occur until you get to 97 or 98 percent \( FIO_2 \), a level difficult to arrive at clinically.

**Dr. T. King:** I agree with that.

**Dr. West:** I would like to make some comments about this because it is an area in which I have been interested for some time. First of all, I would like to say that I enjoyed seeing some Briscoeograms, which I have not seen for some time. On a more serious note, I would agree with you that the profile shown is not consistent with a pure shunt; however, how can you say that it is not a mixture of shunt and ventilation/perfusion abnormalities? In order to do that, you have to take into consideration every possible combination of shunt and ventilation/perfusion abnormality and I can guarantee that you can find such a combination which will fit your data.

**Dr. T. King:** The fact that 100 percent \( FIO_2 \) does not yield 100 percent saturation indicates the changes cannot be due solely to ventilation/perfusion abnormalities.

**Dr. West:** Yes, but that does not mean that you can rule out a combination of shunt and ventilation/perfusion abnormalities giving this picture. Also, though Dr. Weibel showed areas consistent with a low diffusing capacity, there are also various changes in the airways and I find it very difficult to imagine that there are patients who have no shunt. The histologic sections of patients with this much disease almost invariably show various areas of airless alveoli.

**Dr. T. King:** I agree that it is possible that there is a shunt. We have not suggested that there is no shunt, but that our data are not consistent with the hypoxemia being all due to shunt.

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**SESSION IV: INJURY VIA THE CIRCULATION**

**Quantitative Studies of Microaggregate Formation in Vitro and in Vivo**

R. Thomas Solis, M.D.; Creighton B. Wright, M.D.; Mary B. Gibbs, Ph.D.; and Paul M. Stevens, M.D.

Microembolization resulting from blood transfusions,1-4 shock4,5,6,8 and extracorporeal circulation1,9 has been shown to cause functional and structural alterations in the lungs and other organs, which can be prevented by removal of the microemboli either in vivo by the liver5 or in vitro by blood filters.6,4 Although previous studies have suggested that the microemboli are platelet aggregates, quantitation and characterization of this material has relied either on microscopic examination,5,6,8 or on ultrasonic methods,7 or on changes in the screen filtration pressure.1,5,4 The latter method does not distinguish between adhesiveness or aggregation of cells and platelets,1 while the ultrasonic method cannot detect particles smaller than 50 \( \mu \) and measure air bubbles as well as particulate material.1 Because of these limitations, we have utilized a Coulter counter (model T, Coulter Electronics) to measure the size distribution of particles larger than leukocytes in blood.9

Particles ranging from 13 to 80 \( \mu \) were counted simultaneously in nine channels immediately following dilution of blood in saline containing saponin (500 mg/liter). The data are reported as the volume of particles (number of particles counted \( x \) mean volume of particles detected in a given channel) measured in a given size range. We studied two types of particles in blood which have been implicated in the pathogenesis of acute lung injury: (1) the microaggregates that develop in blood during storage;1 and (2) platelet aggregates induced acutely in vitro in blood and in vivo in rats.

Previous studies have shown that platelet aggregation induced in vitro10 and in vivo11 may be reversible within a short time; however, the volume of particles 13 to 80 \( \mu \) in size in ACD-anticoagulated human blood was found to increase progressively during 21 days of storage.

