Platelet Secretion Defect Associated With Impaired Liberation of Arachidonic Acid and Normal Myosin Light Chain Phosphorylation


We describe four patients with impaired platelet aggregation and 14C-serotonin secretion during stimulation with adenosine diphosphate (ADP), epinephrine, collagen, and platelet-activating factor. The response to arachidonic acid was normal in all patients with regard to aggregation and in three of the four with regard to 14C-serotonin secretion. The total platelet adenosine triphosphate (ATP) and ADP content and the ATP to ADP ratio was normal in all patients, thereby excluding storage pool deficiency as the cause of the secretion defect. Studies with 3H-arachidonic acid-labeled platelets revealed that the thrombin-induced liberation of arachidonic acid from membrane-bound phospholipids was impaired in these patients. Further, platelet thromboxane B2 production, measured using a radioimmunoassay, was diminished during stimulation with ADP and thrombin, but was normal with arachidonic acid, indicating that the oxygenation of arachidonic acid was normal and that the diminished thromboxane production was due to a defect in the liberation of arachidonic acid. Release of arachidonic acid is mediated by phospholipases that are Ca2+ dependent. To examine whether these patients may have a defect in making intracellular Ca2+ available, another Ca2+-dependent process, myosin light chain phosphorylation, was studied during thrombin stimulation. Platelets from three of the patients were found to behave the same as normal ones, suggesting that the deficiency in phospholipase activity may not be due to impaired Ca2+ mobilization. Our studies demonstrate a novel group of patients with platelet secretion defects associated with impaired liberation of arachidonic acid from phospholipids. These patients exemplify a congenital defect, other than deficiencies of cyclooxygenase and thromboxane synthetase, by which thromboxane production may be impaired in platelets.

CONGENITAL DISORDERS of platelet function have been classified into disorders of platelet adhesion, primary aggregation, secretion, and platelet procoagulant activities.1 The disorders of secretion are characterized by an inability of the platelets to release their granule contents and are generally identified by the absence of secondary aggregation responses during stimulation of platelet-rich plasma with agonists such as adenosine diphosphate (ADP) and epinephrine. Congenital platelet secretion defects may arise by different mechanisms, and of these, the best characterized is storage pool deficiency, where the contents of dense granules are diminished or absent.2 However, many patients have defects in platelet secretion with normal granule contents. The underlying mechanisms leading to the impaired secretion in the majority of these patients remains to be established. The various abnormalities reported in such patients include deficiencies of the enzymes involved in arachidonate metabolism, such as cyclooxygenase3-5 and thrombox-

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Patient Information

V.H. is a 50-year-old white female with a life-long history of easy bruising associated with occasional bleeding from the gums. She has...
had menorrhagia for over ten years, with a hysterectomy performed in 1982, at which time she was supported with platelet transfusions. In 1975, she fell down a flight of stairs and sustained a head injury. For persistent headache, she took aspirin and subsequently developed altered mental status attributed to a subdural hematoma. Following initial evacuation, she developed reaccumulation of blood, requiring four additional neurosurgical procedures over a period of the next four weeks. Evaluation of the hemostatic system in the subsequent months revealed a marked prolongation of bleeding time (25 minutes). The family history revealed a possible tendency to bruise easily in her daughter, who has not been evaluated by us.

M.G. is a 35-year-old white female with a life-long history of easy bruising and bleeding from the gums on brushing her teeth. She has had epistaxis for at least four years and menorrhagia for over ten years prior to our evaluation. In addition, the bleeding associated with dental extractions was noted to be excessive. Both her daughter and father may also have a history of easy bruising; however, they have not been evaluated. The physical examination on two separate occasions revealed multiple petechiae on her extremities, and she developed numerous fresh ones at the site of the blood pressure cuff. She volunteered a history of marked exacerbation of such lesions each time she took aspirin.

P.P. III is an 11-year-old white male who was evaluated because of excessive postoperative bleeding following repair of an inguinal hernia, which required blood transfusions. He was otherwise an active child, developing ecchymoses only when bumping into objects and had a history of one to two episodes of epistaxis per year over a period of four to five years. His mother and his sister (ages 34 and eight years) are both asymptomatic, with normal bleeding times and platelet aggregation and secretion studies.

