Systemic and transcardiac platelet activity in acute myocardial infarction in man: resistance to prostacyclin

HILTRUD S. MUELLER, M.D., PARINAM S. RAO, PH.D., MARK A. GREENBERG, M.D., PETER M. BUTTRICK, M.D., IRA I. SUSSMAN, M.D., HOWARD A. LEVITE, M.D., RICHARD M. GROSE, M.D., VICTENTE PEREZ-DAVILA, M.D., JANET E. STRAIN, M.D., AND THEODORE H. SPAET, M.D.

ABSTRACT There is increasing evidence that platelets play an important role in the pathogenesis of acute ischemic heart disease. Therefore an understanding of factors that influence platelet performance is important. This study was undertaken (1) to characterize during evolving myocardial infarction platelet activity in the peripheral circulation and across the ischemic/infarcting myocardial compartment, the locus of presumed platelet hyperactivity, and (2) to evaluate the effects of prostacyclin (PGI₂), a most potent antiplatelet agent and vasodilator. A total of 59 patients with evolving myocardial infarction were studied. Twenty-two patients were instrumented with arterial and coronary sinus catheters and received intravenous infusion of PGI₂, 13 ± 4.5 ng/kg/min (mean ± SD), for 90 min. In 15 patients with anterior myocardial infarction, transcardiac platelet function and response to PGI₂ were studied. Plasma levels of β-thromboglobulin (β-TG) and of thromboxane B₂ (TxB₂), in vivo measures of platelet activity, were elevated three- and 10-fold. 6-Keto-prostaglandin F₁₅, the stable end product of PGI₂, was less than 10 pg/ml, reflecting a leftward shift of the TxB₂/PGI₂ ratio. Platelets circulating during evolving myocardial infarction ("ischemic platelets") were hyperaggregable in response to ADP and relatively resistant to PGI₂, both in vivo and in vitro. Concentrations of platelet cyclic AMP and the cyclic AMP response to PGI₂ were diminished. The platelet hyperreactivity, expressed by plasma β-TG, platelet aggregation, and PGI₂-induced inhibition of aggregation, was most intense early during infarct evolution and decreased with time. The increased platelet performance resulted in "platelet fatigue," indicated by decreased contents of β-TG of the ischemic platelet and decreased TxA₂ production in response to collagen. However, the ischemic platelet produced twice normal TxA₂ in response to arachidonic acid (stimulus and substrate), demonstrating a heightened metabolic capacity. TxA₂ was produced across the ischemic/infarcting compartment in 10 of 15 patients with anterior myocardial infarction. The antiplatelet effect of PGI₂ was greatly diminished. In summary, the data define an abnormal pattern of platelet behavior during evolving myocardial infarction, characterized by a proaggregatory environment, heightened platelet reactivity in both the peripheral and coronary circulation, and relative resistance to PGI₂. The clinical consequences of the data are that the patient in the acute phase of myocardial infarction may benefit from suppression of platelet function and requires significantly greater doses of PGI₂ than normal subjects. The data also suggest future directions for therapeutic manipulation of platelet hyperreactivity in the setting of acute myocardial ischemia.

Circulation 72, No. 6, 1336-1345, 1985.

INCREASING EVIDENCE is accumulating that platelets play an important role in the pathogenesis of ischemic heart disease. Previous studies have demonstrated increased circulating platelet release products and platelet aggregates as well as enhanced platelet aggregability in patients with acute myocardial infarction. Clinical and autopsy studies have shown obstructive thrombi and platelet aggre-
gates in acutely occluded coronary vessels. In animal experiments, cyclical flow reduction by platelet aggregates proximal to the obstructive coronary lesion and thromboxane formation distal to the obstruction have been observed. These results have led to the suggestion that increased platelet activity and its consequences contribute to the development of unstable angina, acute myocardial infarction, and sudden cardiac death. Therefore an understanding of platelet function and factors that influence platelet performance is important.

This study was undertaken to characterize both the platelets circulating during acute myocardial infarction and also local and systemic factors that might influence platelet behavior in this setting. Descriptors of platelet function in vivo, such as circulating release products, and platelet responsiveness in vitro to various stimuli were investigated and, whenever feasible, correlated in the attempt to characterize platelet behavior as completely as possible. Transcardiac alterations in platelet function across the ischemic/infarcting myocardial compartment were investigated, since this tissue bed is the locus of presumed platelet hyperactivity.

