested for their ability to stimulate granulocytosis. The agents included common mediators of inflammation and proteinases released by PMNs, extracts of nylon fibers and Tygon tubing, nylon monomers and solvents used in the manufacture of nylon; oxidized and decomplemented plasma; lysates of PMNs and microorganisms.

Normal rat plasma was obtained from 16 rats by exsanguination via the abdominal aorta into syringes containing heparin. Following centrifugation at 2,000 x G for 30 minutes to separate the plasma, the pooled plasma was divided into two 35 ml aliquots, packed in dry ice, and shipped to Dr. Harvey Bank in South Carolina. One 35 ml aliquot was divided into 19 samples and treated, refrozen, and returned. The thawed plasma was divided into treatment groups as follows:

a. Plasma and balanced salt solutions were tested to determine whether they could extract a substance from nylon capable of inducing

a granulocytosis. Small portions of nylon were aseptically removed from Fenwal Leukopak filters, 10 mg samples were weighed, placed in 15 ml glass test tubes and capped with teflon lined camps. Two ml of plasma heated to 56^{o}C for 30 min or 2 ml of Hanks balanced salt solution (HBSS) were added and the tubes were mechanically shaken at 130 strokes/min for 30 min at 37^{o}C. The plasma or balanced salt solution was then decanted and frozen on dry ice.

b. Monomers and solvents and plasticizing agents used in the formulations of a typical nylon (6-6) including cyclohexane, adipic acid, or hexamethylenediamine (3 ul/ml) were added to the plasma which was then refrozen.

c. Tygon tubing and polyvinylchloride (filter casing material) were removed from a Fenwal Leukopak TM filter and cut into pieces two mm in diameter. Samples (10 mg) were placed in test tubes and extracted with plasma as described above to determine if a substance capable of inducing a granulocytosis could be extracted from these plastics.

d. Buffy coat was isolated from Sprague-Dawley rats by dextran sedimentation and the cells were washed twice in HBSS. The cell concentration was adjusted to 10^{8} cells/ml and the cells were disrupted by three cycles of immersion in liquid nitrogen followed by rapid thawing in a 37^{o}C water bath. Lysate from to $5x10^{6}$ cells was added to each ml of plasma.

e. Three common biological mediators of inflammation and two typical PMN proteases were investigated. Specifically, plasma was enriched with pharmacologically active amounts of prostaglandin E_2 (5 ng/ml), bradykinin (5 ng/ml), collagenase (5 ug/ml), histamine (3 ug/ml), or trypsin (5 ng/ml).

f. Extracts of bacteria and yeast which might be present in contaminated filters or result from careless handling of the preparations were investigated. Early log phase cultures of E. coli. Streptococcus intermedius and Bakers yeast were grown in Luria batch on trypticase-soy media, harvested during early log phase growth ($\sim$0.4 OD) and disrupted in a Tekmar homogenizer. The disrupted cell suspension was added to the plasma (50 ul/ml).

g. Control plasma (unmodified) and plasma through which oxygen had been bubbled to oxidize serum proteins were tested. The other 35 ml aliquot sample was simply thawed and refrozen and was used as a control. The untreated and modified plasma was returned on dry ice to Massachusetts. The samples were stored in the gas phase of a liquid nitrogen refrigerator ($\sim$150^{o}C) until use. Immediately prior to use, the samples were thawed by vigorous shaking under running tap water equilibrated at $\sim$40^{o}C. Typically, four rats were given only induction anesthesia, 60 mg/kg Nembutal intraperitoneally. The plasma (1.0 ml/kg) was injected intravenously into anesthetized rats.

Each of the samples was tested in a double blind fashion. Specific samples were identified by a letter and the code was broken after all results were measured and tabulated. WBC and differential counts were

obtained from all animals as soon as they were anesthetized, and at hourly intervals thereafter. Since injection of each set of rats took two minutes, there was little difference in the length of the experiment between those injected with plasma, modified or control, and those receiving no plasma injection. The number of circulating PMNs was calculated as described above.

(1) Controls

The PMN counts of 21 control (noninjected) animals were measured along with each test sample to account for changes in environmental and other undefinable factors over the course of the experiments. A group of 12 animals which received normal plasma was tested as a single group so that the method of study of these controls would be analogous to that of each of the experimental groups.

(2) Statistics

For the group which received the various fractions, significance between the treated groups and each of the control groups was measured by the Wilcoxon rank-sum test (12). This test is appropriate for small sample sizes or when the data may not be normally distributed. For analysis of the groups with larger sample size, the standard error of the differences between the means was calculated to determine statistical significance (13).

2. Pretreatment with Postpheresis Plasma of Rat Donor Undergoing Filtration Leukapheresis

In a previous annual report to ONR (4), we had presented some preliminary data regarding the optimal time to pretreat rats with post-

pheresis plasma prior to undergoing filtration leukapheresis. At that time, some data were available for pretreatment one and two hours before pheresis. During this reporting period, we have added additional experiments to those time periods and have provided data obtained following pheresis preceded by PPP infusion three hours before initiation of the procedure.

Sprague-Dawley rats weighing 350 to 450 grams were leukapheresed as described previously (1). Following pheresis, the rats were exsanguinated and the plasma was separated by centrifugation of the whole blood. The plasma was frozen at -20°C for future use. Additional groups of rats were treated as follows:

a. Two rats were pretreated with 1.5 ml/kg of PPP given intravenously one hour before initiation of pheresis [11]*.

b. Four rats were pretreated with 1.5 ml/mg of PPP given intravenously two hours before initiation of pheresis [8].

c. Eight rats were pretreated with 1.5 ml/kg of PPP given intravenously three hours before initiation of pheresis [8].

Before any treatment (injections of PPP or pheresis) were carried out, cannulae were inserted into a jugular vein and carotid artery, and the animals were anticoagulated with heparin-sodium, 400 I.U. per animal. Injection of PPP was done via the jugular vein cannula. An arterio-venous shunt was then constructed by connection of the arterial and venous cannulae. At the appropriate time after injection of the

*The numbers in brackets are the total numbers of experiments, including those previously reported.

PPP, a nylon wool filter was inserted into the system in the standard manner.

In all groups, following two hours of pheresis, the filters were removed from the system and the granulocytes were eluted with 35 ml of phosphate buffered saline enriched with bovine albumin. The yield of granulocytes was calculated following determinations of the total white blood cell count, the differential WBC count, and the volume of eluate.