P.P. Jr (36 years old) is the father of P.P.-III and has no features to suggest a bleeding diathesis. He has had multiple surgical procedures, including removal of a stone from the ureter and, as a child, tonsillectomy. He has had surgical removal of several subcutaneous nodules as well. There is no knowledge of excessive bleeding in association with any of these procedures. However, his brother was thought to be a "bleeder" on the basis of laboratory tests obtained as preoperative evaluation for a tonsillectomy.

Procedures

Blood was collected in one tenth volume of 3.8% sodium citrate from the patients and normal donors who denied ingesting any drugs for at least ten days. It was centrifuged at 180 g for ten minutes at room temperature to obtain platelet-rich plasma (PRP). The remaining blood was centrifuged at 3,000 g for 30 minutes at 4°C to obtain platelet-poor plasma (PPP), used for coagulation studies. The normal donors were healthy, laboratory personnel and medical students between the ages of 20 and 55 years, who had denied ingesting any medications for at least ten days. They were equally divided between males and females. During each patient evaluation, a normal donor was concurrently studied. Platelet aggregation and 14C-serotonin secretion studies were performed in the normal controls, also, to assess their platelet function.

The bleeding time test was performed using the template method (Simplate II, General Diagnostics, Morris Plains, NJ).

Platelet Aggregation and 14C-Serotonin Secretion

PRP from the patients and normal donors was incubated for 30 minutes with 0.5 μmol/L, 5-hydroxy [side chain-2-14C]tryptamine creatine sulphate (50 μCi/mmol/L) obtained from Amersham, Arlington Heights, IL. The aggregation responses on stimulation of PRP with a number of agonists was monitored using a dual-channel aggregometer (Payton Associates, Buffalo, NY). The extent of aggregation was determined by setting the light transmission of PRP as 0% and that of PPP as 100%. The secretion of 14C-serotonin was assessed as the percent of total 14C-serotonin incorporated that is secreted upon stimulation with the various agonists.23 The different agonists used in these studies included ADP (Sigma, St Louis), epinephrine (Sigma), collagen (Hormon-Chemie, Munchen, Germany), and arachidonic acid (Sigma). The arachidonic acid was neutralized with 0.1 N sodium hydroxide before use. In addition, the response to the following agonists was also tested. The divalent cation ionophore, A23187 (Sigma) was dissolved in dimethylsulfoxide (DMSO, Fisher Scientific, King of Prussia, Pa) and stored at –20°C at concentrations of 25 mmol/L and 50 mmol/L. No more than 0.5 μL of DMSO containing the A23187 was added to the PRP sample (500 μL). The response to the thromboxane A2/endoperoxide analog, U46619 (kindly donated by Dr J. Pike, Upjohn Co, Kalamazoo, MI), was tested in P.P.-III, P.P.-Jr., and V.H., and the response to 9,11-azao PGH2 (kindly donated by Dr D. Harris, Squibb Institute for Biological Research, Princeton, NJ) was tested in M.G. and V.H. The response to platelet activating factor (PAF), a phospholipid derivative (obtained from Avanti Polar Lipids, Birmingham, Ala), was tested in all four patients.

Measurement of Platelet Content of ATP and ADP

The total platelet content of ATP and ADP was measured in ethanolic extracts of PRP using the firefly luciferase–luciferin system described by Holmsen et al.22

Thrombin-Induced Secretion of ATP + ADP and Acid Hydrolases

Platelet-rich plasma was gel-filtered using 2B Sepharose (Pharmacia, Uppsala, Sweden) and Ca2+-free Tyrode's solution containing glucose (5 mmol/L) and albumin (0.2%), as described by Lages et al.23 Aliquots (1 mL) of platelet suspensions were stimulated with bovine thrombin (Parke Davis, Morris Plains, NJ) at final concentrations of 0.1 and 5.0 U/mL for five minutes at 37°C. The percent of total cell-bound ATP and ADP secreted upon thrombin stimulation was determined as described elsewhere.24 Briefly, concentrations of ATP and ADP were measured in ethanolic extracts of the platelet suspensions and the supernatants of thrombin-stimulated platelets by the firefly luciferase–luciferin method using the Luminescence Biometer (Dupont, Wilmington, DE).25 Secretion of two acid hydrolases was also monitored during thrombin stimulation using the above system. The amounts of β-hexosaminidase and β-glucuronidase were measured in the platelets and supernatants using methyl umbellifere derivatives, as described by Dangelmaier and Holmsen.25