In addition to these empiric studies, the effect of a potent antiplatelet agent, prostacyclin (PGI₂), on platelet behavior during acute myocardial infarction was evaluated. This prostaglandin is of interest for several reasons: first, it is considered the most potent antiaggregating agent yet described; second, it is synthesized by vascular endothelium and is postulated to provide endogenous biological protection against platelet microthrombi; and third, it has a short serum half-life, making it an attractive agent for the treatment of patients with unstable coronary disease. From this study it was hoped that an approach to the manipulation of platelet function in acute myocardial infarction would emerge.

Methods

Patients. Two groups of patients with acute transmural myocardial infarction were studied: group I patients received only a peripheral venous line, and group II patients were instrumented with two intravascular catheters. Characteristics of the two groups are shown in table 1.

Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>37</td>
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</tr>
<tr>
<td>Age (yr)</td>
<td>62 ± 10</td>
<td>62 ± 11</td>
</tr>
<tr>
<td>Sex (No. of men)</td>
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<td>Infarct site</td>
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<tr>
<td>Anterior/lateral</td>
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<td>Inferior/posterior</td>
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<td>7</td>
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<td>Infarct size (CK₅₀  g-Eq/m²)</td>
<td>--</td>
<td>44 ± 18</td>
</tr>
<tr>
<td>Infarct onset to study (hr)</td>
<td>7.0 ± 3.4</td>
<td>8.5 ± 2.8</td>
</tr>
</tbody>
</table>

TABLE 1

Patient characteristics

been given morphine in the emergency room. All patients received lidocaine as bolus and continuous intravenous infusion (2 mg/min). Two patients in group I and one patient in group II had taken propranolol (not exceeding 10 mg tid), which was discontinued before the study. None of the patients had received calcium-channel blockers. None of the patients showed evidence of cardiac failure based on clinical findings and chest roentgenogram.

Procedure. The protocol was approved by the Institutional Review Board for Clinical Research, Montefiore Medical Center. All patients gave written informed consent.

In group I, blood was obtained by a single 19 gauge needle stick, with the first few milliliters discarded. Patients in group II were instrumented with an intra-arterial and a coronary sinus catheter: (1) a No. 6F catheter (USCI, Billerica, MA) was inserted via a femoral artery into the descending aorta in 14 patients, and a No. 20 gauge Angiocath (Deseret Medical Inc., Sandy, UT) was inserted via a radial artery in eight patients; (2) a No. 7F Baim catheter (Electro-Catheter Co., Rahway, NJ) was inserted via the right internal jugular vein into the coronary sinus and advanced into the great cardiac vein. In seven of 15 patients in whom transcardiac platelet studies were performed, the catheters were coated with heparin (provided by supplier). The catheters were positioned in the coronary care unit by means of radioolucent beds and portable fluoroscopic equipment. They were flushed by a constant infusion pump with isotonic saline without heparin. A multichannel oscilloscope VR 6 (Electronics for Medicine) was used for recording. Measurements of arterial blood pressure, heart rate, and coronary blood flow and blood samples were obtained at baseline, during the end of a 90 min infusion of PGI₂, and 30 min after discontinuation of the infusion.

Plasma thromboxane B₂ (TXB₂) and β-thromboglobulin (β-TG) were determined from blood samples obtained under the following conditions: before instrumentation by venipuncture and shortly after through the catheter (n = 5), after approximately 40 (n = 5) and 90 min (n = 5), and simultaneously by venipuncture and through the catheter (n = 4).

Specific methods. Thromboxane A₂ (TXA₂) and PGI₂ in plasma were assayed by measuring the stable end products, TXB₂ and 6-keto-prostaglandin F₁α (6-keto-PGF₁α), with a radioimmunoassay kit (New England Nuclear RIA-NEK007/008). The method was modified by us to as follows: To avoid falsely elevated TxB₂ levels caused by the buffer matrix and nonspecific impurities introduced during the extraction procedure, we measured TxB₂ directly in plasma without ethyl acetate extraction, and we used human plasma control, 0 ng/ml TxB₂ (New England Nuclear) instead of 50 mM phosphate buffer. To increase the sensitivity, 20 pg of TxB₂ or 6-keto-PGF₁α in human plasma control was added to duplicate specimens. Normal values were 7.3 ± 6.9 (SD) pg/ml TxB₂ and less than 10 pg/ml 6-keto-PGF₁α (n = 20). The reproducibility of
the assay for duplicate determinations was 5.6%. Our normal values for TxB$_2$ and 6-keto-PGF$_{1\alpha}$ are comparable to those reported in the literature using direct assays$^{17-19}$ or extraction followed by purification with reverse-phase high-pressure liquid chromatography.$^{20}$

$\beta$-TG was assayed in plasma with a commercially available radioimmunooassay kit according to the manufacturer's instruction (Code IM 88; Amersham Corp., Arlington Heights, IL). Normal values averaged 34 ± 10 ng/ml (n = 20).

