The relationship among platelet survival, vessel injury, and thrombosis is of interest because of the role that platelets play in the development of atherosclerosis and its thromboembolic complications.

Blood platelets do not adhere to normal endothelium. However, when a blood vessel is injured, platelets adhere to the injury site and, if blood flow is disturbed, thrombi can form on the injured area. In arteries, fresh thrombi are composed largely of aggregated platelets and are stabilized by fibrin, whereas in veins, although thrombi may be initiated by a mass of aggregated platelets in a valve pocket, the thrombi are mainly composed of red blood cells in a fibrin network. In large normal arteries that have not been previously injured in which blood flow is laminar, only a thin layer of platelets coats the subendothelium when the endothelium is lost. If flow is disturbed, for example at vessel orifices and branches, so that platelets are brought into close contact with each other, thrombus formation will occur because of the accumulation of aggregating agents released from the platelets and thrombin that is generated at the injury site. Thrombi are not static structures, but have been shown to undergo episodic formation and dissolution. Several processes contribute to the dissolution of thrombi. These include the force of flowing blood, platelet deaggregation, and lysis of fibrin. Theoretically, if injury to blood vessels is extensive or repeated, resulting in consumption of a significant proportion of the circulating platelets, a reduction in platelet survival would be expected.

Thus, it is not surprising that vessel wall injury is a common feature of many conditions in which platelet survival is shortened (table 1). Although there are other circumstances in which platelet survival is shortened, in this article we shall concentrate on platelet survival in conditions in which vessel injury has been experimentally induced or occurs as a result of vascular disease.

Measurement of Platelet Survival

Radioisotopic methods are used most extensively for measurement of platelet survival and in general are preferable to the nonisotopic methods. Although a number of radioisotopes have been used, until recently most studies have used 51Cr-labeled platelets. When 111Indium was introduced as a platelet label several years ago, a number of investigators adopted it because it permits external quantitative imaging of platelet localization in thrombi and in sites of sequestration. Neither label is lost from platelets when they discharge their granule contents, and these labels are apparently undiminished in individual platelets until the platelets are removed from the circulation. These radioisotopes are not re-utilized. It is possible to achieve a higher specific radioactivity with 111Indium than with 51Chromium, and hence smaller quantities of blood have to be removed for labeling. The International Committee for Standardization in Hematology has established a standard method for 51Cr-labeling of platelets and for platelet survival studies. Many of the recommendations are also applicable to platelet survival studies with 111In-labeled platelets. Regardless of the label used, blood samples for measuring the amount of radioactivity in the circulation must be taken at predetermined times if comparisons are to be made among platelet survival curves. Because platelet survival curves can vary from linear to exponential, it is essential that they be analyzed in an unbiased fashion. The “gamma function” described by Murphy and his colleagues provides a computerized analysis of platelet survival data using a mathematical formula with a continuous variable that is sensitive to the variations in the patterns of platelet disappearance. With these total population labels, the values obtained are mean platelet survival times. The Workshop of the Interna-

Vessel Injury, Platelet Adherence, and Platelet Survival

Raelene L. Kinlough-Rathbone, Marian A. Packham, and J. Fraser Mustard

From the Department of Pathology, McMaster University, Hamilton and the Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada.

Address for reprints: Dr. R. L. Kinlough-Rathbone, Department of Pathology 3N26, McMaster University, 1200 Main Street West, Hamilton, Ontario, L8N 3Z5, Canada.

Received July 6, 1983; accepted August 1, 1983.

(Arteriosclerosis 3:529–546, November/December 1983)
Table 1. Conditions in which Platelet Survival is Shortened

