Cardiopulmonary Bypass
Microembolization and Platelet Aggregation

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George P. Noon, M.D., and Michael E. De Bakey, M.D.

SUMMARY
Particulate microemboli and in vitro platelet aggregation were studied in blood of patients during cardiac operations with an electronic particle size analyzer. A small gradient of microemboli developed on passage of blood through a bubble oxygenator but not through a 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. 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 shows that membrane oxygenation reduces particulate microembolization and preserves platelet function in patients undergoing cardiac operations when compared to bubble oxygenation.

Additional Indexing Words:
Cardiopulmonary bypass
Membrane oxygenator
Blood filtration
Microembolization
Platelet aggregation
Adenosine diphosphate

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.1–4 This blood trauma results in hemolysis as well as microembolization of platelet aggregates and alterations in blood platelet concentration and function.1, 4, 12 In order to quantitate particulate microembolization and platelet function in the blood of patients undergoing cardiac operations with bubble oxygenation, we have used an electronic particle size analyzer.13, 14 The purpose of the present study was to determine whether use of a microporous Teflon membrane oxygenator causes less particulate microembolization and alteration in platelet function than does the bubble oxygenator.

Methods
The use of the electronic particle size (Model T, Coulter Electronics, Hialeah, Fla.) analyzer to measure particulate microembolization and platelet aggregation in blood of patients undergoing cardiac operations has been described elsewhere.13, 16 Briefly, the particle size analyzer counts particles 13 to 80 μ in diameter in nine channels after dilution of blood in a diluent containing a hemolyzing solution (Isoton and Zap-Isoton, respectively, Coulter Electronics). The volume of particles is calculated by multiplying the number of particles counted in each channel by the mean volume of particles detected in that channel.10 The mean size in μ of platelets in platelet-rich plasma prepared from blood drawn into sodium citrate (9:1 by volume, 0.32 mg % final concentration, Fisher Scientific, Fairlawn, N.J.) was measured with a 70μ aperture by dividing the total volume of particles 1.3 to 3.2μ in diameter by the total number counted. The mean size of platelet aggregates in blood was measured electronically with a 200μ aperture by dividing the total volume of particles 13 to 80μ in diameter by the number counted. The total volume of platelets in blood was calculated by multiplying the mean size of platelets in citrated platelet-rich plasma by the platelet count determined in blood drawn into ethylenediaminetetraacetate (EDTA Vacutainer, Beckton-Dickenson, Rutherford, N.J.) using the phase hemocytometry method.17 The hemoglobin concentration was measured with a spectrophotometer (Model 182, Co- oximeter, Instrumentation Laboratories, Boston, Mass.). Platelet aggregation studies were performed on arterial blood drawn from patients before induction of general anesthesia and at the termination of cardiopulmonary bypass with either bubble (Variflo, adult bubble oxygenator, Travenol Laboratories, Morton Grove, Ill., N = 20) or membrane (Teflo microporous membrane oxygenator, Travenol Laboratories, N = 20) oxygenators. The details of the extracorporeal circuits have been described elsewhere.13, 18, 20 Both oxygenators were primed with 20 ml per kilogram of body weight of 5% dextrose in lactated Ringer's solution. The time on cardiopulmonary bypass with bubble oxygenation (49 ± 12 min, mean ± sd) did not differ significantly
from that with the membrane oxygenator (58 ± 20 min). Because preliminary studies indicated that membrane oxygenation reduced blood trauma and allowed efficient gas exchange during cardiopulmonary bypass, the more difficult cases tended to be placed on the membrane oxygenator. Accordingly, a greater proportion of patients placed on the membrane oxygenator had an aortic or mitral valve replaced, whereas most of the patients on the bubble oxygenator had coronary artery bypass operations (table 1).

