Effects of Intersept® Micropore Filtration of Blood on Microaggregates and Other Constituents

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Microaggregates form during storage of whole blood. Composed of cells (particularly platelets), cell debris, and fibrin, microaggregates vary in size, consistency, and composition according to the duration of storage.[1,2] Routine administration sets incorporate a woven nylon clot filter with a mesh containing holes 170 μm in diameter that allows free passage of normal cells and also of microaggregates less than 150 μm in size. Studies have demonstrated that function in a variety of organs is impaired when a sufficient number of these particles is arrested in the microcirculation.[3-6] It has been suggested that respiratory insufficiency following massive intravenous blood transfusion may be attributable, in part, to the embolic effects of microaggregates in the pulmonary circulation.[7]

Micropore filters that are capable of removing microaggregates and provide protection from embolization in perfused organs have been introduced.[3,7,8] A previous report from this laboratory compared Bentley PFS-127, Fenwal 4C2417, Ultipore[8]—Pall Corporation, and Swank IL200 filters and demonstrated considerable differences in their abilities to filter and in other properties.[9] Another filter (Intersept,[8] Johnson and Johnson) that incorporates design features offering theoretical advantages over previously examined filters has recently been introduced. This report describes the performance of this new filter.

METHODS

The Intersept filter was evaluated in two experiments. In Study A, single test units of 14-day-old stored blood flowed by gravity across each of six filters to an empty container (Fenwal Transpak) 100 cm lower.

In Study B each of six filters was preloaded by passage of two units of type-specific bank blood (21–26 days old) prior to filtration of a test unit of 21-day-old blood. In this study a constant pressure of 150 mm Hg was applied to assist flow.

In both studies measurements of screen filtration pressure, microaggregate count, volume and size distribution, platelet, erythrocyte, and leukocyte counts, packed cell volume, plasma hemoglobin, proteins (total and fractional), plasma sodium and potassium, and erythrocyte fragility were performed on samples of blood contained in plastic syringes before and after micropore filtration. In addition, the rate of blood flow across the filters was determined, and the wet and dry weights of material removed by the filters were evaluated. The material collected on the screen used for measuring screen filtration pressure was examined by scanning electron microscopy. The 12 test units of blood were obtained from paid, adult, human volunteers after the nature of the study had been explained and a written consent form signed.

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The subjects refrained from taking any drugs for a week, and from smoking for 24 hours, prior to reporting to the blood donor station.

Blood was collected in plastic bags (Fenwal Blood-Pack) to a final concentration of 450 ml blood to 63 ml citrate–phosphate–dextrose (CPD) anticoagulant. The bags were mixed at least five times during the collection and were then stored for 14 (Study A) or 21 (Study B) days at 4 C.

Screen Filtration Pressure (SFP)

A modification of the method of Swank[10] was employed for the measurement of SFP. A plexiglass container holding a nickel screen with pores 20 μm in diameter was filled with physiologic saline solution and connected to a syringe containing 3 ml blood. The syringe was placed in a high-pressure Harvard pump which, when activated, forced blood across the screen at a constant rate of 2 ml/10 sec. A calibrated transducer and Texas writer recorded the pressure generated immediately proximal to the screen. The final pressure reached after 10 seconds of blood flow minus the pressure observed with saline solution was calculated as the SFP. The final value for each blood sample was obtained from the mean of triplicate determinations.

Total Screen Protein

Two screens from each of the above measurements of SFP were washed with 100 ml physiologic saline solution and then placed separately in 2 ml warm 1 N sodium hydroxide. The protein content in the final solution was measured spectrophotometrically.[11]

Microaggregate Counts

The number, size, and volume distribution of microaggregates contained in the blood were measured with a Coulter Model TA Counter. Detailed discussion of Coulter counting techniques for this purpose has been presented by Solis *et al.*[12] In outline, the principles of this method are as follows. A current is passed across a conductive fluid flowing through an aperture between conductive plates. A particle entering the orifice causes a current pulse proportional to its volume. The particles in suspension are, therefore, counted and their sizes determined. The Model TA Coulter Counter with a 200 μm aperture detection tube analyzes individual particles into 16 consecutive size ranges from 2.52 to 101.6 μm in diameter or 8.378 to 549 $\times$ 10^3 μm^3 volume. We used a 200-μm aperture detection tube for these studies to avoid inconsistencies observed in using a 400-μm tube. In addition, we employed Zap-o-globin (Coulter Diagnostics, Inc.) to lyse erythrocytes rather than the saponin suggested by Solis, because the latter varies in potency and contains considerable quantities of particulate material that must be removed prior to use.