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cause</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repeated vessel injury, thrombosis, and thromboembolism</td>
<td>Experimentally induced</td>
<td>60, 61, 63</td>
</tr>
<tr>
<td></td>
<td>Indwelling aortic catheter</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Homocysteine by continuous infusion</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>Diet with large amount of saturated fat</td>
<td>49, 73</td>
</tr>
<tr>
<td>Life style</td>
<td>Smoking</td>
<td>81, 82</td>
</tr>
<tr>
<td></td>
<td>Diet with large amount of saturated fat</td>
<td>73</td>
</tr>
<tr>
<td>Vascular disease</td>
<td>Atherosclerosis, coronary artery disease</td>
<td>83-86</td>
</tr>
<tr>
<td></td>
<td>Myocardial infarction</td>
<td>87, 89</td>
</tr>
<tr>
<td></td>
<td>Angina</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Arterial thrombosis</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>Venous thrombosis</td>
<td>91-93</td>
</tr>
<tr>
<td></td>
<td>Rheumatic heart disease (mitral) with thromboembolism</td>
<td>94</td>
</tr>
<tr>
<td>Metabolic disorders</td>
<td>Diabetes</td>
<td>100-104</td>
</tr>
<tr>
<td></td>
<td>Gout</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>Homocystinemia</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>Hyperbetalipoproteinemia</td>
<td>90</td>
</tr>
<tr>
<td>Prosthetic surfaces and grafts</td>
<td>Prosthetic surfaces and grafts</td>
<td>175, 177, 178</td>
</tr>
<tr>
<td></td>
<td>Heart valves (particularly early types)</td>
<td>91, 161-164, 182, 219</td>
</tr>
<tr>
<td></td>
<td>Grafts</td>
<td>91, 178, 220</td>
</tr>
<tr>
<td>Membrane glycoprotein abnormalities</td>
<td>Congenital</td>
<td>221, 222</td>
</tr>
<tr>
<td></td>
<td>Bernard-Soulier syndrome</td>
<td>223</td>
</tr>
<tr>
<td></td>
<td>Wiskott-Aldrich</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Experimentally induced</td>
<td>138, 224-226</td>
</tr>
<tr>
<td></td>
<td>Neuraminidase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Periodate oxidation</td>
<td>227</td>
</tr>
<tr>
<td></td>
<td>Proteolytic enzymes (chymotrypsin, trypsin, plasmin)</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>Prolonged storage</td>
<td>228</td>
</tr>
<tr>
<td>Associated with disease</td>
<td>Infections with viruses with neuraminidase activity</td>
<td>224, 229</td>
</tr>
<tr>
<td></td>
<td>Cirrhosis of the liver</td>
<td>89, 230</td>
</tr>
<tr>
<td>Various diseases</td>
<td>Metastatic cancer</td>
<td>89, 91, 231, 232</td>
</tr>
<tr>
<td></td>
<td>Myeloproliferative disorders of the bone marrow (with thrombotic tendencies)</td>
<td>233, 234</td>
</tr>
<tr>
<td></td>
<td>Bacteremia</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>Crisis in sickle cell disease</td>
<td>235</td>
</tr>
<tr>
<td></td>
<td>Severe chronic airway obstruction</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>Hemolytic-uremic syndrome</td>
<td>91, 236</td>
</tr>
<tr>
<td></td>
<td>Cirrhosis of the liver</td>
<td>237</td>
</tr>
<tr>
<td>Surgical trauma</td>
<td></td>
<td>3, 89, 91</td>
</tr>
<tr>
<td>Disseminated intravascular coagulation</td>
<td></td>
<td>238</td>
</tr>
<tr>
<td>Immunologic</td>
<td>Isoimmune (resulting from multiple transfusions or from immunization of mother by fetal platelet isoantigens)</td>
<td>241</td>
</tr>
<tr>
<td></td>
<td>Drug-induced, e.g., quinine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Systemic lupus erythematosus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Infections — viral, bacterial, and parasitic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Autoimmune hemolytic anemia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chronic lymphocytic leukemia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lymphocytic lymphomas</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rheumatoid arthritis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hyperthyroidism</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Idiopathic thrombocytopenic purpura (ITP)</td>
<td></td>
</tr>
</tbody>
</table>
tional Committee on Thrombosis and Haemostasis recommended "that the maximum likelihood estimate of the integral-ordered gamma function program for calculating platelet survival be made available in a standardized format to be used in all laboratories studying platelet survival."4 9 10 Gamma function analysis shows that autologous \( {\text{Cr}} \)-labeled platelets have a mean platelet survival of 9.5 ± 0.6 days in normal individuals.3 Platelet survival times in most experimental animals tend to be shorter. Upon infusion into humans or experimental animals, a proportion of the labeled platelets does not circulate immediately and is thought to be sequestered in the spleen;11 12 some of these sequestered platelets subsequently reappear in the circulation. These observations have resulted in the recommendation that the initial samples taken in studies of platelet survival should be collected 30 minutes and 2 hours after injection of the labeled platelets and daily thereafter.4 Platelet turnover is calculated from the platelet count divided by the mean platelet survival. It estimates the rate of platelet removal from the circulation and, in the steady state, also measures the rate of platelet production.

Effect of Vessel Wall Injury in Experimental Animals

Mechanical Removal of the Endothelium with Exposure of the Subendothelium

Removal of the endothelium from the aorta of an experimental animal with a balloon catheter leads to rapid platelet accumulation on the exposed subendothelium.1 13 16 The platelets form a monolayer in most regions, and the platelets in direct contact with the vessel wall discharge the contents of their dense granules.17 The initial layer of platelets rapidly accumulates on the surface, but there is little further platelet accumulation, and fibrin is not observed in association with the platelet monolayer. More severe injury with damage to the media may cause some platelet-fibrin thrombi to form. Some white cells are also found on the injured vessel wall.1 15 In rabbits, the platelets that accumulate initially are gradually lost from the surface so that, by 4 days, the deendothelialized aortic surface has very few platelets associated with it.15 It is not surprising that this type of injury does not cause detectable changes in platelet survival because the proportion of the circulating platelets that interacts with the damaged surface is too small to produce a significant change in the number of platelets in the circulation.15

Platelets that interact with collagen in the subendothelium discharge their granule contents and some of this material penetrates the vessel wall. Goldberg and his colleagues18 have demonstrated directly that the alpha granule constituent, platelet factor 4 (PF4), enters the wall at an injury site. With a single injury of this type, however, the extent of the platelet release reaction is insufficient to produce detectable changes in the circulating concentrations of released platelet constituents. Following the initial injury, the vessel surface quickly becomes nonreactive (Figure 1), but the reasons for this have not been defined.15 19 Possibly, circulating platelets cannot adhere to the platelets that have formed the initial monolayer on the exposed subendothelium because the fibrinogen receptors involved in platelet-to-platelet adherence are not available on the platelets adherent to the injured vessel wall. In vitro experiments have shown that after the initial layer of platelets has coated the subendothelium, exposure to fresh \( {\text{Cr}} \)-labeled platelets does not result in the attachment of these platelets to those adherent to the surface (Grovers HM, Kinlough-Rathbone RL, Mustard JF, unpublished observations). In recent studies we have shown that the vessel wall loses its reactivity within 6 to 8 hours even if the number of platelets adhering to the injury site is significantly reduced by treating the animals with high concentrations of dipyridamole (or by continuous infusion of PG\(_I_2\)).20 21 If the plasma concentration of the drug is allowed to fall within the first few hours after injury, platelets adhere to the same extent as they do in the absence of the drug. This indicates that adsorption of plasma proteins onto the damaged surface is unlikely to be responsible for the loss of vessel wall reactivity since one would expect the damaged wall to be rapidly

![Figure 1](http://atvb.ahajournals.org/)

**Figure 1.** Number of platelets adhering to damaged aortas in a 30-minute period at specific times after injury with a balloon catheter. **Bar on the left.** \( {\text{Cr}} \)-labeled platelets were in the circulation at the time of balloon catheter-induced injury; 30 minutes after injury the aortas were removed to determine radioactivity associated with the surface. **Other bars.** \( {\text{Cr}} \)-labeled platelets were injected at specific times after injury; 30 minutes after the injection of \( {\text{Cr}} \)-platelets, the aortas were removed to determine radioactivity associated with the surface. The number of platelets associated with the surface of the aorta was then calculated. Mean platelet survival values for sham-operated animals and for animals after deendothelialization of the aorta are also indicated.
coated with these proteins. Also, since von Willebrand factor is not rapidly lost from the subendothelium following deendothelialization, it appears that loss of vessel wall reactivity is probably not attributable to loss of von Willebrand factor from the subendothelium during the early period after injury.

