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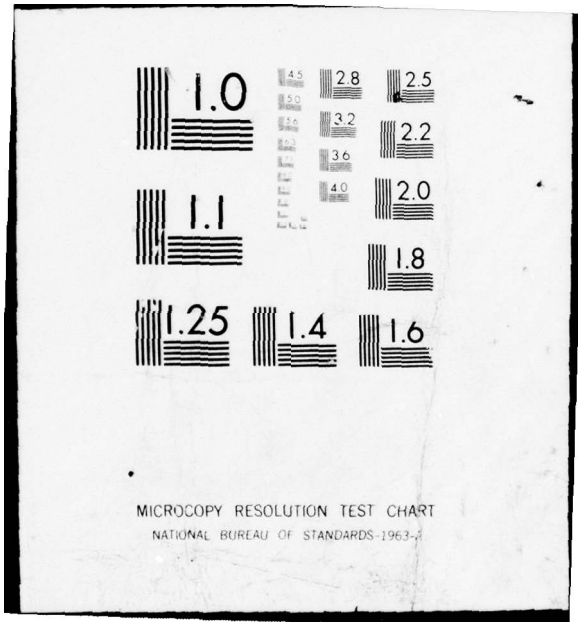
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(6) FURTHER STUDIES INTO THE PATHOGENESIS AND TREATMENT OF POST-TRAUMATIC PULMONARY INSUFFICIENCY WITH SPECIAL REFERENCE TO THE ROLE OF BANKED BLOOD.

(9) Annual Progress Report

Approved for public release; distribution unlimited

(10) Joseph J. Judson/McNamara, M.D.

(11) 30 March 1977

(12) 47p.

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D.C. 20314

Contract No. DADA17-73-C-3040
(15)

Cardiovascular Research Laboratory
The Queen's Medical Center
Honolulu, Hawaii

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
	2b. GROUP

3. REPORT TITLE

FURTHER STUDIES INTO THE PATHOGENESIS AND TREATMENT OF POST-TRAUMATIC PULMONARY INSUFFICIENCY WITH SPECIAL REFERENCE TO THE ROLE OF BANKED BLOOD

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Annual Progress Report

5. AUTHOR(S) (First name, middle initial, last name)

Joseph Judson McNamara, M.D.

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
30 March 1977	53	9

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
DADA17-73-C-3040	
b. PROJECT NO.	
c.	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.	

10. DISTRIBUTION STATEMENT

Distribution of this document is unlimited

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	U.S. Army Medical Research and Development Command

13. ABSTRACT

Studies have focused on two primary areas: 1) microaggregate formation, 2) animal studies on post injury pulmonary insufficiency.

Studies on microaggregate formation have:

1) Established a new and more accurate method for microaggregate counting with an electron particle counter.

2) Established a method for stabilizing in reversible microaggregates such as those occurring in vivo in shock.

3) Completed comparison of Bentley, Pall and Fenwal blood filters.

4) Shown that infused stored platelets have a half-life of about 12 hours in baboons.

5) Began in vivo studies on microaggregate formation.

In this area of post injury pulmonary insufficiency we completed 48-hour shock experiments which demonstrated no significant effect of shock, trauma or stored blood on pulmonary function or hemodynamics.

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General Background

During the past year, we completed studies defining the fate of 10-day old platelets transfused into animals. We continued our work on blood filter evaluation and evaluated the effects of old platelets on fresh platelets. In addition, we were able to investigate the phenomenon of "debris" formation in greater detail, specifically looking at microaggregates of platelets in stored and fresh blood. We also evaluated currently used methods for microaggregate (debris) counting in stored blood and were able to develop a new and much more reliable method. Finally, we completed some of the work for which we were not specifically funded last year. Using general lab funds we purchased animals and our existing technical staff completed a series of shocked, traumatized animals receiving either fresh or stored whole blood.

I. Microaggregate or Debris Formation

It has been well documented by ourselves and others that stored blood develops platelet microaggregates.[1,2] Furthermore, microaggregates develop in vivo in fresh blood in certain situations (i.e., shock, trauma and/or ischemia) and can be induced by a variety of agents (ADP, collagen, epinephrine).[3,4,5] During the past year, we have completed methodological work on microaggregate counting, evaluated a new blood ultrafilter, studied the fate on non-aggregated platelets in stored blood and begun work on methods of measuring platelet microaggregate formation in vivo in certain extreme clinical situations such as hemorrhagic shock.

A. Methodological Studies: Our first efforts this year were directed at establishing a reliable method of counting microaggregates or debris in stored blood. This involved detailed methodologic evaluation of a variety of hemolyzing agents at different concentrations on stored blood and platelet rich plasma. It was shown that saponin, in usual concentrations as described by Solis and co-workers,[6,7] causes the formation of new aggregates in fresh whole blood and platelet rich plasma and reduces volume and number of microaggregates in stored samples. We also devised a method of counting microaggregates in stored blood using an electronic particle counter avoiding the usual method with hemolytic agent. To overcome red cell coincidence counting effects at low sample dilution, two different sized apertures were used. Our method reliably measures microaggregates from 12.7 to 80.6μ diameter. (Appendix A)

B. Microaggregate Formation in Vivo - Determination of Reversible Microaggregates: A further interesting observation made during the above study involves the characterization of microaggregates induced by ADP or epinephrine. For all electronic

particle counting methods of measuring blood microaggregates, dilution of the specimen is essential to minimize coincidence counting errors. We have shown (Appendix A) that microaggregates formed in stored blood are stable and with serial dilution counts remained proportionately unchanged. Microaggregates formed with ADP or collagen, however, are very sensitive to manipulation of the blood and particularly to dilution which produces deaggregation.

C. Filter Evaluation: Another ultrafilter study has been completed comparing the efficacy of the Pall, Bentley and Fenwal filters. We concluded from the data that, of the three filters evaluated, the Fenwal filter provided the most efficient means of removing debris while maintaining adequate flow rates for large volumes of blood. (Appendix B).

D. Fate of Non-Aggregated Platelets in Stored Blood: Five baboons had platelet survival studies performed to determine normal baseline values. Normal half-life in baboons by our methods were 47 ± 9 hours (Fig. 1). Blood was then drawn into plastic bags and stored in CPD solution for 4°C. for 10 days. Platelets were again tagged and reinfused and a half-life of 13 ± 3 hours was determined. Although the platelet survival was markedly shortened ($p<0.01$), half of the stored platelets were still circulating over 13 hours after infusion.

E. Reversible Aggregates: Mounting evidence indicates that in vivo platelet microaggregation occurs commonly in massive injury and shock and that it, furthermore, may be responsible for some of the pulmonary and systemic hemodynamic changes and even specific organ injury. If this were the case, prevention of platelet aggregate formation might prove a significant therapeutic adjunct in the treatment of shock and massive trauma. Certainly, conditions are present in massive trauma and shock which may promote such platelet microaggregate formation. For example, with trauma and large areas of tissue disruption, collagen is exposed to the vascular space. Similarly, with coagulation on raw surfaces and platelet release reaction, ADP levels are increased and, certainly, epinephrine is released in large quantities in response to shock. All these factors promote platelet aggregate formation. Before even considering evaluation of the biologic effect of these aggregates, however, a technique for accurate measurement of in vivo aggregates must be developed and then applied to animals in shock to verify the presence of such aggregates in an experimental model of shock and trauma.

We have established a highly reliable method for microaggregate determination using an electronic particle counter. We have been unable to measure freshly aggregated platelet particles because the dilution necessary for the method produces deaggregation.

Preliminary studies have been completed using different concentrations of formalin or gluteraldehyde to fix platelet microaggregates for counting. Formalin, unfortunately, prompted aggregation in all concentrations studied. Gluteraldehyde in dilute concentrations appears to preserve platelet aggregates without promoting aggregation.

II. Post Traumatic Pulmonary Insufficiency (PTPI)

Considerable work has appeared from our laboratory and others implicating microaggregate debris in banked blood in the pathogenesis of post-traumatic pulmonary insufficiency. This has even resulted in a whole new generation of blood ultrafilters designed to remove such debris. In a series of studies performed over the last 3 hours in baboons infused with stored blood, we have been unable to consistently demonstrate any effect of stored blood debris on pulmonary or systemic hemodynamics or any parameters of pulmonary function.[8] In fact, experimental work by ourselves and others have failed to even produce a satisfactory model of post-traumatic pulmonary insufficiency.[8,9]

During the past year, in an attempt to duplicate the clinical situation which so frequently precedes the onset of PTPI, a group of ten baboons were put into shock using a standard hemorrhage model, subjected to major trauma (a thoracotomy) and reinfused with either their own shed blood (five animals) or 10-day old stored blood (five animals).

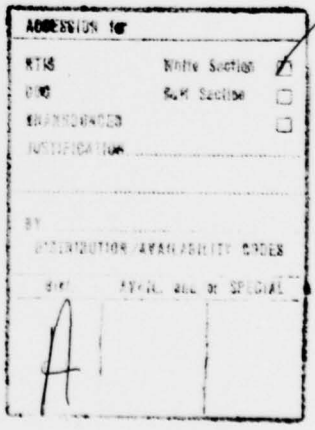
This standard shock model, first described by Moss, has an early mortality of 50%. Our results showed that there was no significant difference between animals who received shed blood infusions and those that received shed blood as regards O_2 consumption, cardiac output, P-VR, TPR, A-aDO_2, V_p/V_T and pulmonary shunt. (Appendix C)

The early mortality was nil. Studies we carried out over 48 hours showed that all animals survived for this period of time. Yet, after this period of close observation and intensive care was over and animals returned to their cages, the ultimate mortality was 50%, all animals dying from 60-78 hours after the period of shock.

All of the animals not surviving the experiment appeared to die with pulmonary insufficiency, although hemodynamic data was not available in the immediate pre-mortem period and not all animals, particularly surviving animals, had careful gross and histologic microscopic data. These data are nonetheless extremely important as they suggest that with longer followup, animals may indeed develop pulmonary insufficiency, yet may not manifest it in short-term studies. Death occurs earlier in

short-term studies and if 50% of the animals expire shortly after the acute experiments, this eliminates the population that may be most at risk to develop PTPI.

Finally, a significant difference between the surviving and non-surviving animals was a VO_2 that increased steadily from 4 hours after resuscitation in all surviving animals and decreased in all non-surviving animals.