Fifty microliters of blood were added to 10 ml of Isoton (Coulter Diagnostics, Inc.) containing 5 μl 25 per cent Zap-o-globin solution. Particle analysis was begun after 15 seconds and completed 15 seconds later; the diluted suspension was stirred throughout. Each final microaggregate analysis, covering ten channels from 10.08 to 80.6 μm in diameter, was obtained as the mean of five separate determinations. The results are presented as numbers and volumes of particles in each size range and their totals.

Cell Counts

Platelet counts were performed in triplicate by phase microscopy, according to the method of Brecher and Cronkite.[13]

Erythrocyte count, leukocyte count, packed cell volume, and total hemoglobin determinations were performed in duplicate by standard techniques with a Model S Coulter Counter.

Specific gravity of blood was measured by the method of Phillips *et al.*[14]

Plasma Electrolytes, Hemoglobin, and Proteins

Plasma was obtained by centrifugation of whole blood at 3,000 rpm for 10 minutes at 4 C. The supernatant was stored in a refrigerator until it was analyzed. Plasma sodium and potassium were measured with a flame photometer (Instrumentation Laboratories Model #343). Plasma hemoglobin was determined by spectrophotometry, utilizing the method of Lindberg *et al.*[15]

Plasma proteins were measured and fractions quantified by electrophoresis (Beckman Microzone System).

Erythrocyte Fragility

Fragility of erythrocytes was tested with a Fragilograph (Elron Electronic Industries Model D-2) by measuring the concentrations of saline solution when hemolysis began and was completed.

Blood Flow Rate

For each test unit the time required for the blood to flow across the filter was measured with a stopwatch. The blood volume was obtained from the weight of each unit, before and after emptying, and from the specific gravity.

The rate of blood flow was calculated for each unit from these volumes and times.

Wet and Dry Filter Weights

The micropore filters were dried for 24 hours in an oven at 60 C and then weighed. A sample (30 ml) of blood from the test unit was injected into each filter, followed by 100 ml of air to eliminate excess blood, and the filter was reweighed. These two measurements provided control wet and dry weights. When filtration of the test unit was completed, 100 ml air were injected to clear the excess blood and the final wet weight was obtained. The filter was flushed with 1 l saline solution to wash the blood from the inside casing and dried in an oven at 60 C until a constant weight was obtained on two consecutive days.

The differences between the initial and final wet and dry weights were divided by the volume of filtered blood; this calculation was used to obtain measurements of the wet and dry material retained by the filters per 100 ml filtered blood. The volume of filtered blood was calculated from the weight of the storage bag, full and empty, and the specific gravity of the blood.

Scanning Electron Microscopy

For each blood sample, following measurement of SFP, one of the nickel screens was flushed with 100 ml saline solution to remove erythrocytes and then fixed in 1 per cent glutaraldehyde. The screen was subsequently dehydrated in graded alcohols and amyl acetate and finally subjected to critical-point drying. A molecular layer of gold was deposited on the surface and the specimen was examined in an JEOL JSM50-A scanning electron microscope. Photomicrographs at 60 and 1,800 × magnification were obtained. In addition, samples from the various components of the micropore filter were treated similarly, examined, and photographed.

The data obtained from these measurements were analyzed for statistical significance by analysis of variance and Student's t test.

RESULTS

The means and standard errors of the data obtained are shown in tables 1–3.

Table 1 shows that in both studies filtration significantly reduced SFP, total screen protein, and microaggregate volume. Platelet and leukocyte counts were reduced, while erythrocyte count, erythrocyte fragility, packed cell volume, and total hemoglobin were not altered.

Results of plasma analyses are shown in table 2. Plasma sodium, potassium, total protein, and albumin were not changed by filtration. In Study A plasma hemoglobin and globulin were unaffected, while significant increases observed in Study B probably reflect the preloading with outdated blood and not an effect of the filter on the test unit.

In table 3 the flow rate of blood by gravity alone (Study A) was 112 ml/min. In Study B, using 150 mm Hg pressure, the mean flow rates for the three successive units were 275, 217, and 213 ml/min, respectively, a decrease that was not significant at the 0.05 level.

