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QUANTITATIVE STUDIES OF MICROEMBOLIZATION IN MAN DURING SURGICAL TRAUMA

ANNUAL REPORT

R. THOMAS SOLIS, M.D.

FEBRUARY, 1977

Supported by

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

Contract No. DADA-17-73-C-3149

Baylor College of Medicine
Texas Medical Center
Houston, Texas 77030

DDC AVAILABILITY STATEMENT

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Microemboli formed in vivo and platelet aggregates induced in vitro in the blood of patients undergoing cardiovascular operations were measured with an electronic particle size analyzer. The effects of extracorporeal circulation, surgical trauma, blood filtration and autologous transfusions on platelet function were assessed. The results demonstrated that assessment of the total volume and size of platelet aggregates induced in blood, when correlated with the total volume of platelets in the blood, provides a unique means of characterizing the mass of functioning blood platelets.

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I. Summary

Microemboli formed *in vivo* and platelet aggregates induced *in vitro* in the blood of patients undergoing cardiovascular operations were measured with an electronic particle size analyzer. The effects of extracorporeal circulation, surgical trauma, blood filtration and autologous transfusions on platelet function were assessed. The results demonstrated that assessment of the total volume and size of platelet aggregates induced in blood, when correlated with the total volume of platelets in the blood, provides a unique means of characterizing the mass of functioning blood platelets.

II. Table of Contents

III. ANNUAL PROGRESS REPORT

Microembolization resulting from microaggregation of platelets and leukocytes has been implicated in the pathogenesis of complications following shock (1-5), trauma (6) and extracorporeal circulation (7-12). Pulmonary embolization of these microaggregates (2-6, 13) as well as those that develop in stored blood (13-18) has been shown to cause alterations in the structure and function of the lungs of experimental animals. Shunting of blood containing these microemboli through the hepatic microcirculation (4) or removal of the microaggregates by Dacron wool (10,18) and 40 μ pore mesh filters (9, 19) prevents these lung changes. In addition, Dacron wool filtration during cardiopulmonary bypass reduces the incidence of systemic non-fat emboli and may lower mortality (11, 12).

Factors which are implicated in microembolic phenomena during resuscitation from trauma, shock, extracorporeal blood oxygenation or intraoperative autotransfusion include: 1) infusion of microaggregates present in stored blood used during the procedure (10, 17, 13-18); 2) formation of platelet aggregates in the patient and/or in the support apparatus (8, 10, 21); 3) incomplete removal of small air bubbles induced by blood oxygenators (22, 23); 4) denaturation of plasma proteins (24); and 5) infusion of fat, fibrin and other foreign material aspirated from the operative site (25-28). Quantitative information on the relative contribution, if any, of each of these possibilities in the etiology of postoperative complications is not available; however, the microaggregates in stored blood, air and fat embolism and platelet aggregation have been considered most important.

Swank has used the screen filtration pressure (SFP) technique to quantitate the microaggregates which develop in stored blood. The SPF is the pressure required to force blood at a constant rate through a 20 μ pore mesh filter (29). He noted that the microaggregates passed through a standard clot filter but were removed by wool filters (14). In subsequent studies, he noted that the high SFP measured in stored blood used during heart-lung bypass was noted to fall to normal levels after passage of the blood through the patient (15). In addition, the SFP was higher in central venous blood samples than in arterial or peripheral venous samples of patients receiving blood transfusions (30). This implies that the material infused is removed by the microcirculation of the recipient and is consistent with findings of multiple microemboli in patients who had recently received massive transfusions (16, 31).

Studies of combat casualties have shown increased hypoxemia after transfusion of over eight units of stored blood (30, 32) and have implicated the microaggregates present in stored blood in the development of pulmonary insufficiency after trauma. However, because of the many complicating factors usually present during massive transfusion in man, such as shock, fat, embolism, overhydration and pulmonary trauma per se, most of the evidence has been based on experimental data obtained from animal studies. Transfusion of stored

blood with a high SFP caused pulmonary hypertension (13, 33) and transient increases in dead space ventilation in dogs (33) and severe alterations in the electroencephalogram in cats (34). Ultrastructural studies of the lungs of dogs receiving large quantities of stored blood filtered with the standard clot filter have shown lesions similar to those noted after hemorrhagic shock (18). These pulmonary lesions which consist of interstitial edema and degeneration of the capillary endothelial and Type 1 alveolar cells (18) and evidence of obstruction of pulmonary blood flow (13, 19) were prevented by effective filtration of the stored blood.