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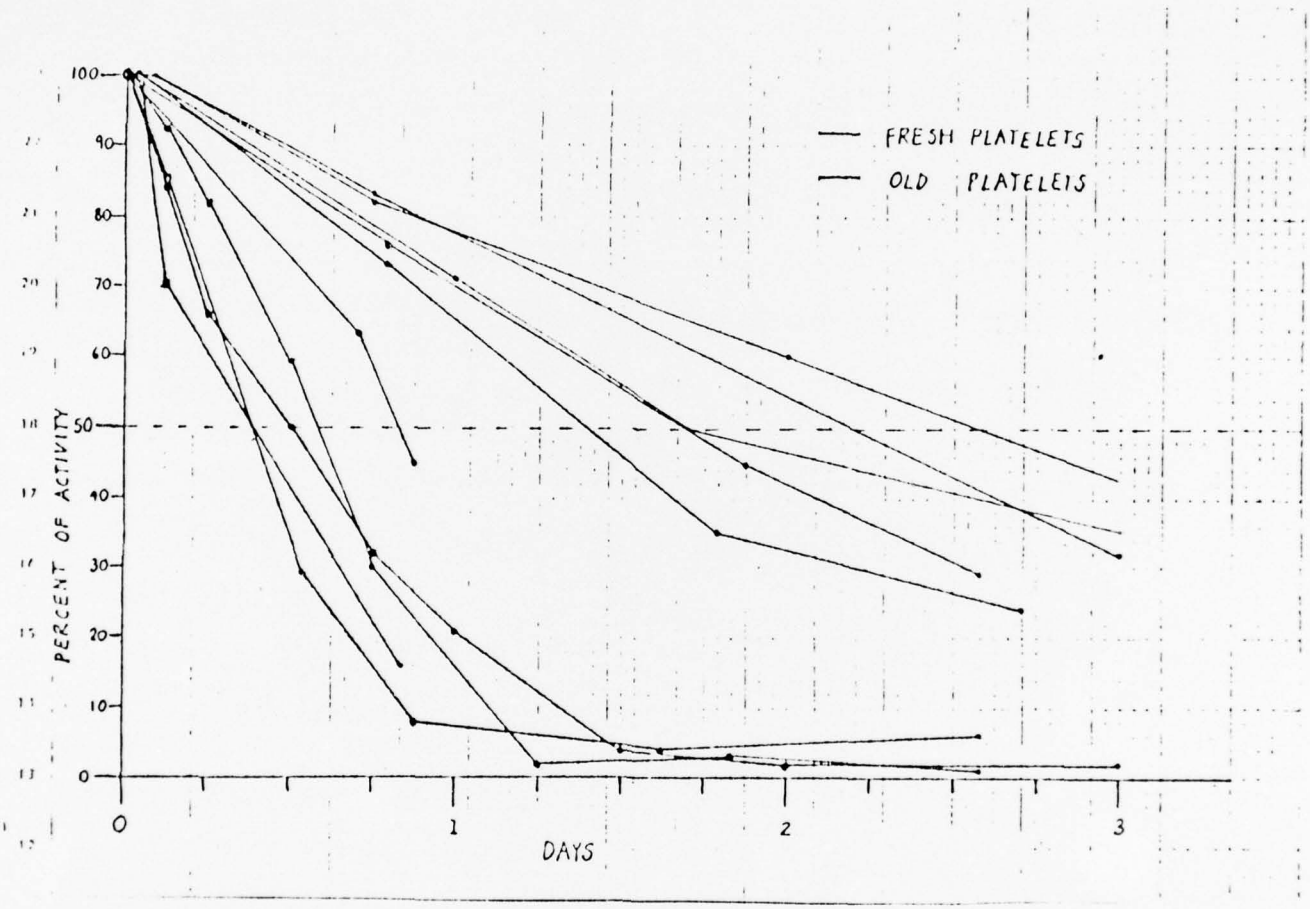

The decrease of radioactivity of Cr^{51}-labeled platelets is shown in percent of the radioactivity of the first blood sample after infusion. Survival curves of platelets from blood stored for 10 days (old platelets) are definitely different than curves of fresh platelets. As the most suitable measure of platelet survival, the platelet half-time is selected. Fresh platelets have a mean half-time of 47±9 hours (n=5). The half-time of old platelets is considerably shortened to 13±3 hours (n=5, $p<0.01$), however, 50% of the platelets from 10-day old blood are still circulating 13 hours after transfusion.

Fig. 1

COUNTING MICROAGGREGATE PARTICLES IN BLOOD

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Abstract

A method is described for measuring microaggregates in stored blood with an electronic particle counter avoiding the usual use of a hemolytic agent. To overcome red cell coincidence at low dilution of the samples two different sized apertures are used. It is demonstrated that our method reliably measures microaggregates from 12.7μ to 80.6μ diameter.

Using this method the effect of hemolytic agents on the volumes and numbers of microaggregates developed in stored blood and PRP are investigated. It is shown that all hemolytic agents produce a concentration dependent shift of the cell population toward smaller volumes and a corresponding increase in number of large microaggregate particles.

The present study demonstrates that the ideal Saponin concentration for microaggregate counting is tenfold more dilute than that currently reported in the literature.

Introduction

Microaggregates formed in vivo due to shock,[6,33,34] trauma[37] and extracorporeal circulation[29,30] during surgery or in vitro in banked blood[2,3,9,32] are believed to cause pulmonary microembolism[6,7,13,14,33] and possibly contribute to the development of respiratory insufficiency.[14,22,34] A reliable method of quantifying this debris is, therefore, of some importance for both clinical and experimental studies. One means of accomplishing this utilizes an electronic particle counter* which both counts and sorts particles by their volumetric displacement. Solis[24,27-30] and others[9,17,41] have repeatedly used this method clinically but there are practical limitations.[16] At too low a blood sample dilution, coincidence occurs, whereupon several small particles passing simultaneously through the machine's aperture field are detected as one aggregate of equivalent volume. On the other hand, at too high a dilution accuracy diminishes due to a decreased total count and background noise becomes more significant relative to actual counts. To avoid a coincidence problem while employing a low dilution, Solis[25,26,29] attempts to selectively hemolyze the erythrocytes without altering the microaggregate debris. The validity of this method has never been adequately tested. Hemolytic substances might lyse microaggregates as well as red cells. Further, due to their surface active properties they might induce platelet aggregation. The current report describes a newly developed method to count microaggregates formed in stored blood and platelet rich plasma (PRP) without the use of hemolytic agents. Using this method, the effects of hemolytic agents in microaggregates in stored blood are investigated.

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* Coulter Electronics, Hialeah, Florida.

Method

1) Electronic Particle Counter

The Coulter Counter Model Ta II* simultaneously sorts particles in an isotonic solution (Isoton) by volume into 16 successive channels. To do this, it measures the change in resistance of an electrical field as the solution is drawn through a glass aperture. The suspending solution is electrically conducting, so any particle passing through the aperture displaces an equal volume of solution which results in a proportional rise in resistance. All volumes measured are automatically converted to equivalent spherical diameters.

Coincidence, which follows a Poisson curve,[16] is dependent on the concentration of the cells and the size of the effective aperture field. For the same cell concentration a smaller aperture has, therefore, less coincidence than a larger aperture. This is because the larger the aperture is, the more probable it becomes that two or more particles will pass through the aperture at the same distance and will be counted as one large particle.

Background noise in the larger particle channels is mainly due to the counter's electrical noise and bubbles in the conducting fluid. While this noise is fairly constant it puts a practical limit on the level of dilution of any cell sample.[16]

2) A Two Aperture Method for Counting Microaggregate Particles

In order to avoid the problem noted above, microaggregate debris is run through both the 70μ and the 200μ diameter apertures. The upper four channels of the 70μ aperture are calibrated to measure a range of 12.7 to 32μ equivalent spherical diameter. For the 200μ aperture the upper four channels range is 32 to 80.6μ diameter. Two ml and 10 ml of solution are drawn through the 70μ and

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* Coulter Electronics, Hialeah, Florida.

200μ apertures respectively after one minute of mixing at the appropriate dilution with or without lysing agent. Debris is defined as the volume and population of all particles which are counted in the upper four channels of both apertures.

Unhemolyzed whole blood (WB) is diluted 1:1000 for the 70μ aperture and 1:100 for the 200μ. Hemolyzed blood is diluted 1:100 for both.

Unhemolyzed and hemolyzed platelet-rich-plasma (PRP) are diluted 1:100.

3) Preparation of Cell Fractions

CPD anticoagulated whole blood is stored at 4 C.

PRP and packed red cells (PC) are both prepared from whole blood. PRP is made by centrifuging fresh blood at 150g's for 15 minutes and is stored stationary at 4 C. or at room temperature in a continually mixed state. Whole blood is spun at 1,200g's for 20 minutes to produce PC which are resuspended in Ringer's-citrate-dextrose solution to hematocrits of 25%, 50% or 100%.

4) Hemolytic Agents Used

.3 ml and .03 ml of a Saponin solution (2.5 gram/100 ml) are the doses used per 10 ml of sample dilutent (Isoton). All samples are continually mixed for one minute at which point the effects of the hemolytic agent have stabilized and remained constant for at least 5 minutes. We have found that during the first minute erythrocyte volume and population distribution shift too rapidly to give reproducible results.

Other hemolytic agents including Zap-Isoton, Cetrimide and Triton X-100, were compared with Saponin to assess the qualitative effects on red cells and platelets.

5) Screen Filtration Pressure (SFP)

Screen filtration pressure (SFP)[32,36] is measured by attempting to force 5 ml of whole blood through a metal screen with 20μ pores at 16.1 per minute.

Results

1) Evaluation of Our Method

No coincidence counts are found in the upper channels which are those used for determination of debris volume and population if packed red cells adjusted in hematocrits between 100 and 25% are used. (Figs. 1a & 1b) Background noice detected in the channels used for debris counts is at these dilutions insignificant for WB and fresh PRP.

The variance of our method was determined by running fresh and stored samples in duplicate. The variance of these duplicate determinations is shown in Figs. 2a-c. The mean value of the difference of the data pairs (n=32) is 6.5 ± 6.5 x $(10^4)\mu^3/mm^3$ for the large aperture. The larger variance of the small aperture is probably due to the higher dilution and smaller volume sampled compared to the large aperture.

The mean changes of debris volume and SFP observed during storage of four CPD blood units are shown in Figs. 3a and 3b. Both SFP and debris volume increased after storage in the same time period. Large standard deviations reflect considerable variations from unit to unit. SFP exceeds the range of quantitative measurements as early as seven days after storage, when the pressure exceeds 500 mmHg. The apparent decrease of debris volume after 21 days of storage indicates that the aggregate size exceeds the range of our method. In fact, visible aggregates are seen at this point.

SFP values lower than 500 mmHg are correlated with volume and number of particles both over the whole range studied (12.7 to 80.6μ) and over a narrower range including only particles larger than 20μ (20.2 to 80.6μ). (Table I). The latter range is studied separately because particles larger than 20μ exceed the size of the holes in the SFP screen. SFP correlates best with total volume of debris and number of particles exceeding 20μ diameter.

2) Qualitative Effects of the Hemolytic Agents

All the hemolytic agents used did not reduce the cell population of either RBC or platelets but reduced their mean volumes causing a shift of the population peak into the smaller sized channels. The degree of size reduction is dependent on the concentration of the hemolytic agents; this is demonstrated in Figs. 4a and 4b with PRP and WB using Zap-Isoton. Examination of these lysed cell fractions by a phase contrast microscope indicate the erythrocytes are converted to "ghosts" and platelet morphology is not significantly altered.

Another consistent observation was that, after hemolysis with all the dilutions and using PRP, PC or WB, there was always an increase in counts of previously free channels in the upper range. (Figs. 5a-c) This result was found to be reproducible and is independent on the hemolytic agent used. It cannot be attributed to coincidence error because as we have previously seen the populations are shifted toward smaller volumes. Therefore, it indicates the formation of a small number of large particles after incubation with any hemolytic agent.

3) Quantitative Effect of Saponin on Debris Counts in PRP and WB

All attempts to form high concentrations of stable aggregates in PRP by appropriate doses of ADP and epinephrine failed because deaggregation occurs within seconds after dilution and, therefore, accurate counts are not possible. Stable aggregates did appear in stored PRP and stored WB. Debris values peaked within 18 to 24 hours in heparinized PRP, in CPD anticoagulated PRP within 7-14 days, WB debris packed latest for all averaging 14-21 days. These aggregates are stable after dilution. The maximal value of the debris volume in WB is 127.2 to 138.1 x $10^4 \mu^3/mm^3$, in PRP 28.5 to 2917 x $10^4 \mu^3/mm^3$.

The correlation of debris volume in PRP and WB as measured with the two aperture method before and after incubation with the two different concentrations of Saponin is shown in Figs. 6a-d. The results represent all measurements of

seven different batches of CPD anticoagulated blood and 13 PRP preparations. Measurements performed immediately after preparation are shown as open circles, measurements at various time after as closed circles.