There has been speculation that the formation of PGI₂ by damaged vessels might prevent further platelet accumulation at or near sites of vessel injury. However, this seems unlikely since it has been reported that the ability of the vessel wall to form PGI₂ is decreased following removal of the endothelium and it is several weeks before the vessels recover their ability to produce large amounts of PGI₂. Furthermore, if animals are treated with aspirin to prevent PGI₂ production, platelet adherence to the subendothelium is not increased in vivo or in vitro, indicating that PGI₂ is not responsible for limiting platelet accumulation with this type of vessel wall injury. However, in a system in which native blood from rabbits was perfused through a deendothelialized rat aorta, Tschopp and his colleagues reported that treatment of the rats with aspirin before removal of the endothelium increased adhesion from 6% to 55% and increased aggregation from 0% to 19%. They concluded that "the smooth muscle cells of the media can produce sufficient amounts of PGI₂ to locally influence the thrombogenicity of a deendothelialized artery." For our in vivo experiments with rabbits, both the platelets and the vessel wall were exposed to aspirin, whereas in the perfusion experiments only the vessel wall was treated with aspirin. The difference in the results, however, cannot be attributed to these experimental designs, because in experiments in vitro, aspirin treatment of deendothelialized vessels did not affect the accumulation of labeled platelets that had not been exposed to aspirin.

In rabbits, it has been reported that aspirin increases the extent of experimental thrombosis and platelet accumulation on the damaged vessel wall, presumably by inhibiting PGI₂ formation. In these experiments, both platelet and vessel wall cyclooxygenase was exposed to aspirin. The conditions in which experimental thrombosis is increased are those that permit extensive thrombin formation, as evidenced by the large fibrin component in the thrombi, and they are often associated with altered blood flow. Thrombin stimulates PGI₂ production, which would limit the contribution of platelets to thrombus formation; in the presence of aspirin, however, the limiting effect of PGI₂ would be abolished.

Among the factors that may influence vessel wall reactivity are the effects of proteolytic enzymes on the surface of the wall. Platelets and white cells are gradually lost from the damaged wall and few additional platelets or white blood cells accumulate. Enzymes that have been released by platelets and white cells or cells of the vessel wall may modify the subendothelial structures so that they become non-reactive to further platelet accumulation. The neointima composed of smooth muscle cells that forms 5 to 7 days after removal of the endothelium in the aorta of rats and rabbits is also not reactive to platelets and does not activate coagulation. Again, its nonreactivity to platelets is not related to PGI₂ production since treatment of the animals with aspirin does not cause platelets to accumulate. It is important to point out that platelet accumulation and interaction with the subendothelium are usually studied in circumstances where there is relatively little disturbed flow except around the vessel orifices and branches.

If a vessel is stenosed and denuded of endothelium, vortices form downstream from the stenosis and in these vortices platelet aggregates may occur because of the accumulation of aggregating agents such as ADP and thrombin. Endothelial damage and thrombus formation may also occur at a site of focal arterial constriction caused by spasm. Although removal of the endothelium from the aortas of rabbits or rats has not been found to shorten platelet survival or increase platelet turnover (Wincour PD, Groves HM, Kinlough-Rathbone RL, Mustard JR, unpublished observations), Ross and Harker found a direct correlation between the amount of endothelium removed from monkey aortas with a balloon catheter and the extent of decrease in platelet survival. There was also a correlation between the time required for reendothelialization and the return to normal platelet survival values. The reasons for the discrepancy between the results obtained with different species is not apparent. It is possible that the subendothelium of monkey aorta does not become quiescent, but remains reactive to circulating platelets; this has not been studied.

**Mechanical Injury to the Neointima**

When platelets interact with an injured surface, they release the contents of their storage granules. Among the substances released is a platelet-derived growth factor that causes smooth muscle cell migration and proliferation in the injured wall. Within 5 to 7 days of removal of the endothelium, a thickened intima composed of smooth muscle cells forms. Platelets do not adhere to these smooth muscle cells at the luminal surface. Thus, reendothelialization is not required for a vessel surface to become non-thrombogenic.

When the neointima that forms following the removal of the endothelium is damaged by passage of a balloon catheter, the interaction between the injured smooth muscle cells and the blood is more complex than that observed when the subendothelium is exposed. Under these circumstances platelet-fibrin thrombi form on some areas of the surface of the vessel; in others, a layer of platelets forms. The platelet layer probably results from platelet interaction with connective tissue and its formation cannot be inhibited by heparin. The thrombi are oriented
in the direction of blood flow, and the distribution around vessel orifices reflects the disturbed pattern of blood flow at such sites (Figure 2). The thrombi that form under these experimental conditions do so under the influence of thrombin since heparin (at concentrations that inhibit coagulation) prevents their formation and reduces platelet accumulation on the injured vessel. In contrast, heparin does not affect the extent of platelet accumulation on the subendothelium in doses that inhibit coagulation.44 In

Figure 2. A. Surface of a rabbit aorta 7 days after the first balloon catheter-induced injury and 30 minutes after the second injury. Linear deposits of thrombi oriented in the direction of blood flow curve around the ostium of an intercostal artery. Blood flow was in the direction indicated by the arrow. B. Detail of the area outlined in A. The surface is largely covered by fibrin and platelet-fibrin thrombi.
areas of extensive thrombus formation, platelets tend to adhere to or be trapped in a fibrin network rather than adhere to the injury site directly.\textsuperscript{43, 52, 54} It seems likely that, when smooth muscle cells are damaged, tissue thromboplastin becomes available so that the extrinsic pathway of coagulation is activated.\textsuperscript{55, 56}

