VITAMIN E and derivatives of vitamin E are compounds that have been frequently suggested as having potential as antithrombotic agents.\textsuperscript{1, 12} However, this group of compounds has had a rather varied history with different workers finding different results, some showing striking effects and others no effect. Furthermore, in some studies there has been the suggestion that therapeutic benefits of vitamin E are not immediate, but begin only after patients have been on the drug for some weeks.\textsuperscript{4} One possible explanation for the variation in clinical benefit and the time delay to onset seen in some studies could be that a metabolite, rather than the original compound, is the active ingredient, and that vitamin E preparations might be variably contaminated by the active metabolite. A metabolite of vitamin E that may be a contaminant in varying degrees of vitamin E preparations is the oxidized form of the vitamin, vitamin E quinone.

In the present investigation, we have evaluated the influence of vitamin E quinone on platelet function and platelet and endothelial cell thromboxane and prostacyclin synthesis. The results demonstrate not only that vitamin E quinone is considerably more potent as an inhibitor of platelet function than vitamin E, but also that vitamin E quinone has very different biochemical actions than vitamin E. The results suggest that previous studies with vitamin E need to be reassessed and that in future clinical and biochemical studies with this agent, it is critical to distinguish between effects of the vitamin and its oxidized metabolite.

**MATERIALS AND METHODS**

**Materials**

Reagents were obtained from the following sources: arachidonic acid (AA) greater than 99\% pure, from NuChek Prep, Elysian, Minn.; nitro blue tetrazolium (NBT), acetylsalicylic acid, citric acid, trisodium citrate, glucose, vitamin E (D-\(\alpha\)-tocopherol), disodium EDTA, and epinephrine from the Sigma Chemical Co., St. Louis, Mo.; 1,4-benzoquinone and 1,4-naphthaquinone from Aldrich Chemical Co.; 1,14\textsuperscript{C}-arachidonic acid (\(\textsuperscript{14}C\)-AA) from Applied Science; 2,3\textsuperscript{14}C-5-hydroxytryptamine (\(\textsuperscript{14}C\)-5HT) from Amersham/Searle; and calf-skin collagen as an acid-soluble collagen from Worthington Biochemical Corp., Freehold, N.J. All other reagents used were of analytical grade.

Vitamin E quinone (VEQ) was prepared by oxidation of a film of vitamin E with concentrated nitric acid at room temperature. The film was rinsed with distilled water until the pH was greater than 5 and then dissolved in absolute ethanol at a concentration of 100 mg/mL. Fresh solutions were required because vitamin E quinone was slowly reduced in ethanol back to tocopherol. The concentration of vitamin E in stock solutions was 100 mg/mL in ethanol. Stock solutions of 10 mM naphthaquinone in ethanol and 10 mM benzoquinone in 10% ethanol were also prepared. When using these reagents to evaluate effects on platelets or platelet biochemistry, ethanol controls were always included, and the concentration of ethanol never exceeded 1%.

Vitamin E quinone was identified by its mass fragmentation pattern determined using an LKB Model 9000 mass spectrometer, ultraviolet absorption spectrum measured in methanol in a Beckman Model 24 spectrophotometer (Table I), and high-pressure liquid chromatographic behavior (Fig. 1). Prostaglandin \(\text{G}_3\), was prepared according to the procedure of Graff et al.\textsuperscript{13} Human umbilical cord endothelial cells were kindly provided by Dr. Charles F. Moldow. These cells were cultured as described before\textsuperscript{16} using the method of Jaffe et al.\textsuperscript{17} and characterized using fluorescein-labeled rabbit antiserum directed against human factor VIII antigen.\textsuperscript{18}

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Table 1. Identification of Vitamin E Quinone

<table>
<thead>
<tr>
<th>Major Mass Fragments in Daltons</th>
<th>Percentage of Maximum Single Ion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VEQ</td>
</tr>
<tr>
<td>446</td>
<td>10</td>
</tr>
<tr>
<td>430*</td>
<td>7</td>
</tr>
<tr>
<td>221</td>
<td>100</td>
</tr>
<tr>
<td>205*</td>
<td>5</td>
</tr>
<tr>
<td>178</td>
<td>74</td>
</tr>
<tr>
<td>165*</td>
<td>25</td>
</tr>
</tbody>
</table>