3. Effect of Sequential Administration of PPP on the Granulocyte Increment

To determine whether two separate injections of PPP separated by three hours were capable of increasing the magnitude of the granulocyte increment, the following groups of animals were studied:

a. Nine normal rats were given a dose of PPP, 1.5 ml/kg. Granulocyte counts were obtained before and each hour for five hours after injection (control series).

b. Nine normal rats were given a dose of PPP, 1.5 ml/kg. A second similar dose was given three hours later. Granulocyte counts were obtained before and each hour for five hours after the first injection (sequential treatment series). The granulocyte increments at three and five hours after the initial injection were compared.

4. Modifications of Elution Technique to Improve Granulocyte Yields

a. Elution in the Cold

Klock et al. (14) suggested that mechanical shearing of adherent cells and not adherence per se causes fragmentation of the cells during

elution. They suggested that agents which decrease cell spreading diminish fragmentation. Cold is such an agent. To examine this possibility, two groups of animals were studied:

(1) Six animals were leukapheresed in the usual manner. The filters were kept at room temperature for one hour. The granulocytes were eluted with the usual eluent and the granulocyte yield was calculated.

(2) Five animals were similarly leukapheresed. The filters were kept at 4°C for one hour. The eluent was also kept at 4°C for the same period. The granulocytes were eluted with the cold eluent and their number was calculated.

A comparison was made between the yields from both groups.

b. Saline Prewash to Remove Excess Red Blood Cells

Although the contamination of granulocyte concentrates with red blood cells is not great, there is some concern that transfusion of even a small number of red cells may be harmful in a small number of granulocyte recipients who are sensitive to red cell antigens from ABO-incompatible donors. Washing the filters with saline prior to elution of the granulocytes has been suggested as a means for removing most of the remaining red cells. The very low pH of saline, and its lack of protein and chelating agents suggest that this may be possible. To determine the loss of granulocytes during a saline prewash, the following study was done: Ten rats were pheresed in the usual manner. Saline, 8 ml/filter, was pushed through the filter with gentle pressure and no tapping. This volume was shown to be just sufficient to remove the

red color of contaminating red blood cells from the filter. The granulocytes were then eluted using the standard eluent. The number of granulocytes in the saline wash and in the standard eluate were calculated and compared.

c. Substitution of Hydroxyethyl Starch for Albumin in the Eluent

Plasma proteins in the form of plasma or albumin have been used routinely in the eluting medium for granulocytes in the filtration system. This material is expensive and makes use of a blood product which is often in short supply. Hydroxyethyl starch has many characteristics in common with plasma proteins, is compatible with blood cells, is relatively inexpensive, and is readily available. The following study was done to determine whether hydroxyethyl starch could be substituted for plasma proteins in the granulocyte eluting medium: Eleven animals were pheresed in the usual manner. Six of the filters were eluted in the usual manner with eluent and filters kept for one hour at room temperature. Five were kept with their eluent for one hour at $4^{o}C$. Following elution of the granulocytes, the yields obtained using the two methods were compared.

An additional comparison was made between warm- and cold-eluted cells. The effect of prewash of the filters with saline on the final harvest of granulocytes from the warm and cold-harvested cells was determined.

5. Granulocyte Yield Versus Prepheresis Donor Granulocyte Count

We had shown previously (4) that the higher the postpheresis granulocyte count in the donor the greater the yield. In a further study of the kinetics of granulocyte mobilization, an analysis was made in which the donor prepheresis count was correlated with the granulocyte yield. Four groups were compared.

a. Counts less than 5,000/mm^3 - 29 animals
b. Counts between 5,000 and 15,000/mm^3 - 16 animals
c. Counts between 15,100 and 25,000/mm^3 - 7 animals
d. Counts greater than 25,000/mm^3 - 3 animals

6. Granulocyte Yield Versus Donor Postpheresis Granulocyte Count - Additional Studies

We had shown previously (4) that there is a direct correlation between the donor postpheresis granulocyte count and the granulocyte yield from that donor following a standard pheresis. During this contract period, an additional 55 animals were added to the original 33 described previously. An analysis was made to determine whether the relationship described two years previously could still be demonstrated and the magnitutde of any change, if any, by the addition of substantially more animals to the series. The following groups were analyzed.

a. Postpheresis counts less than 5,000/mm^3 - 2 additional
b. Postpheresis counts between 5,000 and 15,000/mm^3 - 29 additional
c. Postpheresis counts between 15,100 and 25,000/mm^3 - 15 additional
d. Postpheresis counts greater than 25,000/mm^3 - 9 additional

7. Postpheresis Granulocyte Increments Versus Prepheresis Counts - Stimulated Rats

We had shown previously (4) that there was a direct correlation between the prepheresis granulocyte count and the postpheresis increment in the granulocyte donors. In the earlier study, however, we were dealing with nonstimulated animals with granulocyte counts generally between 2,000 and 5,000/mm^3. Since that time, stimulation of animals before pheresis with postpheresis plasma has resulted in prepheresis counts as high as 20,000 or more. We have now had the opportunity to consider the effect of greater prepheresis counts on granulopheresis. The following comparisons were made:

a. Counts less than 5,000/mm^3 - 30 animals
b. Counts between 5,000 and 10,000/mm^3 - 11 animals
c. Counts between 10,100 and 15,000/mm^3 - 3 animals
d. Counts between 15,100 and 20,000/mm^3 - 6 animals
e. Counts greater than 20,000/mm^3 - 6 animals.

C. RESULTS

1. Perturbations of Granulocyte Counts During Filtration Leukapheresis

Injection of the lysate of freeze-thawed PMNs produced a significant increment of the PMN count when compared with both groups of control animals (Table 1). The plasma enriched with cyclohexane, prostaglandin E_2, or trypsin, extracted with polyvinylchloride, as well as nylon-extracted HBSS, caused a somewhat smaller but still statistically significant granulocytosis.

