Cumulative inhibitory effect of low-dose aspirin on vascular prostacyclin and platelet thromboxane production in patients with atherosclerosis

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ABSTRACT The relationship between the antithrombotic and antiplatelet effects of aspirin is complex, since aspirin influences other systems that protect against thrombosis as well as inhibiting platelet function. We investigated possible cumulative effects of low-dose aspirin on vascular production of prostacyclin in patients with documented atherosclerotic cardiovascular disease. Candidates for coronary artery vein graft bypass ingested 20 mg of aspirin daily during the week before surgery, and platelet aggregation, platelet formation of thromboxane A\textsubscript{2} (TXA\textsubscript{2}), aortic and saphenous vein production of prostacyclin (PGI\textsubscript{2}), and hemostatic status were measured at the time of the bypass surgery. Low-dose aspirin markedly inhibited platelet aggregation responses and reduced TXA\textsubscript{2} generation by greater than 90%, effects similar to those observed with much higher doses of aspirin. Both aortic and saphenous vein production of PGI\textsubscript{2} were inhibited by 50% compared with PGI\textsubscript{2} produced by vascular tissues of control subjects who received no aspirin preoperatively (51 ± 10 pg 6-keto-PGF\textsubscript{1α}/mg aortic wet weight [mean ± SEM] in aspirin-treated subjects vs 130 ± 16 pg/mg in control subjects, and 71 ± 8 pg/mg saphenous vein wet weight vs 131 ± 17 pg/mg). Blood loss at surgery was not significantly increased by preoperative low-dose aspirin as measured by chest tube drainage (754 ± 229 ml in aspirin-treated subjects vs 645 ± 271 ml in control subjects), hematocrit nadir (31.2 ± 1.9% vs 31.8 ± 1.7%), or transfusions (2.2 ± 1.3 units of red blood cells vs 2.2 ± 1.7 units). Endothelial recovery from aspirin-induced inhibition of PGI\textsubscript{2} formation was complete at 24 hr after the last dose, suggesting that cyclooxygenase turnover in endothelium may be more rapid than that in vascular smooth muscle. These findings indicate that preoperative low-dose aspirin can inhibit platelet function without augmenting perioperative blood loss and that partial inhibition of PGI\textsubscript{2} formation by blood vessels accompanies even very low doses of aspirin. However, the rapid recovery of PGI\textsubscript{2} synthetic capacity by vascular endothelium may permit use of a relatively selective antiplatelet schedule of low-dose aspirin administration.


MANY STUDIES of the antithrombotic efficacy of aspirin have been conducted, yet controversy over an optimum dosage remains active. Moreover, the relationship between the antiplatelet effects of aspirin and its antithrombotic efficacy has not been clarified. Aspirin inhibits platelet aggregation, decreases the release of vasoactive substances from platelets, and prolongs the bleeding time. These effects result from irreversible inactivation by aspirin of the enzyme, fatty acid cyclooxygenase, in platelets, blocking enzymatic conversion of arachidonic acid into prostaglandin endoperoxides and thence into prostaglandins and thromboxanes.\textsuperscript{1} Since platelets lack protein synthetic capacity, they do not produce new cyclooxygenase after aspirin exposure and remain functionally inhibited throughout their lifespan in the circulation.\textsuperscript{2} Cyclooxygenase in vascular tissue is also inhibited by aspirin, although the blood vessel cells recover the capacity for prostaglandin production within hours via new cyclooxygenase synthesis.\textsuperscript{3, 4} In addition to inhibiting vascular production of prostacyclin (PGI\textsubscript{2}), aspirin also prevents release of vascular plasminogen activator.\textsuperscript{5} Because aspirin can prevent vascular release of these antithrombotic factors at conventional doses

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Supported under a Specialized Center of Research in Thrombosis Grant from the NHLBI (HL 18228) and a Faculty Research Award from the American Cancer Society to Dr. Weksler.

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Received Sept. 5, 1984; revision accepted Nov. 15, 1984.
known to inhibit platelet formation of prothrombotic thromboxane A₂ (TXA₂), it is important to ascertain whether a lower, specifically antiplatelet dose of the drug exists.

