Platelet Aggregation: Effects of Cardiopulmonary Bypass*


This study was designed to determine whether reduction in platelet aggregate microembolization during the first 30 minutes of cardiopulmonary bypass is due to thrombocytopenia or to decreased ability of platelets to aggregate. The total volume of platelet aggregates induced in blood by adenosine diphosphate (ADP) was measured with a Coulter counter. The volume of platelets in blood was calculated by multiplying hemocytometry platelet counts by the mean platelet volume. Immediately before cardiopulmonary bypass, the total volume of aggregates induced in blood by ADP (2 μM) was reduced when compared to normal donors because of (1) a slight fall in the volume of platelets, and (2) 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 indicates that reduction of platelet aggregate formation during cardiopulmonary bypass is due to thrombocytopenia. It also suggests that anesthesia, surgical trauma and heparinization alter platelet reactivity more than cardiopulmonary bypass.

Alterations in platelet function resulting from extracorporeal circulation of blood may contribute to hemostatic or thromboembolic complications following heart surgery. In addition, microembolization of platelet aggregates may cause pulmonary, cerebral and other complications which occur during cardiopulmonary bypass. In order to measure the quantity and size of microemboli in the blood of patients during cardiac operations, we have utilized an electronic particle size analyzer. Although some of these microemboli were fat globules, their concentration, by volume, 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. In view of the known 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. The present study was designed to determine whether this apparent reduction in platelet aggregation during cardiopulmonary bypass was due to thrombocytopenia or to a decreased ability of the platelets to aggregate.

Methods

Quantitative studies of platelet aggregation were performed on blood obtained from 20 adult patients during elective cardiac operations and from 20 normal blood donors. Nine of the patients had coronary artery bypass procedures, eight had mitral and/or aortic valve replacement, and three had combined coronary artery and valvular replacement surgery. Blood was obtained from the patients immediately before and 30 minutes after the onset of cardiopulmonary bypass. The sample before bypass was obtained after general anesthesia, median sternotomy and heparinization (300 units/kg sodium heparin, Upjohn, Kalamazoo, Michigan). The bubble oxygenator (Variflo, Travenol Laboratories, Morton Grove, Illinois) was primed with 20 ml/kg of 5 percent dextrose in lactated Ringer's solution (Travenol Laboratories). The arterial hemoglobin was determined spectrophotometrically (model 117 CO oximeter, Instrumentation Laboratories, Boston, Massachusetts). The details of the operative conditions have been described.

Blood drawn into plastic syringes was diluted in siliconized test tubes containing sodium citrate (9:1 by volume, Fisher Scientific, Fairlawn, New Jersey, ethylene-diamine tetraacetate (EDTA Vacutainer, Becton-Dickinson, Rutherford, New Jersey) or heparin (9:1 by volume, beef lung, Upjohn) to give final concentrations of 0.32 or 0.15 grams or 10 units per 100 ml, respectively. Platelet-rich plasma (PRP) was prepared by centrifugation of blood at 25°C for ten minutes at 180 × g. Platelets were counted by phase hemocytometry in duplicate after dilution of blood drawn into EDTA in ammonium oxalate (Unopette, Becton-Dickinson) as described by Brecker and Cronkite.

The mean size of platelets in PRP was measured with an electronic particle size analyzer (model T, Coulter Elec-
tronics, Hialeah, Florida) using a 70 μ aperture. The operation of the instrument and the analysis of the data have been described elsewhere. Briefly, the instrument counts particles in 15 channels, each of which detects particles twice the volume of the previous channel. The volume of particles detected in each channel is calculated by multiplying the number counted by the mean size in cubic microns detected in the channel. PRP was diluted in a modified Eagles' solution (Isoton, Coulter Electronics) to a count of less than 2,000 per 0.1 ml. The volume of platelets was calculated by dividing the total volume of particles measured in the four channels detecting particles 3.0 to 23.7 μm³ by the total number of particles counted. With this technique, the mean volume of platelets determined within 30 minutes of drawing the blood of the normal donors into sodium citrate (7.4 ± 0.9 μm³, mean ± 1 standard deviation) was significantly lower than similar measurements in PRP prepared from blood simultaneously drawn into EDTA (8.3 ± 0.8 μm³, P < .01 for paired “t” test). Spontaneous clumping of platelets excluded similar measurements of platelets in PRP prepared from heparinized blood. The total volume of platelets in blood which were available to aggregate, was calculated by multiplying the hemocytometry platelet count by the mean volume of platelets in citrated PRP.

