Platelet Aggregation in Partially Obstructed Vessels and its Elimination with Aspirin

John D. Folts, Ph.D., Edward B. Crowell, Jr., and George G. Rowe

SUMMARY In 35 open chest anesthetized dogs coronary and aortic blood flow were measured with electromagnetic flowmeters while aortic and distal coronary blood pressure and an epicardial ECG were recorded. A fixed amount of stenosis (60–80%) was produced in the coronary artery by an externally applied plastic cylinder. In 24 of the 35 dogs the coronary blood flow showed cyclical reductions to near zero, with a sudden spontaneous return to near control levels. During reduced flow the epicardial ECG showed ST-segment depression suggestive of ischemia, and ventricular premature beats were often noted. Six animals died acutely during episodes of reduced flow.

After 35 mg/kg of aspirin were given intravenously the cyclical reductions in coronary blood flow were abolished and the in vitro platelet aggregations were reduced from a control of 62.1 ± 15 units (Born technique) to an average of 23.7 ± 12 units.

Electromagnetic flowprobes were placed on the ascending aorta and either the circumflex or anterior descending coronary artery or both. The vessel utilized was chosen to provide a long uniform segment, tying small side branches whenever necessary. A 23 gauge silastic catheter was placed in the distal coronary artery for pressure measurement using the method of Khouri and Gregg. A 2-0 silk ligature was placed loosely around the coronary artery below the flowmeter to permit temporary complete occlusion for measuring the reactive hyperemic response, and for checking baseline stability (fig. 1).

A cardiac catheter was passed down the carotid artery, with its tip in the ascending aorta near the aortic valve, for blood pressure measurement. Two ECG leads were sutured to the epicardium in the areas supplied by the circumflex and left anterior descending coronary arteries, respectively, as shown in figure 1. Control aortic and coronary blood pressure and flow, and electrocardiograms were recorded on a Brush Gould direct writing recorder. In all cases, there was no measurable pressure gradient between the aorta and the distal coronary artery prior to narrowing of the coronary artery.

A plastic cylinder 2.5 mm in length, designed to produce a 60–80% narrowing of the vessel, was then placed around the coronary artery between the flowprobe and the coronary artery catheter (fig. 1). We have previously shown that an average of 72% narrowing abolishes the reactive hyperemic response. Thus the dogs in this study will have only a small reactive hyperemic response, if any, with 60–80% narrowing, but should have near normal coronary blood flow. When appropriate, the percent decrease in coronary flow was calculated as follows:

\[
\text{(Control flow - Reduced flow)} \times 100 \over \text{Control Flow}
\]

Material and Methods

Thirty-five healthy adult mongrel dogs of both sexes were anesthetized with morphine sulfate (3 mg/kg) followed one hour later by sodium pentobarbital (30 mg/kg). Respiration was maintained using a positive pressure respirator, and the heart was exposed through a left thoracotomy at the fifth intercostal space in the usual fashion.

MANY STUDIES OF MYOCARDIAL INFARCTION AND SUDDEN DEATH from coronary heart disease have been reported. The significance of acute occlusive thrombi as a precipitating cause is widely debated. Acute coronary occlusion by thrombi were found in 21% to 88% of those infarcted hearts examined at autopsy. These data vary with the investigative technique and material examined. In many patients with coronary artery disease and sudden death no acute thrombus or other mechanism for producing acute coronary insufficiency is demonstrated at post mortem examination and an episode of transient ischemia precipitating arrhythmia is often postulated.

Platelet aggregation is involved in the formation of arterial thrombi and is not prevented by coumarin or heparin anticoagulant therapy. Mechanical damage of platelets can induce platelet aggregation in vitro and thrombosis occurs in areas of turbulent flow. It has been postulated that the turbulent flow produced at the sites of atherosclerotic plaques may play a role in thrombus formation by inducing platelet aggregation. It has also been postulated that sudden coronary death may result from platelet aggregates in the epicardial arteries of man. In many species of animals, sudden death from coronary occlusion has been reported.