As with exposure of the subendothelium, the surface of the damaged neo-intima rapidly becomes nonreactive to further platelet accumulation.\textsuperscript{43} Again, the factors responsible for this rapid loss of reactivity have not been established. Since aspirin treatment of these vessels does not increase platelet accumulation, the loss of reactivity to platelets cannot be attributed to PGI\textsubscript{2} produced by the smooth muscle cells.\textsuperscript{44} Loss of reactivity of the injury site because of the effects of proteolytic enzymes from the white cells or platelets may occur with this form of injury also.\textsuperscript{56-58} The stimulation of blood coagulation also appears to be transient. Piepgras and his colleagues\textsuperscript{57} have demonstrated in cats that endarterectomy of the carotid artery is associated with the formation of platelet-fibrin thrombi that can be prevented by heparin treatment. If the heparin treatment is sustained for 6 hours and then stopped, thrombi do not form on the injured vessel wall. It may be that the damaged cells on the surface are lost over the 6-hour period of heparin protection and that the healthy cells that remain can no longer stimulate thrombin formation.

Injury to the neo-intima of previously damaged rabbit aortas does not shorten platelet survival.\textsuperscript{43} This observation is in keeping with the relatively short time during which the surface is reactive to circulating platelets and the relatively small proportion of the circulating platelets that interact with the damaged smooth muscle cells during the acute phase of thrombus formation. Thus, acute extensive injury of the neo-intima of the rabbit aorta with thrombus formation involving the generation of thrombin does not shorten platelet survival.

**Repeated Mechanical Injury of the Aorta with an Indwelling Catheter**

Platelet survival is reduced by repeated mechanical injury to the vessel wall by an indwelling aortic catheter.\textsuperscript{58-63} In our experiments, we used the technique developed by Moore\textsuperscript{64} in which an indwelling aortic catheter is inserted through a femoral artery into the aorta of rabbits or rats to injure the vessel wall repeatedly.

Although macroscopic thrombus formation was not induced by catheters of the size and type (polyethylene) used in these experiments with rats,\textsuperscript{59, 62, 63} other investigators have produced macroscopic thrombi using tubing with a greater diameter introduced through the carotid artery.\textsuperscript{50} In rabbits, indwelling aortic catheters cause thrombus formation around the catheters or on the vessel wall.\textsuperscript{59, 62, 63} While the catheter remains free in the aorta it can repeatedly damage the vessel wall so that the injured surface remains reactive to platelets (Table 2). We have shown that when \textsuperscript{51}Cr-labeled platelets are infused into animals that have had catheters in situ for 6 days there is still extensive platelet interaction with the vessel wall. More than 90% of the platelets are associated with the vessel wall and less than 10% become associated with the catheter,\textsuperscript{59} indicating that although the catheter may contribute slightly to the reduction in platelet survival, most of the reduction can be attributed to platelet interaction with the vessel wall. Morphological studies of vessel walls in these experiments show that the catheter injures the endothelium and removes it in some areas; these sites are covered by platelets and white cells (Figure 3). The neo-intima that forms in response to the early injuries is repeatedly damaged by the movements of the indwelling catheter.

If aortic catheters are left in situ in rats, platelet survival remains shortened for at least 48 days and the extent to which freshly infused, labeled platelets adhere to the wall is similar throughout this time (Table 3). Reduction in platelet survival is related to the length of the aortic catheter.\textsuperscript{59} Platelet survival is shorter with the longer catheters, probably because they injure a larger area of the endothelium. Thus, repeated or continuous injury to vessel walls shortens platelet survival. Although in rabbits with indwelling aortic catheters, shortened platelet survival is associated with thrombus formation, the results of the experiments with rats indicate that reduction in platelet survival does not depend upon thrombosis, but is caused by repeated vessel wall injury.

**Chemical Injury of Vessel Walls**

Continuous or repeated vessel wall injury produced by chemical means also shortens platelet survival. Harker and colleagues\textsuperscript{65} showed that the continuous intravenous infusion of homocysteine (which damages the endothelium and in some areas

**Table 2. Platelet Accumulation on Indwelling Aortic Catheters and Damaged Aortas in Rabbits**

<table>
<thead>
<tr>
<th>Days after insertion of catheter when \textsuperscript{51}Cr-labeled platelets were injected</th>
<th>No. of animals</th>
<th>\textsuperscript{51}Cr associated with thrombus and aorta at 24 hrs* (% \textsuperscript{51}Cr-platelets injected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>15</td>
<td>1.6 ± 0.24</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>1.3 ± 0.64</td>
</tr>
<tr>
<td>6</td>
<td>16</td>
<td>0.96 ± 0.10†</td>
</tr>
</tbody>
</table>

*Values are means ± se. (From Winocour et al., Arteriosclerosis 2:458–466, 1982, with permission.)
†p < 0.02, compared with 0 days.
removes it) is associated with shortened platelet survival in baboons. Although Harker and colleagues\(^6\) and Roulaud and co-workers\(^6\) have reported reduced platelet survival in humans with homocystinemia, two other groups have been unable to confirm this observation.\(^6\)\(^8\) The reason for the discrepancy has not been established, although Hill-Zobel and co-workers\(^7\) have suggested that differences in the methods used to analyze the platelet survival curves may be responsible.

Ross and Harker\(^4\) have reported that feeding monkeys diets enriched in cholesterol injures the vessel wall, particularly around vessel orifices and branches, and shortens platelet survival. Recently Armstrong and co-workers,\(^5\) in similar experiments in pigs, demonstrated endothelial injury at sites of predilection for the development of atherosclerosis and observed increased platelet accumulation at these sites 10 and 100 days after the pigs were started on the hypercholesterolemic diet. These animals on the hypercholesterolemic diet also showed short-
Vessel Wall Injury Caused By Smoking

In addition to the effects of dietary polyunsaturated fats on platelet survival, other lifestyle factors have been shown to reduce platelet survival. Although it is likely that some constituents of cigarette smoke damage vessel walls, 76-77 the causative factor has not been identified. Nicotine, carbon monoxide, and tobacco antigen have all been suggested as being responsible for the injury. 78-80 Recent studies with rats exposed to cigarette smoke have shown that the endothelium is altered and platelets are adherent to the surface of the aorta around vessel orifices. 79 Two studies have demonstrated that smoking shortens platelet survival in humans. 81, 82 and it seems probable that this is attributable to continuous vessel wall injury.