Platelet aggregation was induced within 10 minutes after drawing the blood by adding 0.1 ml of phosphate-buffered (pH 7.3) saline solution containing adenosine diphosphate (ADP, final concentrations: 0.2 or 2 μM, disodium salt, Sigma Chemical, St. Louis, Missouri) to 0.9 ml aliquots of blood in 12 × 75 mm sterile polystyrene test tubes (Falcon Plastics, Oxnard, California). The test tube was shaken continuously by hand at room temperature for 60 seconds, at which time 0.5 ml was transferred with a plastic 1 ml serologic pipette to 50 ml of dichlorodiluoromethane (Isoton) containing four drops of a hemolyzing solution (Zap-Isoton, Coulter Electronics). When hemolysis of the erythrocytes was grossly evident (3 to 5 seconds after dilution), particles were counted in nine different channels of the instrument as 2 ml of the continuously stirred suspension passed through a 200 μ aperture. The mean size of particles detected in these nine channels, which is reported as the diameter of a sphere having a volume equal to the mean volume of particles counted in each channel, varied from 13 to 80 μ.

The size distribution of platelet aggregates induced in aliquots of citrated blood by the two concentrations of ADP was determined in duplicate within 10 minutes of drawing the blood. Similar control measurements were made in citrated blood after addition of buffered saline solution containing no ADP. Data are reported as the total volume of particles 13 to 80 μ in size measured by the instrument, as the percent by volume of those particles which were larger than 32 μ, or as the mean and modal size. The mean platelet aggregate size was determined by dividing the total volume of particles 13 to 80μ in size by the number of particles detected within this size range. The modal size of the aggregates was the particle size in cubic microns detected by the channel which counted the largest volume of aggregates.

Measurements of platelet aggregates induced by ADP (2 × 10⁻⁷ M) in the blood of the normal donors drawn into citrate or heparin demonstrated that the total volume of particles 13 to 80 μ did not differ (2.08 ± 0.48 vs. 2.20 ± 0.52 × 10⁶ μ³/mm³ respectively, mean ± 1 standard deviation). However, aggregates induced in the heparinized blood were larger (percent by volume of aggregates larger than 32 μ = 31.3 ± 21.5 percent with citrate; 63.8 ± 14.7 percent in the heparin, P < 0.001). Although the patients were fully heparinized, citrated blood was used to allow comparison to similar measurements of aggregates induced in the blood of the normal donors. Statistical calculations were performed as described by Snedecor and Cochran.

### RESULTS

The mean size of the patients' platelets before and after cardiopulmonary bypass was not different from that of the normal donors (Table 1). However, the number and total volume of platelets in the patients' blood before bypass were significantly lower (P < .01 for non-paired “t” test) than in the normal donors' blood and fell even further after 30 minutes on bypass. In 17 patients in whom arterial hemoglobin was also measured, the hemoglobin concentration (grams/100 ml) fell from 11.5 ± 1.4 before bypass to 8.7 ± 1.0 30 minutes after the onset of bypass. When compared to the