Treatment with aspirin (160 mg to 1.8 g daily) alone or in combination with dipyridamole in controlled clinical trials has been observed to decrease arteriovenous shunt thrombosis, prevent stroke or transient ischemic attacks, lower the incidence of myocardial infarction after unstable angina, decrease graft occlusions after coronary artery bypass surgery, inhibit platelet accumulation in prosthetic vascular grafts, prevent pulmonary embolism after hip surgery, and decrease emboli after heart valve replacement. Although 325 mg of aspirin daily decreased the incidence of myocardial infarctions in patients with unstable angina, none of many large clinical trials testing aspirin (1 to 2 g daily) for prevention of recurrent myocardial infarction in survivors of a prior infarct showed clear benefit from the drug. It remains an open question whether nonplatelet effects of higher aspirin dosage might detract from antiplatelet benefits.

Very small doses of aspirin suffice to inhibit platelet aggregation as well as platelet synthesis of TXA₂, the potent vasoconstrictor and platelet aggregant substance. In normal subjects 100 mg of oral aspirin taken once inhibited platelet production of TXA₂ by more than 95%. We reported that a single dose of 80 mg aspirin administered to patients with coronary artery disease produced 95% inhibition of platelet TXA₂ generation, inhibited arachidonate-induced platelet aggregation, and abolished the second wave of ADP- or epinephrine-induced aggregation. Moreover, normal subjects or patients with recent cerebral ischemia given only 40 mg/day aspirin had full inhibition of platelet aggregation and 90% suppression of TXA₂ within a few days of starting treatment, indicating that low-dose aspirin has a cumulative inhibitory effect on platelets.

This study was undertaken to ascertain whether very small repeated oral doses of aspirin administered to patients with atherosclerosis would exert a cumulative inhibitory effect on vascular cyclooxygenase as well as platelet cyclooxygenase. We previously showed that vascular cyclooxygenase production in patients with atherosclerosis was almost completely inhibited by a single "ordinary" dose of aspirin (325 mg) but was not affected by a single dose of 40 mg. Therefore 20 mg of aspirin was administered daily to patients with documented atherosclerotic coronary artery disease before scheduled coronary artery bypass graft surgery, a procedure in which early postoperative aspirin therapy appears to be beneficial. The effects of low-dose aspirin on platelet aggregation and thromboxane production, on the production of PGI₂ by blood vessels, and on hemostatic function were monitored and compared. We found that this low-dose aspirin regimen inhibited platelet function similarly to larger doses and that a cumulative inhibition could also be demonstrated on vascular PGI₂ production.

Methods

The study population comprised 20 male patients with angiographically documented coronary artery disease who were scheduled for elective coronary artery vein graft bypass surgery. Exclusion criteria were limited to history of gastrointestinal intolerance to aspirin, active gastrointestinal bleeding, allergy to aspirin, or current aspirin use. Control subjects were 20 male patients with similarly documented coronary artery disease who were admitted to the cardiovascular surgery service during the same period and were known not to be taking aspirin. Subjects were not randomized to treatment groups but were entered under a case-control design to equalize risk factors. Informed consent was obtained from all subjects, under a study design approved by the institutional human rights committee.

After giving informed consent, subjects took one 20 mg capsule of aspirin each morning during the week before scheduled bypass surgery. Capsules were prepared and supplied by the New York Hospital Pharmacy. Patients continued all other scheduled medications but were cautioned not to take any other aspirin-containing preparation during this time. At hospital admission on the day before surgery, baseline studies of bleeding time and complete blood count were obtained. All subjects underwent platelet aggregation studies and measurement of serum thromboxane levels at least once before surgery. During the surgical procedure, fragments of freshly harvested saphenous vein and aorta were placed into iced HEPES-buffered (0.01M) RPMI-1640 tissue culture medium (Gibco, Grand Island, NY), pH 7.35, containing 10% newborn calf serum and 10 μg/ml paniparine and were transported to the laboratory for studies of PGI₂ synthesis. These studies were obtained from 17 of 20 aspirin-treated subjects and from all control subjects. Postoperative blood loss was monitored for each subject by blood counts, volume of chest tube drainage, and transfusion requirements.