Studies With 1H-Arachidonate-Labeled Platelets

PRP was incubated with 0.01 μmol/L. 1H-arachidonic acid (100 Ci/mmol; Code TRK, Amersham) for 60 minutes at 37°C. The platelets were transferred by gel filtration26 to a Ca2+-free Tyrode's solution containing 5 mmol/L glucose and 0.2% human albumin (crystallized and lyophilized; Sigma). Aliquots (1 mL) of gel-filtered platelets were stimulated with saline or multiple concentrations of thrombin (0.06 to 5.0 U/mL) for five minutes at 37°C without stirring. The samples were extracted according to Bligh and Dyer.26 One volume of the incubation mixture was mixed with 3.6 vol of chloroform:methanol (1:2). H2O (1.2 vol) and chloroform (1.2 vol) was added with vigorous mixing, and the mixture was centrifuged at 200 g (room temperature) to enhance phase separation. The bottom phase was evaporated under nitrogen, the residue dissolved in 25 μL of chloroform and subjected to thin-layer chromatography using aluminum sheets coated with silica gel 60 (Merck, cat. No. 5538, without fluorescent indicator) and the upper phase of ethyl
acetate/acetic acid/2,2,4-trimethylpentane (iso-octane)/H₂O (90:20:50:100) as the solvent system, as described by Hong and Levine. In this system, the phospholipids remain at the point of application, while free arachidonic acid (RF = 0.88), HETE (12 hydroxy-5,8,10,14-eicosatetraenoic acid, RF = 0.74), HHT (12 hydroxy 5,8,10-heptadecatrienoic acid, RF = 0.64), thromboxane B₂ (RF = 0.30), and phosphatidic acid (RF = 0.05) migrate. The plates were sprayed with 10% phosphomolybdic acid in ethanol and were heated at 100 °C for ten minutes. The spots produced were scraped from the aluminum sheets, and the radioactivity in the different fractions was determined by liquid scintillation counting using a Beckman 350 LS scintillation counter. The radioactivities in the phospholipids and free arachidonic acid fractions are expressed as a percent of total radioactivity on the plate. In control experiments, the recovery of the radioactivity in the various fractions on the plate was greater than 90%.

**Thromboxane B₂ Production**

The production of thromboxane B₂, a stable metabolite of thromboxane A₂, was measured after stimulation of PRP with ADP (8 μmol/L), thrombin (1 and 5 U/mL), and arachidonic acid (1 mmol/L) using a radioimmunoassay. One-milliliter aliquots of PRP were incubated with the agonist for five minutes at 37 °C with stirring, and thromboxane B₂ levels were measured in the supernatants. In addition, whole blood (1 mL) was allowed to clot spontaneously at 37 °C for 30 minutes, and the thromboxane B₂ levels were measured in the serum. Authentic thromboxane B₂ was a gift from Dr John Pike (Upjohn Laboratories).

**Myosin Light Chain Phosphorylation During Thrombin Stimulation**

Platelet suspensions were prepared in Tyrode's solution, as described above, and were stimulated with thrombin (0.06 U/mL) for various time intervals up to 60 seconds. The platelet pellets were dissolved in a buffer containing 10 mol/L urea, 24 mmol/L Tris-glycine, pH 8.6, and subjected to alkali-urea polyacrylamide gel electrophoresis (PAGE), as described elsewhere. It has been shown by one of us (J.L.D.) that phosphorylation of the 20,000-D myosin light chain results in a more rapid migration of this protein on alkaline-urea PAGE and that thrombin stimulation causes a dose-dependent shift from the native to phosphorylated state. The extent of myosin phosphorylated was determined by scanning of the gels with a Schoeffel 5300 scanner at 550 nm/L, followed by peak area analysis using a TRS-80 model III microcomputer and digitizer.

**RESULTS**

The bleeding time was prolonged in three of the patients, being 15 minutes in V.H. and P.P.-III and greater than 15 minutes in M.G. (normal two to eight minutes). In P.P.-Jr, it was eight minutes.