Vascular Disease

It is not known whether advanced atherosclerotic lesions are continuously reactive to the circulating blood or whether their reactivity is only intermittent and occurs as a result of injury to the endothelium. It is possible that the surface of injured, diseased vessels, like normal vessels, rapidly becomes quiescent in terms of stimulating thrombus formation. However, if the stimulus that initiated the atherosclerotic process persists or recurs repeatedly, the frequent episodes of injury may lead to extensive atherosclerosis. Thus, shortened platelet survival in association with advanced atherosclerosis may be related more closely to the frequency and severity of vessel injury than to the extent of atherosclerosis. Shortened platelet survival has been observed by a number of investigators in association with the clinical manifestations of atherosclerosis, coronary artery disease, myocardial infarction, angina, or arterial thrombosis. 83-91 The studies of platelet survival have shown the greatest difference to be between control subjects who are nonsmokers and also have a negative family history, and individuals who have had clinical complications, have a positive family history, and smoke. 82, 84 Individuals who have had clinical complications of atherosclerosis, but are nonsmokers and have a negative family history tend to have platelet survival values similar to controls.

Platelet survival may also be reduced in patients with recurrent venous thrombosis 85-93 and in some patients with rheumatic heart disease with thromboembolism. 84 At present, it is not possible to determine whether the subjects with shortened platelet survival are those who have frequent injuries to the vessel wall.

Shortened platelet survival appears to be correlated with abnormally high concentrations of β-thromboglobulin in plasma 85, 86. This has been observed in patients with coronary artery disease or rheumatoid heart disease, but the same type of relationship has been noted in normal subjects. 85-89

Metabolic Disorders

It has been shown that platelet survival is shortened in several metabolic diseases in which vessel wall injury seems to be the most likely cause. Homocystinemia has been discussed above. Patients with Type II hyperbetalipoproteinemia, 86 diabetes, 100-104 and gout 105 have been reported to have shortened platelet survival. In diabetics, the observation that the concentration of von Willebrand factor in plasma is abnormally high 106-109 may indicate that the endothelium is being stimulated or injured. 107 The tendency for diabetics to develop atherosclerosis and clinical complications of vascular disease, 110, 111 is also in keeping with the shortened platelet survival observed in diabetic patients. 100-104 In a recent study of patients with diabetic retinopathy, shortening of platelet survival seemed to be associated with this condition. 104

Mechanisms Responsible for Shortened Platelet Survival

The mechanisms responsible for the shortened platelet survival associated with repeated or continuous vessel injury could be irreversible incorporation of platelets into thrombi and thromboemboli that are subsequently incorporated into the vessel wall 112 the formation of thrombi that are eventually lysed, which frees modified platelets into the circulation from whence they are rapidly cleared; or the interaction of platelets with the surfaces of injured vessels followed by their return to the circulation and rapid clearance. 62

The irreversible incorporation of platelets into thrombi would probably not be extensive enough to have a significant effect on platelet survival, although it could contribute to the reduction in platelet survival caused by other mechanisms. However, in disseminated intravascular coagulation or repeated, multiple thrombotic episodes associated with extensive vascular disease, platelet survival may be shortened.

It seems likely that some of the platelets that are freed from a thrombus, or from an injury site on a vessel wall, may have been released by the action of proteolytic enzymes such as plasmin or leukocyte elastase. 38, 62 The survival time of these platelets...
may be shortened since it has been shown that treatment of platelets with plasmin and other proteolytic enzymes, such as chymotrypsin and trypsin, shortens their survival time.\textsuperscript{113} The finding that epsilon aminocaproic acid, an inhibitor of plasmin and other proteolytic enzymes, prolongs shortened platelet survival caused by continuous vessel wall injury\textsuperscript{62} is in keeping with this theory. It should be pointed out that some of the main aggregating agents to which platelets may be exposed during thrombus formation (ADP and thrombin) do not shorten platelet survival.\textsuperscript{114,115} It is also clear that activation of the arachidonate pathway in platelets does not shorten their survival since thrombin, which causes the formation of large amounts of thromboxane by platelets, has no effect on platelet survival. The release of granule contents from platelets does not shorten platelet survival, since platelets that have released more than 90\% of their amine storage granule contents in response to treatment with thrombin survive normally when they are returned to the circulation of an experimental animal.\textsuperscript{115,116} Cleavage of major membrane glycoproteins is associated with the action of most proteolytic enzymes on platelets, with the exception of thrombin, which apparently only cleaves a minor glycoprotein.\textsuperscript{117-120} Thus, there appears to be a close relation between removal of glycopeptides from major membrane glycoproteins and reduced platelet survival.

Platelets that have been aggregated by ADP or thrombin can undergo deaggregation.\textsuperscript{115,121} They do this most readily if they have been aggregated for only a short time.\textsuperscript{116} It is now established that fibronogen receptors become available on platelets upon exposure to aggregating agents\textsuperscript{122-124} and that fibrinogen is bound during aggregation and dissociates from the platelets during deaggregation.\textsuperscript{125} It is not yet known whether the binding of platelets to fibrin during the process of thrombus formation involves the same receptors on the platelet surface, or whether this binding is reversible. If the same receptors are involved, then it seems possible that platelet deaggregation and dissociation from fibrin could occur and that platelets could be freed from a thrombus due to the forces of blood flow without alteration of membrane glycoproteins and, therefore, without any changes in platelet survival. The turnover of platelets in thrombi has been described by a number of investigators in both experimental animals and humans.\textsuperscript{7,58,126-129} We have found that thrombus formation and the turnover of platelets in the thrombi can occur without demonstrable shortening of platelet survival under experimental conditions in which extensive vessel wall injury is not induced.\textsuperscript{59} Under these circumstances, it may be that the thrombi form largely through the action of aggregating agents that do not affect platelet survival.