Wavelength of Absorbance

Peaks (nm)

Sample | Found  | Reported* |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E quinone</td>
<td>269</td>
<td>269</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>292</td>
<td>292</td>
</tr>
</tbody>
</table>

Preparation of Platelet-Rich-Plasma

Platelets for these studies were obtained from healthy adult volunteers after informed consent. Blood drawn from an antecubital vein into plastic syringes was mixed immediately with 100 mM sodium citrate buffer containing 1.36 mM glucose, pH 6.5, in a ratio of 9 parts blood to 1 part anticoagulant. Platelet-rich-plasma (C-PRP) was separated by centrifugation at 200 g for 20 min at room temperature. Platelet-poor plasma was prepared by centrifugation of anticoagulated blood at 1500 g for 20 min.

Platelet Aggregation and Secretion Studies

Aggregation of C-PRP was monitored using a dual-channel Payton aggregometer set at a stirring speed of 1000 rpm. A variety of aggregating agents (Table 2) were studied. For each of these agents, a single concentration chosen to give full aggregation was used. Samples of C-PRP were usually incubated without stirring for 10 min at 37°C with vitamin E, vitamin E quinone, or ethanol before addition of the aggregating agent on the aggregometer. In a few experiments, C-PRP prewarmed to 37°C was stirred with vitamin E, vitamin E quinone, or ethanol for 1 min before addition of aggregating agent. For vitamin E and vitamin E quinone, the inhibitory concentration (IC50) was determined as that concentration required to reduce, by half, the maximum extent of the change in light transmission achieved on stirring the aggregating agent with C-PRP preincubated with the ethanol alone. In experiments with adenosine diphosphate (ADP), aspirin was added to the platelets before incubation. However, trial experiments suggested that the same IC50 values would have been obtained without the addition of aspirin. In experiments with prostaglandin G2 (PGG2), PGG2 in ethyl acetate was placed in the aggregometer cuvettes, evaporated under a stream of nitrogen, and prewarmed C-PRP (with or without inhibitors) added immediately, and aggregation monitored. Secrecion of 14C-SHT was measured using a modification of the method of Jerusalmy and Zucker. Endogenous serotonin content of platelets was evaluated spectrophotometrically as described earlier. Platelet nucleotides were assessed using high-pressure liquid chromatography, except for cAMP levels which were measured by a protein-binding assay. Platelet ultrastructure was evaluated on fixed samples as described previously by White.

Reversibility of Inhibition

To evaluate the reversibility of inhibitors, C-PRP incubated with inhibitor for 10 min at 37°C was diluted 1:1 with the citrate

Table 2. Effect of Vitamin E (VE) and Its Quinone Form (VEQ) on the Release of Serotonin From Platelets

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Without</th>
<th>With Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VE</td>
<td>VEQ</td>
</tr>
<tr>
<td>Control</td>
<td>5.7 ± 0.2</td>
<td>—</td>
</tr>
<tr>
<td>Collagen (30 μg/ml)</td>
<td>50 ± 1</td>
<td>45 ± 1</td>
</tr>
<tr>
<td>Epinephrine (5.5 μM)</td>
<td>32 ± 2</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>ADP (5 μM)</td>
<td>49 ± 2</td>
<td>50 ± 1</td>
</tr>
<tr>
<td>Arachidonic acid (780 μM)</td>
<td>60 ± 1</td>
<td>55 ± 1</td>
</tr>
<tr>
<td>Thrombin (0.2 U/ml)</td>
<td>49 ± 1</td>
<td>47 ± 1</td>
</tr>
<tr>
<td>Phorbol myristate acetate (10 M)</td>
<td>32 ± 1</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>A23187 (10 M)</td>
<td>67 ± 1</td>
<td>65 ± 1.7</td>
</tr>
</tbody>
</table>

Mean and the standard error (n = 3).

*Platelet-rich plasma was incubated with 2 mM VE or VEQ for 10 min at 37°C prior to testing with releasing agents.
Vitamin E Quinone and Platelet Function

anticoagulant solution described above, pelleted by centrifugation for 20 min at 700 g, and resuspended in Hanks' balanced salt solution at half the original C-PRP volume. An equal volume of fresh citrated platelet-poor plasma was added and the platelet suspensions were processed exactly as indicated for inhibitor-treated samples served as the control.