The wet weight of material retained by the filter in Study A was more than that obtained after passage of the test unit of blood in Study B. The dry weight of obtained material was measured in Study A only, and amounted to 58 mg/ml.

The significant effects on blood of filtration by the Intersept filter are those reflecting microaggregates. Coulter Counter analyses of

TABLE 1. Effects of Filtration on Microaggregates and Blood-cell Components

	Study A		Study B	
	Pre-filtration	Post-filtration	Pre-filtration	Post-filtration
Screen filtration pressure (mm Hg)	407 ± 50	26* ± 5	514 ± 50	28* ± 4
Total screen protein (μg/screen)	142 ± 43	9* ± 5	106 ± 59	5* ± 1
Microaggregate volume (× 10^4 $\mu m^3/mm^3$)	42.8 ± 6.2	3.4* ± 1.4	60.0 ± 10.7	5.1* ± 1.9
Erythrocyte count (× $10^6/mm^3$)	4.4 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.1
Platelet count (× $10^5/mm^3$)	0.64 ± 0.14	0.23* ± 0.03	0.34 ± 0.06	0.31 ± 0.06
Leukocyte count (× $10^3/mm^3$)	3.2 ± 0.3	2.5 ± 0.3	2.3 ± 0.3	1.9 ± 0.3
Erythrocyte fragility: NaCl (per cent) Onset	0.57 ± 0.01	0.59 ± 0.02	0.64 ± 0.01	0.63 ± 0.01
Complete	0.18 ± 0.01	0.19 ± 0.01	0.27 ± 0.01	0.25 ± 0.01
Packed cell volume (per cent)	35.7 ± 3.7	41.6 ± 1.4	38.1 ± 0.6	38.6 ± 0.5
Total hemoglobin (g/100 ml)	12.3 ± 1.3	14.2 ± 0.4	13.2 ± 0.3	13.4 ± 0.2

* Indicates post-filtration value significantly different from pre-filtration value, $P < 0.05$.

ten channel diameters from 10.08 to 80.6 μm for both studies are shown in figure 1. The Intersept filter removed particles over the whole range of sizes. The numbers of particles counted in these ten channels were 105.35 per mm^3 and 198.43 per mm^3 before filtration, and 6.65 per mm^3 and 37.75 per mm^3 after filtration, in Studies A and B, respectively.

TABLE 2. Effects of Filtration on Plasma Components

	Study A		Study B	
	Pre-filtration	Post-filtration	Pre-filtration	Post-filtration
Plasma hemoglobin (mg/100 ml)	17.6 ± 5.3	21.3 ± 5.7	28.1 ± 8.2	196.6* ± 61.6
Plasma sodium (mEq/l)	158 ± 2	159 ± 2	159 ± 1	157 ± 1
Plasma potassium (mEq/l)	21.1 ± 19	21.4 ± 1.7	24.6 ± 1.4	25.8 ± 1.3
Albumin (g/100 ml)	3.6 ± 0.1	3.6 ± 0.1	3.9 ± 0.1	3.9 ± 0.1
Globulin (g/100 ml)	2.3 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	2.8* ± 0.2

* Indicates post-filtration value significantly differs from pre-filtration value, $P < 0.05$.

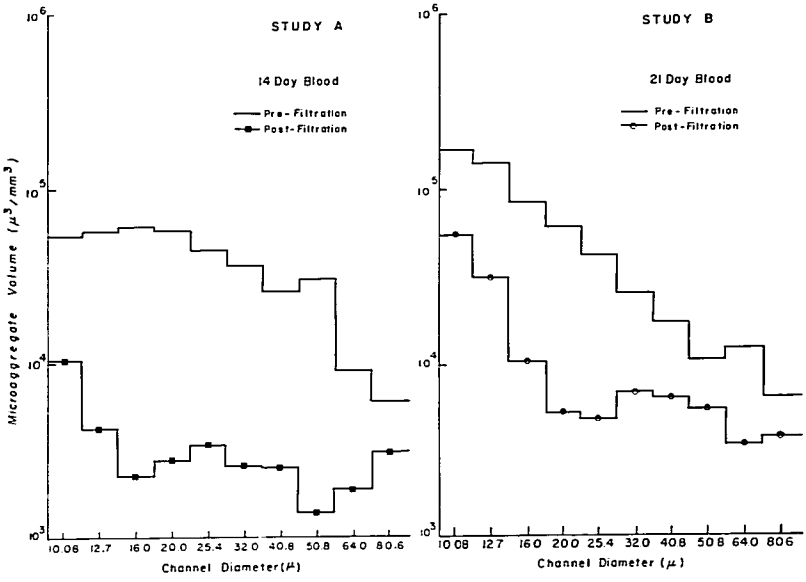

FIG. 1. Microaggregate volumes in each channel size from the Coulter counter analysis. Study A in the left panel and Study B in the right. The means and standard errors from the six observations are shown for the pre- and post-filtration data. The effect of filtration is to remove most of the microaggregates of all sizes.