We present experimental evidence here that a fixed stenosis in a narrowed coronary artery promotes periodic in vivo platelet aggregation which transiently increases obstruction and decreases coronary blood flow. The possible significance of these observations to coronary disease in man is discussed.

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stability. Blood flows, pressures, and ECGs were then monitored continuously for one hour. Thirty minutes after the partial obstruction was produced, and during the one hour continuous recording, a 20 ml sample of venous blood was drawn into a plastic syringe for platelet count and platelet aggregation studies.

At the end of the procedure the percent obstruction produced was determined as follows: The vessel was ligated, first distal and then proximally. It was then excised, fixed in 10% formalin, embedded in paraffin, and sectioned. Microscopic sections were stained with Carstairs platelet stain prior to examination. The percent reduction of lumen diameter was determined by measuring with a calibrated microscope eyepiece and averaging three diameters of the vessel immediately proximal to the site of the constrictor and three diameters of the narrowed vessel within the constricted area. The percent reduction in lumen diameter was then calculated in the following way:

\[
\frac{\text{proximal diameter} - \text{narrowed diameter}}{\text{proximal diameter}} \times 100
\]

All animals were studied in the same fashion initially. They were then subjected to three different protocols to clarify the results.

**Group I — (Heparin and Aspirin).** After the one hour observation period, 1 mg/kg of heparin was given intravenously to 20 dogs and systemic and coronary flow, pressures, and ECGs were recorded continuously for 30 min. Then, 35 mg/kg of acetylsalicylic acid dissolved in 150 ml of saline was given intravenously over a 15 min period. The same parameters were then monitored continuously for 1/2 hours more. Forty-five minutes after the aspirin was given a second 20 ml venous blood sample was obtained for platelet aggregation studies.

**Group II — (Aspirin Only).** In ten dogs no heparin was given. After the 1/2 hour observation period 35 mg/kg of acetylsalicylic acid was given as described above, continued hemodynamic observations were made, and the second 20 ml sample of venous blood was drawn 45 min later for platelet aggregation studies.

**Group III.** In five more dogs prepared as described above and in which cyclical reductions in coronary blood flow were noted neither aspirin nor heparin was given. The animals were observed carefully and at a time when coronary flow had reached a low point, and as best as we could judge by observations of previous cycles at a time immediately prior to when the flow should suddenly rise back to the control level, the vessel was carefully ligated distally, then proximally and then excised and immediately placed in 10% buffered formalin. The section was processed and lumen diameter measured as previously described.

**Platelet Aggregation Studies**

The 20 ml of venous blood was drawn into a plastic syringe and diluted with one part 3.8% trisodium citrate to nine parts blood in a polystyrene tube. Platelet rich plasma (PRP) was obtained by centrifuging this mixture for 15 min at room temperature. If the platelet count in the PRP was above 300,000/mm² it was adjusted to that figure with autologous platelet poor plasma (PPP); if the count was less than 300,000/mm² the plasma was used unmodified. Aggregation studies were done by a modification of the method of Born in a Chrono-Log Platelet Aggregometer (Chrono-Log Corporation, Broomall, PA). Four-tenths ml PRP was placed at 37°C in a siliconized cuvette in the aggregometer with a siliconized stirring bar. The aggregometer was adjusted to give 10 units and 90 units readings on a Heath recorder with PPP and PRP respectively. One tenth ml of aggregating substance (ADP 50 μg/ml) was added and the change in light transmission measured to the point where the tracing reached a plateau or began to rise. The aggregation tendency of each test sample was then reported in units ranging from 10 to 90 units, higher numbers indicating a greater aggregation of platelets by ADP.

**Results**

There were cyclical reductions in coronary blood flow and distal coronary artery pressure in many of the dogs. An example of a reduction in coronary flow is shown in figure 2, followed by spontaneous return to near control levels at point x. The circumflex coronary blood flow had decreased to near zero, and the circumflex epicardial ECG shows ST-segment deviation suggestive of ischemia in panel C.