**Platelet Aggregation and ¹⁴C-Serotonin Secretion in Platelet-Rich Plasma**

The platelet aggregation and secretion of ¹⁴C-serotonin in response to the various agonists is shown in Fig 1. During stimulation with ADP (8 μmol/L), shape change and primary wave of aggregation were noted in all four patients. The secretion of serotonin was impaired in all of them. With epinephrine (8 μmol/L), PAF (19 μmol/L) and collagen (2.5 μg/mL) both aggregation and secretion were uniformly impaired in all the patients. In contrast, during stimulation with arachidonic acid (1 mmol/L), the aggrega-
DEFECT IN PLATELET ARACHIDONATE RELEASE

Table 1. Total ATP and ADP Content of Platelets

<table>
<thead>
<tr>
<th></th>
<th>ATP (μmol/10^11 plat)</th>
<th>ADP (μmol/10^11 plat)</th>
<th>ATP/ADP Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>V.H.</td>
<td>5.63</td>
<td>3.21</td>
<td>1.75</td>
</tr>
<tr>
<td>M.G.</td>
<td>4.96</td>
<td>3.71</td>
<td>1.34</td>
</tr>
<tr>
<td>P.P. III</td>
<td>6.21</td>
<td>3.96</td>
<td>1.57</td>
</tr>
<tr>
<td>P.P. Jr</td>
<td>5.73</td>
<td>4.21</td>
<td>1.26</td>
</tr>
<tr>
<td>Normals (N = 16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>5.84</td>
<td>3.49</td>
<td>1.74</td>
</tr>
<tr>
<td>Range of values</td>
<td>3.07–8.80</td>
<td>2.25–4.90</td>
<td>0.69–3.20</td>
</tr>
</tbody>
</table>

tion response appeared normal in all. The secretion of serotonin was normal in platelets from M.G., P.P. Jr, and P.P. III, but strikingly impaired in V.H. The response to U46619 (1 μmol/L) was tested in two patients (P.P. Jr and P.P. III) on two occasions each. Only reversible aggregation was noted in P.P. III, whereas it was irreversible on one of two studies in P.P. Jr. The serotonin secretion in P.P. III (2% and 17%) and P.P. Jr (1% and 11%) was impaired (normal 27% to 51% in three studies). The aggregation and secretion responses to 9,11-azo PGH2 (0.5 μmol/L) were normal in M.G. but impaired in V.H. (results not shown).

Total Platelet ATP and ADP Content and Thrombin-Induced Secretion of ATP + ADP and Acid Hydrolases

In order to examine whether storage pool deficiency was the cause of the secretion defect, the total platelet ATP and ADP content and the ATP to ADP ratio was measured and found to be normal in all patients (Table 1).

Secretion of ATP + ADP during stimulation with a high concentration of thrombin (5 U/mL) was normal in P.P. III and P.P. Jr and decreased in M.G. It was normal in V.H. on one of two occasions studied (Table 2). However, with a lower concentration of thrombin (0.1 U/mL), it was normal only in P.P. Jr and was abnormal in the other three. Secretion of the acid hydrolases appeared to be normal during stimulation with the higher thrombin concentration in all patients. However, in three patients, β-glucuronidase secretion results were at the lower limits of the values found in the normals. With lower thrombin concentration, acid hydrolase secretion was abnormal in several of the studies: secretion of both enzymes was impaired in M.G. and on one of the two occasions studied in V.H. Overall, these studies suggest that dense granule and acid hydrolase secretion in response to a strong stimulus (thrombin 5 U/mL) was generally normal in these patients, whereas a weaker stimulus (thrombin 0.1 U/mL) was associated with impaired secretion on multiple occasions.

Studies on Arachidonate Metabolism

Studies involving the 3H-arachidonate-labeled platelets revealed abnormalities in all four patients. In the resting state, the total radioactivity incorporated into the platelets from the patients was similar to that in normal platelets. Shown in Fig 2 are the changes in the percent of total radioactivity present in the phospholipid and free-arachidonic acid fractions following stimulation of platelets with increasing concentrations of thrombin. In the platelets from the normal subjects and the patients, over 95% of the total radioactivity was present in the phospholipid fraction prior to stimulation. Following stimulation of normal platelets with thrombin (5 U/mL), there was a decline in the radioactivity in the phospholipid fraction to 77% ± 2% (mean ± SE). This was associated with an increase in the radioactivity in the free-arachidonic acid fraction from 1.5% ± 0.3% in unstimulated platelets to 14.1% ± 1.4% following thrombin (5 U/mL) stimulation. In contrast, in platelets from the patients, over 85% of the radioactivity was still in the phospholipids following thrombin (5 U/mL) stimulation. Furthermore, the