Scanning electron microscopy of the SFP screen (fig. 2) shows a considerable quantity of material obtained from 2 ml blood and the absence of this material on the screen after Intersept filtration. In figure 3 the material at greater magnification can be seen to be composed of degenerate platelets, amorphous material, and fibrin strands.

DISCUSSION

Micropore filtration of stored blood prior to transfusion has become routine in many institutions. However, the conflicting claims of manufacturers and investigators have made the selection of an appropriate filter confusing. The purpose of these studies was to provide information on which a rational selection could be based.

The Intersept filter had no effect on erythrocyte count, total hemoglobin, packed cell volume, or plasma hemoglobin and, therefore, did not remove or grossly damage erythrocytes. Filtration did not alter plasma albumin, globulin, sodium, or potassium, but did remove some leukocytes and platelets and, therefore, should not be used if, for

TABLE 3. General Characteristics of the Intersept Filters (Means ± SE)

	Study A	Study B
Blood flow rate (ml/min)	112 ± 11	213 ± 39
Wet weight retained (mg/100 ml)	1356 ± 582	928 ± 422
Dry weight retained (mg/100 ml)	58 ± 20	—

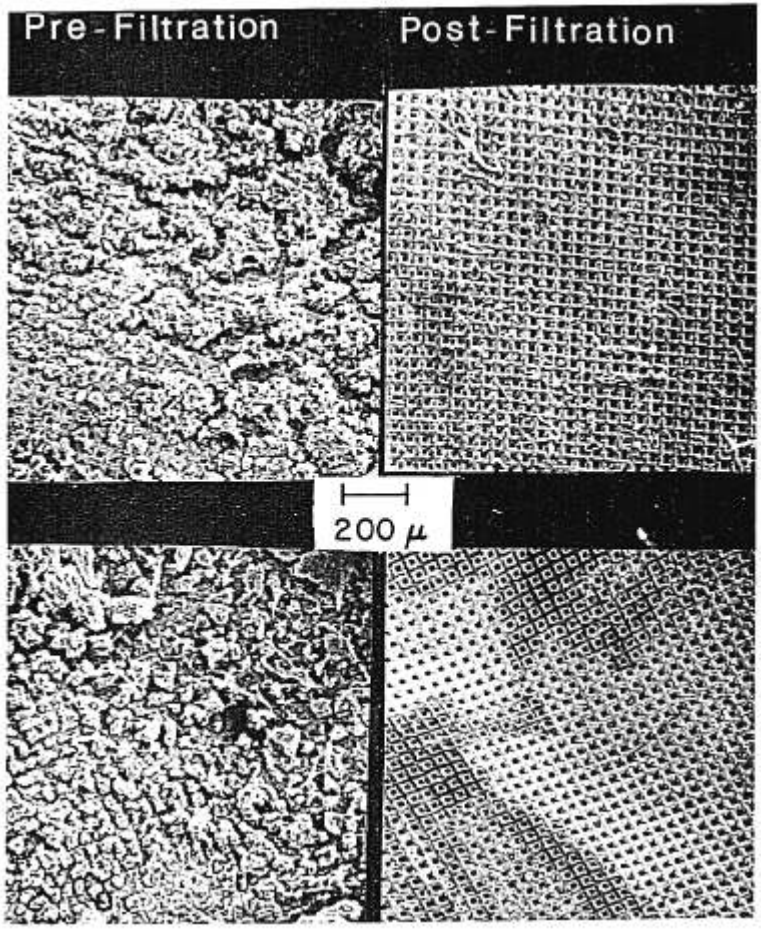

FIG. 2. Scanning electron photomicrographs of representation screen from measurements of SFP, showing microaggregates obtained from blood before and after filtration in Study A (*above*) and Study B (*below*) (Magnification ×60.)

example, platelet transfusion is desired. These observations are essentially identical to previous findings[9] with the Bentley, Fenwal, Pall, and Swank filters, but important differences are apparent with regard to their effects on microaggregates.