Platelet aggregation was induced within 10 min of drawing the blood by adding 0.1 ml of adenosine diphosphate (ADP, final concentration 0.2 or 2 μM, disodium salt, Sigma Chemical, St. Louis, Mo.) dissolved in phosphate-buffered saline (pH 7.3) to 0.9 cc citrated blood in siliconized test tubes. The platelet aggregation studies in the patients were compared to similar measurements performed on blood obtained from 32 normal blood donors. The effect of reduction of the initial platelet concentration on the mean size of aggregates induced in vitro by the two concentration of ADP in blood of an additional seven normal donors was also determined. Serial dilutions were made of unfiltered blood with autologous blood (3:1, 4:2, 3:3, and 2:4) from which the platelets had been removed by passage through small Dacron wool filters. The platelet concentration varied from 217 ± 30 × 10⁶/mm³ in unfiltered blood to 69 ± 6 × 10⁶/mm³ after mixture of 2 ml of unfiltered blood with 4 ml of filtered blood.

In order to measure microemboli generated during membrane oxygenation, blood of 19 additional patients was drawn into 1 ml plastic syringes and particles 13 to 80μ in diameter were measured as previously described. Venous and arterial blood was drawn from T-connectors in the tubing bringing blood to and from the membrane oxygenator. Cardiomyocyte blood was drawn from T-connectors in the tubing draining blood from the cardiomyocyte reservoir to the venous reservoir of the oxygenator. The cardiomyocyte reservoir blood sample was drawn during periods when extravasated blood was returning through the coronary suction system, since extravasated blood which is autotransfused contains the greatest volume of particulate microemboli. Measurements during membrane oxygenation were compared to similar measurements previously reported during bubble oxygenation of 35 patients.

Results

Measurements of microemboli in the blood of patients before and after passage through the bubble and membrane oxygenators during the initial 10 minutes on cardiopulmonary bypass are shown in figure 1. With bubble oxygenation, a small but significant (P < 0.05 for paired t-test) increase in the volume of microemboli was detected in arterial blood when compared to that of the venous measurements. In contrast, similar measurements in venous and arterial blood during membrane oxygenation were not significantly different.

Although particulate microembolization did not result from passage of blood through the membrane oxygenator, microemboli were detected in cardiomyocyte reservoir blood (fig. 2). In contrast to the small volume of particles in venous and arterial blood measured during the initial 30 minutes on bypass with membrane oxygenation, there was a marked and sustained increase in the volume of microemboli in arterial blood.

<table>
<thead>
<tr>
<th>Type of Cardiac Operations</th>
<th>Membrane Oxygenator</th>
<th>Bubble Oxygenator</th>
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</thead>
<tbody>
<tr>
<td>Coronary artery bypass</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>Aortic valve replacement</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Mitral valve replacement</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Left ventricular aneurysm resection</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Atrial septal defect closure</td>
<td>1</td>
<td>—</td>
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</tbody>
</table>

Figure 1

Particulate microembolisation during extracorporeal oxygenation. Volume of particles 13-80 μ in diameter in venous and arterial blood during first 10 minutes on cardiopulmonary bypass with bubble (N = 35) and membrane (N = 19) oxygenation (mean ± 1 se).

Figure 2

Particulate microembolisation during coronary suctioning. Volume of particles 13-80 μ in diameter in venous, arterial, and cardiomyocyte return line blood measured during first 30 min on cardiopulmonary bypass with membrane oxygenation (N = 19) (mean ± 1 se).
increase in the volume of particles in cardiotomy reservoir blood drawn during coronary suctioning. This differs from similar measurements previously reported during bubble oxygenation in that there was a significant reduction in the volume of particles after the first 10 minutes on cardiopulmonary bypass with the bubble oxygenator.

Hematological measurements in normal donors and patients before and after cardiopulmonary bypass with membrane and bubble oxygenation are shown in table 2. The percent reduction in the hemoglobin concentration and in the total volume of circulating platelets after cardiopulmonary bypass with either the membrane or the bubble oxygenators did not differ significantly (fig. 3). However, with both oxygenators, the reduction in the volume of platelets was significantly greater ($P < 0.001$) than that of the hemoglobin concentration. Since the mean size of the platelets measured after bypass with both oxygenators did not change significantly when compared to the preoperative measurements, this reduction in the volume of platelets was due to thrombocytopenia.