In contrast, platelet survival can be shortened by continuous vessel injury under conditions in which gross thrombi do not form. It may be that platelets that have adhered to the damaged sites can only be freed by the action of proteolytic enzymes that hydrolyze major membrane glycoproteins. Platelets on which the membrane glycoproteins have been altered in this way would be rapidly cleared from the circulation.\textsuperscript{113}

The proteolytic enzymes that could be involved are plasmin, leukocyte elastase, and proteolytic enzymes released by the platelets themselves.\textsuperscript{36-42} If these enzymes are partly responsible for shortened platelet survival, one must postulate that high local concentrations are present for a sufficient time to cleave a significant proportion of the membrane glycoproteins. Only plasmin has been studied for its effects on platelet membrane glycoproteins and platelet survival.\textsuperscript{113} A number of studies have shown that plasminogen activator becomes available when the endothelium is damaged.\textsuperscript{130,131} This may be particularly important in the microcirculation which has a large surface area and is capable of producing large amounts of tissue plasminogen activator. Plasminogen can also be activated at injury sites as a result of the activation of factor XII by exposed collagen or aggregating platelets.\textsuperscript{2-132-134} In addition, vascular plasminogen activator can be released from the endothelium by activated protein C. This also could lead to plasmin formation at sites of thrombin generation since thrombin activates protein C.\textsuperscript{135,136} Since cigarette smoking, which may alter the vessel wall,\textsuperscript{69-72} has been shown to increase fibrinolytic activity,\textsuperscript{137} it may be that the shortened platelet survival observed in cigarette smokers can be attributed to cleavage of platelet membrane glycoproteins by plasmin.

It has not been established how platelets from which membrane glycoproteins have been cleaved are recognized as 'foreign' and cleared from the circulation. Platelets that have been treated with neuraminidase to remove membrane sialic acid are also rapidly removed.\textsuperscript{138} It may be the lack of sialic acid, removed either as sialic acid per se or as glycopeptides, that leads to their clearance. If so, the extensive observations of shortened red cell survival upon treatment with neuraminidase may be analogous to the clearance of these altered platelets.\textsuperscript{139-143} Immunoglobulins have been implicated in the clearance of neuraminidase-treated red blood cells.\textsuperscript{139-143} In immune thrombocytopenias in which platelet survival is short, it has been shown that abnormally high amounts of IgG are associated with the platelets.\textsuperscript{134,145} It may be that the IgG acts as an opsonin in the interaction of the cells with the reticuloendothelial system.\textsuperscript{146} Least dense platelets, which have been shown by several investigators to be enriched in older platelets,\textsuperscript{147-152} have less sialic acid per unit of surface area than most dense platelets that are enriched in young platelets.\textsuperscript{151,152} Least dense platelets have been found to have a shorter survival than most dense platelets.\textsuperscript{151} It has also been reported that least dense platelets have more IgG associated with them than most dense platelets.\textsuperscript{153} These observations are in accord with the hypothesis that as plate-
lets age, sialic acid is lost from their surface and increased amounts of IgG bind to the surface, which facilitates the removal of the platelets from the circulation. In conditions in which continuous vessel injury is occurring, the accelerated removal of sialoglycopeptides would then be responsible for shortened survival by this mechanism.

The relationships among platelet density, platelet age, exposure to aggregating agents, and platelet survival are complex. Although several groups of investigators have obtained evidence that platelet buoyant density decreases as platelets age in the circulation, there is some controversy on this point. 147-152, 154, 155 (McDonald JWD, Ali M, personal communication). Exposure of platelets to ADP, thrombin or plasmin decreases their density, 156, 157 but this is not necessarily related to loss of granule contents because ADP does not cause the release of amine storage granules in a medium with a physiologic concentration of ionized calcium. 158, 159 In rabbits with indwelling aortic catheters, which cause extensive thrombus formation and shorten platelet survival, 58, 59 a higher proportion of platelets is found in the least dense platelet fraction 160 in keeping with the concept that the platelets that take part in reversible thrombus formation will have been exposed to ADP and thrombin. If platelets taken from these animals after 7 days are infused into recipient rabbits, they survive longer than platelets from untreated animals. It seems likely that in association with shortened platelet survival, platelet turnover is increased resulting in a high proportion of young platelets in the circulation of animals with indwelling aortic catheters.

However, when extensive vessel injury is induced in rats without the formation of macroscopic thrombi, a greater than normal proportion of the platelets in the most dense fraction, 60 probably because platelets that have interacted with the damaged wall are rapidly cleared when they are freed into the circulation and thus are not found in the least dense platelet fraction. In these circumstances, one would predict that there would be a higher proportion of young, most dense platelets that would have a longer survival time and unaltered buoyant density because they would not have been exposed to ADP and thrombin. We have demonstrated that when platelets taken from these rats at 6 days are injected into normal rats, their mean survival time is significantly longer than that of platelets from rats without indwelling aortic catheters. 63

Thus, indwelling aortic catheters shorten platelet survival whether or not they cause thrombi. The platelets that are in the circulation after equilibrium is established survive longer when they are infused into normal animals, indicating that they are enriched with young platelets. However, platelet buoyant density may be shifted either toward a higher proportion of less dense platelets or of more dense platelets; it may be that the different shifts in buoyant density are related to whether or not macroscopic thrombi form.

### Prosthetic Surfaces and Grafts

#### Heart Valves

Platelet survival is not shortened in most patients who have received homografts unless extensive thrombosis occurs. 161, 162 Steele and colleagues 163 have reported that platelet survival is normal in patients with directly sewn aortic homografts, whereas platelet survival is shortened in patients with stented homografts (i.e., the homograft aortic valve was placed on a metal stent before its placement in the aortic ring).