Figure 3 shows cyclical decreases in mean coronary flow and distal coronary pressure followed by spontaneous recovery. This occurs in spite of a constant mean arterial blood pressure and cardiac output. The zero reference point of the flowmeter was periodically checked by mechanical occlusion of the vessel and found to be accurate. This insures that the changes observed in coronary flow were not due to baseline shift.
**CYCLICAL REDUCTIONS IN CORONARY FLOW/Folts, Crowell, Rowe**

**Figure 2.** Panel A shows normal phasic coronary blood flow in the left anterior descending and the phasic flow pattern in a 70% stenosed circumflex coronary artery. Also shown in panel A is the blood pressure in the aorta and in the circumflex coronary artery distal to the obstruction. There is a pressure gradient of 40 mm Hg across the fixed mechanical stenosis. In panel B, at slow paper speed mean flows and pressures are shown. The circumflex coronary flow is decreasing and the pressure gradient across the circumflex coronary artery has increased to 82 mm Hg. The left anterior descending coronary flow has increased from 45 ml/min to 60 ml/min, possibly due to increased collateral flow. At point x in panel C the circumflex coronary flow spontaneously rises and in panel D, the circumflex flow has risen to above control and the pressure gradient across the stenosis is only 36 mm Hg. This rise in flow above control levels represents a small reactive hyperemic response.

**Group I.** In 13 of 20 dogs there were recurring simultaneous reductions in coronary blood flow and distal coronary artery pressure suggesting periodic increases of obstruction in the lumen of the stenosed vessel. These 13 dogs are listed as group I, part A, in table 1.

These cyclical flow reductions in group I, part A, produced a 47% average decrease in coronary flow from control levels and occurred an average of eight times per hour (table 1). In all cases in which cyclical flow reductions occurred, the administration of heparin did not alter the magnitude or frequency of the flow reductions. However in all cases the cyclical flow reductions were completely abolished by aspirin. Other hemodynamic parameters such as cardiac output and arterial blood pressure did not show a

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**Table 1. Coronary and Hemodynamic Effects after Coronary Obstruction in Dogs**

<table>
<thead>
<tr>
<th></th>
<th>% stenosis of lumen</th>
<th>Average number of cyclical reductions in coronary flow/hour</th>
<th>Control coronary blood flow</th>
<th>Average % reduction in flow from control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SEM</td>
<td>Before stenosis</td>
<td>After stenosis was produced, not during cyclical reductions</td>
</tr>
<tr>
<td><strong>Group I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Part A</td>
<td>73 ± 5</td>
<td>8.2 ± 4</td>
<td>42.5 ± 23</td>
<td>34.9 ± 11</td>
</tr>
<tr>
<td>(N = 13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Part B</td>
<td>75 ± 12</td>
<td>0</td>
<td>49.8 ± 18</td>
<td>37.7 ± 16</td>
</tr>
<tr>
<td>(N = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Group II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Part A</td>
<td>76 ± 8</td>
<td>6 ± 2</td>
<td>48 ± 8</td>
<td>43 ± 8</td>
</tr>
<tr>
<td>(N = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Part B</td>
<td>78 ± 7</td>
<td>0</td>
<td>51 ± 9</td>
<td>40 ± 7</td>
</tr>
<tr>
<td>(N = 4)</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Group I** = dogs given heparin and aspirin.
**Group II** = dogs given aspirin alone.
**Part A** = dogs that showed cyclical reductions in coronary flow.
**Part B** = dogs that did not show coronary flow reductions.
specific trend after the heparin and aspirin were given although the heart rate was reduced (group I, table 2). The percent reduction in lumen diameter produced by the rigid constrictor averaged 73% for those dogs showing flow reductions (group I, part A) and 75.0% for those not showing cyclical reductions of coronary blood flow, (group I, part B, table 1). These differences are not significant.

**Group II.** Six of ten dogs showed cyclical reductions in coronary blood flow (part A in group II). They had an average percent reduction in coronary lumen of 76% ± 8 as shown in table 1. Those dogs not showing cyclical reductions had an average of 78 ± 7% stenosis (table 1).