Table 2. Thrombin-Induced Secretion of Adenine Nucleotides and Acid Hydrolases

<table>
<thead>
<tr>
<th>Patient</th>
<th>ATP + ADP (0.1 U/mL, %)</th>
<th>5.0 U/mL (%)</th>
<th>β-Hexosaminidase (0.1 U/mL, %)</th>
<th>5.0 U/mL</th>
<th>β-Glucuronidase (0.1 U/mL, %)</th>
<th>5.0 U/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>V.H.</td>
<td>10/14*</td>
<td>13/37</td>
<td>0/31</td>
<td>29/40</td>
<td>0/24</td>
<td>15/36</td>
</tr>
<tr>
<td>M.G.</td>
<td>12</td>
<td>23</td>
<td>5</td>
<td>50</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>P.P. III</td>
<td>3</td>
<td>64</td>
<td>17</td>
<td>65</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>P.P. Jr</td>
<td>63</td>
<td>67</td>
<td>5</td>
<td>53</td>
<td>15</td>
<td>40</td>
</tr>
<tr>
<td>Normals</td>
<td>Mean</td>
<td>35</td>
<td>46</td>
<td>21</td>
<td>42</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Range of values</td>
<td>18–66</td>
<td>28–73</td>
<td>8–37</td>
<td>18–65</td>
<td>3–33</td>
</tr>
</tbody>
</table>

Aliquots (1 mL) of platelet suspensions were stimulated for five minutes at 37 °C with thrombin (0.1 U/mL or 5 U/mL).

*Patient V.H. was studied on two occasions.
†The figures in parentheses indicate the number of normal controls studied.
Studies on Myosin Light Chain Phosphorylation

Phosphorylation of myosin light chain during thrombin stimulation was examined in platelets from P.P. III, P.P. Jr, and M.G. and in four normal controls. In all three patients, both the extent and the time course of phosphorylation were within normal limits (Fig 3).

DISCUSSION

Stimulation of platelets results in a number of responses, including shape change, aggregation, secretion of granule contents, and liberation of arachidonic acid from phospholipids. We described four patients in whom platelet dense granule secretion in response to ADP, epinephrine, PAF, and collagen was markedly impaired despite normal dense granule contents of ATP and ADP. In response to arachidonic acid, aggregation was normal in all four patients; secretion was normal in three and abnormal in V.H. The primary aggregation in response to ADP stimulation was normal in these patients, thereby excluding Glanzman's thrombasthenia. Direct measurements of the platelet ATP and ADP content revealed that these patients did not have a storage pool deficiency to explain the secretion defect. However, it was noted that thromboxane B2 production in PRP in response to ADP and thrombin (1 U/mL) was diminished, whereas with arachidonic acid, it was normal. Further studies using platelets labeled with 3H-arachidonic acid revealed that the liberation of arachidonic acid

percent of total radioactivity in free-arachidonic acid fraction was much less than in the normals, following stimulation with 1 and 5 U/mL of thrombin. These results indicate that the platelets from the patients incorporate arachidonic acid normally into platelet phospholipids, but have impaired liberation of this fatty acid upon stimulation with thrombin.

Thromboxane B2 production, measured by using a radioimmunoassay during stimulation of PRP with ADP and thrombin (1 U/mL), was impaired in all patients (Table 3). However, at a higher concentration of thrombin (5 U/mL), it was abnormal only in V.H. and normal in the other three. In whole blood serum thromboxane levels were decreased in V.H. and M.G., but normal in P.P. III and P.P. Jr. During stimulation of PRP with arachidonic acid (1 mmol/L), thromboxane production was normal in all four patients.