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**FIGURE 1.** Mean volume of particles (± SE) 32-80 \( \mu \) (top panel) and 13-25 \( \mu \) (bottom panel) measured at various times after dilution (1:101) of blood in saline containing saponin (500 mg/liter). Aliquots of fresh human blood (0.9 ml) drawn into sodium citrate (0.32 mg percent, final concentration) were diluted following addition of 0.1 ml of phosphate-buffered (pH 7.3) saline (control) or saline containing adenosine diphosphate (ADP, \( 2 \times 10^{-6} \)M final concentration) and subsequent agitation for 60 seconds at room temperature. Similar measurements were made following dilution of ACD human blood stored 25 days at 4°C (stored) \( n=8 \).
at 4°C. The differences in the forces holding these two types of microemboli together is illustrated by the fact that platelet aggregates which can be induced in vitro in blood by adenosine diphosphate10 (Fig 1) and in vivo in rats dissociate rapidly when diluted in saline containing saponin. In contrast, the microaggregates that form in stored blood remain relatively stable in such a suspension (Fig 1). Coincident with the development of the microaggregates in stored blood, there was a gradual reduction in the absolute granulocyte count, while the lymphocyte count remained constant during the 21-day storage period. The platelet count decreased only during the first week of storage. Light1 and electron microscopic12 studies demonstrate that during the first week of storage, the microaggregates consist almost entirely of platelet aggregates, but that subsequently granulocytes and the nuclear material of the granulocytes adhere to the platelets. Fibrin and lymphocytes do not contribute to the formation of these particles.12 After storage of various blood components, such as platelet-rich plasma, buffy coat-rich plasma and saline-washed red cells, the extent of development of microaggregates is found to depend on the amount of platelets and leukocytes present at the beginning of the storage period. These in vitro studies suggest that the microaggregates in stored blood, which are tightly held together, would be more damaging to the tissue to which they embolized than loosely aggregated platelets. It is anticipated that increased use of blood components, such as frozen erythrocytes, platelets, and granulocytes, will reduce the microembolization associated with blood transfusions.

Studies were made of the volume size distribution of platelet aggregates induced during hemostasis following extravasation of aortic blood into the peritoneal cavity of previously heparinized, ether-anesthetized rats.13 Within 15 seconds after extravasation there was a marked increase in the volume of particles detected in the extravasated blood in comparison to measurements made of arterial blood, but most of the aggregates were smaller than 40 μ in size. After 45 seconds, the aggregates reached their maximal size (40-80 μ) and concentration by volume (41.2 ± 3.5 versus 0.4 ± 0.1 × 10^6/μm^3 in arterial blood) and remained stable until 90 seconds. During the subsequent three minutes the platelet aggregates became smaller and gradually dissociated. Figure 2 shows the effects of injection of prostaglandin E1 (PGE1)14,15 a potent inhibitor of platelet aggregation, on the volume of aggregates formed 45 seconds after extravasation of aortic blood. There was progressive inhibition of aggregation as the dosage of PGE1 was increased, with complete inhibition occurring after injection of 100 μg/kg. The bottom panel of this figure shows the effect of increasing the time between injection of 100 μg/kg of PGE1.

*In conducting the research described in this report, the investigators adhered to the Guide for Laboratory Animal Facilities and Care, as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

**Supplied by The Upjohn Co., Kalamazoo, Mich.

Figure 2. Changes in mean volume of particles (± SE) 13-80 μ in rat blood 45 seconds after extravasation as dose of PGE1 injected (IV) was increased 5-10 seconds prior to arteriotomy (top panel) and as the time between injection of 100 μg/kg of PGE1, and arteriotomy was increased (bottom panel) (n=10).

Figure 3. Mean volume of particles 13 to 80 μ (± SE) in rat extravascular blood (hemostasis), rat venous blood following ADP injection (in vivo ADP) and human ACD blood stored 21 days at 4°C (stored blood) before and after passage through various blood filters (n=10).
PGE$_1$ and arteriotomy on similar measurements. If arteriotomy was delayed by 90 seconds or more after injection, there was virtually no inhibitory action on platelet aggregation. These results confirm previous studies of the time course of inhibition of in $vivo$ platelet aggregation by PGE$_1$ and demonstrate that platelet aggregates formed in vitro can be quantitated by electronic particle measurements.