Bleeding time. Bleeding time was measured on the day before surgery approximately 10 hr after the most recent dose of aspirin. Measurements were made with longitudinal incisions on the volar surface of the forearm with a Simplate II apparatus (General Diagnostics, Morris Plains, NJ).

Platelet studies. Venous blood was drawn on the morning of surgery in all subjects before induction of anesthesia and on the day before surgery when possible. Citrated (1/10 volume 3.8% trisodium citrate) venous blood was used to prepare platelet-rich plasma. Platelet aggregation responses were measured within 2 hr in a Payton dual-channel aggregation module (Payton, Inc., Buffalo, NY) after stirring for 4 min at 1000 rpm and 37°C with a stimulus (final concentrations): 250 μM sodium arachidonate (NuCheck, Elysian, MN), 1 μM adenosine diphosphate (Sigma Chemical Co., St. Louis), 0.4 μg/ml collagen (Hormon Chemie, Munich, F.R.G.), or 1 μM epinephrine (Parke Davis, Chicago). If no aggregation response to arachidonate was observed, the test was repeated with twice the initial concentration and, in addition, the response to the endoperoxide analog, U44069 (2.9 μM; gift of Dr. John Pike, Upjohn Co., Kalamazoo, MI), was measured. The occurrence of spontaneous platelet...
let aggregation in stirred platelet-rich plasma was also monitored.

**Serum thromboxane.** Blood drawn into a plain glass tube was allowed to clot at 37°C for 1 hr, after which the serum was separated by centrifugation at 4°C and frozen at −40°C until measurement of released thromboxane B2 (TXB2) by radioimmunoassay as previously described.25 Serum thromboxane elicited in this manner represents the maximal thromboxane production by platelets stimulated by thrombin released by clotting, and is a good measure of platelet capacity to form TXA2.21

**Studies of PGI2 synthesis by vascular fragments.** Vascular fragments were weighed, washed, and incubated at 37°C in HEPES-buffered saline containing divalent cations in the presence or absence of 25 μM sodium arachidonate for 15 min to obtain stimulated or basal production of PGI2.22 PGI2 in the supernatant fluid was measured by radioimmunoassay of 6-keto-PGF1α as previously described.25 When available, additional saphenous vein specimens were also placed in a template chamber, endothelial side uppermost, and incubated with HEPES buffer or buffer containing arachidonate at 37°C to elicit endothelial production of PGI2. The chamber excludes cut tissue edges from study and has previously been shown to measure release of PGI2 released from endothelium rather than from smooth muscle layers of the vessel wall.26 The chambers were designed and made available by Dr. Eric Grabowski.

**Histologic study.** Vascular fragments used for the assays were fixed in buffered 10% formalin and embedded in paraffin, and 6 μm sections were stained with hematoxylin and eosin. The degree of intimal thickening in aortic sections was used as an index of atherosclerosis and was expressed as the ratio of intimal to medial area. The latter was determined by planimetry by means of a Zeiss MOP-3 image digitizer equipped with a camera lucida attachment. A normal intimal-medial ratio was considered to be 5% or less.

**Statistics.** Statistical analysis of data was performed with Student’s unpaired t test for comparison between patient groups and with the Kruskal-Wallis test for multiple comparisons. Values for test results are presented as means ± SEM unless otherwise noted. Statistical significance was considered to be demonstrated at p < .05.

**Results**

**Comparison of subject groups.** The group of subjects who took the prescribed course of low-dose aspirin and the group of untreated controls were comparable on the basis of age, severity of atherosclerosis, other associated diseases, medications, and preoperative laboratory values (tables 1 and 2). The groups also did not differ significantly in duration of cardiopulmonary bypass or number of grafts placed.