Table 3. Measurement of Thromboxane B2 Production

<table>
<thead>
<tr>
<th>Whole Blood</th>
<th>Platelet-Rich Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Thrombin (1 U/mL)</td>
</tr>
<tr>
<td>(pmol/10^9 plat)</td>
<td>(pmol/10^9 plat)</td>
</tr>
<tr>
<td>V.H.</td>
<td>44</td>
</tr>
<tr>
<td>P.P. III</td>
<td>248</td>
</tr>
<tr>
<td>P.P. Jr</td>
<td>115</td>
</tr>
<tr>
<td>M.G.</td>
<td>29</td>
</tr>
<tr>
<td>Normals</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>223</td>
</tr>
<tr>
<td>Range of values</td>
<td>94-407</td>
</tr>
</tbody>
</table>

Thromboxane A2 levels were measured by radioimmunoassay for thromboxane B2 in serum from spontaneously clotting whole blood and in the supernatants from platelet-rich plasma stimulated with ADP, thrombin, and arachidonic acid. The figures in parentheses reflect the number of normals studied.
defect in arachidonate liberation were briefly
discribed a patient with impaired liberation of anachidonic acid from the phospholipids. However, in this
described in an abstract.3 Also, Rendu et al32 have
impaired liberation of anachidonic acid from mem-
brane phospholipids has not been adequately docu-
mented previously. Three other patients with a similar
defect in arachidonate liberation were briefly
described in an abstract.31 Also, Rendu et al32 have
described a patient with impaired liberation of arachidon-
acid from the phospholipids. However, in this
patient, unlike in our cases, the uptake and incorpora-
tion of the radioactive arachidonic acid into the mem-
brane phospholipids was also abnormal. Moreover, this
patient had the Hermansky-Pudlak syndrome, with
the associated storage pool deficiency, making it diffi-
cult to evaluate the major mechanism leading to the
impaired platelet function. None of the patients
reported by us had storage pool deficiency or impaired
arachidonate oxygenation and, thus, constitute a novel
group of patients with platelet secretion defects asso-
ciated with impaired liberation of arachidonic acid.

In the resting platelet, virtually all of the arachi-
don acid is present esterified in the phospholipids. Thus, a major requirement and rate-limiting step in
the synthesis of thromboxane A2 and endoperoxides is
the release of free arachidonic acid from phospho-
lipids, which may occur through several mechanisms.
One general mechanism is mediated by phospholipase
A2 (may be of various specificities) with the break-
down of phosphatidylincholine, phosphatidylethanol-
amine, phosphatidylinositol, and phosphatidic acid to
yield free arachidonate.11,12 Another mechanism
involves the phosphatidylinositol–phosphatidic acid
cycle; phosphatidylinositol is broken down by phos-
pholipase C to yield diacylglycerol, which is acted upon
either by diglyceride lipase to free arachidonic acid or
by diglyceride kinase to produce phosphatidic acid.13
There is evidence that phosphatidic acid enhances the
activity of phospholipase A2.14 Thus, impaired liberation
of arachidonic acid from free phospholipids may
be due to a deficiency in one or more of these involved
enzymes. However, there is evidence that the activities
of phospholipase C15 and phospholipase A216 are depen-
dent on the availability of calcium. It has been postu-
lated that an early step during platelet activation is
mobilization of intracellular Ca++ , which is required in
platelet mechanisms such as the liberation of arachi-
donic acid, phosphorylation of myosin light chain, and
secretion.33,34 Therefore, in the patients described,
there may be a defect in platelet Ca++ mobilization,
leading to defects both in secretion and arachidonate
release. To obtain evidence for such an abnormality,
we examined myosin light chain phosphorylation,
which is a Ca++-dependent process.18 In three patients
(M.G., P.P. Jr, and P.P. III), the aggregation and
secretion responses were normal during stimulation
with arachidonic acid, as was myosin phosphorylation
during thrombin stimulation. Perhaps in these
patients, the platelet defect in the liberation of arachi-
donic acid is due to a deficiency in the involved
enzymes rather than secondary to a defect in intracel-
lar Ca++ mobilization. However, the patients may
have a partial defect in Ca++ mobilization, such that the
Ca++ concentrations achieved are adequate to
support myosin phosphorylation but not arachidonate
liberation. This is consistent with the normal primary
aggregation in these patients, as myosin phosphoryla-
tion appears to be associated with shape change and
primary aggregation responses in platelets.35 In patient
V.H., despite a normal aggregation response to arachi-
donic acid, 14C-serotonin secretion was markedly
impaired, suggesting that, in addition to the defect in
mobilization of arachidonic acid, she has a defect in
mechanisms governing secretion. In her platelets, a
primary defect in a generalized process such as Ca++
mobilization, leading to both diminished liberation of
arachidonic acid and impaired secretion, may be pres-
ent. Results of the measurement of thromboxane B2
production during stimulation of platelets with arachi-
donic acid clearly indicate that in V.H., the secretion
defect is not due to diminished thromboxane produc-
tion related to impaired liberation of AA; the secretion
was impaired despite normal thromboxane production
upon incubation with this fatty acid. Even though
additional studies examining Ca++ mobilization in
platelets need to be carried out, our observations
suggest that, despite the common defect in the release