The filtration characteristics of the various micropore filters depend on two different principles. The Pall filter consists of a folded, woven polyester screen with 40-μm-diameter holes designed to remove microaggregates by simple sieving. The Bentley PFS-127, Fenwal

FIG. 3. Scanning electron photomicrographs of a screen from SFP measurement indicating the platelets, debris, and fibrin that compose microaggregates. Study A (magnification ×1,800).

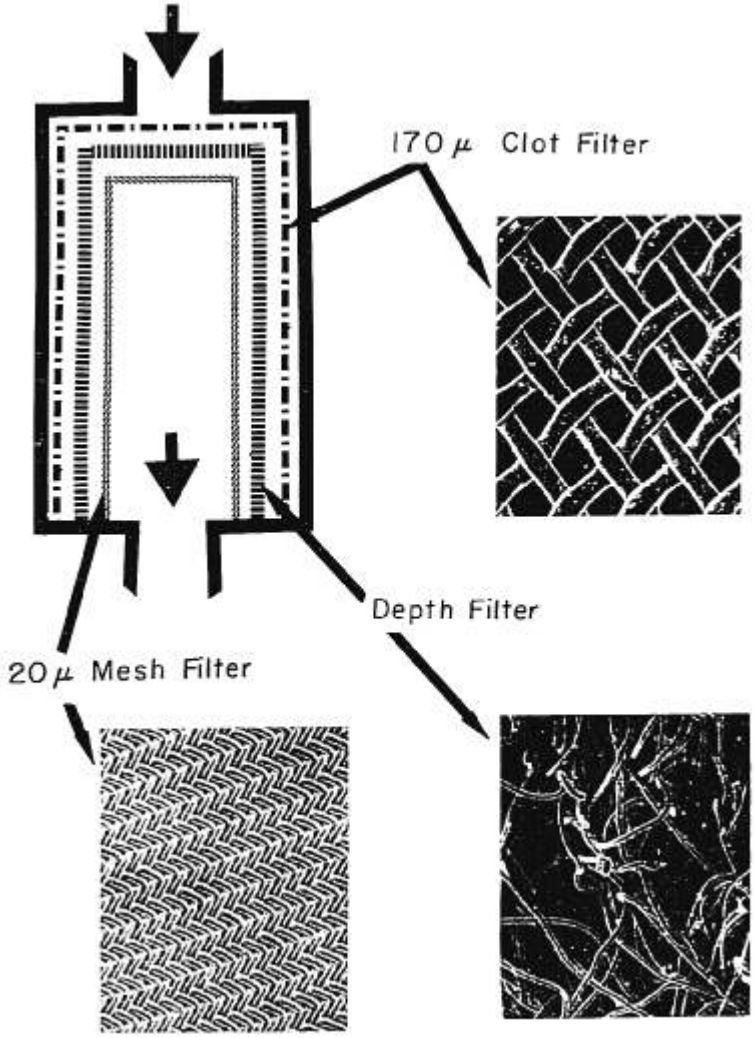

FIG. 4. Characteristics of the Intersept filter. Upper left panel shows cut-away diagram of filter. The 170-μm clot filter, woven Dacron fiber depth filter, and 20-μm mesh filter are located in the diagram and viewed by scanning electron microscopy in the remaining three panels at ×52 magnification (reduced from ×60).