**Studies with platelets.** Platelet aggregation in response to arachidonate acid stimulation was completely inhibited in all subjects who ingested 20 mg of aspirin daily for 1 week, except for a single subject who was found to have been taking ibuprofen, a drug that can interfere with the protracted effects of aspirin on platelets. Similarly, aggregation responses to collagen, ADP, and epinephrine were decreased in all other aspirin-treated subjects. Collagen-induced aggregation averaged 39% of the maximum aggregation response achieved with the endoperoxide analog U44069, which bypasses the aspirin block. The second wave of aggregation in response to ADP and epinephrine was inhibited with preservation of the primary wave. The aggregation pattern observed in these subjects was indistinguishable from that of patients given much higher doses of aspirin, and reflected the typical effects of aspirin on platelets. The extent of inhibition of responses to aggregating agents was similar whether the blood for aggregation studies was drawn 3, 12, or 24 hr after the last dose of aspirin. Slight spontaneous platelet aggregation was observed in three subjects who had taken the low-dose aspirin and in eight control subjects. All subjects in the control group showed full aggregation responses to sodium arachidonate, collagen, ADP, and

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**TABLE 1**

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>Aspirin group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>53 ± 9</td>
<td>57 ± 9</td>
</tr>
<tr>
<td>Previous MI</td>
<td>47</td>
<td>42</td>
</tr>
<tr>
<td>Hypertension</td>
<td>24</td>
<td>42</td>
</tr>
<tr>
<td>Diabetes</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Smoking</td>
<td>59</td>
<td>71</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Blocker</td>
<td>88</td>
<td>81</td>
</tr>
<tr>
<td>Calcium-channel blocker</td>
<td>59</td>
<td>52</td>
</tr>
<tr>
<td>Nitrates</td>
<td>94</td>
<td>100</td>
</tr>
<tr>
<td>No. of Grafts</td>
<td>2.5 ± 0.8</td>
<td>2.8 ± 0.6</td>
</tr>
<tr>
<td>Pump time (min)</td>
<td>76 ± 16</td>
<td>92 ± 26</td>
</tr>
</tbody>
</table>

Values are given as percent incidence or as mean ± SD. No significant differences were found between the two groups in any parameter listed.

**TABLE 2**

<table>
<thead>
<tr>
<th>Hemostatic status</th>
<th>Aspirin group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>44.4 ± 4.1</td>
<td>41.4 ± 6.9</td>
</tr>
<tr>
<td>Platelet count (× 10^3)</td>
<td>237 ± 37</td>
<td>246 ± 50</td>
</tr>
<tr>
<td>Bleeding time (min)</td>
<td>6.1 ± 0.5^A</td>
<td>4.4 ± 0.2</td>
</tr>
<tr>
<td>Platelet aggregation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(% of max response)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arachidonate</td>
<td>7A</td>
<td>100</td>
</tr>
<tr>
<td>Collagen</td>
<td>39^A</td>
<td>81</td>
</tr>
<tr>
<td>Postoperative tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit nadir (%)</td>
<td>31.2 ± 1.9</td>
<td>31.8 ± 1.7</td>
</tr>
<tr>
<td>Platelet nadir (× 10^3)</td>
<td>141 ± 28</td>
<td>147 ± 45</td>
</tr>
<tr>
<td>Chest tube drainage</td>
<td>300 ± 229</td>
<td>315 ± 119</td>
</tr>
<tr>
<td>8 hr (ml)</td>
<td>754 ± 424</td>
<td>644 ± 260</td>
</tr>
<tr>
<td>Total</td>
<td>2.2 ± 1.3</td>
<td>2.2 ± 1.7</td>
</tr>
<tr>
<td>Transfusion (units red cells)</td>
<td>2.2 ± 1.3</td>
<td>2.2 ± 1.7</td>
</tr>
</tbody>
</table>

Values given as means ± SD. ^A p < .001.
epinephrine, confirming their lack of aspirin ingestion.