Two different effects of the high Saponin concentration can be seen in both PRP and WB. Debris volume decreased in smaples with high debris volume (mainly old PRP and WB). In samples with very low debris volume (mainly fresh PRP and WB) debris volume increases after incubation with Saponin. The magnitude of these effects can be seen from the calculated regression line. The slope of the regression line indicates the decrease of debris volume, the y-intercept gives the increase in debris for statistical debris-free sample. (Table II) The magnitude of both Saponin effects is different if Saponin is applied to PRP or WB. In WB debris volume is reduced to approximately 33% and in PRP to approximately 57%. The increase of debris for statistical zero amount is $3 \times 10^4 \mu^3/mm^3$ in PRP.

If a tenfold lower Saponin concentration is used, only the reduction of pre-existing debris in WB is significant.

The high correlation coefficients indicate that the effect of Saponin can be explained with high confidence by the calculated regression lines. This is not true for the effect of the high Saponin concentration of PRP. A larger variance and an unsatisfactory correlation coefficient of 0.668 points out that another variable affects the results. Therefore, the effect of Saponin become highly unpredictable in PRP.

The effect of the concentration of Saponin on debris population follows the similar pattern to that already seen for debris volume. (Table II) The slope of the regression line indicates that the number of debris particles is also more reduced in WB than for PRP. The y-intercept is positive for both WB and PRP. However, more particles are formed in WB whereas the volume is greater in PRP.

For corresponding Saponin concentrations the relative decrease in debris population always exceeds the relative decrease in volume.

Discussion

It is well established that units of banked blood anticoagulated with Heparin, ACD or CPD develop various amounts of microaggregates during storage.[3,9,28,32,38] Blood platelets most probably initiate the formation of these microaggregates[3,32,35] and they represent their major component.[26,34,36]

Usually, hemolytic agents are applied in order to avoid red cell coincidence error while counting these microaggregates with an electronic particle counter at low dilutions of the samples.[9,17,26,27,29] We introduced two different sized apertures as a new and more reliable method to avoid red cell coincidence errors, and it is demonstrated that the upper channels of these two aperture are free of coincidence counts and background noise even if packed cells adjusted to 100% hematocrit are counted.* An increase of microaggregate volume in stored blood is shown to parallel an increase in SFP. Correlations of microaggregate volume and population with SFP confirm that similar information is obtained with both methods. SFP correlates better with the population than with the volume of microaggregates greater than 20μ equivalent spherical diameter, which indicates that the number of particles occluding the holes in the SFP screen is important. The better correlation of SFP to the total volume of particles than the volume of particles exceeding the size of the holes in the screen indicates that the adhesiveness of the debris might influence the SFP results.[32,34,36] However, further studies are necessary to evaluate the regression function to delineate range and significance of both methods and define the role the adhesiveness of the microaggregates might have in determining SFP values.

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* Therefore, it is demonstrated that our method measures microaggregates formed in stored blood reliably and reproducibly. By duplicate counts of the same samples it is shown that the variance of our method is insignificant.

It is demonstrated that the two aperture method reliably measures microaggregates down to 12.7μ particle diameter. Thus, our method covers the same range as the method of Solis which is most frequently used.[8,9,17,26,29] The quantitative effects of hemolytic agents on microaggregate volume and population, therefore, can be evaluated accurately by applying our method before and after incubation with hemolytic agents.

The qualitative effects of every hemolytic agent on PRP and WB is a concentration dependent shift of the cell population toward smaller volumes. Additionally, a reproducible increase of counts in previous, empty, higher channels indicate the formation of microaggregates due to the application of any hemolytic agent to either PRP, PC or WB. Similar observations using Saponin are reported by Solis,[26] who finds a reduction in debris volume and Talstad[39] who notes an increase in platelet aggregates.

The quantitative effects of the hemolytic agents were studied with two different concentrations of Saponin. The higher concentration is the same as used frequently by Solis[26,27,28] and the second concentration was tenfold more dilute. The use of the two aperture method and the handling of the samples should not account for any differences between our values and previous values obtained with Saponin as lysing agent. Furthermore, judging from the lysing potential of Zap-Isoton, a lysing agent currently used in recent publications,[6] the quantitative effects of this agent in the concentration used can be expected to be similar to the high concentration of Saponin.

Our results demonstrate that two effects of Saponin on both PRP and WB can be distinguished:

1) New microaggregates are formed.

2) Pre-existent microaggregates are lysed.

Both effects are much more distinct and of greater magnitude if the higher Saponin concentration is applied and both effects can be seen if debris volume or debris population is studied.

The formation of new microaggregates is seen mainly in fresh PRP and WB. The increase in debris volume is greater in PRP, the increase in debris population greater in WB, i.e., the microaggregates formed are larger in PRP. Together these findings most likely indicate the microaggregates are formed in PRP and WB by platelet aggregation induced by the surface active properties of Saponin. Fresh PRP and WB give better response, because platelet reactivity is still preserved. Larger aggregates are developed in PRP because platelet concentration and, therefore, collision frequently is higher in PRP. The effect of Saponin is dependent on the concentration, the tenfold lower concentration produces no significant microaggregate formation.

The reduction in debris volume and debris population by Saponin is of greater magnitude in stored WB than stored PRP, i.e., microaggregates in PRP are more resistent to Saponin. The maximal amount of debris developed in WB is greater than in PRP. The reason for this must be that platelet aggregates form and behave differently in the two suspensions. The hypothesis which could account most consistently for our data would be a proportional inclusion of red cells within the microaggregates formed in stored blood.

The reduction in debris population is somewhat greater than the reduction seen in debris volume. This should be due to the uneven distribution of the debris particles in the channels measured. A high number of small particles and a low number of larger particles is always observed.[27,28] So, a relatively small numerical decrease in the large aggregates causes a proportionally greater change in volume.

Although new microaggregates are formed and pre-existing microaggregates are reduced by methods previously reported, using hemolytic agents in higher

concentration,[26,27] the results of previous papers remain valid insofar as they concern studies of stored blood. The formation of new aggregates is small in relation to the large amount of aggregates developed during storage of WB. The decrease in debris is considerable but the results can be described with high confidence by a linear regression function indicating a consistent proportionality which makes reported methods reliable for comparative purposes if not for absolute values.

This is probably not true for data relating to microaggregates formed in vivo after various interventions like anesthesia, trauma and extracorporeal circulation.[24,29,30,40] The amount of debris is reported to be low,[24,29,30] therefore, the formation of new debris is likely to influence results further; the amount of new debris formed might be dependent on the concentration and the adhesive tendency of platelets in the sample. The use of hemolytic agents in higher concentrations which have been used might, therefore, change debris volume in a non-predictable manner as we noted in PRP and give highly misleading data.

Microaggregates which develop in stored PRP and WB are stable upon dilution with Isoton, a procedural necessity.[28] Aggregates produced by aggregating agents in PRP are not.[31] However, the conclusion that stable aggregates, therefore, have a more severe pathophysiological impact than reversible microaggregates[28,30] is not documented. This conclusion does not reflect the capacity of reversible platelet aggregates for release of aggregating, vasoconstrictive and procoagulatory substances[8] which cause further aggregation, vasoconstriction and the formation of microthrombi. Reports of morphologic and functional changes after in vivo platelet aggregation present evidence that the platelet release reaction is of more pathophysiologic significance than the phenomenon of the mechanical obstruction by platelet aggregates.*[20] Previous methods as well as our method cannot quantitate

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* Reversible microaggregate produced by ADP in vivo are shown to produce pathomorphological damage.(II) Further recent reports of experimental infusion of stored blood to both healthy and shocked primates could not demonstrate any significant pathophysiological changes which are due to the infusion of stable microaggregates.[19]

the reversible in vivo aggregates with the Coulter Counter. Therefore, further attempts to develop a method to measure reversible, circulating microaggregates formed in vivo are necessary.

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Legends

Figure 1 - Particle counts with (a) 70μ aperture and (b) 200μ aperture with varying hematocrit.

Figure 2 - Constancy of measured debris volume values as illustrated by paired runs using the (a) 70μ aperture, (b) 200μ aperture and (c) combined range of both.

Figure 3 - Development of microaggregate debris in stored blood determined by (a) electronically determined debris volume and (b) SFP.

Figure 4 - Effect of hemolytic agent on particle size.

Figure 5 - Effect of hemolyzing agent on (a) packed cells, (b) whole blood and (c) PRP.

Figure 6 - Alteration of debris volume by hemolyzing with Saponin.

(a) Stored or fresh blood, 0.03 ml Saponin.

(b) Stored or fresh blood, 0.3 ml Saponin.

(c) Stored or fresh PRP, 0.03 ml Saponin.

(d) Stored or fresh PRP, 0.3 ml Saponin.

COMPARISON OF SFP TO:		SAMPLE POPULATION n	CORRELATION COEFFICIENT r
WHOLE BLOOD DEBRIS VOLUME	12.7-80.6 μ	16	.929
	20.2-80.6 μ	17	.646
WHOLE BLOOD DEBRIS POPULATION	12.7-80.6 μ	17	.565
	20.2-80.6 μ	17	.808

Table I

CELL FRACTION	MODE	SAPONIN DOSAGE (ml)	SAMPLE POPULATION n	CORRELATION COEFFICIENT r	Y-INTERCEPT (10^4) μ^3/mm^3 a_o	SLOPE ± 95% CONFIDENCE LIMITS b ± 95 C.L.
WHOLE BLOOD	VOLUME	.03	30	.904	-0.9	.673 ± .123
		.3	30	.896	3	.326 ± .063
	POPULATION	.03	34	.771	6.148	.393 ± .116
		.3	34	.802	0.738	.260 ± .069
PLATELET RICH PLASMA	VOLUME	.03	44	.968	0.4	.908 ± .075
		.3	44	.668	6.5	.565 ± .196
	POPULATION	.03	44	.957	-0.451	.842 ± .079
		.3	44	.706	5.108	.505 ± .124

