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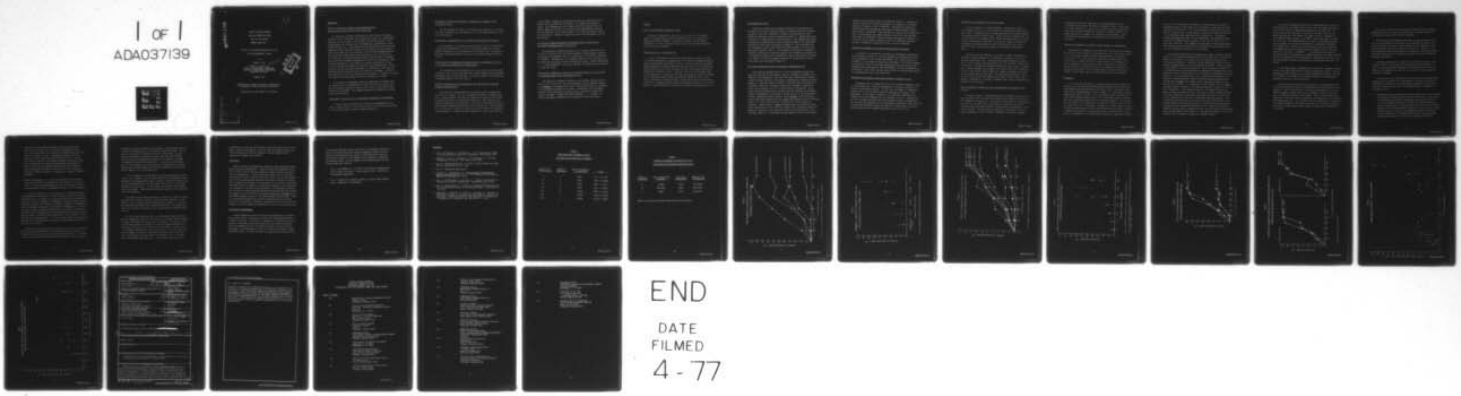

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ANNUAL REPORT NO. 1

Kinetics of Neutrophil-Releasing Activity of Post-Leukopheresis Plasma

by

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METHODOLOGY

Effect of Injection of Plasma from Leukopheresed Rats (Post-pheresis Plasma [P.P.P.]) into Normal Rats

To confirm our previous observations (1) that infusion of adequate amounts of P.P.P. into normal rats consistently results in a marked granulocytosis in the recipient, the following study was carried out. Adult Sprague-Dawley rats weighing 350 to 450 grams were leukopheresed as described previously (2). White blood cell and differential counts were done immediately before and after pheresis and the change in total granulocyte count in the donor rats was calculated. The rats were exsanguinated and the plasma was obtained aseptically and frozen at -20°C for future use. At a later date the plasma was thawed under flowing tap water at approximately 40°C with continuous shaking until the last ice disappeared. Doses of plasma appropriate to the study were injected intravenously via the femoral vein which was exposed by excision of the overlying skin. Before injection and each 30 minutes thereafter for three hours, a WBC count and differential were obtained from the recipients of the plasma. A total of 188 recipients of P.P.P. are included in this analysis. A variety of periods of phereses and plasma dosages were used, depending on the study.

As a means for determining statistical significance of changes seen as a result either of leukopheresis of donor rats or in rats given P.P.P., the pre-treatment granulocyte counts of the first 177 rats used in these studies were calculated. The standard deviation of the mean of all these counts was calculated.

Correlation of Dose of P.P.P. to Granulocyte Increment in the Recipient

In these studies, the duration of pheresis was standardized at two hours. A dosage of 0.38, 0.75, 1.50 or 3.00 ml of P.P.P./kg of body weight or 1.50 ml/kg of normal (control) plasma was injected.

Correlation of Duration of Pheresis to Granulocyte Increment in the Recipient of P.P.P.

In these studies, the duration of pheresis was varied (5, 15, 30, 60, 120, 180, 240 minutes) and the dosage of P.P.P. was standardized at 1.5 ml/kg of body weight.

Correlation of Granulocytosis in the Donor to the Duration of Pheresis

The increments in the granulocyte count of all rats in the study which received the standard dose of 1.5 ml P.P.P./kg body weight were plotted against the duration of the leukopheresis of the donor rats. An analysis was made to determine whether there was any correlation between these two factors.

Correlation of Pre-treatment Granulocyte Count in the Recipient of P.P.P. to Its Ability to Respond with a Granulocytosis

The increments in the granulocyte count of all rats in the study which received 1.5 ml P.P.P./kg body weight were plotted against the pre-treatment granulocyte count of the recipients. An analysis was made to determine the relationship between these two factors.

Ability of Granulocytes to be Mobilized by the Same Dosage of the Same Plasma on Successive Days

Donor rats were leukopheresed for two hours using the standard mini-filter (9 cm in length; 1 cm o.d.; containing 2.20 grams of scrubbed nylon wool). Before pheresis, the filter was flushed with saline and the system was filled with blood, as described previously (2). After pheresis, the plasma was obtained as described above. The plasma was divided aseptically into two equal aliquots and frozen at -20°C for future use. After freezing for up to four days, aliquots of plasma from several animals were thawed in the usual manner. Recipient rats were injected i.v. with 1.5 ml P.P.P./kg

of body weight. Changes in the granulocyte count were obtained each 30 minutes for three hours after injection. The animals were allowed to awaken. The following day, these animals (which were identified by indelible numbers at the base of the tail) were re-anesthetized, injected with the same dose of the second aliquot of the identical plasma received the previous day. Changes in granulocyte counts were determined as on the previous day.

Correlation of Donor Post-pheresis Granulocyte Count to the Titre of Neutrophil Releasing Activity of the P.P.P.

The granulocyte increments observed in all recipient animals after injection of 1.5 ml of P.P.P. were plotted against the post-pheresis granulocyte count of the pheresed donor rat, regardless of the duration of pheresis of the donor. The medians of the increments observed in the recipients were calculated for each group of donor post-pheresis counts: 0-5000, 5001-10,000, ...35,001-40,000/mm^3. An analysis was made to determine the relationship of these two factors.

Correlation of Granulocyte Increase in the Pheresed Donor to the Increment in Granulocyte Count of the Recipients of P.P.P.

The granulocyte increments observed in all recipient animals after injection of 1.5 ml of P.P.P. were plotted against the post-pheresis granulocyte increment of the granulocyte count of the pheresed donor rat regardless of the duration of pheresis of the donor. The medians of the increments observed in the recipients were calculated for each group of donor post-pheresis increment: <1,000, 1000-1999, 2000-2999, ...>30,000. An analysis was made to determine the relationship of these two factors.

RESULTS

Mean of Pre-treatment Granulocyte Counts

The mean of granulocyte counts in 177 rats prior to any treatment other than the induction of general anesthesia by the i.p. injection of Nembutal was 3956 ± 2186/mm^3. A significant increase in count (with a probability of <0.05), calculated on the basis of two standard deviations from the mean, was 4372/mm^3.