**Serum TXB₂.** Serum levels of thromboxane TXB₂, representing the maximal generation of TXA₂ by platelets during blood clotting at 37°C, were significantly lower (p < .001) for all subjects who ingested 20 mg of aspirin daily than serum TXB₂ levels of control subjects and averaged 7% of the TXB₂ level of control subjects. The observed serum TXB₂ level varied with the interval between the last ingestion of aspirin and blood sampling. Lowest levels were obtained in blood sampled 3 to 5 hr after the last dose, with higher levels in samples taken 12 and 24 hr after the last dose (figure 1). At 24 hr the serum TXB₂ levels in aspirin-treated subjects averaged 10% of those in control subjects, whereas at 3 hr they averaged 3% of control values.

**PGI₂ production by vascular tissue.** Aortic fragments removed from aspirin-treated subjects and incubated with sodium arachidonate produced 78 ± 25 pg 6-keto-PGF₁₀⁻/mg of aortic tissue when the last aspirin dose was taken 24 hr earlier and 40 ± 14 pg/mg when the last dose was taken 3 hr earlier. The mean value for all aspirin-treated subjects was 51 ± 10 pg/mg. Subjects who did not take aspirin preoperatively averaged 130 ± 16 pg/mg of aortic tissue (figure 2). The aortic 6-keto-PGF₁₀⁻ level was significantly lower than the control level at both intervals after low-dose aspirin ingestion (p < .001 for all subjects, p < .02 for those receiving aspirin 3 hr before surgery). The weight of aortic fragments was similar in the treated and control groups, 19.0 ± 7.8 mg for aspirin-treated subjects and 16.5 ± 9.5 mg for control subjects.

Segments of saphenous vein removed from aspirin-treated subjects and incubated with sodium arachidonate produced 71 ± 8 pg 6-keto-PGF₁₀⁻/mg tissue when the last dose of aspirin was taken 24 hr earlier and 39 ± 7 pg/mg when the last dose of aspirin was taken 3 to 5 hr before tissue sampling. Saphenous vein fragments from control subjects produced 139 ± 17 pg/mg tissue (figure 3). The saphenous vein 6-keto-PGF₁₀⁻ level for both intervals after low-dose aspirin was significantly

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**FIGURE 1.** Effect of low-dose aspirin (ASA) ingestion on serum TXB₂. Data are means ± SEM. Decrease in serum TXB₂ is dependent on the interval between the last dose of aspirin and measurement in clotted blood. The TXB₂ levels for all aspirin-treated subjects are significantly lower than those for control subjects (p < .001, Kruskal-Wallis) and also differ from one another (p < .01).

**FIGURE 2.** Effect of low-dose aspirin (ASA) ingestion on production of PGI₂ by aortic fragments. Data expressed as picograms of 6-keto-PGF₁₀⁻ (mean ± SD) per milligram wet weight of aortic tissue per 15 min incubation. The aspirin-induced decrease in 6-keto-PGF₁₀⁻ is dependent on the interval between the last dose of aspirin and time of tissue removal. All treated groups had significant (p < .001) decrease from control and differed also from one another.
lower than that in control subjects ($p < .005$ for all aspirin-treated subjects, $p < .025$ for those receiving aspirin 3 hr before surgery). The weights of the saphenous vein fragments were similar in aspirin-treated and control groups, $156.8 \pm 16.9$ mg for aspirin-treated subjects and $211.9 \pm 26.5$ mg for control subjects ($p > .3$).

The production of 6-keto-PGF$_{1\alpha}$ by vascular tissue during incubation with buffer alone was also significantly lower in aortic fragments from subjects who had ingested 20 mg of aspirin daily than in aortic fragments from control subjects and averaged 50% of the latter values. Unstimulated aortic fragments produced $24 \pm 6$ pg/mg compared with $42 \pm 3$ pg/mg for control subjects ($p < .02$). No significant difference was found in the amount of 6-keto-PGF$_{1\alpha}$ produced in unstimulated venous fragments from aspirin-treated subjects ($16 \pm 3$ pg/mg vs $19 \pm 3$ pg/mg for control subjects).