<table>
<thead>
<tr>
<th>Group</th>
<th>Platelets (x10⁹/mm³)</th>
<th>Mean Platelet Size (μm³)</th>
<th>Volume of Platelets (x10⁶ μ²/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal donors</td>
<td>309 ± 75</td>
<td>7.4 ± 0.9</td>
<td>2.26 ± 0.47</td>
</tr>
<tr>
<td>Patients, before bypass</td>
<td>235 ± 45</td>
<td>8.0 ± 0.8</td>
<td>1.87 ± 0.47</td>
</tr>
<tr>
<td>Patients, after bypass</td>
<td>145 ± 43</td>
<td>8.0 ± 0.7</td>
<td>1.15 ± 0.35</td>
</tr>
</tbody>
</table>

In the heparinized blood, the total volume of platelets in blood (PLATELETS) was calculated as shown in Table 1. The total volume of particles 13 to 80 μ in diameter was measured after addition of two concentration of ADP (ADP 2 μM; ADP 0.2μM). Similar control measurements of particles 13 to 80 μ in diameter (CONTROL) were made after addition of buffered saline without ADP (mean ± SE, n=20).

Figure 1. Platelet aggregation before and 30 minutes after the onset of cardiopulmonary bypass. The volume of platelets in blood (PLATELETS) was calculated as shown in Table 1. The total volume of particles 13 to 80 μ in diameter was measured after addition of two concentration of ADP (ADP 2 μM; ADP 0.2μM). Similar control measurements of particles 13 to 80 μ in diameter (CONTROL) were made after addition of buffered saline without ADP (mean ± SE, n=20).
measurements before bypass, the percentage of re-
duction in the platelet counts of these patients dur-
ing bypass (61.4 ± 14.9 percent) exceeded that of
the hemoglobin concentration (75.8 ± 6.5 per-
cent, mean ± standard deviation, P < .01 for paired
“t” test).

The relationship between the reduction in the total
volume of platelets and in the total volume of plate-
et aggregates induced in blood during cardio-
pulmonary bypass is shown in Figure 1. The total
volume of aggregates induced in the blood of the
normal donors by 2 and 0.2 μM ADP did not differ
and represented 94 ± 3 and 93 ± 3 percent, re-
spectively (mean ± 1 standard error) of the total
volume of platelets. In contrast, the total volume of
aggregates induced by both doses of ADP in the
patients’ blood before bypass represented a
significantly lower percentage of the total volume
of platelets which were available to clump (79 ± 3
and 69 ± 4 percent respectively, P < .005) than
was noted in the normal donors’ blood. In addition,
the total volume of aggregates induced by 0.2 μM
ADP in the patients’ blood was lower than that
induced by 2 μM (P < .005). After 30 minutes
on cardiopulmonary bypass, there was a further
reduction in the total volume of aggregates induced
by 2 μM ADP; however, this was entirely due to
thrombocytopenia, since the percentage of platelets
(91 ± 4 percent) which clumped to form aggregates
13 to 80 μ in size actually increased (P < .05 for
paired “t” test). The total volume of aggregates
induced by 0.2 μM ADP also fell in proportion
to the reduction in the volume of platelets; how-
ever, the percentage, by volume of the platelets
which formed aggregates (68 ± 4 percent) re-
ained unchanged when compared to the mea-
surements before bypass.

In all studies, the total volume of particles mea-
sured in the control samples of blood treated with
saline solution containing no ADP was markedly
lower than measurements made after exposure to
ADP (Fig 1). However, the total volume of par-
ticles detected in the normal donors’ control blood
was greater than that detected in the patients’
blood (P < .05).

Although the total volume of platelet aggregates
induced in the blood of the normal donors by the
two concentrations of ADP did not differ, those
formed in response to the higher concentration
were larger (Table 2, Fig 2). The size of the plate-
et aggregates induced in the blood of the patients
before bypass did not differ from that of the nor-
mal donors (Table 2). However, after 30 minutes
on cardiopulmonary bypass, the aggregates were
smaller (Fig 3).