In contrast to valves made from tissue, prosthetic heart valves such as the early Starr-Edward’s ball valve prostheses cause a significant reduction in platelet survival associated in some patients with thromboembolism. 161, 163-165 The shortened platelet survival could result from repeated thrombosis caused by the prosthetic material, platelet interaction at injury sites where the graft meets cardiac tissue, or from altered hemodynamics that might mechanically damage the blood cells. Since red cell hemolysis has been reported in some individuals with the early type of ball valve, 166-168 it seems likely that other cells such as platelets could be similarly damaged or modified so that they could be rapidly cleared from the circulation. 166 Platelet survival is less affected by the new models of Starr-Edward’s aortic valves 160 and porcine heterograft valves. 170 As pointed out by Turpie and colleagues, 171 some workers reported that shortened platelet survival time correlated with thromboembolism and with cardiac valve replacement, although other workers have had difficulty confirming these results. 162, 172

#### Prosthetic Vascular Grafts and Artificial Surfaces

Platelet survival has been studied by several groups in animals with prostheses made from a variety of synthetic materials. Harker’s group 173, 174 has demonstrated a quantitative relationship between platelet consumption and the amount of prosthetic surface exposed to circulating blood. They found that platelets from baboons with femoral arteriovenous cannulae survived normally in normal recipients, whereas normal platelets showed accelerated removal in animals with these shunts. 175 These results were interpreted as indicating that “thrombotic platelet consumption in this model is an all-or-none phenomenon rather than a process of cumulative platelet injury that progressively compromises platelet viability.”

Hanson and colleagues 176 found in baboons that Teflon-Silastic surfaces do not shorten platelet survival, whereas shunts composed of polycrylamide grafted Silastic or biomer polyurethane do shorten platelet survival. 177 They observed 177 that the shortened platelet survival was associated with continuous turnover of platelets on the graft surface. With grafts composed of material such as woven dacron
which eventually become covered with endothelium, platelet survival is initially shortened but gradually lengthens over a period of weeks or months. This has been observed by Harker’s group in studies with baboons, by Clagett and colleagues in dogs, and by Didisheim and co-workers in dogs. Harker’s group also showed progressive normalization of platelet survival in patients within 9 months of aortofemoral fabric graft surgery. Thus, it appears that when an artificial surface with which blood comes in contact remains reactive, platelet survival is shortened, whereas surfaces that have lost their reactivity because of reendothelialization do not shorten platelet survival. In addition, graft material that shows little interaction with circulating platelets does not shorten platelet survival. It is not clear what happens to platelets that interact with the surface of a graft. If platelets are lost from the surface of the graft and return to the circulation, there would have to be some mechanism that breaks the bonds through which platelets adhere to the surface. It is possible that proteolytic enzymes activated by the contact of plasma with the surface, or released from the platelets, are responsible for this. However, there have been no studies directed at answering these questions.

**Effect of Drugs on Platelet Survival**

A number of drugs have been shown to lengthen platelet survival, particularly in conditions in which platelet survival is abnormally short. Drugs that have been most extensively investigated for their effects on platelet survival include dipyridamole, dipyridamole plus aspirin, sulfinpyrazone, clofibrate, and ticlopidine. Surprisingly, aspirin does not affect platelet survival, although its inhibitory effects on platelet function tested in vitro are much more apparent than those of the drugs that do prolong platelet survival. The mechanism by which these drugs prolong platelet survival is not established and, indeed, the drugs may act in different ways. Among the suggestions that have been advanced are inhibition of platelet interaction with the injured vessel wall, reduction in the extent of thrombosis and thromboembolism; reduction in the rate at which glycoproteins are removed from membrane glycoproteins or fragments of platelet membrane are removed from the surface of the platelet; interference with the clearance of the altered platelets by the reticuloendothelial system. Hanson and Harker, however, were unable to confirm George and Lewis’ observation that aspirin plus dipyridamole reduced the rate of loss of labeled membrane fragments from platelets in vivo. It seems unlikely that the species difference between rabbits and baboons could account for this discrepancy.

In venous thrombosis, heparin or warfarin restores shortened platelet survival values toward normal in some patients. The effects of the “antiplatelet” drugs on platelet survival do not correlate with their beneficial effects, as shown by large-scale clinical trials. Peto’s analysis of the six trials with aspirin indicated a 16% reduction in mortality and a 21% reduction in reinfarction in postmyocardial infarction patients. In contrast, in the Antitrust Reinfarction Study (ARIS), sulfinpyrazone reduced the incidence of sudden death in the first 6 months following myocardial infarction. In the ARIS trial, however, there was a significant reduction in the incidence of reinfarction in the sulfinpyrazone-treated group. Thus, the relationship between the effects of these drugs on the complications of vascular disease and platelet survival is complex and is not necessarily related to their effects on platelet function in vitro.

Dipyridamole or dipyridamole plus aspirin have been shown to prolong shortened platelet survival in patients with arterial thrombosis, prosthetic heart valves, arteriovenous Silastic cannulas, prosthetic aortic grafts, vasculitis, or coronary atherosclerosis. The combination of aspirin and dipyridamole also prolongs shortened platelet survival in patients with diabetes mellitus. However, in patients with coronary artery disease in whom platelet survival was not shortened, the combination of dipyridamole and aspirin did not affect platelet survival.

Dipyridamole has been shown to prolong platelet survival that has been shortened by the infusion of homocysteine into baboons. Furthermore, in baboons with femoral arteriovenous cannulas, dipyridamole reduces platelet consumption and prolongs shortened platelet survival. In several studies the effect of high doses of dipyridamole could be duplicated by the combination of a much lower dose of dipyridamole with aspirin, although in nearly all the studies aspirin alone was found to have no effect on platelet survival. The only authors who have reported a slight prolongation of shortened survival by aspirin in patients with coronary artery disease are Steele and co-workers. One way by which dipyridamole may prolong shortened platelet survival is by inhibition of platelet adherence at sites of vessel injury. High concentrations of dipyridamole inhibit platelet adherence to injured vessels or vascular grafts. In similar experiments in which a wide range of concentrations of aspirin were used, platelet adherence to injured vessels was not affected. Thus, the effects of these drugs on platelet survival do not correlate with their effects on platelet aggregation and release of granule contents in vitro since the inhibitory effects of aspirin administered in the usual doses are much more apparent than those of drugs such as dipyridamole that do prolong shortened platelet survival. Dipyridamole has little effect on platelet aggregation in humans.