As an additional comparison, five patients were studied who had been taking 325 mg of aspirin daily before coronary artery vein graft bypass surgery. The last aspirin dose was 12 to 24 hr before tissues were obtained. In these subjects aortic 6-keto-PGF$_{1\alpha}$ level averaged $7 \pm 3$ pg/mg and saphenous vein synthesis was $18 \pm 9$ pg/mg in arachidonate-stimulated tissues. Serum TXB$_2$ level was $4.3 \pm 1.6$ ng/ml.

**Estimation of endothelial production of prostacyclin.** The capacity of endothelium to release 6-keto-PGF$_{1\alpha}$ as a function of the interval after the last dose of aspirin was compared for four subjects whose tissues were obtained 3 hr after the last aspirin dose and for four subjects whose tissues were obtained 24 hr after the last aspirin dose, with the use of segments of saphenous vein tested in a template chamber designed to exclude the contribution of PGI$_2$ produced by smooth muscle layers of the vessel. As seen in table 3, endothelial production of 6-keto-PGF$_{1\alpha}$ was inhibited by 58% in veins removed 3 hr after aspirin but was similar to control production in veins removed 24 hr after aspirin.

**Hemostatic data.** The template bleeding time before surgery was $6.1 \pm 1.7$ min for aspirin-treated subjects and $4.4 \pm 1.0$ min for control subjects. These values both lie within the accepted normal range (2 to 7 min); however, the value for the aspirin-treated group is significantly longer ($p < .001$).

Perioperative blood loss was similar in aspirin-treated and in control subjects (table 2). Chest tube drainage at 8 hr after completion of surgery was almost identical in the two groups ($300 \pm 229$ ml [mean $\pm$ SD] in aspirin-treated subjects and $315 \pm 119$ ml in control subjects); total chest tube drainage was slightly greater ($754 \pm 424$ ml) in aspirin-treated subjects, with a wider scatter than in control subjects ($645 \pm 260$ ml), but the difference was not statistically significant ($p > .4$). Total duration of chest tube drainage did not differ between the groups. Transfusion requirements were similar in the two groups ($2.2 \pm 1.3$ units of packed red cells in aspirin-treated subjects and 2.2

### Table 3

<table>
<thead>
<tr>
<th>Effects of low-dose aspirin on endothelial production of PGI$_2$</th>
<th>Saphenous vein 6-keto-PGF$_{1\alpha}$ (pg/cm$^2$/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aspirin group</td>
</tr>
<tr>
<td>Last dose 24 hr preoperatively</td>
<td>$2067 \pm 424$</td>
</tr>
<tr>
<td>Last dose 3 hr preoperatively</td>
<td>$735 \pm 494^A$</td>
</tr>
</tbody>
</table>

Values given as mean $\pm$ SD.

$^A p < .0025$. 

CIRCULATION
± 1.7 units in control subjects [mean ± SD]). The postoperative nadirs of hematocrit and platelet count were similar for aspirin-treated and control groups, with hematocrit falling an average of 13% in aspirin-treated group and 10% in control subjects, and the platelet count decreasing by 96,000 and 99,000, respectively (table 2).

Pathologic evaluation of atherosclerosis. Histologic examination of the aortic fragments showed that the degree of atherosclerosis was mild, with an average intimal-medial area ratio of 13.0 ± 6.9% for aspirin-treated subjects and 15.3 ± 10.7% for control subjects (p = NS). This represented mainly intimal thickening, with minimal cellular infiltration. Cholesterol deposits were rare and cholesterol clefts were not encountered. No correlation was observed between the degree of intimal thickening and the amount of 6-keto-PGF$_1_α$ formed by the tissue. No correlation was observed between the amount of intimal thickening and the serum cholesterol level for either group of subjects.