Injection of P.P.P. into Normal Rats

A total of 188 normal rats were given post-pheresis plasma. Of these, 174 (93.5%) demonstrated a granulocytosis of at least two standard deviations above the mean within three hours ($p < 0.05$). Of the 14 which did not demonstrate significant increment, three received less than 1.5 ml of plasma from animals pheresed for two hours and eight received 1.5 ml plasma from donors pheresed for 15 minutes or less. Therefore, only 3 of the 14 animals which did not demonstrate a significant increment in granulocyte response following P.P.P. injection received plasma which would be expected to have a high titer of neutrophil releasing activity. On this basis, 98.3% of the animals given P.P.P. in adequate dosage obtained from donors pheresed for a sufficient period of time demonstrated a significant increment in the granulocyte count.

Dose Response of P.P.P.

Injection of plasma into 32 normal recipient rats from normal rats pheresed for two hours invariably induced a leukocytosis within 60 minutes. In general, there was a progressive increase in the granulocyte count during the three-hour period of observation (Figure 1). Maximum increments occurred in all dosage groups in 120-150 minutes. The animals given plasma from normal, non-pheresed rats induced varying responses in the individual recipients by three hours post-pheresis (Figure 2). Both the median and mean increments, however, were lower than those observed in all the treatment groups (Figures 1 and 2). As the dosage of post-pheresis plasma was increased, there was an increased response in the recipients as measured by both the mean increments of the granulocyte count at each period of observation (Figure 1), and in the median increments of the granulocyte count at three hours after transfusion (Figure 2).

Duration of Pheresis and Titre of Neutrophil Releasing Activity

Rats given the standard dose of 1.5 ml of post-pheresis plasma from donor rats pheresed for five minutes elicited a response as measured by an increase in the granulocyte count over three hours, which was not significantly different from that of rats given a similar dose of normal rat plasma (Figure 3). When the period of pheresis in the donor rats was increased to 15 minutes, there was a measurably greater mean increment of granulocyte count in the recipients of P.P.P. at each period of observation. The response was more pronounced when the donor rats were pheresed for 30 minutes. Further, prolongation of the period of pheresis for up to four hours demonstrated a measurably greater response, on the average, in the recipients, but the differences measured were not significantly greater than that obtained using P.P.P. from rats pheresed for 30 minutes ($p > 0.05$ by analysis of variance). However, when the median increments were calculated three hours after transfusion of P.P.P., there was a more direct correlation between the duration of pheresis of the donor and the degree of granulocytosis in the recipient (Figure 4). This became even more apparent as P.P.P. from donors

pheresed for the longer periods was transfused (Figure 4). Doubling the period of pheresis to 60 minutes resulted in an increase in the median granulocyte response by 57%, to 5800 mm^3. Increasing the duration of pheresis four-fold (120 minutes) increased the median response approximately 400%, to 16,000/mm^3. When the period of pheresis was extended six-fold (to 180 minutes), the median response to the P.P.P. was increased about 600%, to 24,000/mm^3. However, further prolongation of pheresis to four hours did not further increase the titer of neutrophil releasing activity as measured by injection of P.P.P. into rat recipients.

Granulocyte Increments in the Donor Versus Duration of Pheresis

In general, as the duration of pheresis was prolonged, the granulocytosis was greater (Table 1). The mean increment of the granulocyte count in rats pheresed for five minutes was 846/mm^3, with a range from -174 to +2752/mm^3. As the length of pheresis was increased to 15, 30 and 60 minutes, a significant increase in the donor granulocyte count was observed which averaged between approximately 4000 and 6000/mm^3. Further prolongation of the pheresis to two, three and four hours resulted in a marked mean increase in the granulocyte count of the donor rats.

Recipient Pre-Granulocyte Count Versus Ability to Respond to P.P.P.

Preliminary data (3) suggested that the pre-treatment granulocyte count of the recipient was a factor in determining its ability to respond to injection of post-pheresis plasma. At that time, the numbers of comparable animals were too few to make a definitive statement with regard to this phenomenon. As the number of animals in the study were subsequently increased, the results suggested that the response of rats to a standard dose of P.P.P. obtained from rats which had undergone filtration leukopheresis for two hours was independent of the pre-treatment granulocyte count (Table 2). No significant differences among the responses of three groups of rats divided according to pre-treatment granulocyte count were observed.

Mobilization of Granulocytes on Successive Days

As seen in Figure 5, the mean increments of granulocyte counts of rats treated with P.P.P. or controls were not significantly different on days 1 and 2. Parenthetically, a slight granulocytosis was again observed following injection of normal plasma. Actual statistical analysis was carried out only for the observations at 180 minutes after injection of P.P.P. Of the 18 animals which received P.P.P., only two showed a statistically significant difference between the observations done on days 1 and 2. On each of these occasions, the granulocyte increment was greater on day 2.

Consistent with the findings of the previous study, the granulocyte increments observed appeared to be independent, in general, of the pre-treatment granulocyte count. The mean pre-treatment granulocyte counts on day 1 of the 18 animals pre-treated with P.P.P. was $4136/mm^3$. There was a 57% increase in the mean pre-treatment granulocyte count on day 2 (to $6507/mm^3$). The mean granulocyte increment seen at three hours after transfusion of P.P.P. was, however, almost identical. Figure 6 represents two studies of five animals each one week apart. The mean initial granulocyte counts in each of these groups were significantly greater on day 2 than on day 1. However, the granulocyte increments observed on day 1 were not substantially different on the two days.

Donor Post-pheresis Granulocyte Count and Neutrophil Releasing Activity of P.P.P.

As shown in Figure 7, the median granulocyte increment observed in rats which received P.P.P. from donors with a post-pheresis granulocyte count of 3 to $5000/mm^3$ was significant (an increase of more than two standard deviations (S.D.) calculated from a large series of normal untreated rats), although several individual animals in the group did not demonstrate significant increases. In the groups receiving P.P.P. from donors with post-pheresis granulocyte counts of 5001 to $30,000/mm^3$, the median increments

observed were even more significant - lying approximately 6.5 S.D. ($p < 0.005$) from the mean. The median increments of each of these groups were almost identical. It was not until plasma from donor animals with counts greater than $30,000/mm^3$ was used that a further increase in the median granulocyte increments was observed in the recipients. These increments were approximately 11 standard deviations from the mean ($p < 0.001$).

Granulocyte Increment in the Donor Versus Increment in the Recipient

The granulocyte increase in the donor which resulted from the pheresis was related to the increase in the granulocyte count of rats which received P.P.P. The analysis shown in Figure 8 demonstrated that injection of 1.5 ml/kg of P.P.P. from donors which exhibited increased granulocyte counts from 0 to 3999 resulted in a steady increase in the median increment of granulocyte counts observed in the recipients of that plasma. Further increases in the donor count did not substantially increase the granulocyte increments in the recipients until the donor increment was 30,000 or greater (and that group is represented by only five animals).