Although sulfinpyrazone is thought to inhibit the same enzyme in platelets (cyclooxygenase) as aspirin, in nearly all reported studies it has prolonged shortened platelet survival. In fact, sulfinpyrazone was the first drug shown to pro-
long shortened platelet survival in animals and humans. It is not known whether this is the result of an effect on platelet membrane lipids or of reduction in vessel wall injury. Ross and Harker carried out matched crossover platelet survival experiments between six normolipidemic and six hyperlipidemic monkeys. They found that platelets from hyperlipidemic monkeys survived normally in normolipidemic animals, whereas platelets from normolipidemic animals infused into hyperlipidemic animals had a decreased survival time. They interpreted these results as being consistent with increased platelet consumption on exposed subendothelium resulting from the injurious effect of hypercholesterolemia. Thus, the effect of clofibrate may be attributable to its effects on serum lipids.

Several investigators have shown that ticlopidine prolongs shortened platelet survival in experimental animals. In most of these studies, very high doses of ticlopidine were necessary to demonstrate this effect (ranging from 50 to 400 mg/kg/day in several species). However, in humans, ticlopidine (500 mg/day) was found to be without effect on reduced platelet survival in patients following reconstructive arterial surgery. The way in which ticlopidine or the active metabolite that has been reported in rats affects platelet function in unknown. Ticlopidine does not inhibit platelet adherence to damaged vessel walls in rabbits.

Suloctidil has been shown to prolong shortened platelet survival in patients with prosthetic cardiac valves. This drug has been reported to be an antithrombotic agent in humans and experimental animals, but its mechanism of action is not known and does not seem to be related to its effects on serotonin in platelets.

Recently, nafazatrom has been shown to be a potent antithrombotic agent in experimental animals and to normalize platelet survival that has been reduced by an indwelling aortic catheter. This drug has been shown by some investigators to increase PG12 production in vitro and in vivo. It does have this effect, platelet adherence would be inhibited. This would account for prolongation of shortened platelet survival.

It thus seems reasonable to conclude that drugs that influence platelet survival may not necessarily influence platelet interaction with the damaged vessel wall or thrombin. As indicated in the introduction to this section, drugs may influence such aspects as vessel injury, modification of platelet membrane glycoproteins, or removal of altered platelets from the circulation.

Conclusions

Interactions of platelets with repeatedly damaged or diseased vessel walls shorten platelet survival even if macroscopic thrombi do not form. The reason why platelet survival is shortened is not established. Shortened platelet survival has been observed in a number of clinical conditions that are associated with vessel injury, vascular grafts, and thromboembolism, but does not occur in all patients with these conditions. Shortening of platelet survival in association with repeated vessel injury could result from consumption in thrombi or turnover on the vessel wall. If proteolytic enzymes are responsible for freeing platelets that have adhered to the injury sites, cleavage of membrane glycoproteins and subsequent clearance of the altered platelets by the reticuloendothelial system may account for their shortened survival. Although a number of drugs have been shown to prolong shortened platelet survival, in most cases this is not closely correlated with their effects on platelet aggregation and the release of the contents of platelet granules in vitro, nor with the clinical effects of the drugs. Drugs could influence platelet survival by reducing platelet consumption in thrombi, decreasing platelet adherence to the damaged vessel wall, or decreasing the rate of clearance of altered platelets by the reticuloendothelial system. Although some drugs may influence platelet survival by decreasing platelet adherence to the damaged vessel wall, generalizations are difficult because the mechanism responsible for shortened platelet survival is not established and the drugs that prolong shortened platelet survival may do so in a variety of ways.

References

VESSEL INJURY AND PLATELET SURVIVAL
Kinlough-Rathbone et al. 541

46. Karlto T, Goldsmith HL. Aggregation of human platelets to collagen on the walls distal to a tubular expansion. Microvasc Res 1979;17:239–262
115. Harfenist EJ, Packham MA, Mustard JF. Reversibility of the association of fibrinogen with rabbit platelets exposed to ADP. Blood 1980;56:169–176
129. Jancik JM, Schauer R, Andres KH, von Düring M. Sequestration of neuraminidase-treated erythrocytes. Studies on its topographic, morphologic and immunologic as-


185. George JN, Lewis PC. Membrane glycoprotein loss from

186. 159. 158. 157. 156. 155. 154. 153. 152. 151. 150. 149. 148. 147. 146. 145. 144. 143. 142. 141. 140. 139. 138. 137. 136. 135. 134. 133. 132. 131. 130. 129. 128. 127. 126. 125. 124. 123. 122. 121. 120. 119. 118. 117. 116. 115. 114. 113. 112. 111. 110. 109. 108. 107. 106. 105. 104. 103. 102. 101. 100. 99. 98. 97. 96. 95. 94. 93. 92. 91. 90. 89. 88. 87. 86. 85. 84. 83. 82. 81. 80. 79. 78. 77. 76. 75. 74. 73. 72. 71. 70. 69. 68. 67. 66. 65. 64. 63. 62. 61. 60. 59. 58. 57. 56. 55. 54. 53. 52. 51. 50. 49. 48. 47. 46. 45. 44. 43. 42. 41. 40. 39. 38. 37. 36. 35. 34. 33. 32. 31. 30. 29. 28. 27. 26. 25. 24. 23. 22. 21. 20. 19. 18. 17. 16. 15. 14. 13. 12. 11. 10. 9. 8. 7. 6. 5. 4. 3. 2. 1. 0.
circulating platelets: Inhibition by diprydamole and aspirin. Thromb Haemost 1977;38:111


218. MacIntyre DE, Salzman EW. Effects of Bay g 6575 on platelets and on vascular PGL2 production. Thromb Haemost 1981;46:192


Index Terms: vessel injury • platelet adherence • platelet survival • prosthetic surfaces • antiplatelet drugs • atherosclerosis