Norepinephrine and epinephrine in plasma were determined by high-pressure liquid chromatography (HPLC, Waters 6000A) with an LC-4 electrochemical detector.$^{21}$ Standards containing 200 pg/ml of norepinephrine and epinephrine were run in parallel. Plasma contents in normal volunteers, obtained after 20 min rest in a supine position, were 168 ± 41 pg/ml for norepinephrine and 47 ± 14 pg/ml for epinephrine (n = 13). The coefficient of variation of 30 duplicate determinations was 2.1 ± 1.2% (norepinephrine) and 4.5 ± 4.2% (epinephrine).

Platelet aggregation in response to ADP (Sigma Chemical Co.) and collagen (Bio Data Corp.) was measured by methods standard in our laboratory.$^{22}$ In brief, blood was carefully drawn and transferred into polypropylene tubes containing 3.2% sodium citrate. This was then centrifuged at room temperature for 2 min 45 sec at 1000 to obtain platelet-rich plasma (PRP). Platelet-poor plasma (PPP) was obtained by centrifuging the remaining pellet at 2200 g for 10 min at +4°C. Platelet cell counts were determined in an uniflow blood counter (Clay Adams), and final PRP concentration was adjusted to 250 to 400 × 10$^6$ platelets/ml with autologous PPP.

Aggregometry was performed in a Payton dual-chamber aggregometer at 37°C with constant stirring (1000 rpm). Serial studies using various concentrations of ADP (0.5 to 8 µM) and of collagen (0.5 to 8 µg/ml) were done to generate dose-response curves. Results are expressed as aggregation to 50% (AG$_{50}$; µM ADP or µg/ml collagen), indicating the amount of ADP or collagen required to achieve 50% change in optical density. Normal values averaged 1.46 ± 0.35 µM ADP (n = 20) and 1.26 ± 0.53 µg/ml collagen (n = 11). Total time from initial phlebotomy to aggregation was greater than 30 min, and all studies were performed within 120 min. Duplicate studies were done at the beginning and end of this study interval to verify internal consistency.

Inhibition of platelet aggregation (INH$_{50}$): Maximal aggregation in response to ADP was determined as described above. PRP was exposed in the aggregometer to varying concentrations of PGE$_2$ (0.5 to 4 ng/ml) for 3 min, followed by addition of 4 µM ADP, and aggregation was determined. Results are expressed as inhibition to 50% (INH$_{50}$; ng/ml PGE$_2$), indicating the amount of PGI$_2$ required to inhibit the maximal change in optical density by 50%.

$\beta$-TG release was measured after 5 min exposure to ADP (0.5 to 8 µM) or collagen (0.5 to 8 µg/ml) in the aggregometer under the conditions described above. Release of $\beta$-TG was stopped by the addition of 10 µM PGE$_2$ and 8 µM indomethacin. The specimen was subsequently centrifuged at 9000 g for 2 min and the supernatant diluted in saline buffer containing 10 µM PGE$_2$ and 8 µM indomethacin. $\beta$-TG was assayed with an Amersham radioimmunooassay kit (see above). Normal values averaged 34 ± 8.7 µg/10$^9$ platelets after ADP exposure (n = 15) and 37 ± 10 µg/10$^9$ platelets after collagen exposure (n = 10).

Platelet $\beta$-TG content: PRP was frozen at −20°C for 15 min and thawed (room temperature) six times.$^{23}$ The lysate was diluted (100×) with saline containing 10 µM indomethacin and 10 µM PGE$_2$. A 50 µl volume of the solution was assayed for $\beta$-TG with an Amersham radioimmunooassay kit (see above). Normal values were 44 ± 9.3 µg/10$^9$ platelets (n = 15).

Platelet thromboxane production was determined according to the method of Ludlam et al.$^{24}$ Briefly, samples of PRP were stimulated in the aggregometer as described above with 50 to 500 µg/ml arachidonic acid (AA), (Bio Data Corp) for 8 min or with 0.5 to 8 µg/ml collagen for 5 min and then centrifuged at 900 g for 2 min. A 50 µl aliquot of supernatant was then transferred into phosphate buffer containing 10 µg/ml diclofenac sodium. TxB$_2$ contents were measured as indicated above with the exception that phosphate buffer was used as a matrix system. Results are expressed as maximum TxA$_2$ production; normal values were 2.5 ± 0.9 ng/10$^9$ platelets after arachidonic acid exposure to AA (n = 20) and 1.0 ± 0.35 ng/10$^9$ platelets after collagen (n = 10).