TABLE 1

EFFECT OF VARIOUS PROCEDURES ON THE GRANULOCYTE COUNT OF NORMAL RATS

Sample Treatment	No. of Animals	Granulocyte Increase Three Hrs Postinjection ($\times 10^3$)	Significance Compared to* Noninjected Animals	Plasma Injected Animals
1. (a) normal plasma	10	5.0	-	-
(b) oxidized plasma	4	1.8	-	-
2. Enriched with enzymes				
(a) prostaglandin E_2	4	5.2	-	+
(b) bradykinin	4	5.2	-	-
(c) histamine	3	3.7	-	-
(d) collagenase	4	4.5	-	-
(e) trypsin	4	4.5	-	+
3. Enriched with extracts of				
(a) granulocytes	4	9.0	+	+
(b) E. coli	2	6.8	-	-
(c) Bakers yeast	3	2.7	-	-
(d) streptococcus	4	2.1	-	-
4. Enriched with plastic components				
(a) adipic acid	3	2.3	-	-
(b) hexamethylendiamine	4	1.2	-	-
(c) cyclohexane	4	7.8	+	+
5. Nylon extracted with				
(a) plasma	4	4.2	-	-
(b) decomplemented plasma	4	1.8	-	-
(c) HBSS	4	5.6	+	+
6. Plasma extracted				
(a) tygon tubing	4	3.8	-	-
(b) polyvinylchloride	4	6.9	-	+

*By the Wilcoxon rank-sum test

In the control groups there was a slight increase in PMN count ($\sim$2,000/mm^3) in the group which received plasma and the group which was only anesthetized. The increments in the two groups were essentially identical (Fig. 1).

There was no significant difference between the average number of circulating PMNs in noninjected controls (which were studied over several months) and in control animals injected with normal plasma on the same day.

2. Pretreatment of Donors with Postpheresis Plasma

Twenty-nine rats pheresed for two hours without pretreatment with postpheresis plasma yielded a mean granulocyte yield of 8.7 x 10^7 granulocytes (Table 2). When 11 rats were given 1.5 ml PPP/kg of body weight one hour before pheresis, the mean yield of granulocytes was 12.9 x 10^7, an increase over the nontreated controls of almost 50%. Pretreatment with PPP two hours before a two hour pheresis resulted in a mean yield of 13.5 x 10^7 granulocytes. When the time between PPP injection and initiation of pheresis was extended to three hours, the yield was 9.6 x 10^7.

When these figures were added to those obtained previously, the yields from animals not pretreated and those pretreated one, two, and three hours before pheresis were 8.9, 14.4, 14.2, and 9.6 x 10^7, respectively. The yields in animals pretreated one, two, and three hours before pheresis were 62, 60, and 8 percent greater than those not pretreated when using the combined figures.

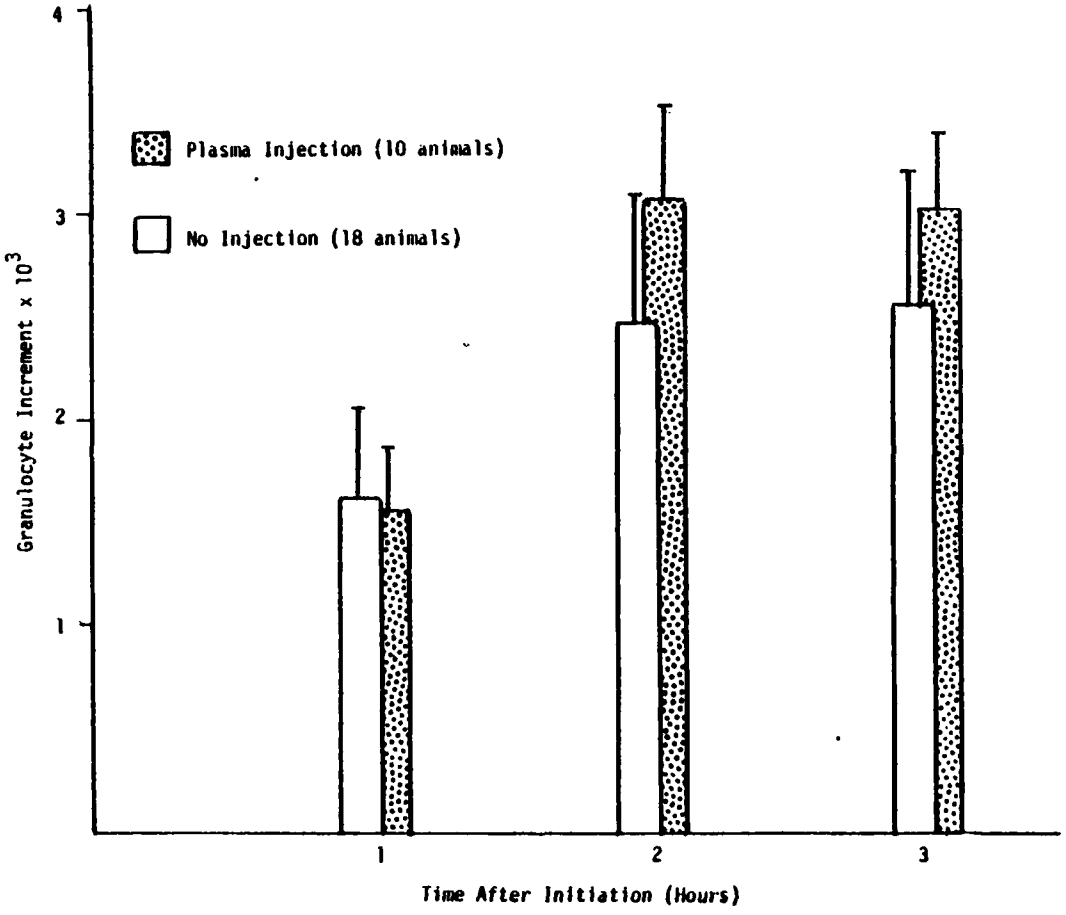

FIGURE 1

EFFECT OF PLASMA ON CIRCULATING GRANULOCYTES

TABLE 2

PRETREATMENT WITH POSTPHERESIS PLASMA OF RAT DONORS UNDERGOING FILTRATION LEUKAPHERESIS

		Pheresis	Pretreatment before Pheresis					
	No.	Alone	No.	1 Hour	No.	2 Hours	No.	3 Hours
Previous Group	8	9.69	9	16.34	4	15.53	0	-
Present Group	29	8.7	11	12.9	8	13.5	9	9.6
Combined Groups	37	8.9	20	14.4	12	14.2	9	9.6

3. Sequential Administration of PPP

In nine animals given a single dose of 1.5 ml/kg of PPP, the granulocyte increment after three hours was 9,134/mm^3 and after five hours was 10,919. This represented the control group (Table 3). The granulocyte increment in nine animals given two sequential doses of 1.5 ml/kg of PPP was 8,639 three hours after the initial injection. The increment two hours after the second dose (given three hours after the first dose) was 7,332/mm^3.