In order to localize the microemboli within the circulation during intravascular aggregation of platelets, inferior vena caval and descending aortic blood samples were drawn from rats for particle analysis 5 to 180 seconds after either intravenous or intra-arterial injection of adenosine diphosphate (ADP, 50 mg/kg). Immediately (three to five seconds) after injection of ADP the cumulative volume of particles 13 to 80 $\mu$m in size in venous blood was 17.03 $\pm$ 1.55 vs 0.23 $\pm$ 0.09 $\times$ 10$^4$ $\mu$m$^3$/mm$^3$ in control rats ($p$<.001). The volume of particles in venous blood was not significantly different after either intravenous or intra-arterial injection of ADP and did not return to control levels until 180 seconds. In contrast, the volume of particles in arterial blood never rose significantly above control levels regardless of the site of ADP injection. Immediately (three to five seconds) after ADP injection, inferior vena caval blood pressure fell from 1.9 $\pm$ 0.3 to 6.9 $\pm$ 0.6 mm Hg and descending aortic blood pressure fell from 124 $\pm$ 7 to 48 $\pm$ 4 mm Hg. These hemodynamic parameters then returned to control levels over the next three to five minutes, paralleling the disappearance of particles in venous blood.

The persistence of the aggregates in the venous blood and their absence in arterial samples along with the hemodynamic changes suggest pulmonary vascular obstruction and are in agreement with previous studies, which demonstrate that platelet aggregate embolization to the lung may cause structural and functional abnormalities. However, because of the rapid clearance of these particles from the bloodstream and the transient nature of their physiologic effects, their clinical importance remains to be determined.

Although microembolization can be prevented by manipulation of blood during storage or by the action of pharmacologic agents in $vivo$, the most convenient means of removal of microemboli is by blood filtration. The effectiveness of various blood filters in removing platelet aggregates induced in $vivo$ in rats or microagggregates formed in stored human blood is shown in Figure 3. Particle measurements were made before and after passage of blood through small filters made with mesh of 120 $\mu$m (Bentley Cardiomyotomy Reservoir, Bentley Corporation) and 40 $\mu$m (Barrier, Extracorporeal Blood Filter, Johnson and Johnson) and with tightly packed wool (obtained from the Blood Transfusion Filter, Pioneer Filters). The results demonstrate that the commonly used mesh blood transfusion filters with pore sizes larger than 120 $\mu$m are ineffective in removing these microemboli. Reduction of the pore size of the mesh to 40 $\mu$m increased the amount of microaggregates removed from stored blood, but did not remove acutely induced platelet aggregates. In contrast, the Dacron wool filter was extremely efficient in removing microemboli, but this filter was also the only filter that removed a significant number of platelets.

In considering the clinical usefulness of these newer and more effective blood filters, the possible harmful effects of the microemboli must be weighed against the thrombocytopenia and other complications that may result. Recent clinical studies suggest that effective filtration of stored blood lowers the incidence of pulmonary insufficiency after massive transfusions. Similarly, use of Dacron wool filters during cardiac surgery reduces the pulmonary ultrastructural alterations which develop after cardiopulmonary bypass. However, the extent to which intravascular microaggregation associated with conditions such as trauma and shock contribute to the development of acute lung injury in man remains to be determined.

REFERENCES

9 Solis RT, Gibbs MB: Filtration of the microaggregates in stored blood. Transfusion 12:245-250, 1972
Discussion

Dr. Loury: I believe this work emphasizes an important point to be considered in the transfusion of injured patients. I would like to make these additional points. First, ADP is the most potent endogenous aggregator of platelets. It is released in high concentrations from injured tissue. Although Dr. Solis has shown us that the aggregates formed by the release of ADP break up rather quickly, it should be noted that in the injured patient ADP is released continuously and that aggregation may be a continuing phenomenon. Secondly, it should be noted that platelets have several specific binding sites and that the other endogenous activators, including epinephrine, norepinephrine and serotonin—all of which are released in the case of massive trauma—act upon these other binding sites even when the platelets and ADP binding sites are saturated with ADP. Thus, there may be further tendency toward aggregation in the injured patient. And third, we should note that these aggregates will obviously be delivered to the gravity-dependent part of the lungs, particularly in the presence of hypotension when the PA pressure falls and the aggregates are preferentially delivered to the gravity dependent areas by the zone I theory of West. This implies that changing position of the injured patient may be beneficial in preventing injury to the lung.