Table 2—Changes in the Size of Platelet Aggregates
Induced by ADP in Vitro during the Initial 30 Minutes
of Cardiopulmonary Bypass (mean ± standard error,
n=20)

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean size (x10^4 μ^2)</th>
<th>Modal size (x10^4 μ^2)</th>
<th>% of aggregates larger than 32 μ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal donors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADP, 2 μM</td>
<td>12.5 ± 1.0</td>
<td>51.3 ± 7.6</td>
<td>67.4 ± 2.9</td>
</tr>
<tr>
<td>ADP, 0.2 μM</td>
<td>6.1 ± 0.7</td>
<td>15.2 ± 2.8</td>
<td>31.3 ± 4.7</td>
</tr>
<tr>
<td>Patients, before bypass</td>
<td>11.9 ± 1.0</td>
<td>54.8 ± 9.9</td>
<td>64.2 ± 4.4</td>
</tr>
<tr>
<td>ADP, 2 μM</td>
<td>5.8 ± 0.8</td>
<td>15.7 ± 3.5</td>
<td>33.7 ± 5.2</td>
</tr>
<tr>
<td>ADP, 0.2 μM</td>
<td>4.2 ± 0.6</td>
<td>6.4 ± 1.9</td>
<td>22.6 ± 3.7</td>
</tr>
</tbody>
</table>

**Discussion**

The thrombocytopenia which develops during
cardiopulmonary bypass has been attributed to
hemodilution with oxygenator prime, dilution with
stored blood, and trauma resulting from contact of
blood with foreign surfaces and gas in the oxygena-
tor or cardiotomy return system.1-3,5-6,18 Experi-
mental studies have demonstrated a partially re-
versible sequestration of platelets in the hepatic cir-
culation during the initial few minutes on cardio-
pulmonary bypass.24 This has been attributed to
trapping by the patients’ microcirculation of plate-
et aggregates induced during the procedure10-13,18
and is consistent with the finding that a signifi-
cant gradient of particulate microemboli across the
oxygenator is noted only during the first ten min-
utes on cardiopulmonary bypass.26 In the present
study, the thrombocytopenia which developed dur-
ing bypass exceeded that which would have been
expected from hemodilution, as reflected by the
reduction in arterial hemoglobin. However, hemo-
dilution accounted for most of the reduction in the
platelet concentration.

Younger platelets, which are more active func-
tionally and metabolically, are larger than older
platelets.35,36 The lack of change in the mean size
of the patients’ platelets during the development
of thrombocytopenia while on cardiopulmonary
bypass, suggests that the rate of removal of larger
and smaller platelets was equal. Experimental error
due to selection of a nonrepresentative population
of platelets during preparation of PRP or to errors
in the electronic measurement could also be re-
sponsible for this lack of change. The sizing of platelets
with the model T is probably less precise than with
a model B Coulter counter, which has narrower
channel widths.27 However, the finding that the
FIGURE 2. Frequency distribution by volume of platelet aggregates induced by two concentrations of ADP (ADP 2 μM and ADP 0.2 μM) in blood of normal donors. The data represent the percent of the total volume of particles 13 to 80 μ in diameter detected in each of the nine channels which measure particles within this size range (mean ± SE, n=20).

Platelet size was increased in EDTA-anticoagulated PRP indicates that the model T is capable of detecting alterations in platelet size which are known to occur.

Most quantitative studies of in vitro platelet aggregation have utilized the turbidimetric method. Although the fraction of platelets which aggregate in plasma can be calculated from changes in the optical density, these measurements cannot relate the mass of platelets in blood to the mass of aggregates formed either in vitro or in vivo. Assessment of platelet aggregation in vivo has therefore relied on either observation of the microcirculation or on changes in the circulating platelet concentration. The screen filtration pressure and various platelet retention tests can measure platelet function in blood, but these techniques detect adhesive as well as aggregated platelets. In contrast, electronic particle measurements can quantitate the size distribution of platelet aggregates formed in vitro and in vivo.