Cyclic AMP (cAMP) contents in PRP alone and in PRP exposed to 1 ng/ml PGI$_2$ at 37°C for 3 min in the aggregometer were measured by a standard radioimmunoassay kit (DuPont, NEK-0033) modified by us as follows: PRP after stimulation was precipitated with equal volumes of 10% trichloroacetic acid, centrifuged at 9000 g for 2 min, and the supernatant washed four times with fivefold excess volume of ether. The aqueous phase was dried under nitrogen and the residue was resuspended in 1 ml of sodium acetate (0.5 M). cAMP determinations were made with a nonacetylated technique. Normal values in unstimulated platelets were 8.8 ± 3.7 pmol/10$^9$ platelets (n = 20).

Infarct size was measured by serial determinations of plasma creatine kinase–MB (CK-MB) sampling every 2 hr for 12 hr, every 6 hr for 48 hr, and every 12 hr thereafter, until baseline values were reached. Analysis of plasma CK-MB was performed with the ESKACHEM Kit for CK-MB (No. 86239; Smith Kline Co., Sunnyvale, CA), with an immunoinhibition assay at 30°C. Infarct size was calculated according to the formula of Sobel et al.$^{25}$ applying a constant rate of disappearance (Kd). The following values were used for calculation: plasma volume, 4.4% of body weight; Kd, 0.0017/min; CK-MB content of normal myocardium at 30°C, 170 U/g wet weight, of infarcted myocardium, 46 U/g.

Coronary blood flow was measured by thermodilution technique and a Baim double-lumen catheter.$^{15}$ The design of the catheter allows measurement of both coronary blood flow and blood substances.
PGI₂ was kindly provided by Upjohn Co., Kalamazoo, MI. Intravenous infusion of PGI₂ was begun at 2 ng/kg/min and increased at 3 min intervals at increments of 2 ng/kg/min. Limiting end points for the infusion were: (1) increase in heart rate of 20% of baseline and/or absolute heart rate greater than 115 beats/min, (2) decrease in mean arterial pressure of 20% below baseline and/or absolute mean blood pressure less than 65 mm Hg, (3) side effects such as nausea, vomiting, headache, or excess anxiety. The maximum dose tolerated by patients with myocardial infarction for 90 min was 13 ± 4.5 ng/kg/min.

Data analysis. Statistical analysis was performed with Student’s t test for paired and unpaired data. Data with three study points on the same patient were compared by analysis of variance. A p value < .05 was considered significant. Data were expressed as mean ± 1 SD. To reduce the impact of the skewness of the original thromboxane levels in the artery and great cardiac vein on test statistics, which assumes some degree of normality, and to adjust for the exaggeration of the impact of small negative great cardiac vein–arterial differences with large arterial levels (see below), we used percent change and log transformation of the data. Statistical significance of the log of the ratios was calculated by t test. The use of the log and reciprocal functions for calculating relationships between time and the measurements for AG50, INH50, and plasma β-TG concentration was based on analyzing several different types of data transformation and displaying those that provided the best linear fit.

Results

Platelet function in peripheral blood

Group I (not instrumented). Data of 37 patients with myocardial infarction and of 20 normal volunteers are shown in figures 1 and 2. In the patients with infarction, plasma contents of β-TG and TXB₂ were significantly elevated compared with normal. Plasma contents of 6-keto-PGF₁₀ (not shown) averaged less than 10 pg/ml in both groups. Thus the TXB₂/PGI₂ ratio was shifted toward thromboxane. The platelets circulating in the acute phase of infarction (“ischemic platelets”) were highly aggregable: significantly less ADP was required for 50% aggregation compared with normal. Furthermore, the ischemic platelets were relatively resistant to PGI₂; twice the amount of PGI₂ was required for inhibition of aggregation by 50%. Concentrations of cAMP, a biochemical mediator for the inhibitory effect of PGI₂ on aggregation, were decreased in the PRP of patients with infarction compared with normals. PGI₂ (1 ng/ml) increased the cAMP concentrations to 10 (p < .001) and 14 (p = .001) pmol/10⁸ platelets, respectively. The PGI₂-induced changes were similar in both groups, although the maximal cAMP levels in the infarct group remained lower than in normal subjects. The maximal thromboxane production by the ischemic platelet in response to AA was twice that of normal. Plasma contents of norepinephrine and epinephrine (not shown) were approximately fourfold elevated in the infarct group (725 ± 282 vs 176 ± 49 pg/ml, p < .001; 140 ± 112 vs 41 ± 14 pg/ml, p < .001). Platelet counts were similar in both groups (351 ± 109 [infarct] and 384 ± 121 × 10⁸/μl [normals]).