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**Fig 3.** Myosin light chain phosphorylation in platelets during stimulation with thrombin (0.06 U/mL). Gel-filtered platelets were stimulated with thrombin, extracted, and subjected to alkali-urea polyacrylamide gel electrophoresis. The extent of phosphorylation was obtained by scanning the gels as described in Materials and Methods. The open circles and the bars represent the mean ± SD in four normal controls, and the closed symbols represent the patients: P.P. III (○), P.P. Jr (△), and M.G. (□).
of arachidonic acid in all four patients, the underlying mechanisms in these patients may be different.

The measurements of thromboxane production during stimulation of platelets from the patients with different agonists provide additional information on the nature of the abnormality. Thromboxane B2 production, measured using a sensitive radioimmunoassay, was impaired in all four patients during stimulation with ADP and thrombin (1 U/mL). In contrast, with arachidonic acid (1 mmol/L) stimulation, it was normal in all patients, indicating that the steps involved in the oxygenation of arachidonic acid to thromboxane B2 are intact. Thus, the impairment in thromboxane B2 production noted with the other agonists is related to a decreased ability of these agonists to liberate arachidonic acid. Patients with defects in thromboxane production due to deficiencies of cyclooxygenase \(^ \text{1-5} \) and thromboxane synthetase \(^ \text{6,7} \) have been reported previously. The patients described by us demonstrate another mechanism by which thromboxane production may be impaired. Interestingly, thromboxane B2 production during stimulation with a high concentration of thrombin (5 U/mL) was normal in three of the four patients despite the impaired liberation of arachidonic acid observed at the same thrombin concentration in the studies with \(^ {3} \text{H}-\text{arachidonic acid.} \)

It is known that arachidonic acid is preferentially utilized by the cyclooxygenase–thromboxane synthetase pathway in platelets, as compared to the lipoxygenase pathway. Therefore, the normal thromboxane B2 production noted with a high concentration of thrombin may be related to a preferential oxygenation of the liberated arachidonic acid to thromboxane A2, even though the mobilization of arachidonic acid from phospholipids is to a much lesser extent than in the normals. These observations may also explain the normal secretion noted in most of the studies during stimulation with the high concentrations of thrombin (Table 2).

The divalent cationophore A23187 induces platelet activation independently of extracellular Ca\(^ {++} \), and it has been suggested that this agent activates platelets by mobilizing intracellular Ca\(^ {++} \). We therefore tested the response to A23187 (12 μmol/L) in the four patients and found secretion to be impaired in P.P. III and V.H. However, there is evidence that platelet responses to A23187 are dependent on both close cell contact induced by stirring of the PRP during aggregation studies and on thromboxane production. Therefore, even though platelet secretion was impaired in two patients during stimulation with A23187, it is difficult to draw any conclusions regarding Ca\(^ {++} \) mobilization from these observations.

It is interesting to note that response to platelet activating factor was markedly abnormal in all patients, with only a primary wave elicited. Vargaftig et al. have proposed that PAF induces platelet activation by a pathway independent of thromboxane A2 production and the secreted ADP. Studies from several laboratories, including ours, have demonstrated that the full response of human platelets to PAF is dependent on the feedback mechanisms mediated by thromboxane A2 and ADP. Because the response to PAF is impaired in platelets from patients presented here who have defects in the response to a number of agonists, it suggests that this agent activates platelets by mechanisms that are common to multiple agonists rather than by an independent “third” pathway.

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DEFECT IN PLATELET ARACHIDONATE RELEASE

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Platelet secretion defect associated with impaired liberation of arachidonic acid and normal myosin light chain phosphorylation

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