Dr. Wilson: Have you looked at substances in stored blood other than ACD which might influence microaggregate size?

Dr. Solis: Yes. Certain blood components contain smaller quantities of microaggregates, which may prove to be an additional incentive for their clinical utilization. For instance, frozen blood is virtually free of microaggregates, since they are removed in the process of washing the glycerol from thawed blood. In regard to the different anticoagulants, it is well known that microaggregates develop more rapidly in blood drawn into heparin than in ACD. However, in studies of blood drawn into plastic bags or bottles we have found that the apparent leaching of the plasticizer from the polyvinyl/chloride of the bag does not affect the formation of these particles. Blood drawn into ACD or CPD anticoagulants contains the same amount of particulate material after 14 days of storage, while adding adenine to ACD blood increases the rate of formation of these particles.

Controlled Endotoxemia and the Lung*

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The mortality and morbidity in "septic shock" remains high despite the vigorous use of antibiotic therapy, corticoids, glycosides, etc. One of the reasons for this high mortality and morbidity has been attributed to the development of respiratory complications, which are fatal in a significant number of instances. These complications are characterized by alveolar septal thickening, edema, interstitial presence of fibrin and red cells, impaired surfactant production, leukocyte infiltration and atelectasis.1-4

The purpose of this study was to examine the possible role of circulating endotoxin in the etiology of pulmonary insufficiency by correlating structural and functional changes in the lungs of rabbits.

Materials and Methods

Thirty New Zealand rabbits weighing between 2-3 kg were anesthetized with intravenously administered pentobarbital (30 mg/kg). The right carotid artery and jugular vein were exposed and cannulated. While the animals were breathing room air, arterial blood samples were taken for Po2, PCO2, pH, bicarbonate, oxygen saturation, hematocrit and endotoxin levels. Respiratory rate and temperature were monitored. Endotoxin levels in the liver and cecal content were determined. Endotoxin levels were measured by the limulus lysate technique. This specific method utilizes a property of the lysate of the cell (amebocyte) in the blood of limulus polyphemus, 0.1 ml of which forms a clot on exposure to 0.1 ml of a test fluid containing at least 10-4 µg of biologically active endotoxin per ml.

Twenty-six animals served as experimental models and four served as controls. The experimental animals were injected with endotoxin (J. Rudbach) 100 µg/kg intravenously over a five-minute period. Sampling began 15 minutes following the completion of injection and continued hourly for a period of six hours. In the animals who died before the end of eight hours, postmortem examination of the lungs and sampling of liver and cecal content for endotoxin were done immediately. After 24 hours from the endotoxin injection, the measurements were repeated in the survivors. After killing the survivors, postmortem examination and sampling of liver and cecal content for endotoxin level were carried out in all animals.

Control animals were observed and followed in the same way as in the experimental group.

The lungs were examined in situ for macroscopic changes, after which the lungs and the trachea were removed en bloc. One lung was inflated by 10 percent formalin given as a gravity drip, then floated in formalin; the other lung was inflated by air; one lobe was clamped by a sponge holder while the lung was inflated, and the clamped segment was resected and immersed in formalin. Following a period of fixation, specimens from the formalin-inflated and air-inflated lung were examined microscopically.

Results

Sixteen animals died within 24 hours (group 1); of these, ten died in the first eight hours. Ten animals survived for 24 hours, then were killed (group 2). The mortality rate was 61.5 percent. There were no deaths in the control group. Figure 1 shows the mean values obtained for Po2, PCO2, pH, bicarbonate, hematocrit, plasma endotoxin and respiratory rate of the animals who died. Figure 2 shows these values for the survivors. Figure 3 shows these values for the controls. Blood gas patterns in all the experimental animals showed a significant rise in Po2 (p<0.001) to supranormal value from control values. Simultaneously PCO2 levels decreased significantly (p<0.001). All experimental