Separation of the platelet function data described above according to anterior/lateral (AMI, n = 25) and inferior/posterior infarction (IMI, n = 12) showed no statistically significant differences between the two subgroups: plasma contents averaged for β-TG, 121 ± 56 (AMI) and 107 ± 74 (IMI) ng/ml; for TXB₂, 73 ± 62 and 82 ± 59 pg/ml; for AG50, 0.88 ± 0.28 and 0.93 ± 0.37 μM ADP; for INH50, 1.79 ± 0.93 and 1.98 ± 1.69 ng/ml PGI₂; for maximal TXA₂ production, 4.38 ± 1.64 (n = 19) and 4.70 ± 1.24 (n = 11) μg/ml AA.

In order to evaluate the impact of the increased release of β-TG and production of thromboxane in vivo on the functional capacity of the ischemic platelet, we studied the platelet response to maximal stimulation in vitro (table 2). In response to collagen, the production of thromboxane was reduced by 46% compared with normal. However, if AA was used as stimulus (and substrate) (figure 2), the ischemic platelet produced twice as much thromboxane as the normal. Platelet β-TG contents of the ischemic platelet were half of normal. Maximal β-TG release in response to collagen approximated 80% in both groups. The amounts released averaged 17 and 37 μg/10⁸ platelets.

Group II (instrumented with two catheters). Baseline
data demonstrate that mean values did not significantly differ between groups I and II: plasma contents averaged for \( \beta-TG \), 110 ± 51 (group II) and 116 ± 60 (Group I) ng/ml; for \( TxB_2 \), 89 ± 62 and 78 ± 69 pg/ml; for \( AG_{50} \), 1.0 ± 0.42 and 0.89 ± 0.31 \( \mu \)M ADP; for norepinephrine, 683 ± 307 and 725 ± 282 pg/ml; and for epinephrine 166 ± 126 and 140 ± 112 pg/ml.

Figure 3 compares plasma contents of \( TxB_2 \) and \( \beta-TG \) from blood samples obtained before placement of catheters and shortly after through the catheter, after approximately 40 and 90 min, and simultaneously by venipuncture and through the catheter. The distribution of the data points is similar above and below the line of identity. The mean values for \( TxB_2 \) are 69 ± 37 (venipuncture) and 65 ± 38 ng/ml (catheter) and those for \( \beta-TG \) are 100 ± 42 and 102 ± 50 ng/ml.

**Platelet function in relation to infarct duration.** Platelet aggregability was highest during the early phase of infarction and decreased with time (figure 6, A): \( AG_{50} = 0.54 \times \log_{10} (hr) \), \( r = .71, p < .0001, n = 37 \). Inhibition of aggregation by PGI\(_2\) was least effective in the early infarct phase and improved with infarct duration (figure 6, B): \( INH_{50} = 9.61/hr, r = .67, p < .0001, n = 26 \). Plasma contents of \( \beta-TG \) were highest early after infarct onset: 246 ± 54 \( \times \log_{10} (hr) \), \( r = -.44, p < .005, n = 41 \). There was no relationship between plasma \( TxB_2 \) and infarct duration. When the data obtained within 4 hr (n = 7) and within 10 to 15 hr (n = 9) after clinical onset of infarction were separated, the differences became accentuated. The average data for \( AG_{50} \) were 0.54 ± 0.35 (early) and 1.0 ± 0.13 (late) \( \mu \)M ADP (p < .01) and the data for \( \beta-TG \) were 194 ± 31 and 106 ± 35 ng/ml (p < .01). Thromboxane contents of the two subgroups did not significantly differ.