Platelet Arachidonic Acid Metabolism

Platelet arachidonate release was assessed by measuring the amount released from platelets in which the phospholipids had been prelabeled with 1-14C-arachidonic acid, as described previously. Platelet prostaglandin and thromboxane synthesis was assessed by adding 14C-arachidonic acid to washed platelets, and following the conversion of the labeled fatty acid to hydroxy derivatives and to thromboxane B2, using thin-layer chromatography of the methyl esters on silica gel G with the organic layer of isooctane:ethyl acetate (100:100:50, v/v) as the eluting solvent (system A). The radioactive zones were identified by comparison to those patterns obtained previously in which the substance at each zone was established by gas chromatography–mass spectrometric analysis.

Conversion of labeled arachidonic acid to thromboxane B2 was also evaluated using thin-layer chromatography of the free acids on silica gel G with diethyl ether:methanol:acetic acid (135:5:3, v/v) as the eluting solvent (system B).

Assessment of Platelet Calcium Flux

Calcium flux was studied using membrane vesicles prepared according to Gerrard et al., which is a further modification of the method of Robblee et al. that was first modified by Kaser-Glanzmann et al. These vesicles were equilibrated with calcium 45 in the presence of adenosine triphosphate (ATP) and magnesium for 10 min, at which time a sample was taken to assess uptake of calcium by the vesicles. To prevent further uptake of calcium, 100 mM EDTA was added immediately by 5 mM A23187 to initiate calcium release. After 20 min, a second sample was taken to measure the amount of labeled calcium released from the membrane vesicles. Vitamin E or vitamin E quinone solubilized in soybean oil and ethanol were added at time 0. To the control, without vitamin E or vitamin E quinone, a similar amount (7 µl/ml) of vehicle (30% soybean oil in ethanol) was added at time 0.

Endothelial Cell Prostacyclin (PGI2) Production

Arachidonic acid was supplied exogenously to cultured endothelial cells, and conversion of this fatty acid to PGI2 was estimated from the inhibition of thrombin-induced aggregation of platelets and from the conversion of labeled arachidonic acid to 6-keto-PGF1α, the major metabolite of PGI2. Endothelial cells were supplied as monolayers in 5-cm Petri dishes covered with growth media. For experiments using platelet aggregation to monitor PGI2 production, the media was poured off and 1 ml of Hanks' balanced salt solution (HBSS) or platelet-poor plasma containing 2.3 mM acetyl salicylate was added to 900 µl of a washed platelet suspension, stirred for 1 min on the aggregometer, and then 10 µl containing 0.2 U of thrombin was added. The delay in the initiation of aggregation was taken as a measure of the PGI2 formed.

For experiments in which the conversion of labeled arachidonic acid was evaluated, 2 nmole 14C-arachidonic acid (specific activity 50 mCi/nmole) was incubated with the endothelial cells for 30 min at 37°C. After a 10-min incubation, this solution was replaced with 200 µl of 25 µg arachidonic acid/ml HBSS. After 2 min, 100 µl of this solution was added to 900 µl of a washed platelet suspension, stirred for 1 min on the aggregometer, and then 10 µl containing 0.2 U of thrombin was added. The delay in the initiation of aggregation was taken as a measure of the PGI2 formed.

RESULTS

Platelet Aggregation and Secretion Studies

Vitamin E quinone more effectively inhibited platelet aggregation than vitamin E. The inhibition was greater against all aggregating agents tested, including arachidonic acid, collagen, A23187, thrombin, epinephrine, phorbol myristate acetate, and ADP. A dose-inhibitory response curve for the inhibition of epinephrine-induced aggregation is shown in Fig. 2. The concentration of vitamin E or vitamin E quinone...
required to produce 50% inhibition (IC₅₀) of certain of these aggregating agents is shown in Table 2. The results show clearly that vitamin E quinone is 5–10 times more potent than vitamin E in inhibiting platelet aggregation. Other quinones were also effective; benzoquinone and naphthoquinone had IC₅₀ values against ADP-induced aggregation of 160 and 20 μM, respectively. During these studies it was also noted that vitamin E quinone in low concentrations could completely inhibit the second wave of epinephrine aggregation without affecting the first wave, whereas vitamin E did not inhibit the second wave without also suppressing the first wave. The other quinones also displayed this selectivity against second wave aggregation.