The above studies show that microembolization occurs as a result of blood transfusion and of microaggregation occurring in vivo; however, quantitation of these particles has been limited because of their wide size range and marked instability. The SFP method is sensitive to adhesiveness as well as aggregation of blood cells and platelets (29), but does not indicate the quantity of material which obstructs the 20 μ pores of the filter. Another method of quantitation of the particulate material consists of weighing the amount of material retained by a filter (27-30). In studies of combat casualties receiving blood transfusions, Moseley and Doty (38) found that as much as 5 grams (net weight) of material was retained by the standard blood transfusion filter (170 μ pore mesh) per unit of blood. However, this method does not reflect the smaller sized particles which pass through the filters nor the labile particles larger than the pore size of the filter which break up and pass through mesh filters (19). The ultrasonic method can detect particles in whole blood but is sensitive to air bubbles as well as particulate material (41, 42). Filtration (43) and other techniques (44-46) have been developed for study of platelet aggregation, but these methods are restricted to the study of microaggregates in plasma and do not give quantitative information on the size and number of microaggregates in whole blood.

In studies begun while in the Division of Surgery, Walter Reed Army Institute of Research (WRAIR), we developed a method of quantitating the size and number of microaggregates in whole blood utilizing an electronic particle size analyzer (Model T, Coulter Electronics). Initial studies documented that the instrument could provide reliable measurements of particles larger than leukocytes provided the concentration in the suspension being analyzed does not exceed the established limits of the aperture being used. The microaggregates in stored blood were found to develop progressively during the 21 day storage period in ACD at 4° C. Particles smaller than 25 μ comprise the greater portion by volume of the microaggregates which developed during the first week of storage, while larger particles were found to develop later in storage (48). Filtration of stored blood through the standard infusion filter (170 μ pore mesh) did not significantly remove particles smaller than 80 μ. The 40 μ pore filter (Pall Corporation, Glen Cove, New York) removed particles larger than 25 μ but not those smaller. On the other hand, a Dacron wool filter (Pioneer Filters, Hollsboro, Oregon) removed virtually all particles larger than leukocytes in stored blood (27).

In other studies begun at WRAIR, the electronic particle size analyzer was utilized to measure the size distribution of platelet aggregates induced *in vitro* and *in vivo* by various aggregating agents. *In vivo* microaggregation of platelets was induced by either infusion of adenosine diphosphate (ADP), a known inducer of platelet aggregation, or by extravasation of blood into the peritoneum of ether anesthetized rats. Immediately after injection of ADP, microemboli 13-80 μ in size were detected in the inferior vena cava blood but not in arterial blood of ether anesthetized rats. The increased microemboli in venous blood returned to control levels within three mintues after ADP injection. Immediately after the infusion of ADP the arterial blood pressure and platelet concentration fell and the venous blood pressure increased suggesting that occlusion of the pulmonary microcirculation occurred as a result of the intravascular aggregation of platelets. These alterations in venous and arterial pressure and the platelet counts returned to control levels within three to five mintues as the concentration of microemboli in the venous blood gradually fell to control levels. These studies indicated that the microaggregates of platelets were formed in the peripheral vascular bed and embolized to the lung. The transient alterations in the hemodynamics caused by these particles suggest that they are rapidly broken up *in vivo*. These studies confirmed previous histologic observations of the time course of trapping of platelet aggregates in the pulmonary microcirculation and demonstrated that the electronic particle size analyzer could detect microemboli formed *in vivo*. The inhibition of platelet aggregates induced *in vivo* by adenosine (53) and prostaglandin E_1 (PGE_1, 54) was detected by a decrease in both the cumulative volume and the size of aggregates formed in venous blood following ADP injection (49).