DISCUSSION

It is clear that the effect of injection of post-pheresis plasma into normal rats is both dose-related and duration-dependent up to three hours. The maximum dosage investigated was 3.0 ml/kg. This is equivalent in the human being to approximately one unit of plasma, i.e., the volume of plasma obtained from 500 ml of blood. It is a convenient volume to retain from a human donor following pheresis and is also a convenient volume to retransfuse into a granulocyte donor prior to a subsequent pheresis. On the basis of these studies, we recommend that the dosage of 3 ml/kg be used in clinical studies of P.P.P., if possible. A dosage of 1.5 ml/kg results in granulocyte increments which are 75% of those obtained with 3.0 ml/kg. However, a significant increase in the granulocyte count, as defined above, occurs with the transfusion of 3.0 ml/kg within 60 minutes of transfusion, whereas

the use of 1.5 ml/kg requires 90 minutes. Previous experience with this experimental model (1) has shown that pre-treatment of rats one hour before pheresis with 3.0 ml/kg of P.P.P. obtained from rats resulted in twice the harvest of granulocytes. No improvement in granulocyte yields was observed if the plasma was given immediately before pheresis (4). The present studies also demonstrate that, in this animal model, the granulocyte count continues to increase with all dosages of P.P.P. for at least 2 1/2 to 3 hours after injection. This suggests the possibility of increasing granulocyte yields even more than two-fold by prolonging the period between injection of the plasma and initiation of the pheresis.

Our previous observations have demonstrated a nadir of granulocytopenia within 15 to 30 minutes following initiation of pheresis (2). This was followed by a granulocytosis of greater than 30% above pre-pheresis values occurring at 45 minutes. The present studies demonstrate little or no neutrophil releasing activity after five minutes of pheresis. Within 15 minutes, a slight degree of activity was measured. After 30 minutes of pheresis, at a time when we had previously observed the beginning of granulocytosis in rat granulocyte donors, a significant titer of granulocyte releasing activity was measured. It is interesting that a two- to eight-fold prolongation of the duration of pheresis produces plasma containing granulocytosis-promoting activity which <u>averaged</u> only slightly greater than that produced in rats pheresed for 30 minutes. It is important to note, however, that the <u>median</u> increment appears to provide a more meaningful measure of effect of P.P.P. in this circumstance. In the dosage studies, no such disparity between the median and mean increments was seen. The results of these studies also suggest that prolongation of pheresis beyond three hours does not result in a further augmentation of the titer of neutrophil mobilizing capacity. This is probably because three hours of pheresis results in mobilization of the maximum number of available granulocytes. The slightly reduced granulocyte increments obtained following transfusion of P.P.P. obtained from animals pheresed for four hours as compared with that from three-hour pheresed animals cannot be explained by these studies. It may represent simply a variation in different lots of animals to respond to a similar stimulus.

It has been estimated that about 50% of the granulocytes in the total blood granulocyte pool (TBGP) can be found in the circulating granulocyte pool (CGP) and 50% in the marginating granulocyte pool (MGP) (5). The reserve granulocytes available to be drawn on in time of need are the mature granulocytes in the marrow, the number of which is estimated at four times the number present in the TBGP or about eight times the number in the CGP (5). The average granulocyte count in the peripheral blood of the rats studied in the present experiments is approximately $4000/mm^3$. This suggests that, should all of the granulocyte reserves be mobilized, the maximum granulocyte increment which could appear in the circulation would be of the order of $32,000/mm^3$. It is interesting that in each of the three series of experiments, the maximum granulocyte increments observed were of that magnitude, thus demonstrating a remarkable efficiency of post-pheresis plasma to mobilize the granulocyte reserves under the proper conditions.

In this animal model, leukopheresis for three hours is capable of mobilizing a very large proportion of the marrow granulocyte reserve. The average mean increment observed in the donor following three hours of pheresis is $27,000/mm^3$, or over 80% of the estimated reserves in the marginating granulocyte pool and in the bone marrow.

The injection of normal rat plasma resulted in a granulocytosis which did not reach significant levels as defined above, within three hours after injection. This is consistent with our previous observations (6,7) which regularly showed a similar effect and is presumed to result, at least in part, to repetitive handling of the animals. There is also evidence that injection of heparin can cause an increase in the concentration of peripheral blood granulocytes (8). It is possible that the concentration of heparin in the transfused plasma is sufficient to account for the degree of granulocytosis seen in animals given normal plasma or plasma from rats pheresed for five minutes.

We could not show a direct correlation between granulocyte increment observed in the P.P.P. donor and the granulocyte increment produced by the plasma from these donors into rat recipients.

Post-pheresis plasma obtained from donors for all durations of pheresis from five minutes to 120 minutes, when transfused into animal recipients, produced, in general, a mean increment in the granulocyte count which was greater than that observed in the donor rat. When P.P.P. from rats pheresed for 180 and 240 minutes was transfused, the mean granulocyte increments observed in the recipients were equal to or greater than that observed in the donors.

Additional studies will be required to elucidate the kinetics of the effects of P.P.P. in recipients and to determine the relationship of the titer of the granulocytosis-promoting activity produced in the donor by pheresis to the magnitude of the effect of injection of such plasma into homologous recipients.

The question of the relationship of the recipient pre-treatment granulocyte count to the ability of these animals to respond to injection of post-pheresis plasma was to determine whether granulocyte donors could be selected, in part, on that basis. Three possibilities are apparent in this kind of study:

1. Recipients with low granulocyte counts may either have little endogenous circulating neutrophil releasing activity which could, in turn, lead to two possible physiological alternatives upon injection of P.P.P., i.e., more exogenous neutrophil releasing factor may be required to induce a given granulocyte increment than would be required by animals with more circulating neutrophil releasing activity and thus, a higher initial count; or a low level of endogenous neutrophil releasing activity may make the "releasable" granulocytes in the marginating pool more "sensitive" to the effects of exogenous stimulation;

2. Conversely, a high initial granulocyte count in the recipient could reflect a higher level of circulating endogenous factor. This could result either in a population of marginating cells which have been "primed" to react to exogenous stimulation, and thus greater increases could result upon injection of P.P.P. On the other hand, a high titer of exogenous neutrophil releasing activity may be responsible for a degree of granulocyte release which is being reflected in the higher initial count and the remainder of those potentially releasable cells may be less able to respond to exogenous stimulation because of triggering of a negative feed-back mechanism;

3. Another alternative, i.e., no relation between the pre-treatment count and the ability to respond to P.P.P., appears to be the most likely. The pre-treatment granulocyte count of recipients of P.P.P. has no predictive value in determining the effect of such treatment.

In clinical situations, it is not unusual for a granulocyte donor to be leukopheresed several times - in most instances with one or more days between phereses. It appears to us that there was a need to determine the ability of the body to respond to stimulation of greater intensity than an occasional pheresis. This study was designed to provide the experimental data for increased stimulation by carrying out leukopheresis on two successive days in order to determine whether there is a degree of exhaustion of the body's ability to provide circulating granulocytes following a second pheresis within a relatively short period after a first pheresis. Such exhaustion was not demonstrated. It is not yet known whether the granulocytes mobilized by the second pheresis represent a similar population as those from the first pheresis.

Studies by other groups (5) have suggested that a maximum of approximately 32,000 granulocytes/mm^3 of circulating blood are available in the marginating granulocyte pool and are potentially mobilizable. The dosage of P.P.P. used in these studies mobilized approximately half this number.