Platelets exposed to vitamin E quinone significantly inhibited secretion of platelet serotonin at concentrations where vitamin E alone was ineffective (Table 3). This result was found for all aggregating agents tested and confirms the significantly greater potency of the oxidized vitamin on platelet function.

**Studies of Platelet Ultrastructure**

Thin sections of platelets incubated with vitamin E quinone (VEQ) or alcohol carrier at the highest concentrations employed in this study for intervals up to 2 hr revealed no evident changes in morphology. The platelets retained their characteristic discoid form. Dense bodies, granules, and mitochondria appeared unaffected and were randomly distributed in the platelets after incubation (Fig. 3). The influences of the carrier and VEQ on the physical changes in platelets after stirring with aggregating agents were also examined in the electron microscope. Samples of C-PRP with the same concentrations of alcohol used to dissolve VEQ had no effect on the aggregation induced in C-PRP by thrombin, collagen, A23187, epinephrine, and ADP. Samples fixed 3 min after addition of the aggregating agent on the aggregometer revealed platelet shape change, internal contraction, loss of granule contents, and irreversible aggregation.

These changes were identical to the alterations in control samples without alcohol fixed at the same time after exposure to the same concentrations of aggregating agents and similar to previously described effects of these agents. Samples of C-PRP incubated with VEQ before exposure to aggregating agents on the aggregometer differed strikingly from the response of control platelets in C-PRP with alcohol alone. VEQ-treated platelets appeared completely unresponsive to the same amounts of thrombin, collagen, ADP, A23187, and epinephrine, which produced irreversible alterations in the control samples without VEQ. Stirring with collagen produced a slight shape change in about 20% of the VEQ-treated cells, but the rest of the platelets retained their discoid shape. No changes in discoid form or internal organization were noted in platelet samples containing VEQ after stirring with A23187, thrombin, ADP, or epinephrine.

**Investigation on Possible Chemical and Metabolic Alterations Responsible for Inhibition**

The lack of ultrastructural alterations in platelets inhibited by vitamin E and vitamin E quinone indicates that these agents must alter platelet biochemistry. Nucleotide concentrations along with serotonin levels were first assayed and found within normal limits (Table 4). The possibility that these agents could stimulate adenylate cyclase or inhibit phosphodiesterase was assessed by comparing the concentration of cAMP in platelets incubated 10 min at 37°C with 2 mM vitamin E or vitamin E quinone with that
in platelets stimulated with $10 \text{nM}$ prostaglandin $E_1$ and in the unstimulated control (76, 71, 210, and 81 pmole/10$^9$ platelets, respectively).

The greater potency of vitamin E quinone might result from its oxidative potential. To test this possibility, C-PRP containing 2 mM vitamin E or vitamin E quinone was incubated for 10 min at $37^\circ\text{C}$. The platelets were then pelleted at 600 g for 20 min, the pellet extracted with chloroform, and the concentrations of vitamin E and vitamin E quinone in the extract estimated from their absorbances (250–300 nm wavelength). No oxidation of vitamin E or reduction of vitamin E quinone was detected.

In a second study, the possibility of a permanent chemical modification of the platelets was eliminated by demonstrating that the inhibition was reversed by a single washing of platelets. Platelets incubated with vitamin E or vitamin E quinone regained almost full activity in response to arachidonate and epinephrine (Fig. 4). In the sample treated with vitamin E, platelets retained better responsiveness to ADP than the control after the washing procedure. For platelets treated with vitamin E quinone, full primary aggregation and most of the secondary aggregation was recovered after the single washing.