Similar results were obtained in studies of platelet aggregates formed following extravasation of rat blood (53). Control blood drawn from the aorta or inferior vena cava of rats contained low concentrations of particles larger than 13 μ. However, the volume of particles formed in rat blood within 15 seconds following extravasation were significantly increased. These aggregates were found to gradually increase in cumulative volume and modal size during the first 90 seconds following extravasation. Subsequently, deaggregation was manifested by decrease in these parameters, with return to control levels by 5 minutes after extravasation. In other studies microaggregates formed during hypovolemic shock and in a simulated intraoperative Autotransfusion apparatus were similarly studied (55).

During the initial year of the present U.S. Army Medical Research and Development Command Contract to the Baylor College of Medicine, the validity of the electronic particle size distribution measurements of platelet aggregates formed *in vitro* has been documented and the technique has been used to study microembolization and platelet aggregation in man. The electronic particle size measurements were found to correlate closely with independent measurements of fixed particles and of platelets using optical methods. Platelet aggregates induced in plasma by ADP were used to evaluate the size

measurements of platelet aggregates. These studies indicated that the cumulative volume of aggregates formed in plasma varied linearly with 1) the volume of platelets which were available to clump and 2) simultaneous changes in the optical density of the plasma. The size of the aggregates varied with the dose and time of exposure to ADP, the volume of platelets available to clump and when aggregation was inhibited by adenosine. The manuscript of this study has appeared in the Journal of Applied Physiology (56).

In a subsequent study which appeared in Transfusion (57), the technical aspects of measuring the size distribution of microaggregates in blood were analyzed. This included a description of the calibration of the instrument, of the reproducibility of the measurements, of the coincidence limits of the apertures used, of the effects of hemolyzing agents on microaggregate dissociation after dilution, and of the effect of leukocyte counting on the measurement of smaller microaggregates. The major point made in these studies was that although dilution and exposure to hemolyzing agents accelerates the dissociation of platelet aggregates, completion of the analysis within 15 seconds after dilution allows accurate assessment of the size distribution of platelet aggregates in blood.

This study (57) also described the effects of varying the storage conditions and the types of blood components on the total volume of microaggregates which develop in blood during storage. The microaggregates were found to develop progressively during storage of ACD whole blood at 4 to 6^{0} C. Coincident with this there was a drop in the platelet count during the first week of storage and a progressive reduction in the absolute granulocyte count. Microaggregate development after storage of various components of ACD blood was proportional to the concentration of platelets and leukocytes prior to storage. The microaggregates settled into the buffy coat after centrifugation and became larger. In vitro studies indicated that they were resistant to dissociation in vitro in comparison to plagelet aggregates induced in fresh blood by adenosine diphosphate. Microaggregate formation was greater in CPD than in ACD anticoagulated blood stored at 4 to 6^{0} C for twenty-four hours, but was not different after seven days of storage. A greater volume of microaggregates was formed in aliquots of ACD blood stored at 4 to 6^{0} C than at room temperature, while no differences were noted after storage of blood in plastic bags or glass vacuum bottles.

In another paper (58), further evidence was presented to support the validity of the electronic particle measurements of platelet aggregates in blood. The deaggregation of platelets in plasma was shown to be accelerated by addition of hemolyzing agents to the diluent used to perform the electronic size measurements. However, because of the rapidity of the hemolysis of red cells and of the subsequent analysis immediately after dilution of blood, accurate measurements of platelet aggregates in blood are possible before significant alterations in their total volume occurs. This was shown in an experiment where the initial platelet concentration and the population of

platelets to be studied in plasma and in reconstituted whole blood were held constant by adding on equal volume of either platelet-poor plasma or saline washed red cells to aliquots of platelet-rich plasma (PRP). There was no significant difference in the total volume of the platelet aggregates larger than leukocytes (13 μ and larger) that were induced in the plasma and in the blood.