According to strictly mathematical calculations, an equal number would be available for mobilization if the stimulus is exactly the same. The results of this study are consistent with these calculations. Further studies are required to determine how many successive daily phereses would be required to demonstrate exhaustion of the ability to respond to exogenous neutrophil releasing factor. In any case, we have shown that the rat, at least, has a large reserve of granulocytes, as has been demonstrated for human beings, and that these granulocytes can be stimulated to appear in the circulating blood.

It is not known at this time whether the increased granulocyte count on day 2 is a residuum of the increment obtained by the treatment 24 hours previously with post-pheresis plasma, or whether it may have resulted from a slight localized infection in some animals at the site of the incision overlying the femoral vein. In each animal, the operative site appeared quite clean grossly. Additional microbiological studies will be needed to answer this question.

The reason for the slight granulocytosis consistently observed following injection of normal rat plasma is not known with certainty. It may result, in part, from the constant handling of the animals. It has also been shown (8) that heparin will induce granulocytosis. We estimate that there are 20-40 units of heparin in each plasma injection, whether normal or P.P.P.

It is not surprising that there is a relationship between the post-pheresis granulocyte count and the ability of the plasma from these animals to induce a granulocytosis in appropriate recipients. The degree to which the granulocyte count of the donor rat increases during leukopheresis is obviously a reflection of the elaboration of neutrophil releasing factor. It is somewhat unexpected that over a broad range of both donor count and donor increment, the titer of neutrophil releasing activity varies very little and that only very high counts ($\geq 30{,}000/mm^3$) result in a greater titer of neutrophil releasing factor. At the present time, we have no

explanation to account for this increase at the very high counts or increments. However, this suggests a point at which the maximum possible neutrophil releasing activity is elaborated. Further studies will be required to elucidate this question.

CONCLUSIONS

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

SIGNIFICANT ACCOMPLISHMENTS

We have studied the effects of filtration leukopheresis on an animal donor and the effect of injection of plasma from leukopheresed 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 post-pheresis plasma in clinical studies and suggest a starting dosage of 1.5 ml P.P.P./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 post-pheresis plasma and have suggested possible means for further increasing granulocyte yields in this animal model using the physiological approach of pre-treatment with autologous or homologous post-pheresis plasma. We have submitted the following papers for publication under this contract:

1. Roy, A., and Ramirez, M. The effect of plasma from leukopheresed rats in normal recipients: a dose-response study. Submitted to "Experimental Hematology."

2. Roy, A. Methods for assaying viability of frozen-thawed granulocytes. Submitted to "Cryobiology."

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TABLE 1

MEAN GRANULOCYTE INCREMENTS IN RATS

FOLLOWING VARIOUS DURATIONS OF PHERESIS

Duration of Pheresis (min)	Number of Experiments	Mean of Granulocyte Increment/mm^3	Range
5	6	+846	-174 to +2752
15	4	+4303	+1229 to +10990
30	4	+3624	+3176 to +4374
60	4	+5842	+4654 to +7058
120	15	+17392	+6383 to +39178
180	6	+27206	+16461 to +38583
240	4	+20299	+11185 to +29609

TABLE 2

RELATION OF RESPONSE TO INJECTION OF P.P.P.

TO RECIPIENT PRE-TREATMENT GRANULOCYTE COUNT

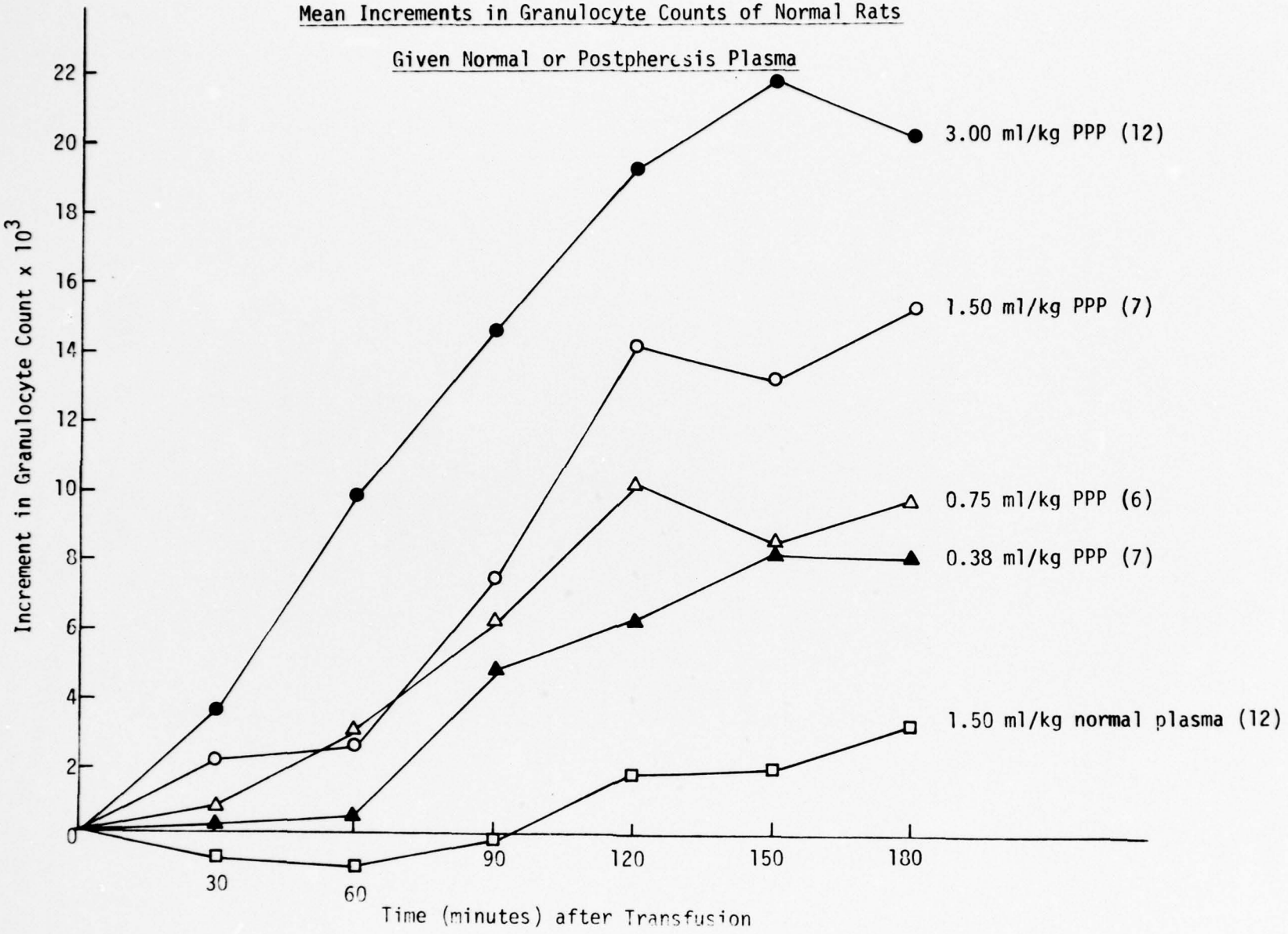
Number of Experiments	Pre-Treatment Gran Count/mm^3	Mean Gran Increment/mm^3	Range of Gran Increments/mm^3
14	0-3000	15152	7652-23129
13	3001-6000	16718	9262-31302
14	>6000	15215	2413-23751

*Mean of granulocyte increments 180 minutes after injection.