### Studies of Platelet Arachidonic Acid Metabolism

The mechanism of the action of vitamin E quinone to inhibit selectively platelet second-wave aggregation was explored by studying the enzymes involved in metabolism of arachidonic acid, including those that release the acid and those that metabolize it: lipoxygenase, cyclooxygenase, and thromboxane synthetase. Vitamin E quinone markedly inhibited platelet arachidonate release when it was stimulated by the calcium ionophore (Table 5). Vitamin E quinone also significantly inhibited, but to a lesser degree, the cyclooxygenase (prostaglandin endoperoxide synthetase enzyme), but it did not inhibit the platelet lipoxygenase (Table 6). Rather, the proportion of 12L-hydroxy-5, 8, 10, 14-eicosatetraenoic acid (HETE) produced was increased due to the inhibition of the cyclooxygenase. Furthermore, no increased production of prostaglandin $E_2$ or prostaglandin $D_2$ was seen under conditions where thromboxane $B_2$ was inhibited, showing that vitamin E quinone did not selectively inhibit thromboxane synthetase. These effects of vitamin E quinone were very different from effects of vitamin E (studied at the same time but reported elsewhere$^{93}$), which had no effect on arachidonic acid metabolism, except to slightly enhance arachidonate release by the ionophore A23187 and thrombin.

### Table 5. The Influence of Vitamin E Quinone on Platelet Arachidonic Acid Release

<table>
<thead>
<tr>
<th>Percent Release of $^{14}$C-Arachidonic Acid From Platelet Phospholipids</th>
<th>Percent Aggregation</th>
</tr>
</thead>
<tbody>
<tr>
<td>VE (2.3 mM) + A23187 (10 $\mu$M)</td>
<td>3</td>
</tr>
<tr>
<td>A23187 (10 $\mu$M)</td>
<td></td>
</tr>
</tbody>
</table>

$^*$Mean ± SEM, 4 replicates.
Table 6. Influence of Vitamin E Quinone (VEQ) on the Metabolism of 14C-Arachidonic Acid (AA) by Washed Platelets

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Condition</th>
<th>Percent of Product*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>HETE</td>
</tr>
<tr>
<td>Experiment 1</td>
<td>Ethanol + AA</td>
<td>3.0,3.8</td>
</tr>
<tr>
<td></td>
<td>VEQ (0.5 mg/ml) + AA</td>
<td>4.5,4.7</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>Ethanol + AA</td>
<td>3.4,4.0</td>
</tr>
<tr>
<td></td>
<td>VEQ (0.5 mg/ml) + AA</td>
<td>5.1,6.5</td>
</tr>
</tbody>
</table>

*Results from duplicate assays are expressed as the proportion of 14C-AA converted to each metabolite, and the proportion remaining as AA.

The Influence of Vitamin E Quinone on Intracellular Calcium Flux

In view of recent evidence suggesting that vitamin E inhibits platelet aggregation by inhibiting calcium release from an intracellular storage compartment,33 a similar study was performed using vitamin E quinone. Vitamin E quinone was slightly more effective than vitamin E in inhibiting A23187-induced calcium release (Table 7).

Table 7. The Influence of Vitamin E Quinone on the A23187-Induced Release of 45Ca From a Platelet Membrane Fraction

<table>
<thead>
<tr>
<th>Condition</th>
<th>Percent Release of 45Ca</th>
<th>Percent Inhibition</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 μM A23187</td>
<td>37.8 ± 6.4*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>0.07 mM VEQ +</td>
<td>37.8 ± 6.4*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5 μM A23187</td>
<td>24.7 ± 2.7</td>
<td>35</td>
<td>NS</td>
</tr>
<tr>
<td>0.7 mM VEO +</td>
<td>24.7 ± 2.7</td>
<td>35</td>
<td>NS</td>
</tr>
<tr>
<td>5 μM A23187</td>
<td>8.8 ± 0.6</td>
<td>77</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>0.07 mM VE +</td>
<td>8.8 ± 0.6</td>
<td>77</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5 μM A23187</td>
<td>31.2 ± 1.6</td>
<td>17</td>
<td>NS</td>
</tr>
<tr>
<td>0.7 mM VE +</td>
<td>31.2 ± 1.6</td>
<td>17</td>
<td>NS</td>
</tr>
<tr>
<td>5 μM A23187</td>
<td>19.7 ± 1.5</td>
<td>48</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*Mean ± SEM.