**Platelet function across the infarcting myocardial compartment.** We obtained transcardiac measurements in 15 patients with transmural anterior wall infarction, sampling from the aorta and great cardiac vein. We did not include patients with inferior wall infarction because of the inability to sample selectively from the infarcting compartment. Platelet counts were similar in the arterial and great cardiac vein blood: 311 ± 70 and 309 ± 85 \( \times 10^3/\mu \)l. Ten of the 15 patients produced thromboxane across the infarcting compartment (figure 4); the \( TxB_2 \) contents averaged 76 ± 101 pg/ml in the artery and 211 ± 273 pg/ml in the great cardiac vein. In the remaining five patients, no changes in transcardiac thromboxane contents were seen. For the entire group, the arterial–great cardiac vein differences did not reach statistical significance (p = .068). The geometric mean value of the great cardiac vein–arterial ratio of the entire group was 1.85 (p < .005). \( \beta-TG \) contents did not change across the infarcting compartment.

**Effect of PGI\(_2\) on platelet function.** PGI\(_2\) was infused for 90 min at the maximal tolerated dose, 13 ± 4.5 ng/kg/min. Data are reported for baseline, after 90 min of infusion, and 30 min after termination of infusion (table 3). PGI\(_2\) caused a sharp rise in the plasma con-

### Table 2

<table>
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<tr>
<th>Measurement</th>
<th>Infarct</th>
<th>Normal</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal ( TxA_2 ) production (ng/10(^7) platelets)</td>
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</tr>
<tr>
<td>Collagen</td>
<td>0.55 ± 0.20 (10)</td>
<td>1.02 ± 0.35 (10)</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>Maximal ( \beta-TG ) release (( \mu )g/10(^7) platelets)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen</td>
<td>17 ± 11 (12)</td>
<td>37 ± 10 (10)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>ADP</td>
<td>10 ± 8.5 (12)</td>
<td>34 ± 8.7 (15)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Platelet ( \beta-TG ) content (( \mu )g/10(^7) platelets)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>22 ± 7.9 (12)</td>
<td>44 ± 9.3 (15)</td>
<td>&lt; .001</td>
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</tbody>
</table>

Data in parentheses are number of subjects.
receiving the agent; the ratios remained essentially unchanged in four patients and increased in two. The mean values of the ratios of the entire group were 2.2 and 1.5 (NS); the geometric mean values were 1.85 and 1.38 (NS), respectively. The great cardiac vein/arterial ratios for $\beta$-TG (figure 5) were distributed essentially around unity at control measurements and during infusion of PGI$_2$. The mean values were 1.1 and 1.2. Blood flow in the great cardiac vein remained essentially unchanged during administration of PGI$_2$: 62 ± 23 ml/min before and 66 ± 26 ml/min during the infusion, excluding a dilutional effect on the transcardiac PGI$_2$ and $\beta$-TG values.

**Discussion**

Our data clearly demonstrate enhanced platelet reactivity during the early hours of myocardial infarction. In vivo, release of $\beta$-TG and formation of thromboxane are increased, and the relationship between thromboxane and PGI$_2$ is strikingly shifted in favor of thromboxane. In vitro, platelet aggregation in response to exogenous stimuli is enhanced. The ADP dose-response curve of the ischemic platelets is left shifted when contrasted with normal. The alteration in platelet behavior in patients with acute myocardial infarction is also marked by a reduced response to exogenous (and probably to endogenous) PGI$_2$. In vivo, infusion of 10 to 20 ng/kg/min PGI$_2$ increases the required amount of ADP for 50% aggregation from 1.1 to only 2.1 $\mu$m, in contrast to normal volunteers$^{27}$ in whom infusion of 10 to 12 ng/kg/min increased the required ADP from 1.8 to 4.5 $\mu$m. In vitro, twice the amount of PGI$_2$ is required to inhibit platelet aggregation by 50%. Similarly, concentrations of cAMP, a biochemical mediator for the antiaggregatory effects of PGI$_2$, are lower in the ischemic platelet compared with normal and remain lower after exposure to PGI$_2$ in vitro.

These results in patients with acute myocardial infarction and endogenously elevated catecholamines support previous observations by us$^{28}$ and others$^{29, 30}$ that catecholamines change the platelet response to PGI$_2$, presumably by altering the bioavailability of platelet membrane receptors.$^{31-33}$ Using platelets from normal volunteers, we have shown$^{29}$ that subaggregatory doses of epinephrine and norepinephrine abolish the inhibitory effect of PGI$_2$ on aggregation and also the cAMP increase in response to PGI$_2$. $\alpha$-Adrenergic blockade reestablishes both PGI$_2$ effects.