**Platelet Aggregation Studies**

**Group I.** Platelet counts on the dogs in group 1, part A, averaged 360,000 ± 110,000/mm³, and those dogs not showing cyclical reductions (group I, part B) had an average platelet count of 310,000 × 95,000/mm³. All were within the normal range for healthy mongrel dogs¹² and the difference between the groups is not significant (table 2).

The average in vitro platelet aggregation with ADP for those dogs showing cyclical flow reduction (part A) was 62 ± 15 units versus 33 ± 16 units for dogs not showing flow reductions (Part B) (P < 0.001). After heparin and aspirin, platelet aggregation averaged 24 ± 12 units for dogs who

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**Figure 3.** Mean coronary and aortic flows and aortic and distal coronary blood pressure recorded at slow (1 mm/sec) paper speed. The coronary artery is stenosed 70%, and the coronary flow gradually decreases, as does distal coronary artery pressure, while aortic blood pressure remains constant. The cardiac output, as measured by mean ascending aortic blood flow and heart rate, also remain constant, indicating that there has been no change in cardiac work. At the points marked x, the flow spontaneously rises, presumably because the added obstruction caused by a platelet aggregate is removed when the aggregate breaks loose and is carried downstream, thereby restoring the lumen to its 70% stenosis.

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**Table 2. Platelet Levels and Hemodynamic Changes after Coronary Obstruction in Dogs**

<table>
<thead>
<tr>
<th>Group</th>
<th>Platelet aggregation</th>
<th>Platelet counts x 10⁹/ml</th>
<th>Heart rate</th>
<th>Arterial blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>After drugs</td>
<td>Control</td>
<td>After drugs</td>
</tr>
<tr>
<td><strong>Group I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Part A</td>
<td>62.1 ± 15</td>
<td>23.7 ± 12</td>
<td>360 ± 110</td>
<td>304 ± 122</td>
</tr>
<tr>
<td>(N = 13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Part B</td>
<td>32.7 ± 16</td>
<td>25.1 ± 12</td>
<td>310 ± 95</td>
<td>301 ± 110</td>
</tr>
<tr>
<td>(N = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Group II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Part A</td>
<td>69 ± 7</td>
<td>20 ± 5</td>
<td>393 ± 127</td>
<td>*</td>
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<tr>
<td>(N = 6)</td>
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<tr>
<td></td>
<td>P &lt; 0.001</td>
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</tr>
<tr>
<td>Part B</td>
<td>42 ± 8</td>
<td>31 ± 7</td>
<td>342 ± 54</td>
<td>*</td>
</tr>
<tr>
<td>(N = 4)</td>
<td></td>
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</tr>
</tbody>
</table>

*Not measured.

See table 1 for explanation of groupings.
had demonstrated transient flow reduction ($P < 0.001$ versus pre-aspirin value) and 25.1 ± 12 units for dogs without reductions (NS).

**Group II.** The average platelet aggregation was 60 ± 7 before the aspirin alone (group II, part A) at a time when cyclical flow reductions were noted, and this decreased to 20 ± 5 after the aspirin was given, and when cyclical reductions in flow were abolished (table 2). The other four dogs (group II, part B) had an average platelet aggregation of 42 ± 8 before the aspirin and did not show any cyclical reductions in coronary flow. After the aspirin alone was given, platelet aggregation decreased to 31 ± 7 units (table 2). The average platelet counts did not differ significantly between group II and group I.

**Group III.** All five dogs in this group showed cyclical reductions in flow, and had hemodynamic data comparable to groups I and II. In all five vessels which were ligated during the low point of a cyclical flow reduction, microscopy showed platelet aggregates in the narrowed lumen, or in the area just distal to the obstruction when the section was stained with the Carstairs stain for platelets. A representative photomicrograph is shown in figure 4. By contrast those vessels which were ligated after heparin and aspirin or aspirin alone, when there were no cyclical flow reductions and flow was near the normal range, did not show platelet aggregates in the narrowed lumen. The quality of the histologic sections was not good enough to determine if there was slight damage to the endothelium. However there was no evidence that the endothelium was grossly damaged by the encircling plastic cylinder.