Although the Army Contract has supported the studies of the electronic measurements of platelet aggregates induced in vitro and of the microaggregates that develop in stored blood, the major objective of the contract has been and continues to be to study particulate microembolization and alterations in platelet aggregation resulting from or during surgical trauma. Because of the large number of patients undergoing heart surgery in Houston, we have utilized patients undergoing cardiopulmonary bypass as a model. In the initial study (52), particulate microemboli were measured in patients' blood drawn before and during cardiac operations. Venous particle measurements did not change. The volume of particles in arterial blood drawn from the bubble oxygenator ($41.3 \pm 4.4 \times 10^3$ $^3/mm^3$, mean $\pm$S.E.) was greater than that in venous blood ($30.7 \pm 4.1 \times 10^3$ $^3/mm^3$, p 0.005) only during the first ten minutes on bypass. In contrast, the volume of particles in blood drawn from the cardiotomy return line was markedly elevated ($903 \pm 121 \times 10^3$ $^3/mm^3$). These microemboli remained elevated throughout the procedure, were most evident when extravasated blood was collected with the coronary suction line, and had a size distribution similar to platelet aggregates which could be induced in vitro. A Dacron wool filter removed 89% of these microemboli, while a 40 μ pore mesh filter and a polyurethane foam filter removed 58 and 64% respectively. The data thus indicate that Dacron wool filtration of blood infused through the cardiotomy system would virtually eliminate particulate microembolism during cardiopulmonary bypass.

The major accomplishment during the second year (February 1975 - January 1976) of the U.S. Army Contract was the correlation of measurements of platelet aggregates induced in blood in vitro by ADP with alterations in the aggregation of platelets that were noted during cardiopulmonary bypass. Although some of the particulate microemboli measured in the cardiotomy return line blood of patients were fat globules, their concentration, by volume and size distribution, was similar to that of platelet aggregates which could be induced in vitro in the patients' arterial blood by adenosine diphosphate (ADP) and fell during the first 30 minutes on cardiopulmonary bypass (52). In view of the presumed effects of cardiopulmonary bypass on the concentration and on the reactivity of platelets, this reduction in microembolization probably resulted from alterations in the formation of platelet aggregates. A subsequent study, which appeared in Chest (59) was designed to determine whether this reduction in platelet aggregate microembolization during the first 30 minutes of cardiopulmonary bypass was due to thrombocytopenia or to a decreased ability of platelets to aggregate. The total volume of platelet aggregates induced in blood by adenosine diphosphate was measured with the electronic

particle size analyzer. The volume of platelets in blood was calculated by multiplying hemocytometry platelet counts by the electronically determined mean platelet volume. Immediately before cardiopulmonary bypass, the total volume of aggregates induced in blood by ADP (2μM) was reduced when compared to normal donors because of 1) a slight fall in the volume of platelets and 2) a reduction in the percentage by volume of platelets which aggregated. After 30 minutes on bypass, the volume of both platelets and aggregates fell, but a greater percentage of platelets aggregated. This indicated that reduction of platelet aggregate formation during cardiopulmonary bypass is due to thrombocytopenia. It also suggested that anesthesia, surgical trauma and heparinization alter platelet reactivity more than cardiopulmonary bypass.

Although the study in Chest (59) was limited to determining the mechanism of the reduction in microembolization during cardiac surgery, the methods utilized may be applicable to the study of other clinical disorders in which platelet aggregation may be of importance. These include thrombosis and hemostatis as well as shock, trauma and pulmonary embolism. The electronic measurements of platelet aggregates induced in blood by ADP in vitro provide quantitative assessment of the mass of functioning platelets in blood. Although the platelet count provides the same relative information, the electronic measurements can be performed more rapidly and easily. When combined with an independent measurement of the number and mean volume of platelets in the blood, the electronic measurement of platelet aggregates induced in vitro can be used to assess the capacity of the platelets to aggregate. The study reported in Chest demonstrated that this provides a unique means of correlating in vitro studies with measurements of platelet aggregates formed in vivo in man.

Coincident with the above mentioned studies of particulate microembolization and platelet aggregation during heart surgery, the Department of Surgery of the Baylor College of Medicine began an extensive evaluation of the clinical usefulness of a new microporous membrane oxygenator for use during heart surgery. Membrane oxygenators have been advocated for use during cardiac operations because of the reduction in blood component trauma due to elimination of the blood gas interface created during bubble oxygenation. In order to assess the effects of membrane oxygenation, particulate microemboli and in vitro platelet aggregation were studied in blood of patients during cardiac operations (60). A small gradient of microemboli developed on passage of blood through a bubble oxygenator but not through the membrane oxygenator. However, with both types of oxygenators, there was a sustained increase in the volume of microemboli in cardiotomy return blood which was much greater than in arterial blood. Immediately after cardiopulmonary bypass with both oxygenators, there was a comparable reduction in the volume of circulating platelets which exceeded that of the hemoglobin concentration, indicating platelet loss exceeded that expected from hemodilution alone. However, the total volume and mean size of platelet aggregates induced in blood of patients after membrane oxygenation was significantly greater than similar measurements

after bubble oxygenation. This study showed that membrane oxygenation reduced particulate microembolization and preserved platelet function in patients undergoing cardiac operations when compared to bubble oxygenation.