Table II

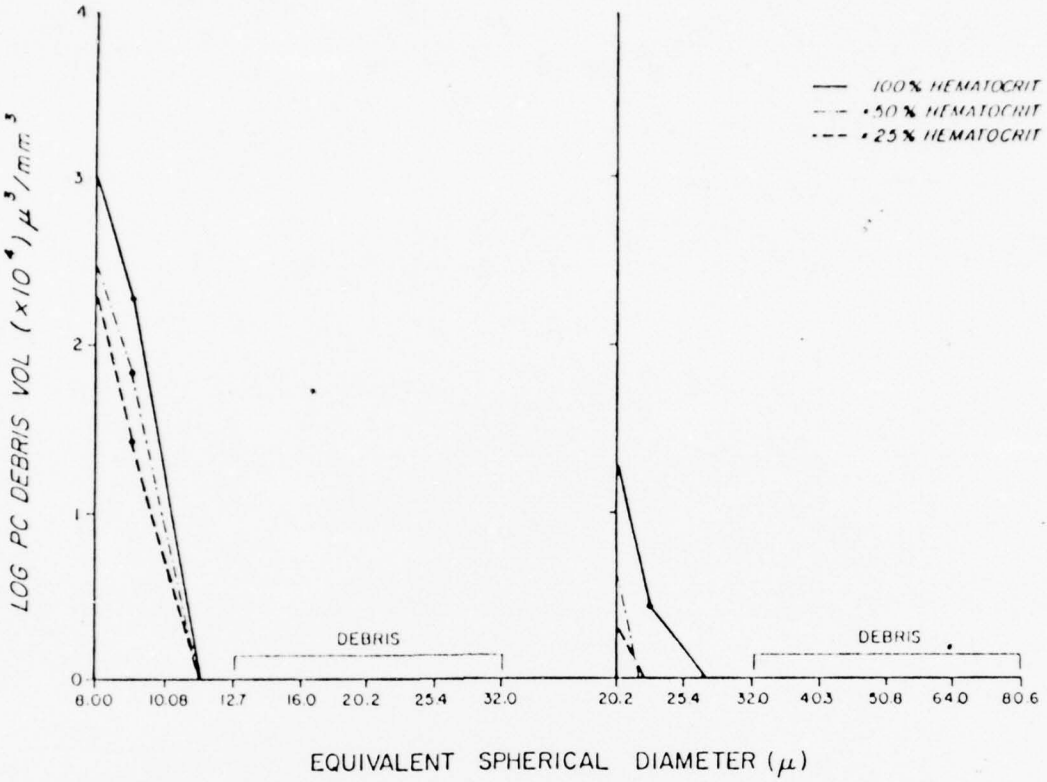


Fig. 1a & b

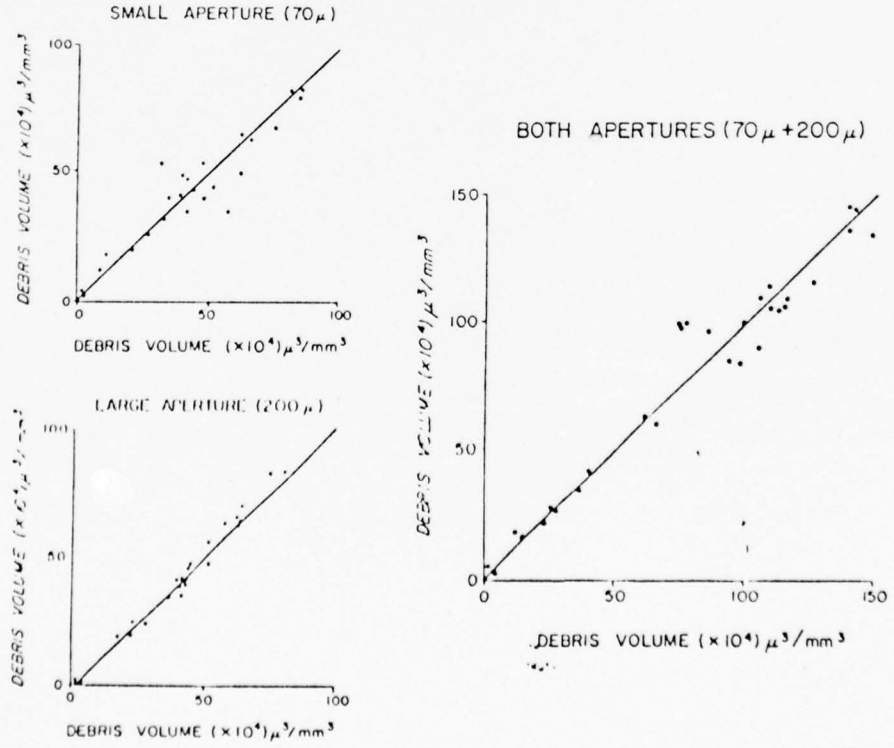


Fig. 2a, b & c

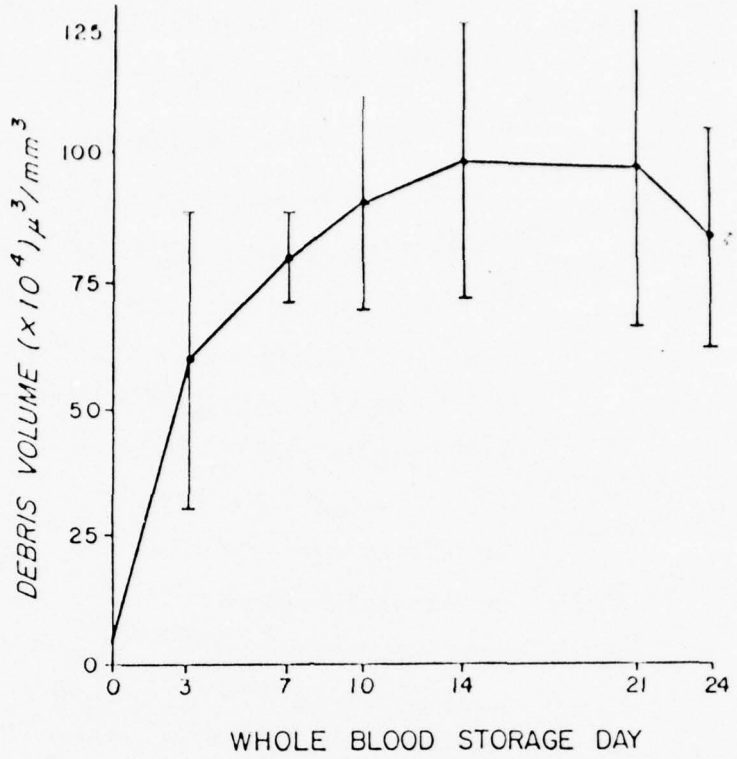

DEBRIS VOLUME (×10⁴)μ³/mm³
125
100
75
50
25
0
0
3
7
10
14
21
24
WHOLE BLOOD STORAGE DAY

Fig. 3a

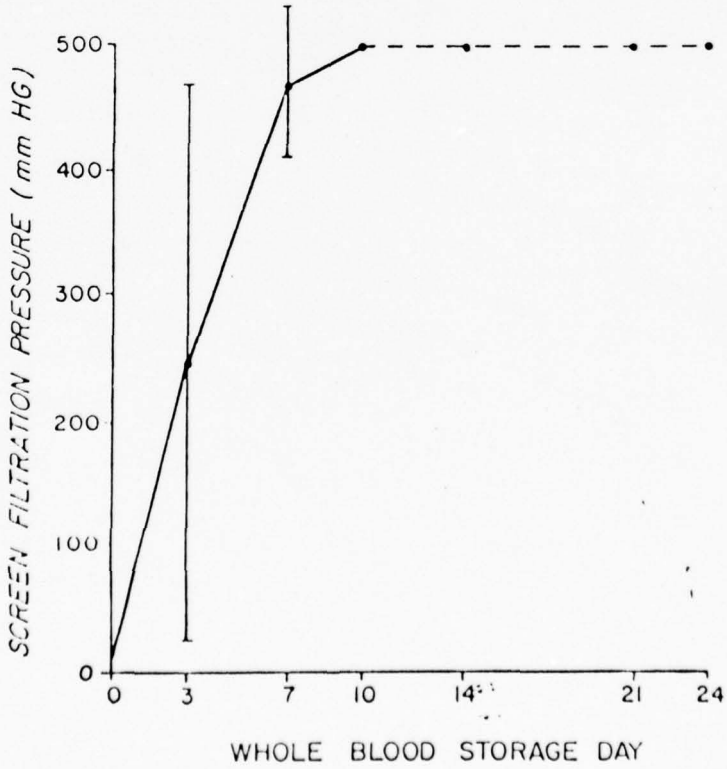

SCREEN FILTRATION PRESSURE (mm HG)
500
400
300
200
100
0
0
3
7
10
14
21
24
WHOLE BLOOD STORAGE DAY

Fig. 3b

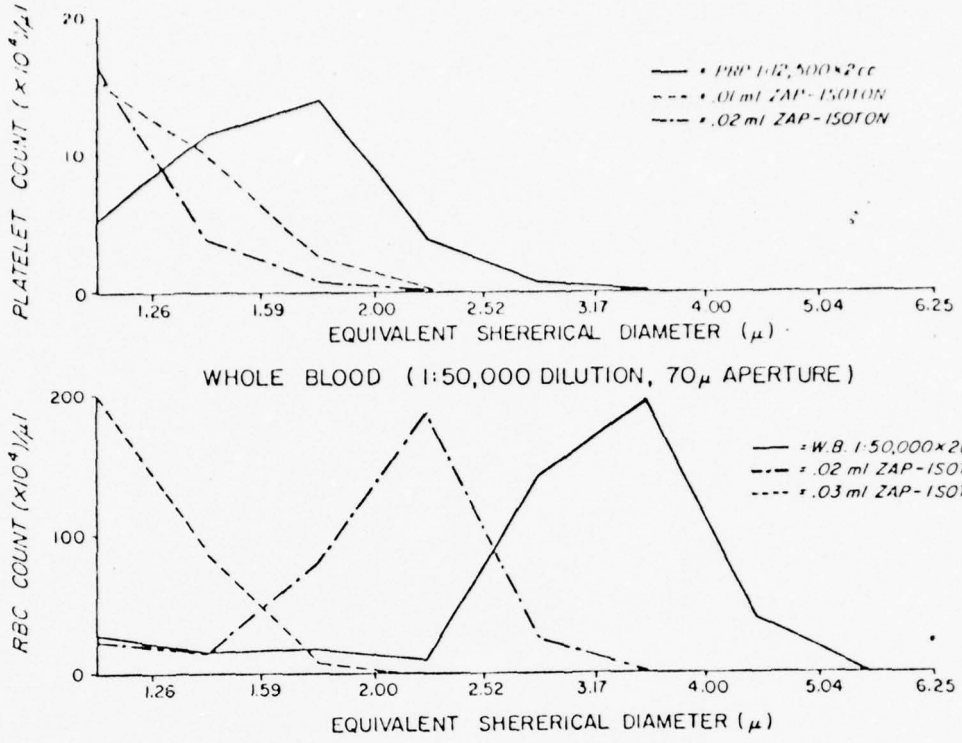


Fig. 4 a & b

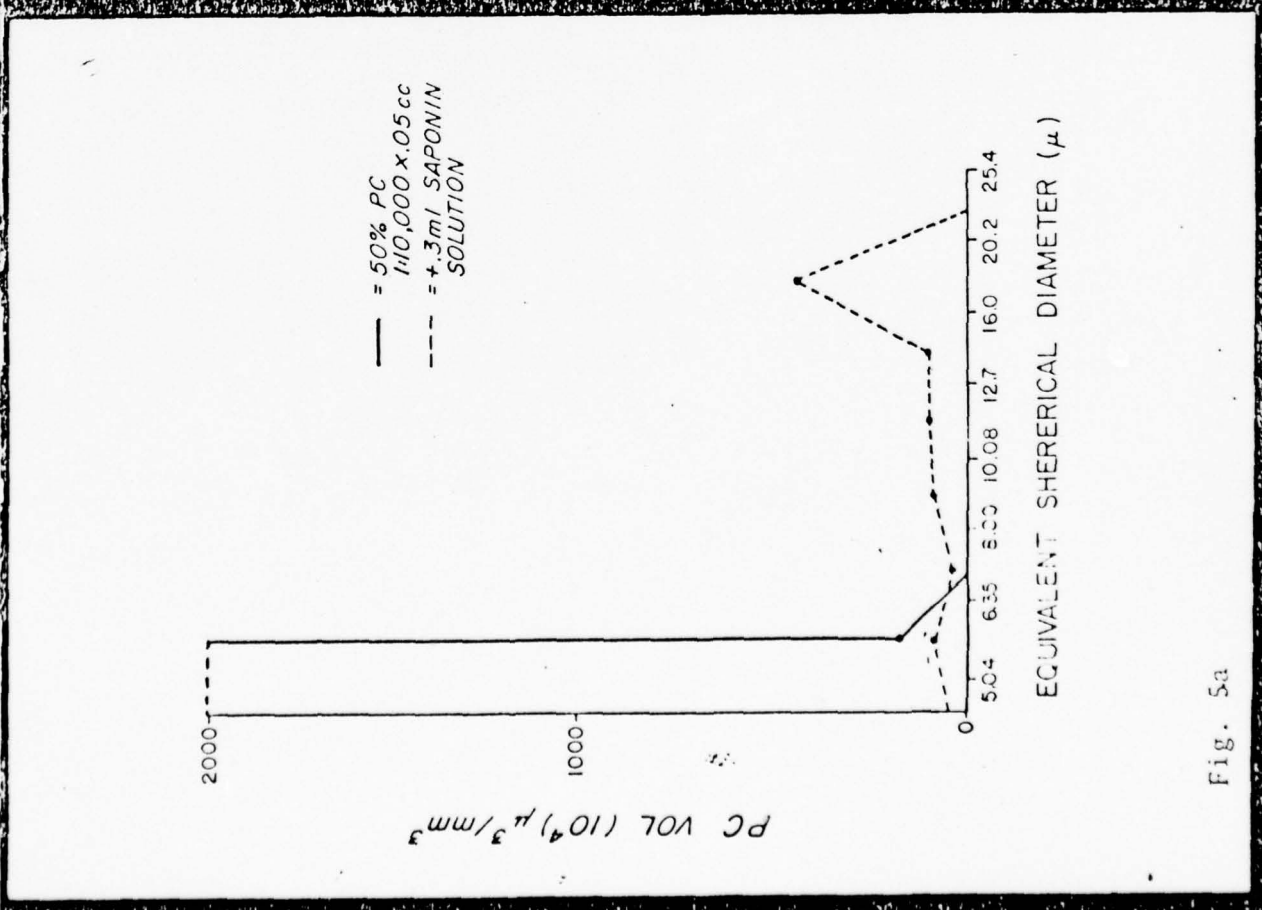


Fig. 5a

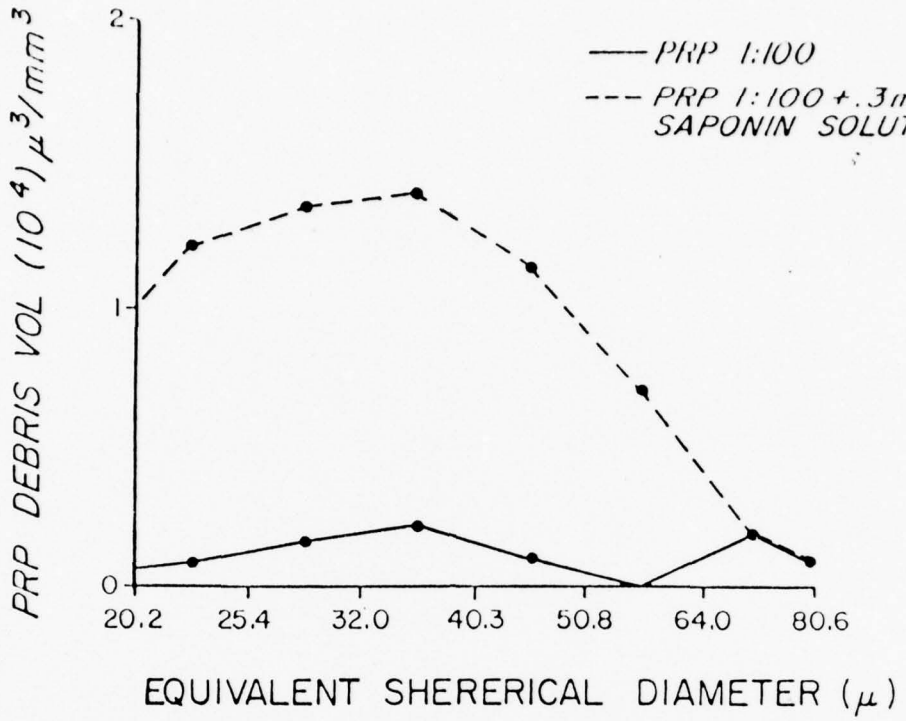

PRP DEBRIS VOL $(10^4)\mu^3/mm^3$
2
1
0
20.2
25.4
32.0
40.3
50.8
64.0
80.6