Although the volume of circulating platelets after cardiopulmonary bypass was comparable in the two groups of patients, the in vitro responsiveness of the circulating platelets to ADP was markedly different. The total volume of platelet aggregates induced by ADP was not significantly different (table 2) in the two groups of patients before anesthesia. However, after cardiopulmonary bypass with the bubble oxygenator, the total volume of platelet aggregates induced in the blood by both concentrations of ADP was markedly lower than after membrane oxygenation ($P < 0.001$). This indicates that although the same total volume of platelets was circulating in the blood after bypass with the two oxygenators, a much smaller percentage of these platelets responded to the ADP after bubble oxygenation (fig. 3).

The mean size of the platelet aggregates induced in vitro after cardiopulmonary bypass with the two oxygenators also differed (table 2). With bubble oxygenation, the mean size of the aggregates induced by ADP was significantly lower when compared to the preoperative measurements ($P < 0.005$). However, this reduction in aggregate size may have been due to thrombocytopenia, since a similar reduction in platelet aggregate size occurred in the blood of normal donors when the volume of aggregates formed in response to both concentrations of ADP was reduced by lowering the initial platelet concentration (fig. 4). In contrast, the mean size of aggregates induced after

Table 2

<table>
<thead>
<tr>
<th>Hematological Measurements in Normal Donors and Before and After Cardiopulmonary Bypass (mean ± 1 SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND (N = 32)</td>
</tr>
<tr>
<td>Hemoglobin (g/100 ml)</td>
</tr>
<tr>
<td>Platelets</td>
</tr>
<tr>
<td>Number (x 10$^9$/mm$^3$)</td>
</tr>
<tr>
<td>Mean size (μm$^2$)</td>
</tr>
<tr>
<td>Total volume (x 10$^4$ μm$^3$/mm$^3$)</td>
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<tr>
<td>Volume of aggregates (x 10$^6$ μm$^3$/mm$^3$)</td>
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<tr>
<td>ADP, 2.0 μM</td>
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<tr>
<td>ADP, 0.2 μM</td>
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<tr>
<td>Mean aggregate size (x 10$^6$ μm$^2$)</td>
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<tr>
<td>ADP, 2.0 μM</td>
</tr>
<tr>
<td>ADP, 0.2 μM</td>
</tr>
</tbody>
</table>

Abbreviations: ND = normal donor; Before = before bypass; After = after bypass; M = membrane; B = bubble; ADP = adenosine diphosphate.
membrane oxygenation was not significantly different from the preoperative measurements (P > 0.05 for paired t-test) despite the development of a comparable thrombocytopenia.

Discussion

Hemostatic and thromboembolic complications following heart surgery may result from alterations in platelet function. In addition, microembolization of platelet aggregates formed during cardiopulmonary bypass has been implicated in the pathogenesis of cerebral, pulmonary, and other complications following cardiopulmonary bypass. The present study shows that particulate microembolization is reduced and platelet function is preserved during membrane oxygenation of blood when compared to bubble oxygenation.

A similar reduction in microembolization during membrane oxygenation was noted using an ultrasonic detector which measured air bubbles as well as particulate material. Since the particle size analyzer counts only particulate material, the combined results of these two studies, as well as those using the screen filtration pressure method, suggest that both air and particulate microembolization are reduced during membrane oxygenation. In contrast, a significant difference in the gradient of platelet aggregate microemboli trapped on 48 μ pore mesh filters was not noted in arterial and venous blood of patients undergoing cardiopulmonary bypass with either membrane or bubble oxygenators. Although this discrepancy may be due to the fact that different types of membrane oxygenators were evaluated, previous studies with the electronic particle size analyzer have indicated that platelet aggregates larger than the pore size may break up and pass through mesh filters. This plus the fact that the 48 μ pores of the mesh filter are larger than the vast majority of microemboli detected by the electronic particle size analyzer probably accounts for the difference in the results.