Figure 1

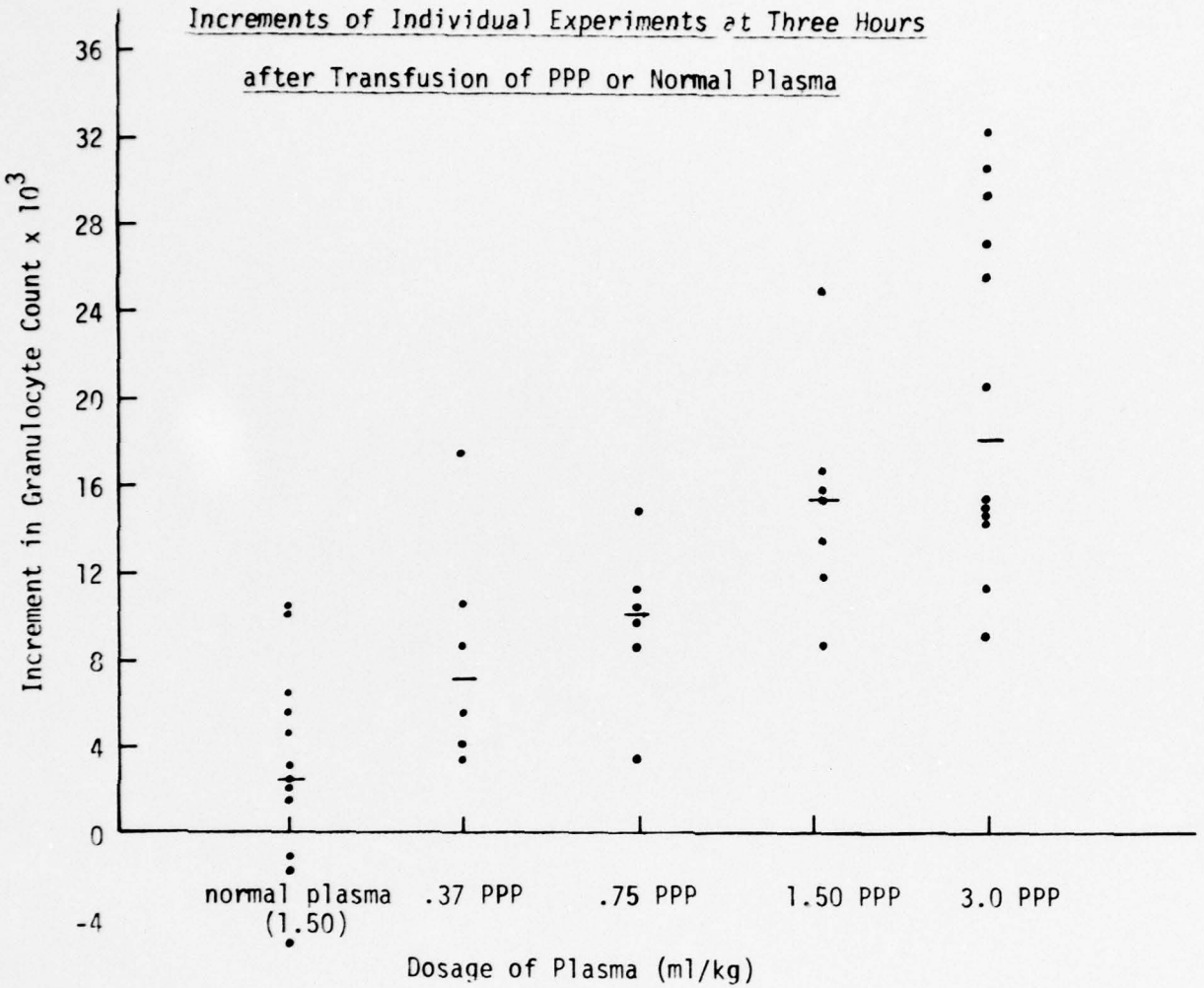
Mean Increments in Granulocyte Counts of Normal Rats

Given Normal or Postpheresis Plasma



( ) The number in parenthesis denotes the number of experiments.

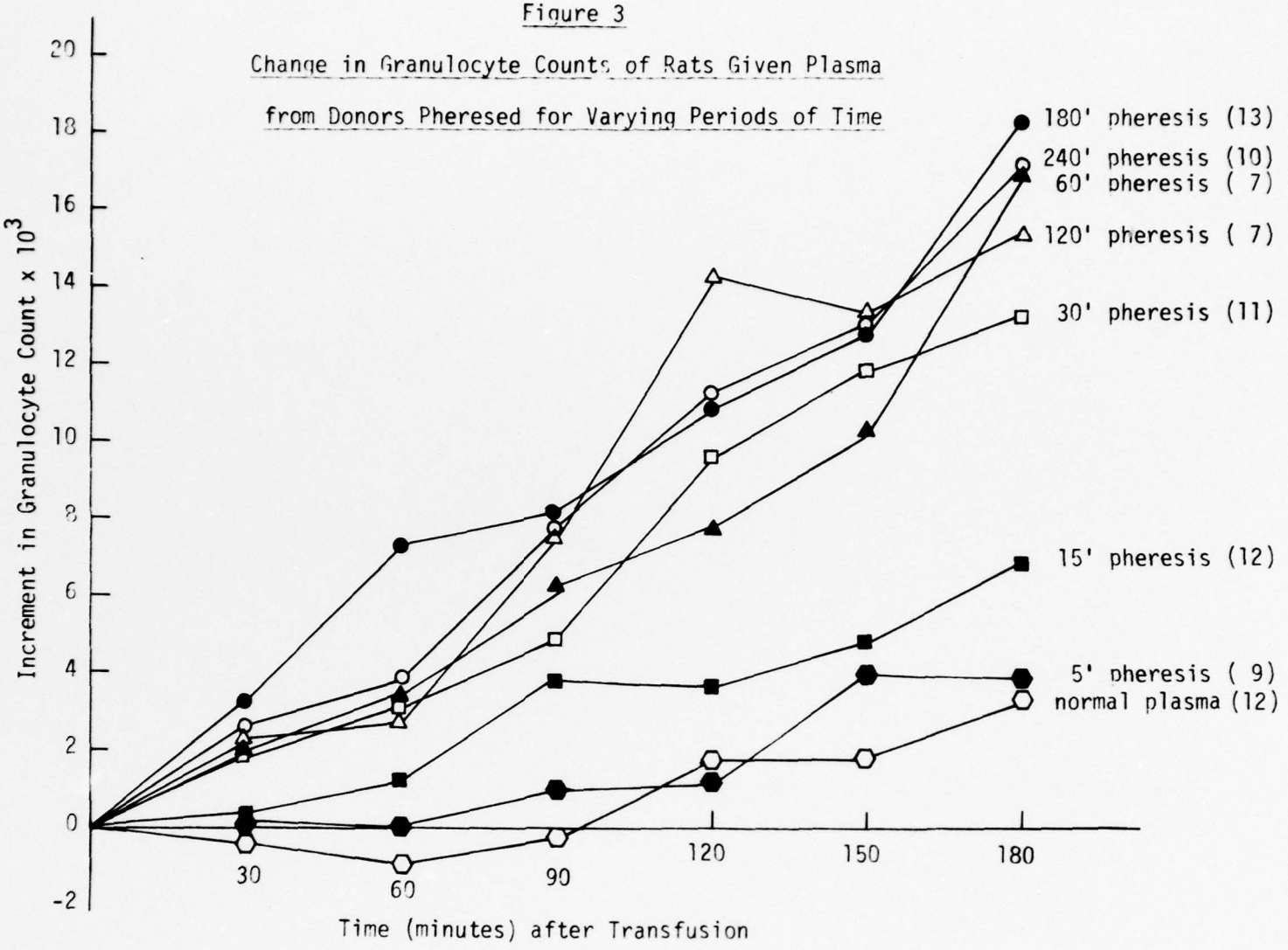
Figure 2



The bar represents the median of each group of experiments.