EQUIVALENT SHERERICAL DIAMETER (μ)
PRP 1:100
PRP 1:100 + .3ml SAPONIN SOLUTION

Fig. 5b

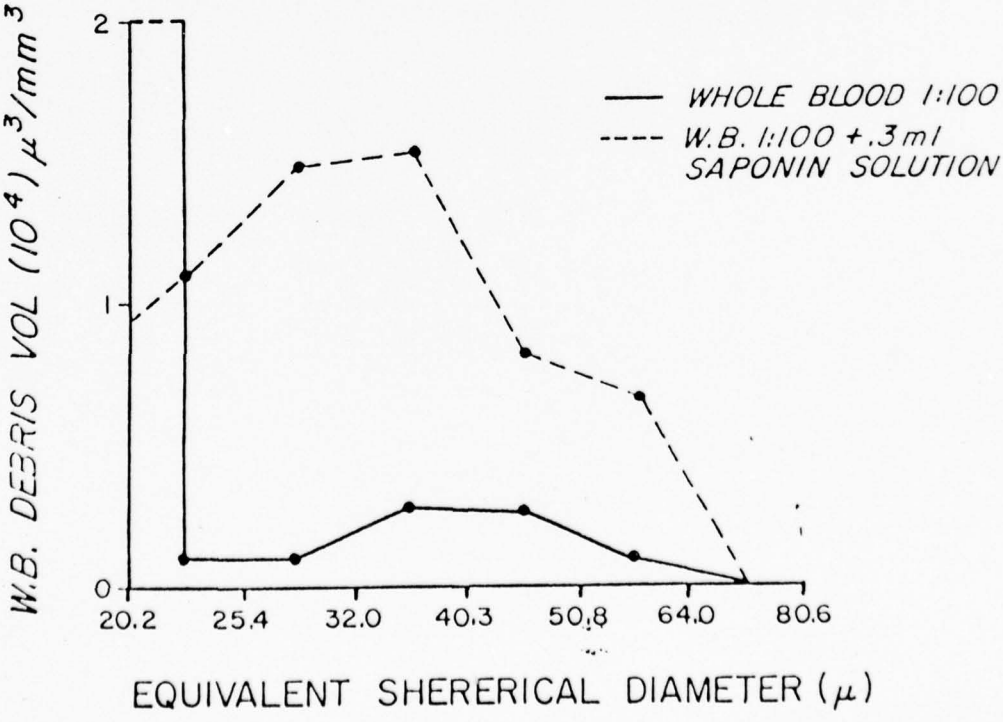

W.B. DEBRIS VOL $(10^4)\mu^3/mm^3$
2
1
0
20.2
25.4
32.0
40.3
50.8
64.0
80.6
EQUIVALENT SHERERICAL DIAMETER (μ)
WHOLE BLOOD 1:100
W.B. 1:100 + .3ml SAPONIN SOLUTION

Fig. 5c

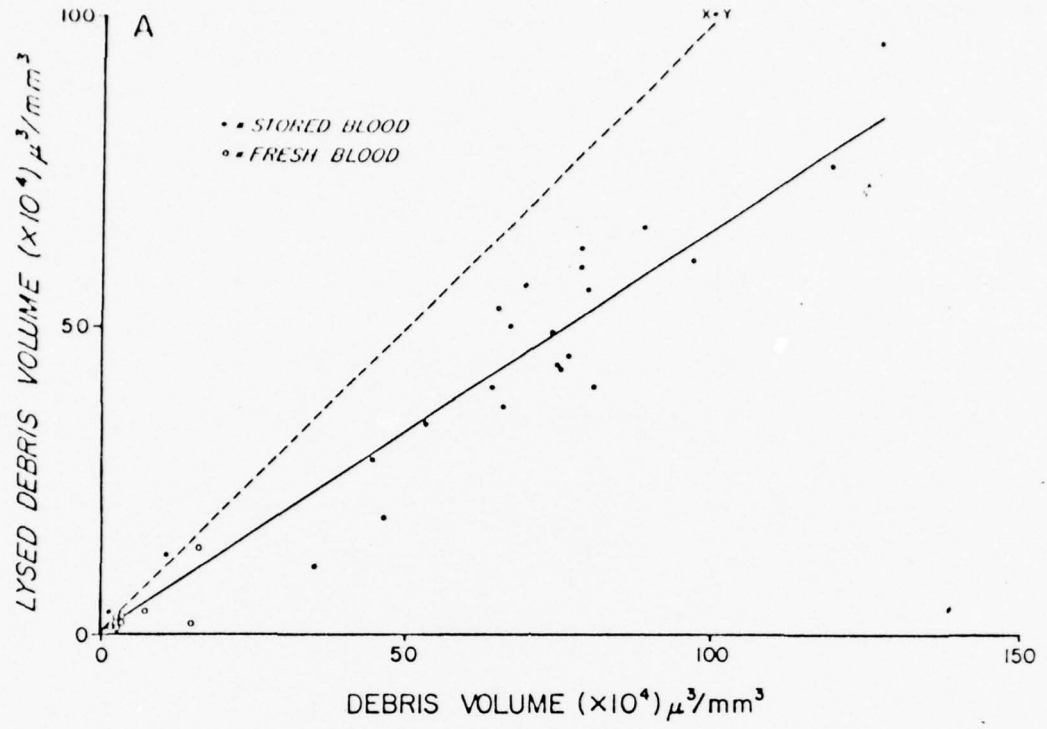

A
100
50
0
X=Y
• = STORED BLOOD
∘ = FRESH BLOOD
LYSED DEBRIS VOLUME (×10⁴) μ³/mm³
0
50
100
150
DEBRIS VOLUME (×10⁴) μ³/mm³

Fig. 6a

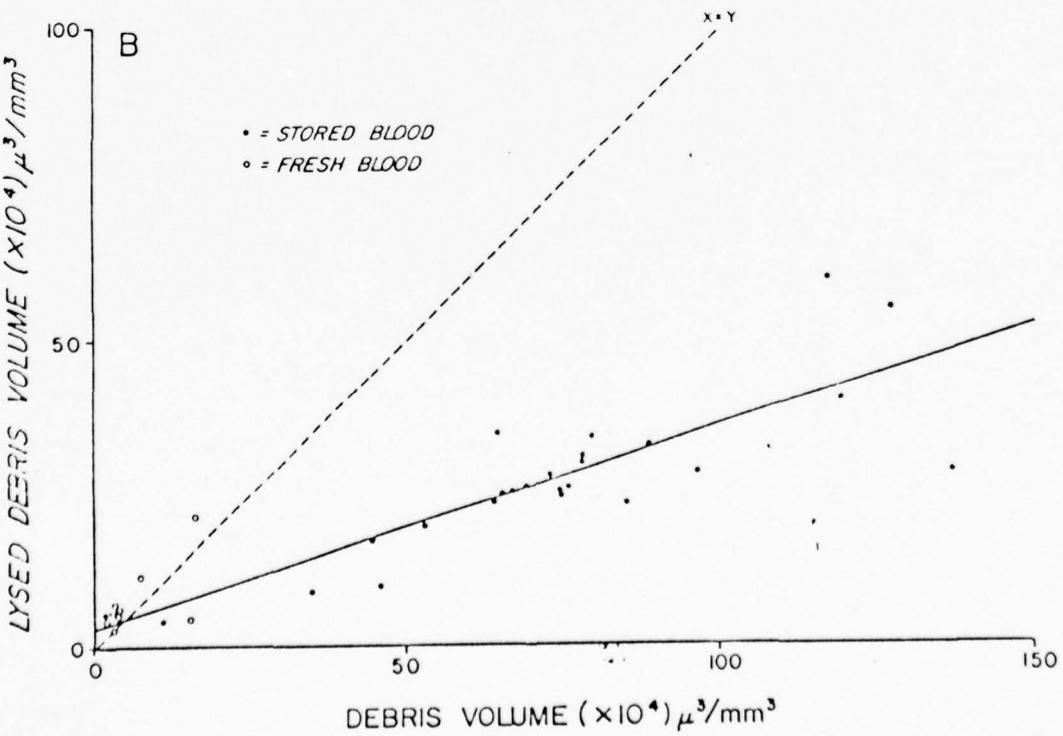

B
100
50
0
X=Y
• = STORED BLOOD
∘ = FRESH BLOOD
LYSED DEBRIS VOLUME (×10⁴) μ³/mm³
0
50
100
150
DEBRIS VOLUME (×10⁴) μ³/mm³