In both groups, there was no significant difference between the three and the five hour count. A second injection of PPP did not further stimulate mobilization of granulocytes.

4. Granulocyte Elution in the Cold

As shown in Table 4, there was only a slight increase (10%) in the yield of granulocytes eluted from cold filters with cold eluents. There are too few experiments to give a definite answer with regard to the advantage of this modified elution method.

5. Saline Prewash

A total of nine experiments was carried out in which the filters were prewashed with saline to remove contaminating red blood cells. As shown in Table 5A, a mean of 34% of the granulocytes was lost by prewashing the filter with saline.

Of the five filters subjected to warm elution, the loss of granulocytes into the saline wash averaged 41% while that lost following cold elution averaged 26% (Table 5B).

TABLE 3

EFFECT OF SEQUENTIAL PPP INJECTION ON THE GRANULOCYTE COUNTS OF NORMAL RATS

Treated Group (Two Injections)			Control Group (One Injection)		
Initial Count x 10^3	3 Hour Count x 10^3	5 Hour Count x 10^3	Initial Count x 10^3	3 Hour Count x 10^3	5 Hour Count x 10^3
2174	17802	16424	843	21040	19453
13701	24141	16240	3736	19824	34669
3808	7164	15680	9790	25174	17713
7277	19662	16434	6401	26074	36611
7249	24953	20789	1629	3588	5180
1001	4853	4280	5938	10164	7938
2017	3072	5134	1931	6195	7030
3885	15556	10785	1187	2601	3001
4227	5894	5560	9795	8794	7919
5038 (Mean)	13677 (Mean)	12370 (Mean)	4583 (Mean)	13717 (Mean)	15502 (Mean)

TABLE 4

COMPARISON OF WARM AND COLD ELUTION PROCEDURES
ON THE GRANULOCYTE YIELD FROM NORMAL RATS

Harvest of Granulocytes ($x10^7$)	
Warm	Cold
6.5	6.3
9.4	7.0
5.7	7.8
7.3	10.7
8.5	13.9
12.7	
8.4 (Mean)	9.1 (Mean)

TABLE 5A

LOSS OF GRANULOCYTES INTO SALINE PREWASH

DURING ELUTION OF GRANULOCYTES FROM NYLON FILTERS

Total Granulocytes Harvested ($x10^7$)	Granulocytes in Saline Wash ($x10^7$)	Granulocytes in Saline Wash (%)
9.39	3.73	40
7.01	1.66	24
5.72	2.94	51
7.84	1.20	15
7.26	3.64	50
10.72	3.21	30
8.46	3.37	40
13.92	4.71	34
12.72	3.33	26
9.22	3.09	34

TABLE 5B

EFFECT OF COLD ELUTION ON GRANULOCYTE LOSS

INTO SALINE PREWASH

Granulocytes Lost in Saline Wash (%)	
Warm Elution	Cold Elution
40	24
51	15
50	30
40	34
26	—
41	26

6. Hydroxyethyl Starch to Replace Albumin in the Eluent

In six experiments using 35% hydroxyethyl starch (Hespander, HES), an average yield of 12.6 x 10^7 granulocytes were harvested per filter following standard two hour phereses. This compares with an average of 8.9 x 10^7 cells from 37 animals using the standard albumin-containing eluent and represents an increased yield of 42% using HES (Table 6).

7. Granulocyte Harvest Versus Prepheresis Count

As shown in Table 7, there was a direct correlation between the donor prepheresis granulocyte count and the granulocyte yield from those animals following a standard two hour pheresis. The average yield from 29 rats with prepheresis counts below 5,000/mm^3 was 7.8 x 10^7. As the mean prepheresis count increased to greater than 25,000/mm^3, the mean granulocyte yield increased to 17.7 x 10^7 (+126%).

8. Granulocyte Harvest Versus Postpheresis Count

As shown previously (4), there was a direct correlation between the postpheresis granulocyte count and the granulocyte yield (Tables 8A, 8B, and 8C). In comparing the extremes, the mean yield of granulocytes in three animals with a postpheresis count less than 5,000 was 2.84 x 10^7. That of 15 animals with counts above 25,000 was 16.05 x 10^7, a 4.7 fold increase.

Figure 2 demonstrates an essentially linear relationship between the postpheresis granulocyte count and the granulocyte yield.

It is important to note that in 24 of 36 (67%) experiments in which a yield of less than 12 x 10^7 cells was obtained, the postpheresis

TABLE 6

HYDROXYETHYL STARCH AS A SUBSTITUTE FOR PLASMA PROTEINS IN ELUENT FOR GRANULOCYTE HARVESTING

	Granulocyte Yields Using	
	Albumin Eluent	HES Eluent
Number of experiments	37	6
Mean Yield x 10^7	8.9	12.6
Range x 10^7	2.00-23.4	7.26-22.39

TABLE 7

CORRELATION OF PREPHERESIS GRANULOCYTE COUNT AND GRANULOCYTE YIELD FROM NYLON FILTERS

	Prepheresis Granulocyte Count			
	<5,000	5,000-15,000	15,100-25,000	>25,000
Number of experiments	29	16	7	3
Mean Gran Yield x 10^7	7.8	10.7	12.5	17.7
Range x 10^7	2.0-18.1	5.7-19.5	5.5-20.1	14.0-20.0

TABLE 8A

CORRELATION OF POSTPHERESIS GRANULOCYTE COUNT WITH GRANULOCYTE YIELDS FROM NYLON FILTERS

	Postpheresis Granulocyte Count (New Experiments)			
	<5,000	5,000-15,000	15,100-25,000	>25,000
Number of experiments	2	29	15	9
Mean Gran Yield x 10^7	3.1	7.5	12.7	15.3
Range x 10^7	2.3-3.9	2.0-15.2	5.5-23.4	8.7-20.0