Figure 3

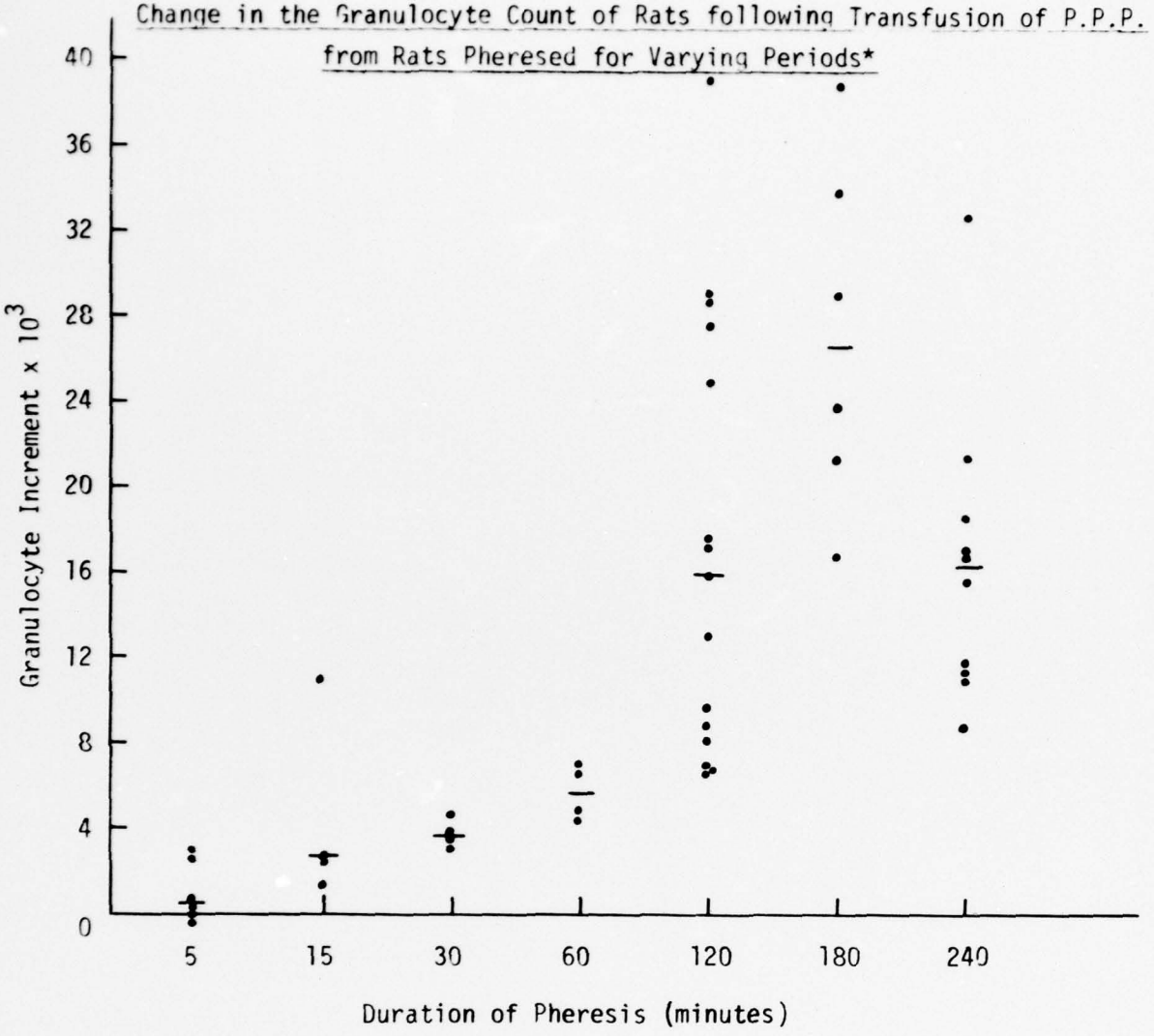
Change in Granulocyte Counts of Rats Given Plasma from Donors Pheresed for Varying Periods of Time



( ) The number in parenthesis denotes the number of experiments.

Figure 4

Change in the Granulocyte Count of Rats following Transfusion of P.P.P. from Rats Pheresed for Varying Periods*



The bar represents the median of each group of experiments.

* Each point represents the granulocyte count in the recipient of P.P.P. 180 minutes after transfusion.