Fig. 6b

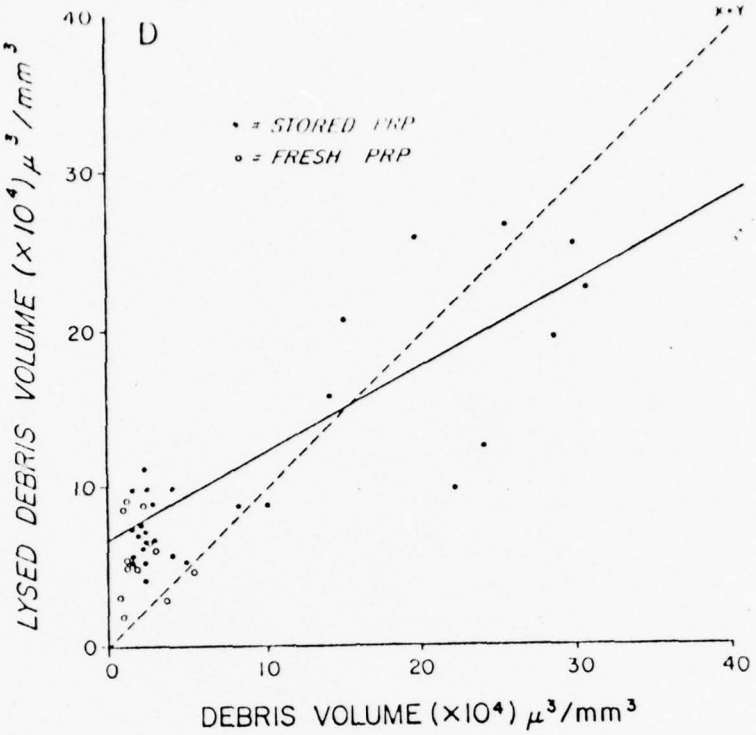

D
• = STORED PRP
∘ = FRESH PRP
X=Y
LYSED DEBRIS VOLUME (×10⁴) μ³/mm³
DEBRIS VOLUME (×10⁴) μ³/mm³
0
10
20
30
40

Fig. 6d

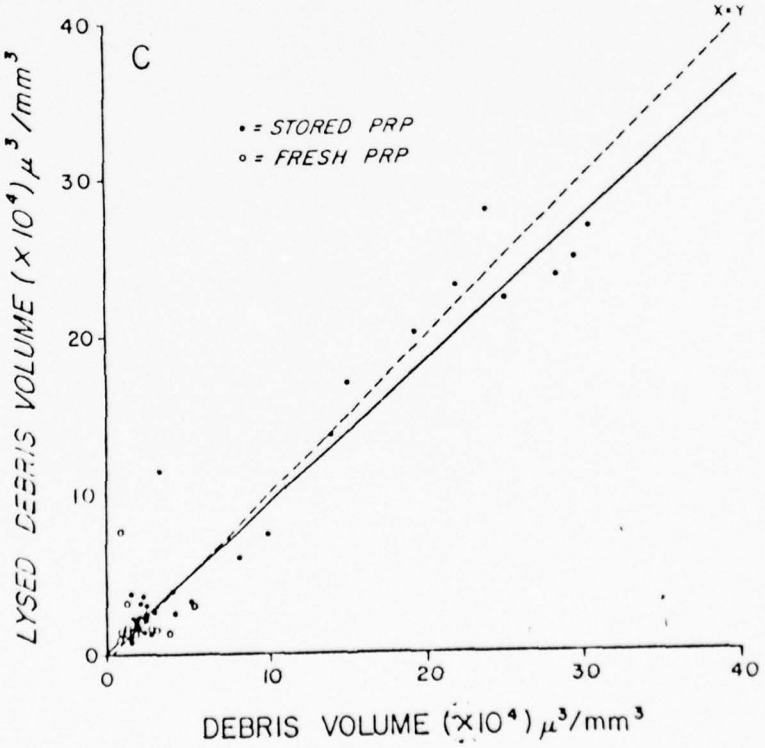

C
• = STORED PRP
∘ = FRESH PRP
X=Y
LYSED DEBRIS VOLUME (×10⁴) μ³/mm³
DEBRIS VOLUME (×10⁴) μ³/mm³
0
10
20
30
40

Fig. 6c

FILTRATION OF DEBRIS FROM BANKED BLOOD

By

Peter Gay

Joanne Thede

Glenn Suehiro

J. Judson McNamara

It is well documented that stored blood develops large quantities of small particles or debris composed of platelet and fibrin microaggregates. Recent studies have implicated pulmonary microembolism of microaggregates, or "debris", infused during massive transfusions of stored blood in the pathogenesis of post-traumatic pulmonary insufficiency. Commercially available micro-pore filters have been developed which remove microaggregates from stored blood with varying degrees of efficiency. The present study evaluates the relative effectiveness of each of these filters.

Measurements of debris weight and screen filtration pressure (SFP) as well as particle size analysis with an electronic particle size analyzer are the methods used for evaluating the size and amount of debris in banked human blood and the efficacy of each of these filters in removing this debris.

Materials and Methods

Outdated banked human blood (21-23 days old), stored in CPD bags at 4°C is obtained from the Blood Bank of Hawaii. All units are agitated by hand for one minute prior to testing. Multiple units of crossmatched blood are used for both gravity flow and 150 mmHg pressure infusions using a Fenwal pressure infusion bag. Samples for testing are drawn from each 85cc increment through the filter for the gravity flow studies and after 100cc increments for pressure infusion studies. Blood was continually collected for 4 units or until the flow rate was sharply reduced as indicated by a rate of less than 1 cc/min. All filters were intially primed to the point where they first began to pass blood. Up to five filters were tested for gravity flow study. One pressure infusion study was performed for each filter.

Standard blood administration filters (V-2950 McGaw Laboratories, Division of American Hospital Supply Corp., Glendale, California), with a 170μ pore size, are placed in-line and precede each test filter. The filters compared are 1) the Bentley Infusion Blood Filter PF-127 (Bentley Laboratories, Inc., Irvine, California, 2) the

Pall Ultipor^{R} Blood Transfusion Filter (Pall Corp., Biomedical Products Division, Glen Cove, Long Island, New York), and 3) the Fenwal Microaggregate Blood Filter 4C2417 (Fenwal Laboratories, Division of Travenol Laboratories, Morton Grove, Illinois).

The Bentley utilizes a 260-300μ polyester screen followed by a depth filter consisting of three successive layers of polyester urethane of 150, 73 and 27 micron pore sizes respectively. The Pall is a surface filter with a coarse clot screen, 1270μ, both above and below a pleated polyester fine mesh screen, 25μ in size. For the Fenwal, a 250μ filtration screen precedes a 150μ capacity reticulated pore structure. This is followed by a depth filter of compressed fiber designed to retain particles of close to 20μ.

Particle size analyses were accomplished with a Coulter Counter Model TAII (Coulter Electronics, Inc., Hialeah, Florida).( ) The machine was adjusted to that with a 200μ aperture; particles with diameters ranging from 3 to 80 microns could be detected. A 1 to 100 dilution of blood in Isoton (Coulter Diagnostics, Hialeah, Florida) was used.

Debris weights and screen filtration pressures were done in a manner previously described,( ) except that control samples were run repeatedly throughout the procedure rather than just initially. SFP is reported in mmHg/cc since most control samples are not able to pass an entire 10cc increment through the SFP apparatus. Debris is given in mg/cc for the same reason.

Fifteen cc of blood is drawn from a point immediately preceding the test filter and another 15cc is drawn from blood that has just passed through each filter. All blood passes first through a standard administration filter since it has previously been demonstrated that it does not significantly lower particle counts or alter debris weights and SFPs.

Values obtained for gravity flow experiments were summated for each filter and graphs constructed from mean values of all 5 units of blood tested for each filter.

The numbers of determinations at each point decrease as longer volumes are passed through the filter reaching the maximum capacity of the filter.

Results

An analysis of blood filters using SFP, debris weight, and the Coulter Counter offers a comprehensive and reliable evaluation technique. Figs. 1 and 2 represent the results for the gravity flow experiments, while Figs. 3 and 4 are data obtained after pressure infusion. All points in Fig. 1 represent the mean from the number of filters achieving a specified volume, as indicated by the numbers printed above each flow rate line in Fig. 1a. In Figs. 2 and 4, the points on the upper line represent the mean particle number for all the pre-filtration samples, while the lower line shows the post-filtration results. Control samples in these figures show high SFPs and debris weights, as well as a considerable accumulation of particles greater than 20μ.

The Pall Ultipor^{R} 25μ is essentially the same design as their earlier 40μ filter (SQ-40), except for a smaller screen size. It was able to maintain the fastest flow rates for the largest volumes. The Pall, however, was not nearly as effective in removing debris as the other filters studied. Pressure infusion showed relatively similar differences in debris removal and flow rates as noted with gravity infusion with observation that the Bentley filter would not accept 1,000cc even under pressure infusion (Figs. 3a-c). The Pall displayed a relatively smaller change in SFP and a significant reduction in debris weight was not evident ($p>.15$) (Figs. 1b and 1c). Similar data was obtained for the Pall after pressure infusion (Fig. 3a-c). Debris weights indicated that differences with pressure infusion, between control and post filter values disappeared for the Pall filter after one unit of blood (Fig. 3c).

The Bentley filter showed a substantial reduction in debris weight and SFP (all $p<.05$) as did the Fenwal (Figs. 1b-c and 3b-c). Flow rate for the Bentley, however, was lowest and the fewest number of units could be passed successfully (Fig. 1a).

Particle counting in gravity flow experiments showed significant removal of particles by the Fenwal down to an 11μ size as the Bentley at 16μ. The Pall, however,

showed no difference in control and post filter particle counts until a size of about 32μ (Figs. 2a-c).

Discussion

Microaggregates of fibrin and aggregated platelets occur in banked blood as early as 2 days and becomes quite substantial after 10 days. This report evaluates the relative efficiency of three blood ultrafilters in removing microaggregate debris from stored human blood.

The Pall filter, though able to maintain high infusion rates, was far less effective in removing debris. This is undoubtedly accounted for by the fact that it is a fundamentally different filter. It is a surface type as opposed to the other two which are depth filters and use multiple layers in succession. The present data again suggest that with higher volumes and pressure infusion, the Pall surface filters lose their efficiency as larger volumes of blood are passed through and that some of the accumulated debris may actually be blown off the screen back into the circulation as we have previously reported.[1]

Particle size analysis did provide an added dimension in allowing analysis of the smallest particles which each filter would remove effectively and is important in considering actual numbers of particles removed by each filter. The different techniques of monitoring filtering efficiency were in close agreement. Both the Fenwal and Bentley filters are highly efficient in removing debris, but the Bently has a much smaller, in fact, inadequate filtering capacity both with gravity flow and pressure infusion.

Summary

Should the infusion of multiple units of outdated banked blood be a primary source of pulmonary microemboli as evidence seems to support, commercially available filters may help to obviate this problem. The combined results of SFP, debris weight and particle size analysis offers a clear means of evaluating different filters. Based on this study, the Fenwal filter provides the most efficient means of removing debris

while maintaining adequate flow rates for large volumes of blood.

The effectiveness of three blood ultrafilters (Fenwal, Bentley and Pall) is evaluated using SFP, debris weight measurement, particle size analysis and determination of filter capacity. Of the filters studied the Fenwal filter provides the most efficient means of removing debris while maintaining adequate flow rates for relatively large volumes of blood.