Although generation of microemboli was reduced during membrane oxygenation, a large volume of microemboli was present in cardiotomy reservoir blood. The increased volume of microemboli in cardiotomy return blood during membrane oxygenation emphasized the continuing need for an effective system for filtration of cardiotomy blood regardless of the type of blood oxygenator utilized. Accordingly, a subsequent investigation (61) was begun to assess the effectiveness of various cardiotomy reservoirs and inline blood filters. Of five cardiotomy reservoirs tested only one was able to remove a significant volume of microemboli larger than 13 μ in diameter; however, its filtration system, which consists of polyurethane foam, was not as effective as the least efficient of the inline blood filters. Less than 10% by volume of microemboli larger than 32 μ remained after passage through all of the inline blood filters tested, but the mean percent ($\pm$ 1 S.E., n=8) of microemboli smaller than 32 remaining varied (25 μ pore polyester mesh 45 $\pm$ 5%; polyurethane foam 36 $\pm$ 3%; woven fabric 13 $\pm$ 2%; Dacron wool 12 $\pm$ 2%). The results of this suggested that the cost and effectiveness of blood filtration during cardiopulmonary bypass could be improved by the design of a cardiotomy reservoir with a more effective filtration system than is currently available. This is of importance because efforts directed at improving the quality of blood salvaged intraoperatively during cardiopulmonary bypass will also be useful in designing systems for use during general surgical procedures.

During the last year of the U. S. Army Contract, studies begun previously were continued in three separate areas: 1) *In vitro* measurements of platelet aggregation using the electronic particle size analyzer, 2) correlation of *in vitro* platelet aggregation studies with alterations occurring *in vivo* in both laboratory animals and in patients undergoing cardiopulmonary bypass and peripheral vascular surgery, and 3) *in vitro* evaluations of blood filtration systems for use during homologous and intraoperative autologous transfusions.

IN VITRO MEASUREMENTS OF PLATELET AGGREGATES

As described above, the early work supported by the U. S. Army Contract helped validate the electronic measurements of platelet aggregates formed in plasma and was published in a manuscript which appeared in the Journal of Applied Physiology in 1975 (56). Since then, subsequent studies have extended this method of studying platelet aggregation such that quantitative assessment of the process in whole blood can be accomplished. These studies were performed in collaboration with Dr. Peter S. Kennedy during his tenure as a Fellow in Oncology at the Baylor College of Medicine. Platelet aggregates induced *in vitro* by adenosine diphosphate (ADP) were used to test the measurements. Initial experiments demonstrated that the electronic measurement of platelet aggregates and platelet-rich plasma were not altered by the addition

of hemolyzing agents to the diluent. As platelets began to clump after exposure to ADP in whole blood, the total volume and size of aggregates increased while there were opposite changes during platelet deaggregation. With higher concentrations of ADP, the total volume and size of aggregates formed during the initial sixty seconds increased; subsequently only their size increased and they dissociated more slowly. As the platelet concentration in blood was increased by serial dilutions, with the hematocrit maintained constant, the mean size of aggregates varied linearly with the total volume of aggregates that were formed (linear correlation coefficient = 0.94). When the platelet count in blood was kept constant, the mean size of aggregates varied inversely with changes in the hematocrit (linear correlation coefficient = 0.98). These studies showed that platelet aggregates induced by ADP in whole blood can be quantitated using the electronic particle size analyzer and defined the effects of changes in the platelet count and in the hematocrit on the resulting measurements. This preliminary work suggested that measurement of the mean aggregate size when corrected for platelet concentration and hematocrit could serve as an index of the responsiveness of the circulating platelets to aggregating agents. The first manuscript resulting from these studies has already been published (62) and a second has been submitted for publication.