FIGURE 5

EFFECT OF SUCCESSIVE DAILY INJECTIONS OF PPP

ON THE GRANULOCYTE COUNTS OF NORMAL RATS

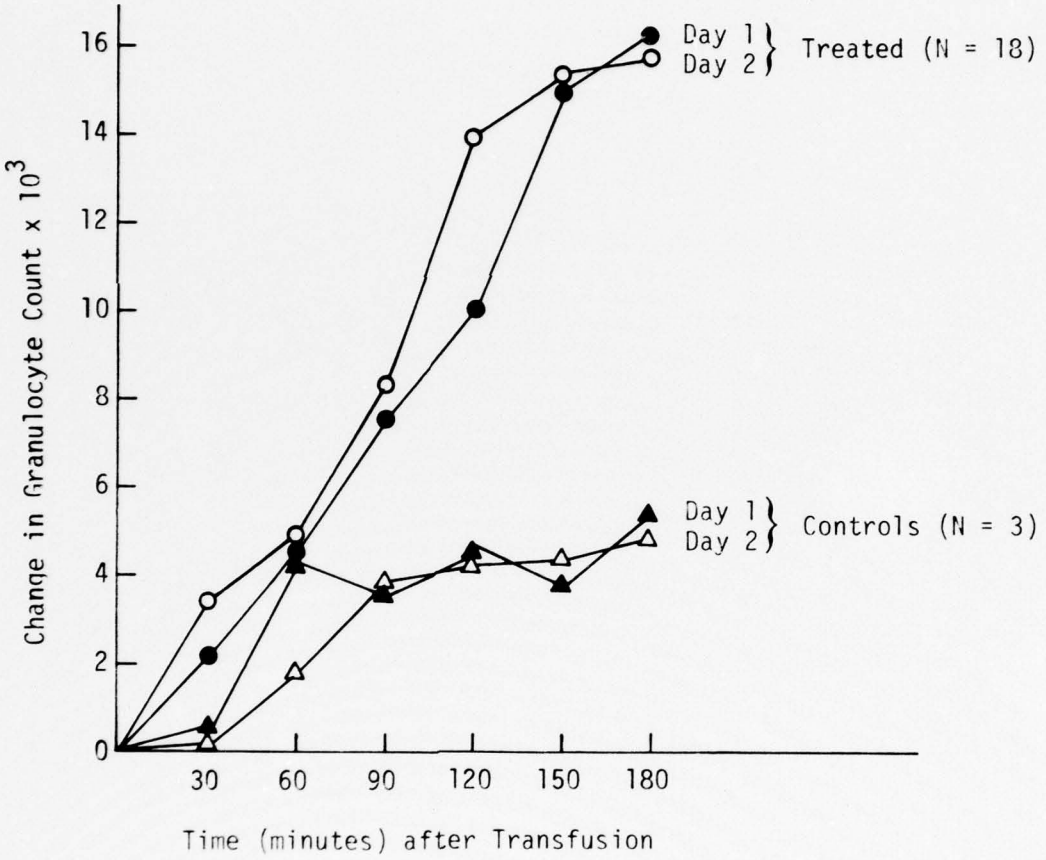
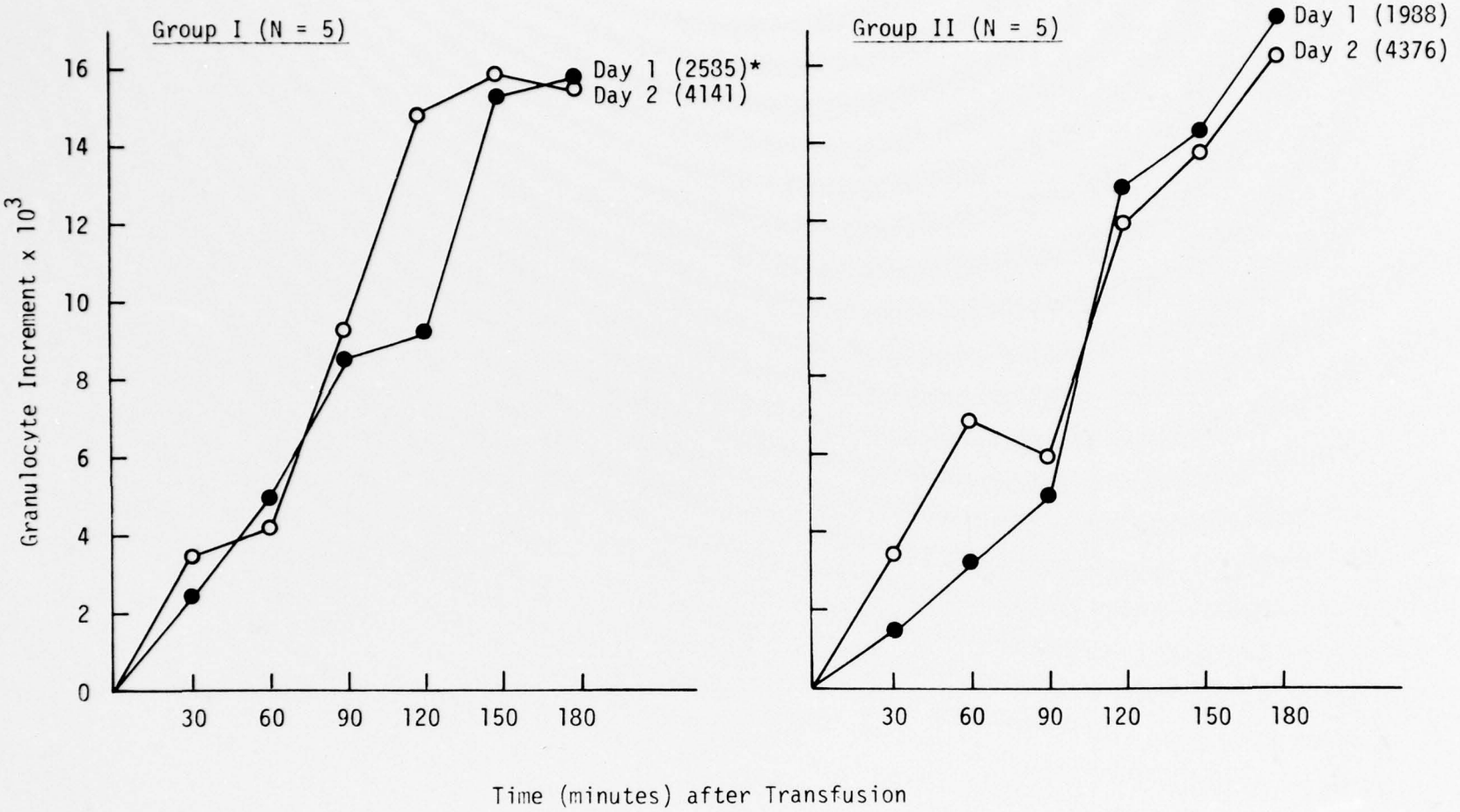


FIGURE 6

INCREMENTS OF GRANULOCYTE COUNTS OF TWO REPRESENTATIVE GROUPS OF RATS TREATED ON TWO SUCCESSIVE DAYS WITH PPP



*Mean initial granulocyte count.