Effect of Vitamin E Quinone on Endothelial Cell Synthesis of Prostaglandin I2

The Influence of Vitamin E Quinone on Endothelial Cell PG12 Production

When endothelial cell PG12 production was assayed by measuring inhibition of platelet aggregation on addition of a high concentration of arachidonic acid to the cells, then no inhibition of endothelial cell PG12 production was seen (Fig. 5). When only a small amount of labeled arachidonic acid was added and basal production of 6-keto-PGF1α was evaluated, small inhibition (32%) was seen. However, 6-keto-PGF1α produced under thrombin stimulation was markedly inhibited (73%).

DISCUSSION

The present investigation has demonstrated that the oxidized form of vitamin E quinone inhibits platelet aggregation and secretion much more potently than vitamin E. Our findings disagree with those of Steiner,34 who said these two agents were equally potent, but he presented no comparative data. Furthermore, in his studies, Steiner found that vitamin E itself was more potent than we have found, and it is possible that part of the effect attributed to vitamin E might have been due to contamination by vitamin E quinone.

Several possible mechanisms by which vitamin E and vitamin E quinone might inhibit platelet function...
were eliminated. Neither agent inhibits by reducing the ATP pool, by stimulating adenylate cyclase, or by inhibiting phosphodiesterase. There was also no evidence that either agent acted through oxidation-reduction reactions. Certainly, no permanent chemical modification, such as oxidation of membrane lipids, occurred since the effect was readily reversed. Also, no reduction of vitamin E quinone or oxidation of vitamin E was detected. The latter results show that the effects of vitamin E in our studies was not derived from its conversion to vitamin E quinone. Furthermore, there was no evidence that the agents acted through oxidation-reduction reactions. Certainly, no permanent chemical modification occurred since the effect was readily reversed. Also, no reduction of vitamin E quinone or oxidation of vitamin E was detected. The latter results show that the effects of vitamin E in our studies was not derived from its conversion to vitamin E quinone. Furthermore, the lack of detectable oxidation of vitamin E combined with the demonstration of the greater potency of vitamin E quinone argue against vitamin E affecting inhibition through its antioxidant capacity. In fact, both agents probably directly alter membrane function since they rapidly inhibit platelets without evidence of chemical modification or demonstrable ultrastructural changes and they are very insoluble in aqueous solutions.

Although both of these agents may alter membranes, vitamin E quinone does produce significantly different effects than vitamin E. In previous studies, vitamin E was found to inhibit calcium release from a platelet subcellular storage organelle. However, vitamin E had no effect on the cyclooxygenase enzyme activity and potentiated arachidonate release. The findings of the present study show that vitamin E quinone blocks calcium release slightly more effectively than vitamin E, markedly inhibits arachidonate release induced by the calcium ionophore A23187, and slightly inactivates cyclooxygenase. The inhibition of calcium flux by vitamin E quinone might be responsible for the prevention of arachidonate release, but this seems most unlikely since vitamin E was nearly as potent as an inhibitor of calcium flux and yet potentiated arachidonate release. Therefore, vitamin E quinone is not only quantitatively more potent but biochemically different from vitamin E in its mechanism of action on platelets.

The present studies are relevant to understanding the potential role for vitamin E and its metabolites as antithrombotic agents. Vitamin E quinone, a natural metabolite, might be responsible for in vivo effects previously attributed to vitamin E, such as the reduction in platelet aggregability. However, since recent evidence suggests that the relative concentrations of prostacyclin and thromboxane A2 modulate platelet-endothelial cell interaction in atherothrombotic disorders and that this cellular interaction is involved in development of thrombosis, it was important to evaluate endothelial cell PGI2 production. The results presented here suggest that-thrombin-induced stimulation of endothelial cell PGI2 production is markedly inhibited by vitamin E quinone, whereas the conversion of arachidonic acid directly to PGI2 is less affected. Since it is unknown at present whether endothelial cell PGI2 is produced continually or only on thrombin stimulation, and which is more important in vivo, we do not know how vitamin E quinone will affect PGI2 production in vivo. Thus, we cannot say with certainty what the overall effect of vitamin E quinone in vivo will be. However, in view of the present results showing marked inhibition of platelet function, the in vivo effects of vitamin E quinone and its production during vitamin E therapy need to be considered and evaluated in future studies of vitamin E and related compounds as antithrombotic agents.

ACKNOWLEDGMENT

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