EFFECTS OF SURGICAL TRAUMA AND BLOOD TRANSFUSION ON PLATELET AGGRESSION

The second major area supported by the Army Contract over the past contract year was correlation of measurements of platelet aggregates formed in vitro with alterations which occur in vivo. As a result of the studies detailed above, we have developed a simple method of determining the functional capacity of the circulating blood platelets to aggregate (59, 60, 64). In a study which appeared in Surgery, Gynecology and Obstetrics, this method was utilized to study the platelet count and the functional capacity of the platelets to aggregate in vitro before and after peripheral vascular surgical procedures during which autologous blood was administered using a simple method of intraoperative autotransfusion (65). This study was performed in collaboration with Dr. George P. Noon of the Cora and Webb Matting Department of Surgery of the Baylor College of Medicine. The paper describes a simplified method whereby extravasated blood is collected from the surgical field in a reservoir which is contained within a housing attached to a vacuum source. After collection of the blood into one reservoir within the system, the blood drains into a second reservoir which can be detached and, after removal of all air within the bag, can be used for administration of blood, either by gravity drainage or by infusion with a standard pressure cuff. The paper described the use of this system which can be readily utilized in any operating room which has a standard wall suction system. In addition to the above mentioned studies of the functional capacity of the patient's circulating blood platelets, the paper describes quantitative measurements of microemboli in the autotransfused blood before and after filtration through various blood filters. The total volume of microemboli measured in the collected autologous blood was comparable to that in stored blood. Particle measurements

cient at removal of the microemboli in the cardiotomy reservoir blood allowed a significantly higher circulating blood platelet count; however, there was no coincident increase in the total volume of circulating platelets which were able to aggregate in vitro. These results suggest that the microemboli are aggregated platelets which subsequently break up and circulate but do not function. The significance of this study is that it utilizes methodology developed by the studies supported by this contract which allow quantitative assessment of the relationship between the mass of circulating blood platelets and the mass of these platelets which are able to aggregate. An abstract (64) of this study will be published and the manuscript is being prepared for publication.

MICROEMBOLIZATION AND BLOOD TRANSFUSION

The third major area of interest of the studies supported by the Army Contract relates to quantitation of the extent of microembolization which occurs in people as a result of surgical trauma and blood transfusion. These studies relate primarily to blood filtration during homologous and autologous transfusion. During this contract year, three studies have been completed and accepted for publication. The above described study of a simple method of intraoperative autotransfusion reported studies of the microaggregates which developed in blood extravasated during peripheral vascular surgical procedures. The microemboli in the extravasated blood had physical and filtration characteristics similar to those previously reported for both stored blood (57) and blood extravasated into the pericardium during cardiopulmonary bypass procedures (53).

A second study which was performed with the support of the Army Contract funding (66) investigated the microemboli which remain in the blood which is contained within the heart-lung bypass circuit after termination of a cardiac operation. This blood is routinely readministered to the patient and in the study reported was found to contain a large quantity of microemboli which were readily removed by a commercially available cell washing system. The results of this study indicated that the use of a cell washer will reduce the total volume of microemboli which would be administered to a patient. This confirmed previous in vitro measurements of the physical characteristics of the microaggregates in stored blood (57) and suggested that a cell washing system which was capable of removing microaggregates from large quantities of either homologous or autologous blood.

The third study relating to filtration of stored blood has been accepted for publication in Transfusion (67). This reports results of an in vitro evaluation of five different commercially available blood filters and reports on their filtration efficiency during gravity and pressure flow, as well as on their flow characteristics. The blood filters tested were 1) the Dacron wool filter (Pioneer Laboratories, Hillsboro, Oregon), 2) the Ultipore filter (a 25 x 40 micron pore mesh polyester filter, Pall Corporation, Glen Cove, New York), 3) the Intercept blood filter (Johnson & Johnson, New Brunswick, New

after filtration with a Dacron wool blood filter (Pioneer Filters, Inc., Hillsboto, Oregon) were reduced to about the same volume as in the control sample of the patient's arterial blood. Experience with over 100 surgical procedures indicated that the need for homologous blood was reduced but was not eliminated. The study indicated that intraoperative autotrnasfusion of blood with this simple system was safe and had distinct advantages over banked homologous blood. The results suggested that the system could be incorporated into any operating room. If such a system became widely accepted, its advantages in a combat situation would be significant.