Fig. 1a

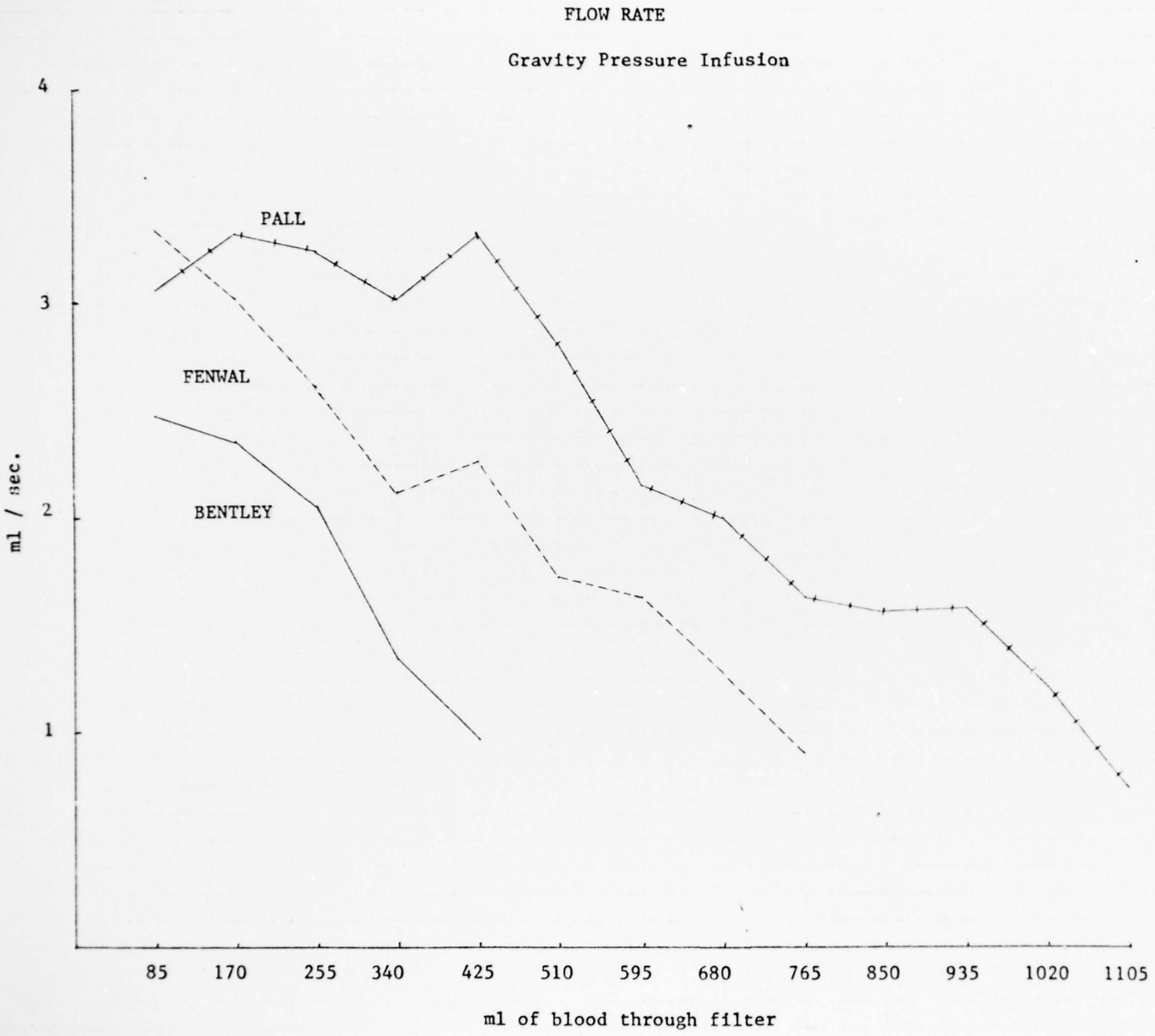

FLOW RATE
Gravity Pressure Infusion
4
3
2
1
ml / sec.
PALL
FENWAL
BENTLEY
85
170
255
340
425
510
595
680
765
850
935
1020
1105
ml of blood through filter

Fig. 1b

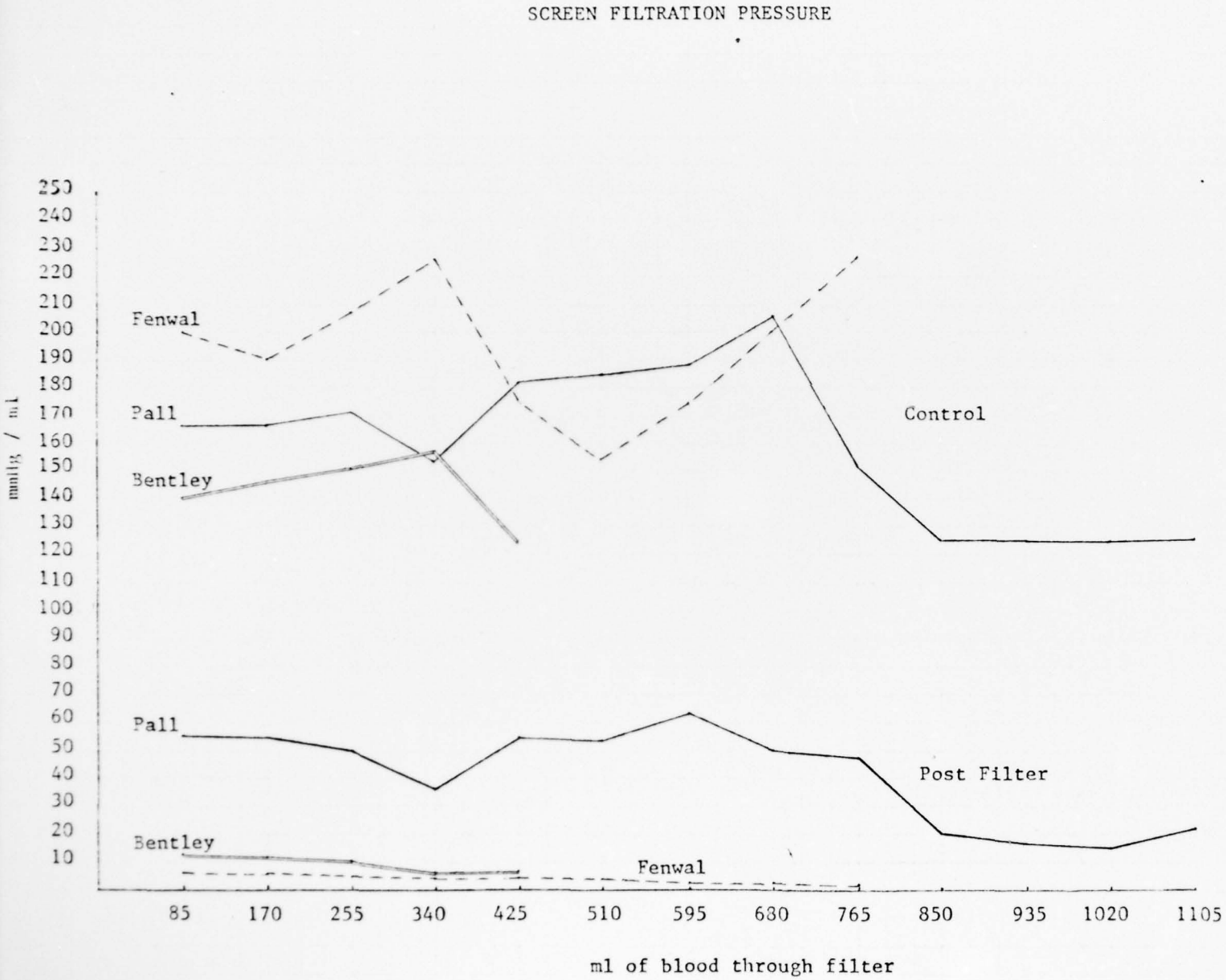

SCREEN FILTRATION PRESSURE
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
mmHg / ml
Fenwal
Pall
Bentley
Control
Pall
Post Filter
Bentley
Fenwal
85
170
255
340
425
510
595
680
765
850
935
1020
1105
ml of blood through filter

Fig. 1c

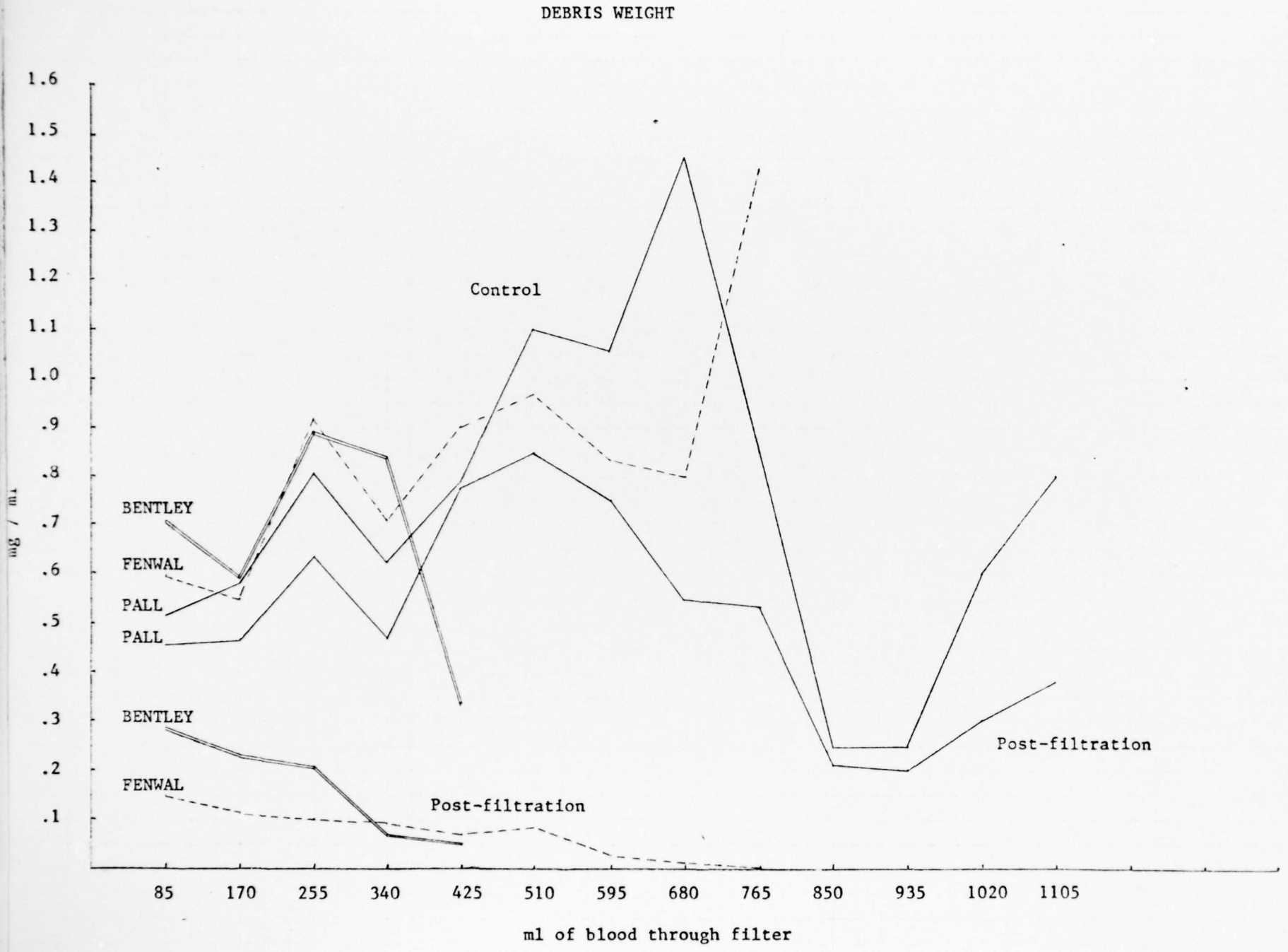

DEBRIS WEIGHT
1.6
1.5
1.4
1.3
1.2
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
mg / ml
Control
BENTLEY
FENWAL
PALL
PALL
BENTLEY
FENWAL
Post-filtration
Post-filtration
85
170
255
340
425
510
595
680
765
850
935
1020
1105
ml of blood through filter