The platelet hyperreactivity in vivo is reflected by alterations of the metabolic capacity of the ischemic platelet as studied in vitro. We realize that in vitro tests may not accurately represent actual physiologic condi-

*FIGURE 4*. Arterial–great cardiac vein differences in plasma levels of TxB$_2$ and $\beta$-TG. Dotted lines represent data obtained with heparin-coated catheters. In two-thirds of the patients, TxB$_2$ levels in the great cardiac vein are twofold to threefold higher. There is no difference in the transcardiac $\beta$-TG levels, except in one patient.

tents of 6-keto-PGF$_{1\alpha}$, the stable metabolite of PGI$_2$. The agent decreased plasma $\beta$-TG and reduced platelet aggregation; the amount of ADP required for 50% aggregation doubled. These PGI$_2$-induced changes were reversible after termination of the infusion. PGI$_2$ tended to decrease plasma TxB$_2$ levels; however, the responses varied widely. Plasma levels of norepinephrine and epinephrine remained unchanged during the infusion.

We evaluated the effect of prostacyclin on platelet reactivity across the infarcting compartment. Data are reported as ratios of great cardiac vein/arterial contents (figure 5). During infusion of PGI$_2$, the TxB$_2$ ratios decreased in six of the eight "thromboxane producers"
TABLE 3
Effect of PGI₃ on platelet function and plasma catecholamines (mean ± SD)

<table>
<thead>
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<th>Measurement</th>
<th>Baseline</th>
<th>90 min on</th>
<th>30 min off</th>
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<tbody>
<tr>
<td>6-keto-PGF₁₀₀₀ (pg/ml)</td>
<td>&lt;10</td>
<td>454 ± 332</td>
<td>39 ± 64</td>
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<tr>
<td>β-TG (ng/ml)</td>
<td>117±51</td>
<td>83±42</td>
<td>133±62</td>
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<tr>
<td>AG₉₀ (μM ADP)</td>
<td>1.09±0.44</td>
<td>2.08±1.09</td>
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<tr>
<td>TxB₂ (pg/ml)</td>
<td>109±87</td>
<td>87±71</td>
<td>103±94</td>
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<tr>
<td>Norepinephrine (pg/ml)</td>
<td>674±278</td>
<td>713±375</td>
<td>577±14</td>
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<tr>
<td>Epinephrine (pg/ml)</td>
<td>139±59</td>
<td>126±57</td>
<td>114±36</td>
</tr>
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</table>

Data in parentheses are p values.

*Compared with baseline values.

Given this limitation, the various measures obtained, however, appear to be consistent with each other. β-TG contents in ischemic platelets are 50% lower than normal. However, the maximal percentage of β-TG released by the two platelet groups is the same, implying that the ischemic platelet has a functionally hyperaggregable in the face of reduced thromboxane formation may imply that additional pathways of platelet aggregation are activated during myocardial ischemia and that antiplatelet therapy aimed only at the cyclooxygenase pathway (such as aspirin) may be less effective than combination therapy in suppressing platelet hyperactivity. Furthermore, it is conceivable that compounds such as TxA₂, serotonin, and catecholamines remain bound to their respective receptors after removal of platelets from the circulation, contributing to the hyperaggregability of the ischemic platelets.

When platelet activity is related to infarct duration, it is evident that the most striking alterations are seen early after onset of infarction. Aggregation (figure 6, A) is most abnormal in the hyperacute phase and tends to normalize as time from clinical onset increases although aggregation remains abnormal. Inhibition of aggregation (figure 6, B) is least effective in the hyperacute infarct phase and improves with time. Plasma contents of β-TG, an in vivo indicator of platelet activity, are highest early after onset of infarction and decline with increasing infarct duration. Whether or not
Thromboxane is produced across the coronary circulation in two-thirds of patients with evolving anterior wall infarction. The reason that this response is not universal is unclear, and no factor can be identified that predicts thromboxane production. Plasma catecholamine contents are elevated in both groups, there is no consistent transcardiac catecholamine increase in the thromboxane producers, and time from onset of infarction to study and size of infarct are not different among groups. We cannot demonstrate transcardiac release of $\beta$-TG, in contrast to thromboxane, perhaps because the amount released at the infarct site is insufficient to influence the high serum concentrations given the long half-life of the compound. It is also possible that $\alpha$-granule function is exhausted in the ischemic compartment, since release is independent of thromboxane production and responds to less potent stimulation.

Transcardiac formation of thromboxane in patients with acute myocardial infarction has been reported previously, although results have varied. Hirsch et al. have shown that thromboxane is produced in patients with unstable angina or recent myocardial infarction but not in patients with stable angina, suggesting that the dynamic character of the coronary lesion may be important. Autopsy studies in patients with sudden cardiac death have identified layers of thrombus of different ages and platelet thrombi in occluded coronary vessels.