TABLE 8B

Previous Experiments

Number of experiments	1	14	12	6
Mean Gran Yield x 10^7	2.34	7.52	11.44	17.17

TABLE 8C

Combined Experiments

Number of experiments	3	43	27	15
Mean Gran Yield x 10^7	2.84	7.51	12.14	16.05

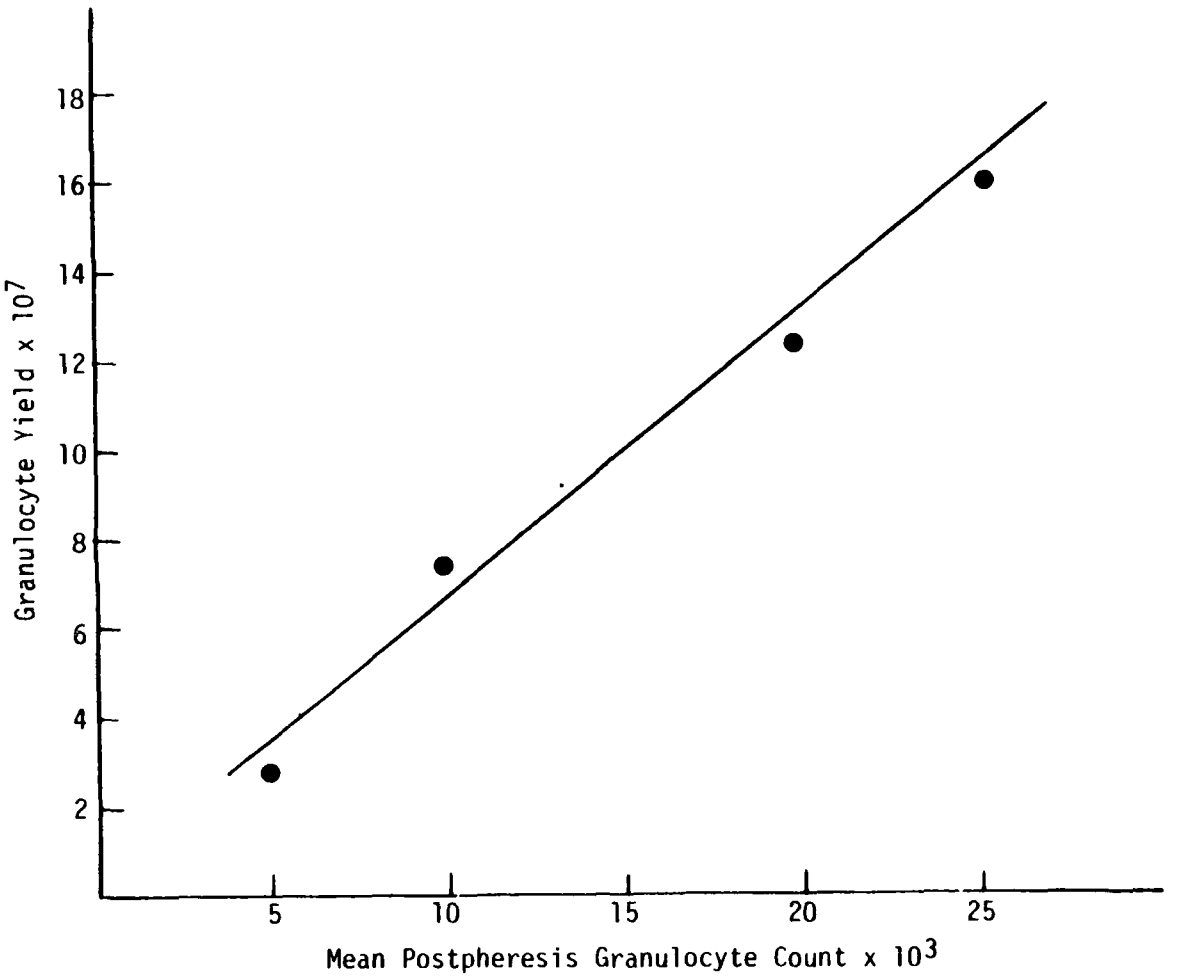

FIGURE 2

CORRELATION BETWEEN POSTPHERESIS GRANULOCYTE COUNT AND GRANULOCYTE YIELD

count was under 14,000/mm^3. On the other hand, in 15 of 19 experiments (79%) in which the granulocyte yield was greater than 12 x 10^7, the postpheresis counts were greater than 14,000/mm^3. These correlations are significant ($p<.005$) by the chi square method of analysis.

9. Postpheresis Increments Versus Prepheresis Counts (Stimulated Animals

As shown in Table 9A, the 31 animals with normal granulocyte counts ($\leq$5,000/mm^3) demonstrated a mean postpheresis increment of over 12,000/mm^3. As the mean prepheresis count increased, the mean postpheresis increment was reduced. Animals with a prepheresis count averaging over 20,000/mm^3 actually showed a mean decline. If one considers the mean postpheresis count (prepheresis count plus end of pheresis increment) we note that in each group this count averages between 15,000 and 20,000/mm^3. Table 9B also shows a similar phenomenon except for the animals with the very lowest counts (<2,000/mm^3).

D. DISCUSSION

Filtration leukapheresis is a rapid and efficient method for harvesting PMNs from the circulating blood (15-18). Shortly after the onset of filtration leukapheresis, a granulocytopenia followed by a pronounced granulocytosis occurs. Upon injection into a homologous recipient, plasma from these pheresed animals (PPP) can induce a granulocytosis (1,19). Comparable effects do not occur when PMNs are harvested by centrifugal pheresis procedures (1,20). This may be related to the slower rate of PMN harvesting by centrifugal methods (1). Potentially, an injection of PPP or neutrophil releasing factor could

TABLE 9A

CORRELATION OF PREPHERESIS GRANULOCYTE COUNT WITH POSTPHERESIS GRANULOCYTE INCREMENT

	Prepheresis Granulocyte Count/mm^3				
	<5,000	5,000-10,000	10,100-15,000	15,100-20,000	>20,000
Number of Experiments	31	11	3	6	6
Mean Postpheresis Increment/mm^3	12,400	7,741	6,889	2,008	-1,070
Range/mm^3	-2,107 to 29,958	301 to 15,320	-763 to 19,482	-9,020 to 11,552	-16,311 to 18,181
Postpheresis Count/mm^3	~17,400	~15,200	~19,389	~19,508	~20,000

TABLE 9B

	Prepheresis Granulocyte Count/mm^3		
	<2,000	2,000-3,999	>4,000
Number of Experiments	12	22	24
Mean Postpheresis Increment/mm^3	9,189	14,556	14,808

be utilized to improve the yield of PMNs by leukapheresis or to mobilize PMNs during episodes of neutropenia (22).