Discussion

Preoperative treatment of coronary artery bypass patients with low-dose aspirin for 1 week resulted in significant inhibition of platelet function and of vascular PGI$_2$ production. Platelet aggregation was inhibited in the pattern typical for aspirin, and platelet formation of TXA$_2$ was only 10% that of untreated control subjects. Both aortic and saphenous vein tissue removed from subjects treated with 20 mg/day aspirin for 1 week produced less than 50% as much PGI$_2$ when stimulated with arachidonate than did tissues removed from subjects who did not receive any preoperative aspirin. These results indicate that a cumulative effect of very low doses of aspirin on both platelet and vascular cyclooxygenase occurs, since in the same clinical preparation a single dose of 80 mg inhibited only venous but not arterial PGI$_2$ production (figure 4) and a single dose of 40 mg failed to inhibit either aortic or venous PGI$_2$ formation.$^{22}$

The inhibition of platelet aggregation and of thromboxane release from platelets in subjects who took 20 mg/day aspirin for a week implies that the platelets of patients with atherosclerotic coronary artery disease are not more resistant than platelets of normal subjects to the inhibitory effects of once-daily, low-dose, oral aspirin. Previous studies in normal young subjects given 20 to 40 mg of aspirin daily indicated that cumulative inhibition of TXA$_2$ synthesis by 95% from pre-aspirin levels was regularly achieved after three to four such daily doses.$^{27}$ In atherosclerotic patients with recent cerebral ischemia$^{28}$ or myocardial infarction, aspirin daily was shown to inhibit platelet function to a similar extent; a daily dose of 80 to 125 was required in two other recent studies of patients with cerebrovascular disease.$^{29,30}$ In all but one of our subjects, platelet aggregation was inhibited to an extent similar to that observed for doses of aspirin of 325 mg or greater.$^{31}$ That single exception was a patient also taking ibuprofen, which can block the acetylation of cyclooxygenase by aspirin. It is possible that the β-adrenergic–blocking drugs, nitrates, or calcium channel–blocking drugs that these patients were taking for control of angina also contributed to the profound platelet inhibition observed after such low doses of aspirin.$^{32,33}$ Although thromboxane generation by platelets after low-dose aspirin therapy was inhibited by 95% at 3 to 12 hr after the last dose of 20 mg of aspirin, it was inhibited by only 90% at 24 hr after such a dose; after a daily dosage of 325 mg or more of
aspirin, TXA₂ synthesis by platelets is more profoundly suppressed. It has been postulated that low-dose aspirin does not inhibit megakaryocyte cyclooxygenase as much as higher dosage.²¹

Despite the marked change in platelet aggregation and TXA₂ production, patients who received 20 mg/day aspirin for the week before surgery averaged no greater perioperative blood loss than subjects who received no aspirin, as measured by chest tube drainage in the first few hours after surgery, total chest tube drainage, fall in hematocrit or platelet count, or requirement for red blood cell transfusion. The bleeding time was not prolonged beyond the normal range. However, four patients receiving the low-dose aspirin, but only one control subject, had total chest tube drainage of more than 1000 ml. These results suggest that preoperative low-dose aspirin producing a marked antplatelet effect is well tolerated in hemostatic terms. Since very early postoperative initiation of aspirin therapy, but not initiation of aspirin 3 to 5 days postoperatively, has resulted in improved bypass graft patency in patients also treated with dipyridamole,¹⁰ the demonstration of hemostatic safety of preoperative low-dose aspirin should permit its inclusion in future drug studies aimed at improving graft patency.

PGI₁ release from saphenous vein fragments (representing normal veins) was inhibited by the low-dose aspirin to a similar extent as that from aortic fragments that showed the atheromatous changes of intimal thickening and cholesterol deposition. This finding is in contrast to our previous observation that a single dose of 80 mg of aspirin partially inhibited aortic 6-keto-PGF₁₀ production without a significant change in saphenous vein 6-keto-PGF₁₀ production.²² The results of the single-dose study suggested that the atheromatous vessel might be more easily inhibited by aspirin. Since we did not find any correlation between the degree of intimal thickening of the aortic fragments studied and the level of 6-keto-PGF₁₀ measured in aortas from control subjects or those who had received aspirin, it appears that early atheromatous changes such as intimal proliferation are not accompanied by a decrease in PGI₂ production capacity of the vessel per se. Such decreases have been reported for atheromatous plaques, perhaps because of their acellular nature.⁴,³⁸ Intimal thickening may increase the pool of cells that produce PGI₂, since neointimal cells after aortic injury in animal preparations produce increased amounts of PGI₂.²³