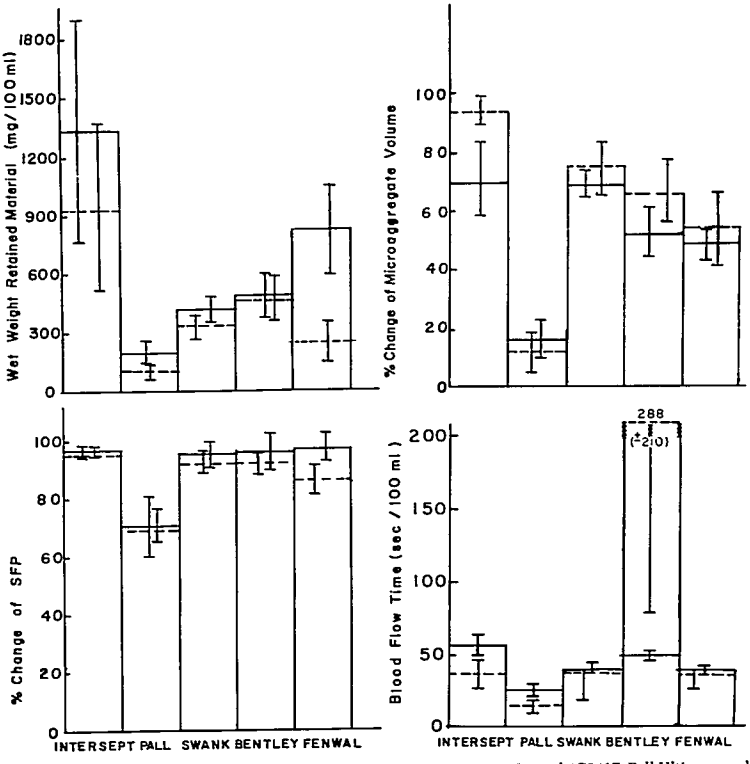

FIG. 5. Comparison of principal effects of Intersept, Bentley PFS-127, Fenwal 4C2417, Pall Ultipore, and Swank IL200 micropore filters on microaggregates and blood flow rate. Solid line indicates Study A and dotted line, Study B. Means ± standard errors from six observations each are shown. Intersept data from this study and data for remaining filters from previous report.[9] See text for discussion. The ordinates (per cent change of SFP and per cent change of microaggregate volume) are calculated from [(value before filtration − value after filtration) ÷ (value before filtration)] 100.

4C2417, and Swank IL200 filters, on the other hand, are depth-type filters containing polyester sponge (Bentley), polyester sponge and nylon fiber (Fenwal), or Dacron fibers (Swank). The huge foreign surfaces presented to the blood provide filtration by adsorption of microaggregates. The Intersept filter combines design features of both types, as shown in figure 4. Blood entering the filter encounters a 170-μm screen filter that removes gross clots, a woven Dacron depth filter providing an adsorption surface, and finally, a screen filter with holes 20 μm in diameter. The folded arrangement of these filter materials provides a large surface area to minimize impediment of blood flow.

In figure 5 the principal effects of these five filters are compared for both Study A and Study B. Blood flow time was shortest with the Pall filter. The Bentley filter became so readily occluded on occasion as to be impractical. There were minimal differences be-

tween the Intersept, Swank, and Fenwal filters, particularly by the third unit of blood (Study B), and the flow rates provided by these filters would appear to be adequate for most clinical situations.

Removal of microaggregates was assessed by several methods, and in each of them the Pall Ultipore filter was significantly less effective; the Intersept filter retained more material than the Fenwal or Swank. From the change in SFP with filtration the Fenwal was slightly less effective in Study B but, otherwise, Intersept, Swank, and Fenwal filters performed similarly. The change in microaggregate volume with the Intersept filter was greater than that with the Fenwal filter in both studies, while the Swank filter was as effective as the Intersept filter in Study A only.

Scanning electron microscopy of the blood emerging from all the filters revealed a quantity of foreign fibers in the effluent from the Swank filter. This material was thought to be cellulose or Dacron fibers, and was not seen with the other filters.

The Intersept filter appears to have advantages over other currently available micropore filters for use with massive transfusion of stored blood.

Summary

Micropore filtration (Intersept®) of whole, stored blood was examined in two studies. In Study A, 1 unit of 14-day-old blood flowed by gravity across the filter. In Study B, the filter was preloaded by passage of 2 units of blood, and the effects on a third, consisting of 21-day-old blood, flowing under 150 mm Hg pressure, were examined. Filtration did not significantly alter erythrocyte count, total hemoglobin, plasma hemoglobin, erythrocyte fragility, plasma sodium, potassium, albumin, or globulin in either study, although some platelets and leukocytes were removed. Microaggregates, assessed by Coulter counting, screen filtration pressure, total screen proteins, wet and dry weights of material retained, and scanning electron microscopy, were satisfactorily removed over the whole range of particle sizes. Comparison with the Bentley PFS-127, Fenwal 4C2417, Pall Ultipore,® and Swank IL200 filters led to the conclusion that the Intersept® is the most efficient filter available at the present time for removing microaggregates during massive blood transfusion.

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