During the course of the previous contract year, another study in the general area of correlation of in vitro and in vivo platelet aggregation was continuation of studies previously reported relating changes in the circulating blood platelets to events which occur during cardiac surgical procedures (59, 60, 64). In previous studies we had demonstrated that membrane oxygenation reduced particulate microembolization and preserved platelet function in patients undergoing cardiac operations when compared to bubble oxygenators (60). In studies recently completed, we evaluated the effect of two separate oxygenators and two inline cardiotomy blood filters on the function of human blood platelets after cardiopulmonary bypass. In initial studies we measured platelet aggregates induced at two hours and twenty-four hours after either membrane or bubble oxygenation. Although the total volume of platelet aggregates induced immediately after bubble oxygenation was lower than after membrane oxygenation, there was no significant difference in the total volume of platelet aggregates or in the blood platelet count at two and twenty-four hours after bypass. This suggested that the defect in platelet aggregation after bubble oxygenation, which was most likely due to the large blood gas interface to which the blood was exposed during bubble oxygenation, corrected itself within two hours after the procedure. In a subsequent study similar measurements were performed on the blood of patients undergoing membrane oxygenation in which either a 25 x 40 micron polyester mesh blood filter (Pall Corporation, Glen Cove, New York) or a Dacron wool filter (Pioneer Filters, Hillsboro, Oregon) was incorporated into the cardiotomy reservoir line. In these patients the preoperative platelet counts and total volume of platelet aggregates induced in vitro were not significantly different. However, at termination of the bypass procedure and at two and at twenty-four hours after the procedure, the platelet count was lower in the group of patients with the Dacron wool filter when compared with the 25 micron pore mesh filter group. At two and twenty-four hours after bypass, the platelet count was 177 $\pm$ 17 and 173 $\pm$ 18 ($X10^3/mm^3$, mean $\pm$ SE) with the Dacron wool filter as compared to 270 $\pm$ 42 and 249 $\pm$ 24 with the 25 micron pore mesh filter. In contrast to these measurements, the total volume of platelet aggregates measured at two and twenty-four hours was not significantly different (1.46 $\pm$ 0.13 and 1.54 $\pm$ 0.12 with the Dacron wool filter; 1.49 $\pm$ 0.16 and 1.58 $\pm$ 0.12 with the 25 micron pore mesh filter). The data indicated that the Dacron wool filter, which was much more efficient at removing the microemboli in the cardiotomy reservoir blood, removed aggregated platelets which were able to aggregate. In contrast, the 25 micron pore mesh filter, which was less effi-

Jersey), 4) the Fenwal microaggregate blood filter (Fenwal Laboratories, Morton Grove, Illinois), and 5) the Bentley blood filter (PF124, Bentley Laboratories, Santa Anna, California). The results of this study demonstrated that the Dacron wool filter was the most effective at removing particulate material from stored blood in a size ranging from 13 to 100 microns. The Intercept, the Bentley and the Microaggregate blood filters were all intermediate in filtration efficiency, whereas the 25 x 40 micron pore mesh filter was the last effective at removing particulate material from the stored blood. However, the 25 x 40 micron pore mesh filter had the best flow characteristics in that it was able to sustain a significant flow rate with gravity pressure after filtration of three units of outdated stored blood, whereas all of the other filters required pressure infusion for maintenance of an adequate flow rate. In addition, this study reported results of the microscopic and electronic particle size analyzer assessment of the amount of particulate material which would be washed off the various commercially available blood filters and which may result in embolization of foreign material to the patient.

These initial studies performed under the present U. S. Army contract have demonstrated that the electronic particle size distribution measurements are able to quantitate the extent of microembolization which develops in man during surgical procedures. They indicate that platelet aggregates contribute significantly to the formation of microemboli in man. In addition, the changes in the mass of circulating platelets which function resulting from surgical trauma, blood oxygenation and blood filtration have been investigated utilizing a new method of assessing platelet function in blood. However, the effect of anesthesia, heparinization and oxygenation of blood, homologous and autologous blood transfusions, as well as shock and respiratory failure, on intravascular platelet aggregation have not been extensively studied. The objective of the future studies under the present contract will be to continue ongoing studies of the effects of these clinical variables on platelet aggregation in man.

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