Although generation of microemboli was reduced during membrane oxygenation, a large volume of microemboli was present in cardiotomy reservoir blood. Previous studies had indicated that lipid and other aspirated material account for some of the particles detected in cardiotomy return blood but that platelet aggregates probably constitute the major portion by volume of these microemboli. The increased volume of microemboli in cardiotomy return blood during membrane oxygenation emphasizes the continuing need for an effective system for filtration of cardiotomy blood regardless of the type of blood oxygenator utilized.

Many studies have demonstrated that thrombocytopenia develops during the first 5 to 10 minutes on cardiopulmonary bypass. The possible causes of this thrombocytopenia include acute hemodilution with the oxygenator prime, dilution with stored blood, filtration of aggregated platelets in the microcirculation of the patient or adhesion of platelets to the surface of the extracorporeal circuit. We had previously noted that there was an excessive reduction in the volume of circulating platelets in comparison to the reduction in hemoglobin concentration after bubble oxygenation. This indicated that there was a disproportionate reduction in the volume of circulating platelets when compared to that which would have resulted from hemodilution with the oxygenator prime alone. The present study shows that use of the membrane oxygenator does not prevent this excessive loss of platelets. The particle measurements suggest that filtration of platelet aggregates formed in cardiotomy return blood either within the extracorporeal circuit or in the microcirculation of the recipient may be the mechanism of platelet loss during membrane oxygenation. Since the mean size of platelets did not change after bypass with both oxygenators, smaller platelets, which have been shown to be older and less responsive to aggregating agents in vitro, were probably removed at the same rate as larger platelets.

The electronic measurements demonstrated that the total volume of platelet aggregates induced in vitro after cardiopulmonary bypass was reduced after both membrane and bubble oxygenation. Since the total volume of circulating platelets also was reduced after both types of extracorporeal oxygenation, thrombocytopenia was the major cause of reduction in the volume of aggregates. Indeed, with membrane oxygenation, thrombocytopenia was the only reason for the reduction in the volume of aggregates, since the percent by volume of the platelets which aggregated...
before and after bypass did not differ significantly. However, after bubble oxygenation, the percent of circulating platelets which aggregated in vitro was reduced indicating that altered platelet reactivity to ADF also contributed to the reduction in aggregation. These findings are consistent with many others which have documented that membrane oxygenation reduces blood trauma when compared to bubble oxygenation.\textsuperscript{1, 2, 4, 29}

The effects of cardiopulmonary bypass on the size of platelet aggregates induced in vitro are difficult to interpret because of the resulting thrombocytopenia. Previous electronic measurements demonstrated that the size of platelet aggregates induced by ADP in vitro in human plasma\textsuperscript{16} or in vitro in experimental animals\textsuperscript{30, 32} increased with the concentration of ADP and decreased with addition of adenosine or prostaglandin E\(_1\), which inhibit aggregation.\textsuperscript{33, 34} This suggests that the size of aggregates is determined by platelet reactivity. However, the size of aggregates induced in plasma also has been found to vary directly with the concentration of platelets which were available to aggregate.\textsuperscript{19} This was also shown in the present study by the reduction in the size of aggregates induced in vitro by ADP in normal donors' blood after induction of thrombocytopenia by dilution with autologous platelet-poor blood. Since the degree of reduction in aggregate size noted after bubble oxygenation was similar to that noted after induction of thrombocytopenia in normal donors' blood, the change in aggregate size after bubble oxygenation may have been due to thrombocytopenia rather than to altered platelet reactivity. However, the lack of change in the mean size of aggregates after membrane oxygenation, despite the development of thrombocytopenia, suggests that platelet reactivity to ADP may have actually increased as has been shown in canine experiments.\textsuperscript{35}

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