Recently, increased urinary excretion of metabolites of 6-keto-PGF₁₀ was documented in patients with peripheral vascular insufficiency, indicating that other factors such as tissue hypoxia rather than extent of atherosclerotic vascular surface may regulate the actual production of PGI₁ in vivo.³⁶ The studies ex vivo in the present report focus on the capacity of vascular tissue to produce prostaglandins under conditions designed to maximize synthesis. The relationship of such studies to steady-state excretion of urinary metabolites of vascular PGI₂ has not been explored. Experimental systems ex vivo generally measure the summed prostaglandin production of endothelium plus that of exposed smooth muscle cells, except in template chamber experiments, which exclude cut edges of tissue from study.

PGI₂ synthesis in vessels from patients treated with low-dose aspirin varied with the interval after the last dose of aspirin, indicating rapid recovery of synthetic capacity by both aorta and saphenous vein, with values of 6-keto-PGF₁₀ ten times more than doubling between 3 and 24 hr after the last dose of 20 mg of aspirin. This finding differs from that of FitzGerald et al.,³⁶ who observed that urinary PGI₂ metabolites remained significantly depressed for several days after cessation of aspirin therapy. It is possible that resynthesis of cyclooxygenase in large vessels, measured here, occurs more quickly than in the microvasculature, which contributes more heavily to the urinary pool of PGI₂ metabolites by virtue of its much larger surface area. An additional explanation is supported by the results of our studies with the template chamber technique, which indicate that resynthesis of cyclooxygenase by endothelium may be more rapid or more extensive after aspirin than resynthesis of cyclooxygenase by vascular smooth muscle. The production of 6-keto-PGF₁₀ by saphenous vein endothelium in the chamber at 24 hr after the final dose of aspirin was equivalent to that produced by tissue from control subjects who had not taken aspirin. In contrast, 6-keto-PGF₁₀ production by vein fragments in which both endothelium and smooth muscle portions were stimulated remained significantly depressed at 24 hr compared with control values. These observations are consistent with increased availability or turnover of cyclooxygenase in endothelium. Indeed, a 20-fold greater content of cyclooxygenase has been observed in bovine aortic endothelium compared with smooth muscle.³⁸ In addition, the experimental design of these studies, with arachidonate stimulation, may better measure a more rapidly renewed pool of cyclooxygenase presumably located in the plasma membrane, as suggested by the tissue culture studies of Willems et al.⁴ The implication of these findings is that normal endothelium has the capacity to "recover" rapidly from daily exposure to
low-dose aspirin in terms of producing PGI₂ at the luminal-blood interface. Whether areas of vessel involved in atherosclerotic plaque formation or lacking normal endothelium might show a different, slower, pattern of recovery of PGI₂ formation remains to be determined.

In conclusion, it appears clear that a low-dose regimen of daily oral aspirin will profoundly inhibit platelet aggregation and thromboxane synthesis in patients with coronary artery disease and at the same time will partially depress vascular PGI₂ production. Administration of low-dose aspirin preoperatively does not appear to compromise hemostasis. Endothelium appears to overcome the aspirin-induced cyclooxygenase block more rapidly than vascular smooth muscle, suggesting another possible antithrombotic feature of the vascular lining. These data strongly suggest that the dosage and schedule of aspirin administration designed as antiplatelet therapy must also be examined for cumulative inhibitory effects on vascular and other tissues.

We wish to express our gratitude to Drs. Paul Stelzer and John McCabe for providing access to their patients, to Dr. S. V. Karwande, and to Dr. Marjorie Topkins of the Department of Anesthesiology and the operating room nursing staff for their assistance. The collaboration of Dr. Eric Grabowski of the Department of Pediatrics in designing the modified template chamber and establishing its use is gratefully acknowledged. We also thank Karen Lamont and Denise Moy for excellent technical assistance.

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Cumulative inhibitory effect of low-dose aspirin on vascular prostacyclin and platelet thromboxane production in patients with atherosclerosis.
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doi: 10.1161/01.CIR.71.2.332

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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