We assessed the relative contributions of a number of chemical factors of filtration leukapheresis which may contribute to the granulocytosis.

Both leukocytes and plasma may interact with the nylon filters (23-25) and induce a transient granulocytopenia when injected into non-leukapheresed recipients (1,19,23,26). Alternatively, the transient granulocytopenia may result from activation of the complement system of the PMNs with a resultant increase in the tendency of the circulating PMNs to marginate (27).

Factors other than the initial granulocytopenia may contribute to the granulocytosis which occurs during filtration leukapheresis. The plasma sample containing a lysate of frozen-thawed PMNs produced a significant PMN increment compared to both controls. This is consistent with the findings of Weisberger that injections of leukocyte lysates induce an initial neutropenia followed by a leukocytosis (28). However, in the rat the leukopenia occurs within a few minutes and the leukocytosis starts 10 to 15 minutes later, while in humans leukocytosis did not occur for more than two hours after induction of the leukopenia (28).

The PMN increment caused by PMN lysates in this study was comparable to that produced by sham-pheresis (9,000 vs. 8,000/mm^3). Other plasma samples which differed significantly ($p<.05$) from the control series were enriched with prostaglandin E_2, trypsin, or cyclohexane.

In addition, polyvinylchloride extracted with plasma and nylon extracted with HBSS produced a significant granulocytosis. These results indicate that products of PMNs are released during the interaction with PVC tubing and/or nylon filter material which may contribute to the granulocytosis observed in filtration pheresed animals. The interaction of plasma with the freshly ground PVC in our studies is somewhat greater than would be expected with intact PVC tubing. Since granulocytopenia and granulocytosis did not occur during centrifugation leukapheresis, which also utilizes PVC tubing, it is unlikely that interaction of PMNs with the PVC tubing is a major cause for the granulocytosis.

Cyclohexane, a solvent commonly used in the manufacture of nylon 6-6, produced a significant granulocytosis. Typically, adipic acid and hexamethylenediamine are dissolved in cyclohexane. Through a condensation reaction, the dicarboxylic acid reacts at the interface with the diamine to form a linear polymer (polyamide) which is then drawn out in a continuous strand. Despite the flushing of the system with normal saline prior to use, some residual solvent may be present. Thus, at least one component of the nylon may contribute to the granulocytosis observed in animals undergoing filtration leukapheresis.

Two mediators or products of inflammation caused a significant granulocytosis. After PMNs are exposed to a phagocytic stimulus, they release nanogram quantities of prostaglandins and trypsin (29,30). Both of these agents produced a statistically significant granulocytosis. Bradykinin, histamine, and collagenase produced a milder granulocytosis which did not differ statistically from the controls.

In this study, the number of circulating PMNs were used as the sole criterion for determining the effect of the plasma samples. The number of circulating PMNs is the sum of the initial circulating PMN levels plus the PMNs mobilized from the bone marrow, vascular lining, and tissue spaces, less those PMNs sequestered in tissues or marginating on the vascular walls or lysed. We cannot conclude from these studies that those plasma samples which did not result in granulocytosis had no biological effect, especially since the intravascular PMN pool is only ~1/50 of the total body PMN pool (23). The effect of biological mediators of inflammation such as bradykinin and histamine may be so transitory that the effect is essentially completed within the first few minutes and thus would have been missed in these studies. Similarly, endotoxins cause a dose-dependent delay prior to the appearance of PMNs in the circulation, possibly due to transient margination (22).

Based on the time course of granulocytosis and the magnitude of the reactions observed, we conclude that some of the biological and chemical agents tested in this study contribute to the granulocytosis occurring after filtration pheresis. However, granulocytopenia and concomitant release of a PMN mobilizing substance(s) during the filtration procedure appear to provide the major stimulus for the observed granulocytosis.

In both the recently studied group and that investigated two years ago, it was clear that pretreatment of rat donors before filtration leukapheresis did increase the granulocyte yields by 50 to 70% if given one hour before initiation of the pheresis. We had shown previously

that there was no advantage in pretreating two hours before. The present study shows a decided disadvantage in pretreatment three hours before pheresis. If the human situation is analogous to that of the rat, we would recommend that should clinical studies of this nature be attempted the plasma should be given one hour before pheresis.

It is interesting that a second injection of PPP given two hours after the first did not further increase the granulocyte count. Assuming that all these plasmas were of equal potency (and there is no assurance that they were), it would appear that the first injection, in general, provided the maximal stimulation possible under the conditions of these experiments. The degree of stimulation seen in the present experiments following injection of a single dose of 1.5 ml/kg of PPP is perfectly consistent with that seen when the original dose-response experiments were done five years ago (15,000/mm^3 vs. ~14,000/mm^3). There is further evidence that there is a limit (~20,000/mm^3) that a group of animals can be stimulated by PPP to mobilize granulocytes, although individual animals may show a considerably greater granulocyte increment upon stimulation by PPP. Doubling the dose to 3.0 ml/kg will mobilize additional granulocytes on the average by only an additional 4,000/mm^3. We would, therefore, recommend, on the basis of these experiments, that a single dose of PPP is as effective in stimulating mobilization of granulocytes over a five hour period as two doses given during that time.

There are too few experiments to decide definitely whether elution of granulocytes from nylon filters in the cold offers any advantage over

warm elution in terms of improving the yield of granulocytes. Although a slight trend in that direction was seen, the difference was not shown to be statistically significant in this small group of animals. There does seem to be an advantage, however, in keeping the filter and eluent cold if one wishes to prewash the filter to remove contaminating red blood cells. It is clear that prewashing the filter with saline does elute a significant portion (about 40% by warm elution and about 25% by cold elution). It would seem that prewashing should not be carried out unless the small number of contaminating red blood cells would pose a problem in a non ABO-matched recipient who is especially sensitive to ABO antigens. The use of hydroxyethyl starch in place of albumin in the cocktail used for elution of granulocytes from the nylon filters is important information. Albumin is expensive. HES is not. If a larger number of experiments using HES substantiates the retrieval of a greater number of granulocytes (>40% in this small series), there would be an additional and even greater advantage in using HES for this purpose.

It is not surprising that animals with higher prepheresis granulocyte counts, when pheresed, provide a greater number of granulocytes than do those with lower counts. It must be emphasized, however, that this is not a linear relationship. A doubling of the prepheresis count does not result in a doubling of the yield. This study does reemphasize our long-held contention that there is an advantage in mobilizing granulocytes before pheresis and the higher this count the higher the granulocyte yields, on the average.