Fig. 2a

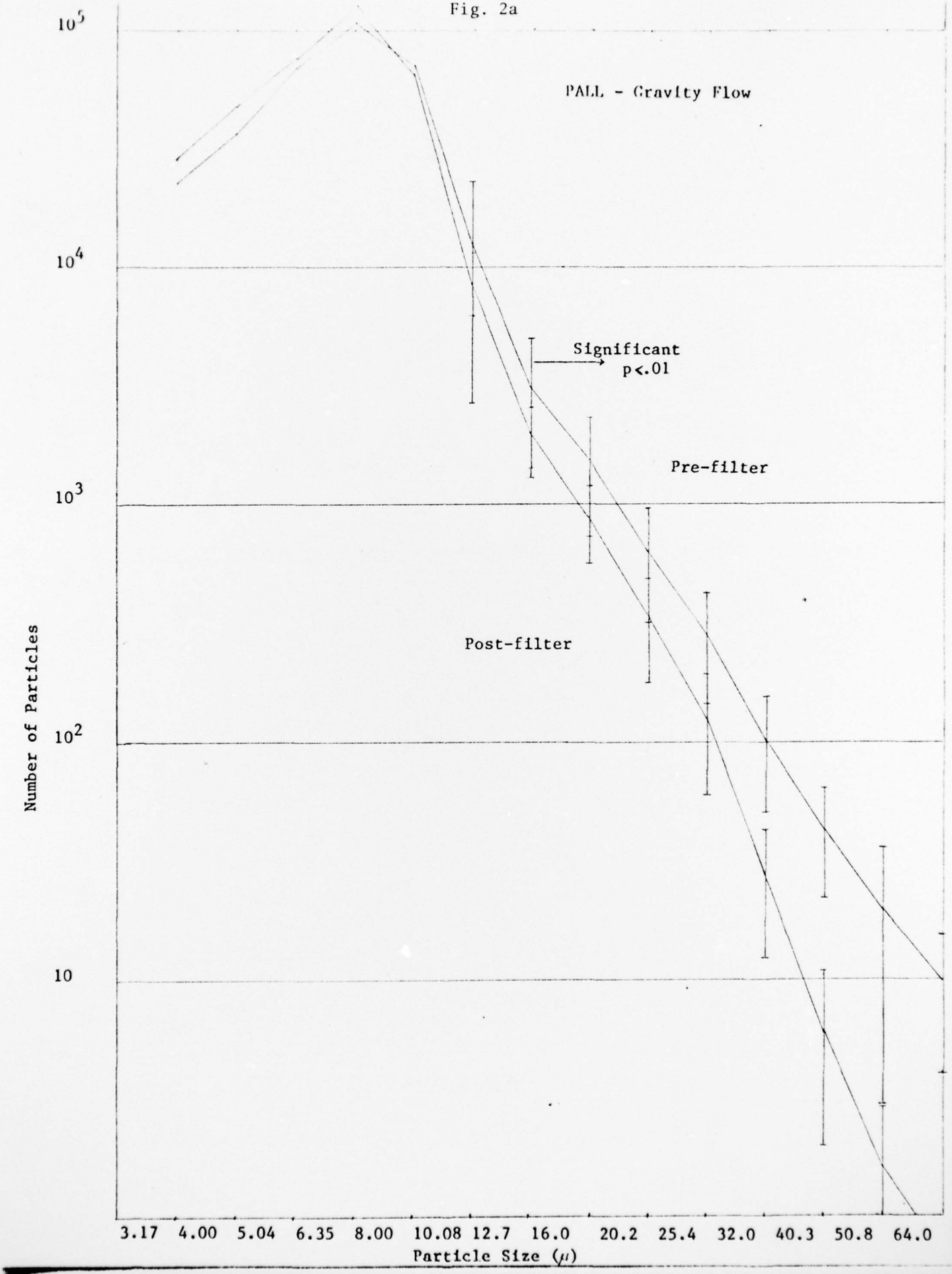
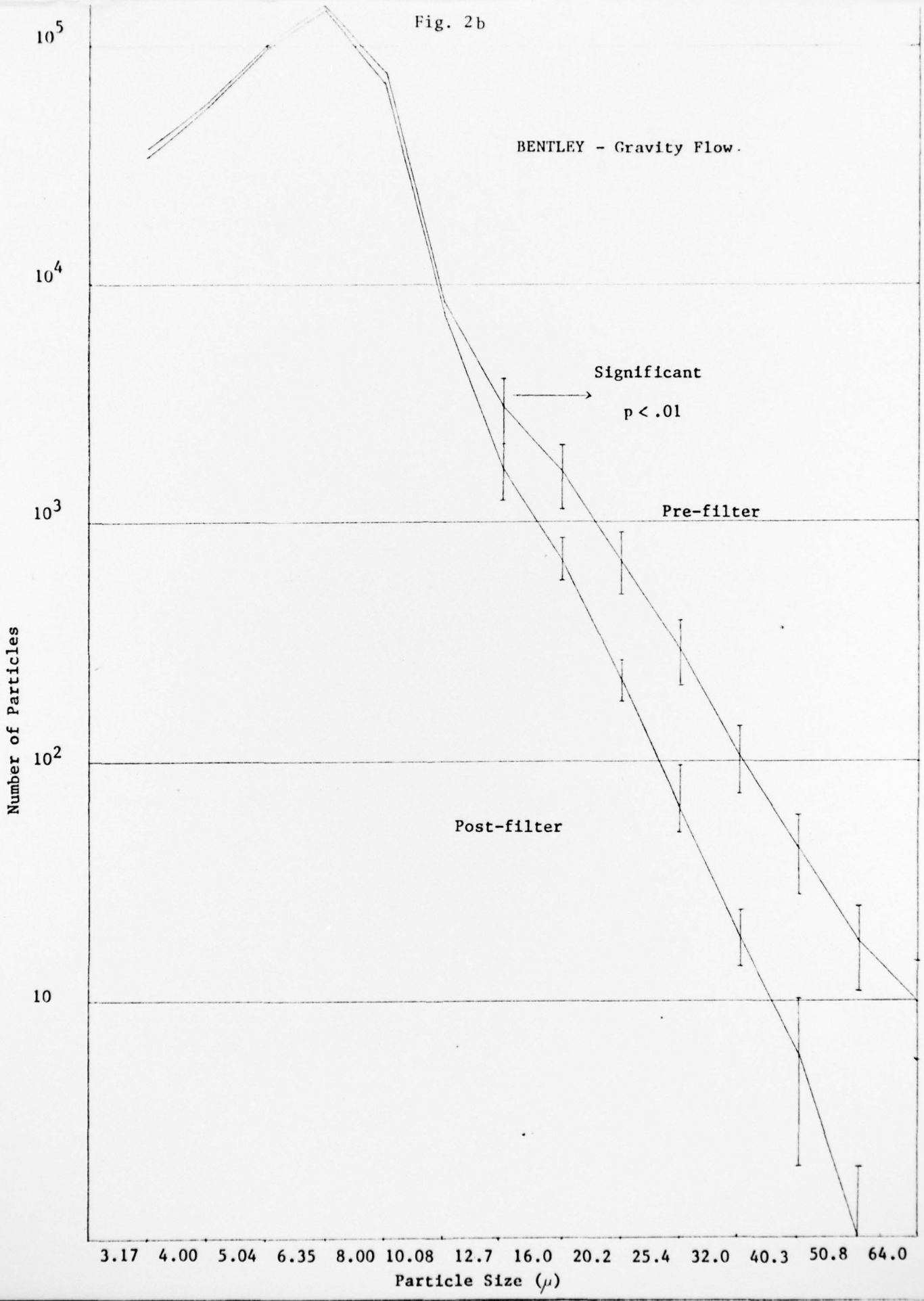

PALL - Gravity Flow
Significant
p<.01
Pre-filter
Post-filter
Number of Particles
10^5
10^4
10^3
10^2
10
3.17 4.00 5.04 6.35 8.00 10.08 12.7 16.0 20.2 25.4 32.0 40.3 50.8 64.0
Particle Size (μ)

Fig. 2b


BENTLEY - Gravity Flow
Significant
p < .01
Pre-filter
Post-filter
Number of Particles
10^5
10^4
10^3
10^2
10
3.17 4.00 5.04 6.35 8.00 10.08 12.7 16.0 20.2 25.4 32.0 40.3 50.8 64.0
Particle Size (μ)

PULMONARY AND SYSTEMIC EFFECTS OF STORED VS. FRESH BLOOD IN TRAUMATIZED, SHOCKED BABOONS

Judy T. McDanal, MD and J. Judson McNamara, MD, FACS

Conflicting reports have been published concerning the role of stored blood debris as the etiologic agent in post-traumatic pulmonary insufficiency (1-5). In previous studies our laboratory found that in anesthetized baboons, in a non-shocked experimental model and with infusion of 35cc/kgm of autologous blood, the infusion of stored whole blood containing microaggregates or of any individual fraction of stored blood does not significantly affect total body oxygen consumption or systemic or pulmonary hemodynamics (1). Based on this data and also in view of recent work by Tobey (2), Soma (4), Garvey (5) and others, there is at present no evidence to show that microaggregates in stored blood produce any significant pulmonary or systemic abnormality in animals not in shock.

The present study was designed to determine if the shocked animal is more susceptible to pulmonary damage induced by stored blood.

MATERIAL AND METHOD

Ten baboons, 10-15 kgms, underwent initial phlebotomy of 35cc/kgm. Three weeks later, the animals were anesthetized (induced with Phencyclidine and maintained with minimal doses of Valium), intubated and ventilated. A catheter was placed in the right femoral artery and a flow probe around the pulmonary artery. The flow probe was monitored through a BL-610 pulse logic flowmeter, and the electrocardiogram and pulmonary artery flow were displayed on a Brush 260 recorder.

From the Department of Surgery, University of Hawaii John A. Burns School of Medicine, at Queen's Medical Center, Honolulu. Supported by a grant from the US Army Medical Research and Development Command DADA17-73-C-3040.

The animals were bled rapidly to a blood pressure of 60 mmHg. One hour later, they were further bled to a blood pressure of 40 mmHg which was maintained for the second hour. The animals were then resuscitated - five animals with autologous shed blood and five animals with autologous 21-day old stored blood. Only a standard filter was used with the infusions.

Studies were made of the following: arterial and venous blood gases, systemic arterial and central venous blood pressure, pulmonary (PVRS) and systemic vascular resistance (TPR), cardiac output (CO), oxygen consumption ($\dot{V}O_2$), A-aDO_2, V_D/V_T and pulmonary shunt.

The parameters were studied pre-bleed, after 1 and 2 hours of shock and 4, 12, 24 and 48 hours after resuscitation with either shed or stored blood. All determinations were in quadruplicate. Studies were done on both room air and 100% O_2.

RESULTS

1. There was no significant difference between the animals who received shed blood infusions and those that received stored blood infusions as regards oxygen consumption, cardiac output, PVR, TRP, A-aDO_2, V_D/V_T and pulmonary shunt. The mortality rates were equal - two animals from each group died and three lived.
2. No animal died during the acute studies or the 48-hour followup period. Four animals died with signs of respiratory failure after 60 to 78 hours, a late mortality of 40%.
3. The most significant difference between the surviving and the non-surviving animals was an $\dot{V}O_2$ that increased steadily from 4 hours after resuscitation in all surviving and decreased in all non-surviving animals.

DISCUSSION

As previously demonstrated in non-shocked animals, we note in our shock animals that stored autologous blood transfusion produced no further pulmonary

or systemic hemodynamic changes than seen with shed blood transfusion. In spite of our 48 hour observation period, which exceeds that of similar studies and covers the time when post-traumatic pulmonary insufficiency most commonly occurs in humans after massive transfusion, no impairment in respiratory function was seen. We feel that there is no good evidence to indicate that any relationship that exists between massive stored blood transfusion and post-traumatic pulmonary insufficiency is of any major clinical importance.

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