**Discussion**

When the phenomenon of cyclic reduction in blood flow was observed in segments of partially but rigidly obstructed coronary arteries it was presumed to be due either to fibrin deposition in the constricted lumen of the coronary artery or to accumulation of platelets at the site of perturbation of flow. If the increased obstruction were produced by fibrin it should have been prevented by heparinization but this did not occur. The possibility that platelet aggregates were responsible for the reduction in flow is supported by the fact that there was significantly greater ADP-induced platelet aggregation in those dogs that developed transient coronary flow reduction when compared with those that did not. Those dogs showing cyclical flow reductions had ADP-induced aggregations averaging 62 ± 15 (group I, part A) and 60 ± 7 (group II, part A). The platelet aggregations were much lower in those dogs not showing cyclical flow reductions averaging 33 ± 16 (group I, part B) and 42 ± 8 (group II, part B). If the transitory flow reductions were due to aggregation of platelets heparin should not affect this process but aspirin should.5 14 Our results show that aspirin abolished the cyclical flow phenomenon whether or not heparin was given, as there were never any cyclical flow reductions after the aspirin was given. The demonstration of platelet aggregates by microscopy in those vessels ligated at a time when flow was at a low point confirms that platelet aggregates are indeed responsible for the cyclical reductions in flow, by producing further narrowing at the constriction in the coronary artery. The absence of platelet aggregates in those vessels narrowed the same amount but examined histologically after the administration of aspirin and when there was not reduction in flow also supports the concept that platelet aggregation was responsible. The latter observation also supports the idea that platelets, having aggregated and obstructed the narrowed artery, can wash free, leaving no real evidence of their previous presence, since these studies were made of constricted areas of vessels which had shown transient flow reduction prior to aspirin administration.

Two factors are required for platelet aggregation.15 First a suitable stimulus for aggregation is needed. ADP or collagen produce in vitro aggregation, and in vivo studies show that damaged or denuded endothelium provokes platelet aggregation.16 17 The second condition needed for platelet aggregation is adequate mixing so that the platelets come into intimate contact with each other. In the present experiments it is postulated that turbulent flow through the narrowed lumen provides both the necessary mixing and the platelet damage to cause ADP release and platelet aggregation in the narrowed segment.

The sudden reduction in obstruction may occur for at least two reasons. First, dog platelets deaggregate in vitro after 6–10 min if viscous metamorphosis does not occur so spontaneous deaggregation may explain the sudden rise in coronary flow we have observed.18 Second, perhaps as the platelets aggregate, increasing obstruction and decreasing

**FIGURE 4.** Histologic section treated with Carstairs stain for platelets. × 70
flow, the pressure gradient across the constriction increases until it is sufficient to displace the platelet plug from the narrowed lumen. These platelets could then pass distally as a mass producing peripheral microemboli, or they could deaggregate as they moved downstream leaving no residue. Further experiments are planned to clarify this phenomenon.

The cause of the marked variability of platelet aggregability in these normal dogs needs further study. Surely it may be due in part to the plasma level of circulating catecholamines or lipids, both of which are known to affect platelet aggregation. Local anesthetics also inhibit in vitro platelet aggregation in man and many factors including general anesthesia may have affected the platelets of these dogs. A survey of the literature has shown a clear relation between many coronary risk factors and increased platelet aggregability in man. It has been shown that cigarette smoking, hyperbetalipoproteinemia, diabetes mellitus, and hyperuricemia with gout all increase in vitro platelet aggregation in man in response to standard stimuli. In addition, patients with elevated catecholamines such as in hypertension and coronary prone behavior pattern would be expected to be at greater risk of sudden death since catecholamines enhance human platelet aggregation.

These relationships are under investigation in our laboratory utilizing the present model in the hope that their significance in coronary disease may be elucidated.

Acknowledgments

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References

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