It is extremely important, from the point of view of the kinetics of granulocyte mobilization, that there is a linear relationship between the postpheresis granulocyte count and granulocyte yields. The postpheresis granulocyte count is, in general, a more consistent predictor of granulocyte yield. This study also points out again the consistency of the results with this animal model. There are no significant differences among four groups of animals compared with similar groups two years apart.

One of the most interesting results obtained was the inverse correlation between prepheresis granulocyte count and postpheresis granulocyte increment. These results suggest that there is a maximum granulocyte count obtainable with filtration leukapheresis which is defined, at least approximately, by these studies. The lower the granuloycte count before pheresis, the greater the number of cells which can be mobilized by the procedure and vice versa. This mean maximum number appears to be on the order of $20,000/mm^3$, although individual animals do show a greater magnitude of increase. These studies suggest a mechanism for maintaining the granulocyte count at a defined level and that the degree of mobilization depends on the count prior to initiation of the stimulation.

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III. CONCLUSIONS

Humoral factors are elaborated by animal and human granulocyte donors during filtration leukapheresis. Plasma obtained from leukapheresed rats (PPP) may be used to increase granulocyte yields in donors when given prior to leukapheresis. In these studies, it appears that (1) the effect of injection of PPP is dose-related and is not an "all or none" phenomenon, (2) the granulocytosis observed in the donor is related to the duration of pheresis for periods up to three hours, (3) the titer of neutrophil releasing activity is related to the duration of pheresis, (4) the granulocyte increments in the donor are proportional to the duration of pheresis, and (5) there is a good correlation between the donor postpheresis granulocyte count, as well as the donor postpheresis granulocyte increment to the neutrophil releasing activity of the plasma obtained from the donor. There is no relationship between the recipient pregranulocyte count and the ability of the recipient to respond to injections of PPP, or the ability of groups of rats to respond to injections of PPP on two successive days.

Plasma obtained from rats which have been leukapheresed can be used to increase the granulocyte yields with leukapheresis procedures in subsequent donors. This results from the activity of humoral factors elaborated by the animal during the pheresis. The neutrophil-releasing activity of postpheresis plasma is stable for up to three weeks at $4^{o}C$. When aliquots of the identical PPP are used in the same animal on

successive days, the increased pretreatment count observed on the second day is probably the result of a subclinical infection at the injection site rather than of a residuum of the previous day's stimulation. The maximum granulocyte increment observed following injection of PPP occurs at three hours and is maintained for at least the following three hours. Intraperitoneal injection of PPP is ineffective in stimulating the release of neutrophils from body reserves for up to three hours after injection. The use of 50% rather than 100% plasma for elution of granulocytes from nylon filter columns results in granulocyte yields which are approximately 20% less. However, an 80% yield may be perfectly adequate for most of the proposed studies. This technique will result in the saving of substantial volumes of plasma. It is possible to use the plasma/saline eluent at least twice in the animal model being used without substantial loss of efficiency. We have shown that the dye inclusion/exclusion method of Dankberg and Persidsky for determining granulocyte viability can be adapted to the study of rat granulocytes but that the "elutriator" is probably not an effective instrument for separating granulocytes from rat blood. Preliminary nonoptimal studies have suggested that whole-body radioautographic methods are useful in determining the viability and fate of a variety of preparations of transfused granulocytes.

The optimal time for pretreating donor rats with postpheresis plasma to improve granulocyte procurement using filtration leukapheresis may be one hour prepheresis. This is suggested by studies involving various pretreatment schedules. A maximum rate of granulocyte mobili-

zation may be the limiting factor. Pretreatment with PPP less than one hour before pheresis will not yield optimal granulocyte harvests. Granulocyte harvest depends directly on the donor granulocyte count, i.e., the higher the donor count, the greater the yield. Low granulocyte counts in potential granulocyte donors, in general, predict a significantly smaller increment in the granulocyte count during pheresis. These studies suggest that the reason for the low counts in these donors is a low endogenous availability of neutrophil releasing factor. Older animals appear to possess a greater capacity for granulocyte mobilization per unit of body mass.

We have successfully replaced plasma with albumin as the major active material in the eluent for harvesting granulocytes from nylon filters and have been able to modify the staining procedures of blood films low in protein media. This had been an important problem heretofore. We have demonstrated the disposition of infused fresh and frozen granulocytes in normal guinea pigs using the technique of whole-body radioautography. Using noncell bound DFP, we have reinforced our impressions that the technique is suitable for this purpose. We have demonstrated that filtration leukapheresis can be carried out in the guinea pig. Analysis of a series of experiments has suggested the limits of granulocyte mobilization.

Several manipulations of the animals are necessary to carry out the procedure of filtration leukapheresis or to study the effect of postleukapheresis plasma in normal animals. They are all anesthetized. Pheresed animals are always anticoagulated with Heparin. The plasma

injected into PPP recipients contains Heparin. Blood is in intimate contact with plastic tubing for extended periods during the filtering process. Recovery from anesthesia can result in granulocyte mobilization. It is not clear from these experiments whether or not Heparin mobilizes granulocytes. However, it would appear that if it does, its contribution to increases in the granulocyte count is small. Of more consequence is the interaction of blood (probably the cellular elements) with the plastic tubing. However, the contributions of all these factors together is less than half of that of granulocyte removal as a cause of granulocyte mobilization. These factors do not in any way minimize the importance of the previously reported observations regarding granulocyte mobilization in leukapheresed animals and those given PPP. However, their potential contribution to granulocyte changes in these systems must be taken into account when interpreting the results of such experiments. One of these mechanisms can be minimized or eliminated as a factor in these studies if desired, i.e., maintenance of deep anesthesia can eliminate lightening of the anesthesia and subsequent stimulation of granulocyte mobilization by this means.

The injection of normal homologous plasma does not mobilize granulocytes but heterologous plasma does. The use of human plasma for any purpouse in these studies is contra-indicated. The glucocorticoid, prednisolone, exerts a granulocyte mobilizing effect in the rat but at a much more rapid rate than is generally seen in the human being.