The present study demonstrates that in vivo factors which influence platelet aggregate formation can be quantified by relating electronic measurements of platelet aggregates induced in vitro to the total mass of platelets in blood. All but about 7 percent by volume of the platelets in the normal donors’ blood formed aggregates 13 μ or larger in response to both concentrations of ADP, with larger aggregates being formed as the concentration of ADP increased. In contrast, a greater percentage of platelets in the patients’ blood before bypass did not form aggregates larger than 13 μ and the total volume of aggregates induced by the two doses of ADP differed. Whether the remaining platelets failed to aggregate or formed aggregates smaller than 13 μ is uncertain, since detection of leukocytes precludes electronic measurement of aggregates within this size range. The cause of this alteration in the patients’ platelets before bypass is not known, but anesthesia, surgical trauma and heparin have all been reported to alter platelet function.

The marked difference in the size of platelet aggregates induced by different concentrations of ADP (Fig 2) suggests that the propensity of platelets to aggregate might be characterized by changes in size independent of the total volume of aggregates formed under given experimental conditions. Similar alterations were noted in the size of platelet aggregates induced in vivo by ADP following pretreatment of rats with prostaglandin E1, a potent inhibitor of platelet aggregation. However, since the size of platelet aggregates induced by the same concentration of ADP varies directly with the initial platelet concentration, alterations in platelet aggregate size may be due entirely to thrombocytopenia. The increase in the percentage of platelets which aggregated in re-
response to 2μM ADP during cardiopulmonary bypass, suggests that the reduction in the size as well as the total volume of the aggregates was due to thrombocytopenia rather than decreased platelet responsiveness to ADP.

When compared to the normal donors, the percentage of the patients’ platelets which aggregated during cardiopulmonary bypass was reduced only in response to the lower concentration of ADP. However, a greater impairment of platelet aggregation was noted before bypass. This suggests that anesthesia, surgical trauma and/or heparinization had a more pronounced acute effect on the function of the circulating blood platelets than cardiopulmonary bypass. The discrepancy between these results and previous findings of reduced ADP-induced platelet aggregation during cardiopulmonary bypass6,9 are difficult to explain in view of methodologic differences. The screen filtration pressure detects platelet aggregates indirectly as a result of increases in the resistance to flow of blood through a 20 μ pore mesh filter. In addition to the size of platelet aggregates, this increased resistance to flow is also determined by the hematocrit.6,7,46 In view of the hemodilution which occurs during cardiopulmonary bypass, the screen filtration pressure of blood may not detect alterations in platelet responsiveness to ADP even when corrected for changes in the platelet concentration. Turbidimetric measurements of platelet aggregation may be influenced by selection of a non-representative population of platelets during preparation of PRP. This, plus differences in species and in the concentration of ADP utilized, may account for the variance between turbidimetric measurements of platelet aggregation reported during cardiopulmonary bypass in pigs9 and the present study.

Although our investigation has been limited to determining the mechanism of the reduction in microembolization during cardiac surgery,26 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 hemostasis as well as shock, trauma, and pulmonary embolism.26,31,36,37 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 present study demonstrates that this provides a unique means of correlating in vitro studies with measurements of platelet aggregates formed in vivo in man.

REFERENCES

The Dance as Religious Ritual

The Bible contains many references to the dance, ranging from Miriam to Salome. A decree of the year 744 was necessary to abolish “dancing places” in and about churches. As a matter of fact, a group of boys called Seizes—because originally six in number—still dance in costume, to music, before the high altar in the Cathedral of Seville, at several church festivals each year. There is a legend to the effect that when a Pope some centuries ago again determined to ban all dances in churches, he was asked particularly to exempt the Seizes. His bull stipulated that they could continue until such a time as their costumes were worn out. The authorities therefore never provided new costumes without sewing on patches off the old, by which, they technically confirm to the decree, and so still enjoy the indulgence of His Holiness.

Cheney, S: The Theatre—Three Thousand Years of Drama, Acting and Stagecraft. New York, David McKay, 1966