There is concern that platelets might be activated by catheter placement, exposure over time, and blood sampling. Our data indicate that catheter-induced platelet activation is unimportant in patients with increased platelet activity before instrumentation. As demonstrated in figure 3, plasma contents of TxB$_2$ and $\beta$-TG do not significantly differ when obtained before and after catheter placement over a period of up to 90 min, or simultaneously by venipuncture and through the catheter. In particular, there is no consistent increase over time. The distribution of the data points is similar above and below the line of identity. Other investigators have failed to show consistent alterations in platelet activity attributable to catheter exposure. Furthermore, we have established the platelet function profile in a matched group of patients with acute myocardial infarction without instrumentation, and the data of the instrumented infarct group are similar to the data of this group.

Intravenous infusion of PGI$_2$ to patients with evolving myocardial infarction produces an attenuated antiplatelet effect. Patients with infarction tolerate higher doses of PGI$_2$ (10 to 20 ng/kg/min) over a longer period
of time than either normal volunteers\textsuperscript{51-53} or cardiac patients without acute myocardial ischemia (8 to 12 ng/kg/min).\textsuperscript{27, 54} The few studies using higher doses report infusions of only 10 to 15 min.\textsuperscript{55, 56} In spite of the higher doses of PGI\(_1\), the inhibitory effect on platelet aggregation is small. At an equivalent infusion rate of 10 to 12 ng/kg/min, the rightward shift of the ADP dose-response curve of the patients with infarction is only 20\% of the shift observed in normal volunteers.\textsuperscript{51} These data obtained during intravenous infusion of PGI\(_1\) support the results in vitro discussed above in which PGI\(_1\) added to PRP of patients with evolving myocardial infarction has an attenuated inhibitory effect on aggregation of ischemic platelets in vitro. The PGI\(_1\)-induced decrease in plasma \(\beta\)-TG (table 3) is moderate, and there is no effect on coronary sinus \(\beta\)-TG levels. PGI\(_1\) does not consistently affect peripheral or transcardiac TxB\(_2\) concentrations.

In summary, we have defined a pattern of abnormal platelet behavior in the acute phase of myocardial infarction. This is characterized by a proaggregatory environment (elevated thromboxane-to-PGI\(_1\) ratio in both systemic and coronary circulation), evidence of heightened platelet activity in vivo (increased circulating \(\beta\)-TG) and increased platelet aggregability in vitro. Platelets from patients with myocardial infarction are relatively resistant to the antiaggregatory effects of exogenous PGI\(_1\). These data and the finding that the platelet abnormalities are most striking in the hyperacute phase of infarction support the contention that platelet aggregation and thrombus formation are important participants in the events of acute myocardial ischemia/infarction.

On the basis of the characterization of the platelet abnormalities, acknowledging the limitations that our patients were studied relatively late after onset of infarction and not allowing a separation of primary from secondary events, future directions for the manipulation of platelet hyperactivity in acute myocardial infarction can be suggested. Conventional antiplatelet agents and PGI\(_1\) are probably insufficient by themselves to influence the proaggregatory environment. Alternative approaches would include agents that decrease the release of catecholamines and thus ameliorate their synergistic effects on endogenous inducers of platelet activity,\textsuperscript{12, 40-42} agents that block the platelet \(\alpha_2\) adrenergic receptors so as to make platelets more responsive to the antiaggregatory effects of PGI\(_1\),\textsuperscript{28-30} agents that potentiate the effect of PGI\(_1\), such as phosphodiesterase inhibitors,\textsuperscript{57} and agents that reduce the effect of thromboxane such as thromboxane synthetase inhibitors or thromboxane receptor antagonists.\textsuperscript{12, 39}

The recognition that the event of myocardial infarction likely involves dynamic thrombus formation, in which both platelets and plasma proteins participate, and that the ischemic platelet has a heightened sensitivity to these plasma constituents might suggest use of both antiplatelet and anticoagulant modalities.

We appreciate the assistance of the nurse specialists, Miss Anne Dyer, Miss Eileen Ferrick, and Mr. Michael Behrens. We thank Miss Joyce Milinowicz for performance of the laboratory tests and Mrs. Virginia Wengerter for preparation of the manuscript.

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1344 CIRCULATION
Systemic and transcardiac platelet activity in acute myocardial infarction in man: resistance to prostacyclin.

Circulation. 1985;72:1336-1345
doi: 10.1161/01.CIR.72.6.1336

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

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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