Smaller (younger?) rats have a lower basal granulocyte level than older rats. This suggests that for the purposes of these studies, larger (older?) rats should be used to obtain the optimal effect whenever the objective is to obtain the greatest number of granulocytes since it has been shown that the higher the prepheresis count, the greater the granulocyte yield. The construction of a mathematical model for measuring the actual degree of granulocyte mobilization will allow more precise measurements of movement of granulocytes within the granulocyte compartments of the body. The "neutrophil releasing factor" described by us as the operating hormonal mechanism for rapid mobilization of granulocytes in animals rendered leukapenic by filtration leukapheresis is probably not "leukagenenol." It would appear that this latter substance is elaborated much later than "neutrophil releasing factor."

IV. SIGNIFICANT ACCOMPLISHMENTS

We have studied the effects of filtration leukapheresis on an animal donor and the effect of injection of plasma from leukapheresed animals into normal recipients. The kinetics of elaboration of neutrophil releasing activity in the donor during that process have been further elucidated. We have recommended the use of postpheresis plasma in clinical studies and suggest a starting dosage of 1.5 ml PPP/kg of body weight in human granulocyte donors. We have determined the granulocyte releasing activity in recipients which may bear on the results to be obtained following injection of postpheresis plasma and have suggested possible means for further increasing granulocyte yields in this animal model using the physiological approach of pretreatment with autologous or homologous postpheresis plasma.

We have extended the studies of the kinetics of granulocyte mobilization following injection of postleukapheresis plasma which were begun in year one of this contract. We have recommended the liquid ($4^{o}C$) storage of PPP for use in clinical studies. We have recommended that in clinical studies pretreatment of granulocyte donors should be carried out as long as possible (but not more than three hours) before leukapheresis. This results from the observations that the mean granulocyte increment occurs at three hours after injection of PPP. We have re-initiated studies of granulocyte harvest in rats under a variety of conditions and have shown that dye inclusion/exclusion methods and whole-

body radioautography can be used effectively to study basic and applied physiology of harvested and transfused rat or guinea pig granulocytes. Preliminary studies have demonstrated that certain proposed studies will require the development of a guinea pig model for filtration leukapheresis.

We have provided additional information regarding the potential use of the neutrophil releasing activity of plasma obtained from granulocyte donors. Hopefully this information will be incorporated with other data previously gathered with that yet to be obtained to suggest optimal clinical studies.

We have improved the practicality of the rat model for filtration leukapheresis by demonstrating the usefulness of a nonplasma containing eluent, as well as by developing a quick and simple method for assessing the granulocyte harvest. We have demonstrated that whole-body radioautography is a valuable new tool for assessing disposition of infused granulocytes, as well as demonstrating the usefulness (and limitations) of the guinea pig for studies of filtration leukapheresis. Basic observations of the kinetics of granulopoiesis have shed additional light on physiological mechanisms of granulocyte mobilization.

Invited lecturer:

Bermuda Medical Society, Paget, Bermuda

Granulocyte Symposium, Uppsala University, Uppsala, Sweden

Royal Postgraduate Medical School, London, England

Royal Army Medical Blood Depot, Aldershot, England

Irish Transfusion Board, Dublin, Ireland

New Zealand Transfusion Service, Auckland, New Zealand

Wellington General Hospital, Wellington, New Zealand

New Zealand Defense Ministry, Wellington, New Zeland

V. TECHNICAL REPORTS

1. Technical Report 1, Kinetics of neutrophil releasing activity of post-leukopheresis plasma, 31 July 1976.

2. Technical Report 2, Kinetics of neutrophil releasing activity of post-leukpheresis plasma, 31 July 1977.

3. Technical Report 3, Kinetics of neutrophil releasing activity of post-leukopheresis plasma, 31 July 1978.

4. Technical Report 4, Kinetics of neutrophil releasing activity of post-leukopheresis plasma, 29 August 1980.

VI. PUBLICATIONS

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2. Roy, A. and M. Ramirez. Mobilization of granulocytes on successive days with plasma from leukopheresed rats: In Blood Leukocytes: Function and Use in Therapy. C. Högman, K. Lindahl-Kiessling, and H. Wigzell, Eds. Almqvist and Wiksell, Int., Stockholm, Sweden, 1977.

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11. Roy, A. Leukocytes: collection and storage in the 1980's. Proc. Symp. on Current Concepts of Combat Casualty Resuscitation, D.O.D., Bethesda, Md., September 15-19, 1980.

12. Roy, A., H. Bank, and W. Howard. Perturbations of granulocyte counts induced by procedural, chemical, and physiological events occurring during filtration leukapheresis. Submitted to Transfusion.

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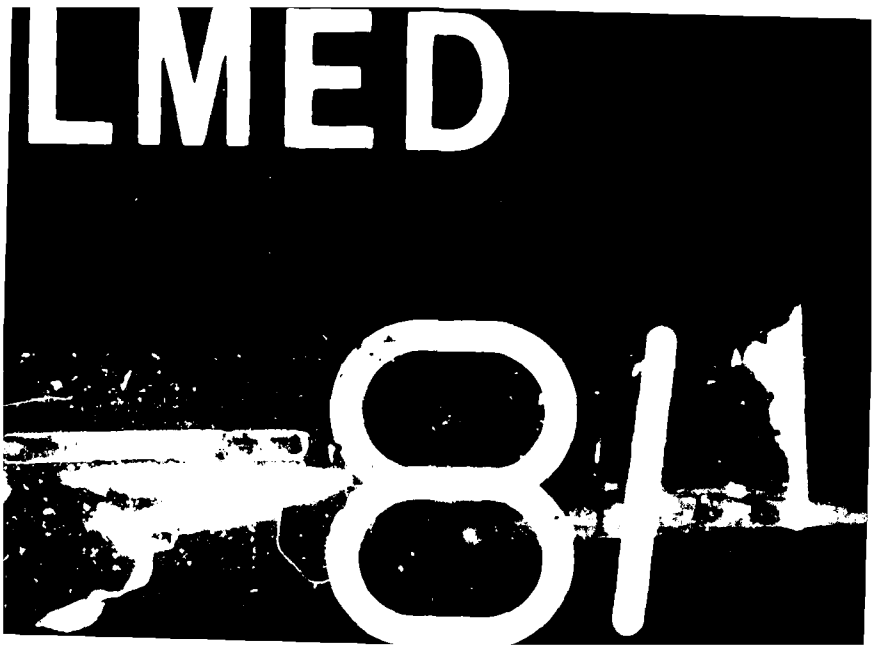
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