FIGURE 7

DONOR GRANULOCYTE COUNT vs. RECIPIENT INCREMENT

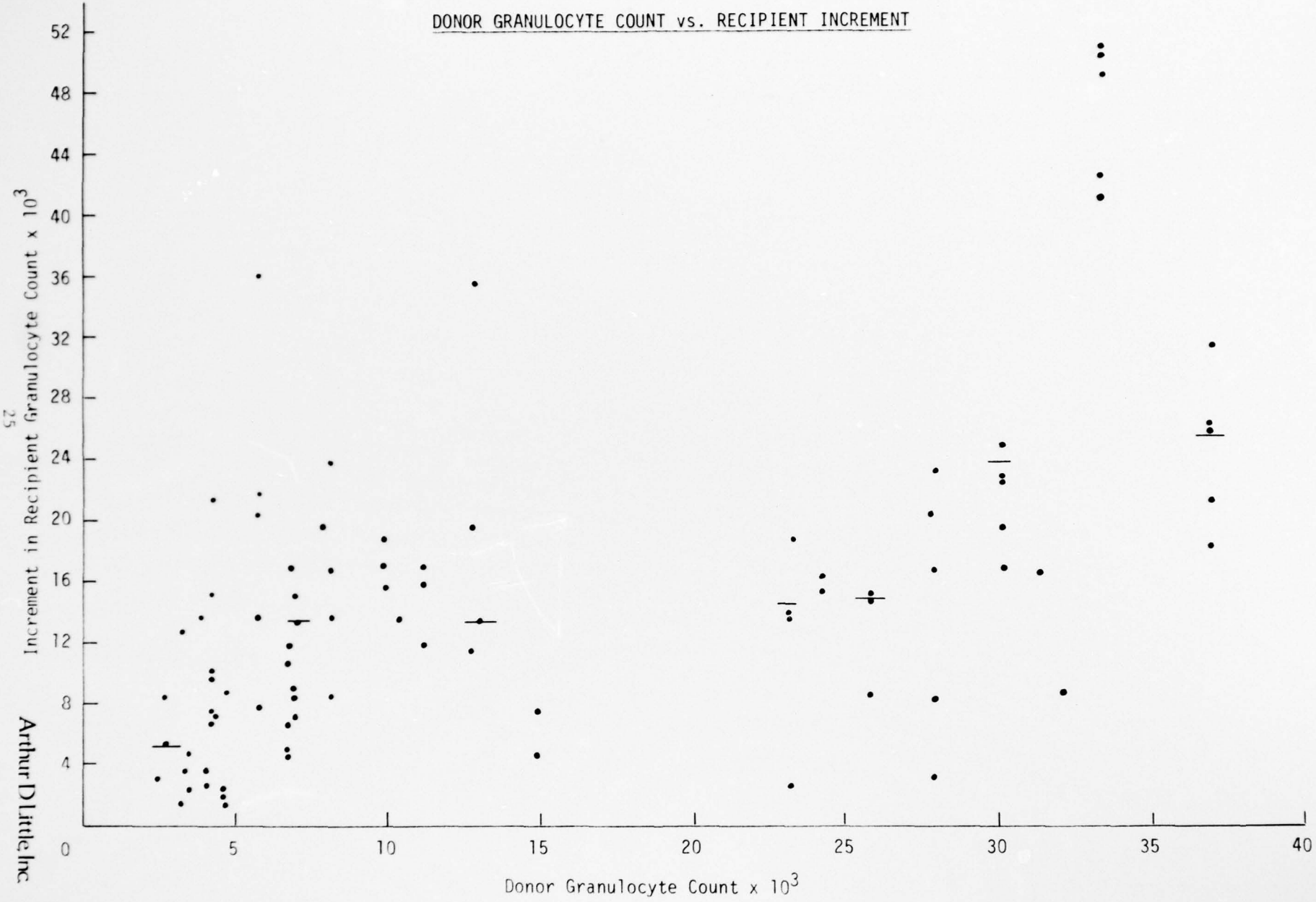
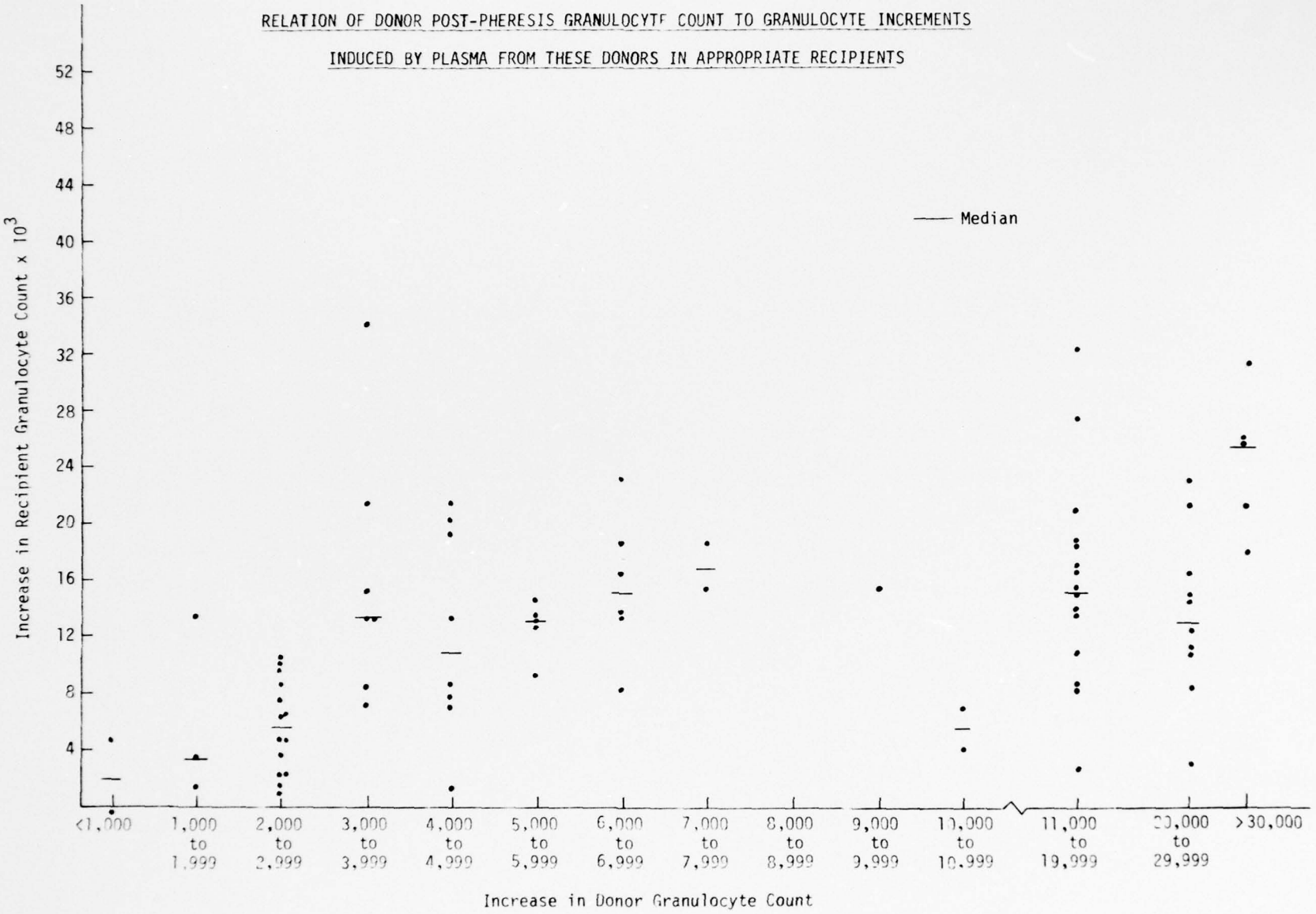


FIGURE 8

RELATION OF DONOR POST-PHERESIS GRANULOCYTE COUNT TO GRANULOCYTE INCREMENTS INDUCED BY PLASMA FROM THESE DONORS IN APPROPRIATE RECIPIENTS



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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Humoral factors are elaborated by animal and human granulocyte donors during filtration leukopheresis. Plasma obtained from leukopheresed rats (P.P.P.) may be used to increase granulocyte yields in donors when given prior to leukopheresis. In these studies it appears that (a) the effect of injection of P.P.P. is dose-related and is not an "all or none" phenomenon, (b) the granulocytosis observed in the donor is related to the duration of pheresis for periods up to three hours, (c) the titer of neutrophil releasing →

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20. Abstract (continued)

activity is related to the duration of pheresis, (d) the granulocyte increments in the donor are proportional to the duration of pheresis, and (e) there is a good correlation between the donor post-pheresis granulocyte count, as well as the donor post-pheresis granulocyte increment to the neutrophil releasing activity of the plasma obtained from the donor. There is no relationship between the recipient pre-granulocyte count and the ability of the recipient to respond to injections of P.P.P., or the ability of groups of rats to respond to injections of P.P.P. on two successive days.

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