Platelet adhesiveness was tested ex vivo in a group of six normal individuals receiving varying doses of α-tocopherol. Adhesion to glass slides coated with fibronectin, collagen, fibrinogen, or plasma proteins was studied by perfusing platelet-rich plasma through a flow chamber that allowed time- and space-resolved observations of platelet adhesion. Platelet adherence was measured in an area of parallel flow lines and low shear rate under standardized conditions before and after dietary supplementation with vitamin E at doses of 200 and 400 IU/d. Platelet adherence differed in magnitude depending on the adhesive surface. There was a distinct preference of platelets to adhere to sites that had been previously occupied. A remarkable decrease in platelet adherence was observed after vitamin E supplementation. The average decrease in adhesion after 2 weeks of 200 IU vitamin E was 75%. After 2 weeks of 400 IU vitamin E, platelet adhesion was reduced by 82%. The inhibitory activity of α-tocopherol was dose dependent and correlated well with the increase in α-tocopherol concentration in platelets after supplementation. Scanning electron microscopy revealed a striking decrease of pseudopodium formation in α-tocopherol–enriched platelets. Our results suggest that vitamin E may also be an effective antiadhesive agent in vivo.

MAJOR EMPHASIS of the pharmacologic modification of platelet activation has been placed on inhibition of aggregation. Thus far very few if any effective inhibitors of platelet adhesiveness have been described that have potential for use in humans. In previous studies we were able to demonstrate that α-tocopherol has a potent antagonistic effect on platelet adhesion to collagen when measured in an EDTA-containing system. These ex vivo studies were performed with platelets of normal individuals who had ingested vitamin E in doses ranging from 400 to 1,200 IU daily. Acetylsalicylic acid, on the other hand, was completely ineffective in this assay system. The effect of these two platelet antagonists on aggregation was completely opposite to their action on adhesion.

In a follow-up investigation of those studies, we now describe the effect of vitamin E administered in increasing doses to a group of normal individuals on platelet adhesion measured in a “dynamic” system. This system is based on the Hele-Shaw flow chamber, which was modified by Richardson et al1 to allow time- and space-dependent observations of platelet adhesion.

We were able to show that α-tocopherol is an effective antiadhesive agent when measured by this device in an area of parallel flow lines and low shear rate. The inhibitory activity of α-tocopherol was dose dependent and correlated well with the increase in tocopherol concentration in platelets after supplementation.

MATERIALS AND METHODS

Preparation of platelets. Blood was obtained from a group of normal, healthy volunteer donors who had abstained from any medication including aspirin and other nonsteroidal antiinflammatory agents for a period of no less than 2 weeks. Whole blood was collected into 1/10 vol of 3.8% sodium citrate by clean venipuncture. Platelet-rich plasma (PRP) was prepared as previously described.3 The platelet count of the PRP was adjusted to 300,000 to 350,000/μL.

Experimental design. A total of six normal, healthy volunteers, three men and three women, were studied. Each individual abstained from all medications for the entire period of the study. After baseline evaluation the volunteers began taking 200 IU d-α-tocopherol acetate for 2 weeks and then 400 IU for another 2 weeks. At the end of each dosage period, platelet adhesion to four different adhesive surfaces was studied. Adhesion was also measured at varying times after the vitamin E supplementation period had ended. Three of the six subjects in our study were tested at intervals up to 6 months after vitamin E administration had been stopped. The age distribution of the volunteers ranged from 28 to 52 years. All subjects were nonsmokers. Informed consent was obtained from all volunteers who participated in this study.

Preparation of test surfaces. The glass slides used for test surfaces were chemically cleaned and dried before coating with protein by using the method of Cazenave et al.4 Slides were soaked for five hours consecutively in detergent, 1 N KOH, and 1 N HCl, rinsed thoroughly with distilled water between each chemical and then air-dried. The following proteins were used to coat slides: type I collagen (from rat tail) in 0.1% acetic acid, 2 mg/mL; fibronectin (human) in 0.05 mol/L Tris-HCl and 0.14 mol/L NaCl, pH 7.2 (TBS), 0.4 mg/mL; and fibrinogen (human) in distilled water, 5 mg/mL. A few experiments were also performed with bovine serum albumin (BSA) in TBS, 5 mg/mL. The glass slides were dip-coated, allowed to dry completely on a flat surface, and stored upright at −80°C. All of the proteins were analyzed by sodium dodecyl sulfate–polyacrylamide gradient gel electrophoresis5 and by reacting the gels that had been transblotted onto nitrocellulose with specific antibodies that were recognized with gold-conjugated antirabbit IgG. There was no recognizable contamination of fibrinogen with fibronectin and vice versa. von Willebrand factor did not appear to be a contaminant of our proteins. The sensitivity of this method should have allowed us to detect contaminating proteins at a concentration ≥2%. Two hours before the adhesion experiments, the slides were removed from cryogenic storage and allowed to equilibrate to room temperature in a Petri dish. The storage period of a typical coated slide was approximately 1 week. Uncoated glass slides were also used. Cleaning and storage conditions were as stated earlier.

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**Flow chamber.** The flow chamber basically consists of a Hele-Shaw channel between two flat parallel surfaces and has been described in detail elsewhere. The chamber is 43 mm long and 14.2 mm wide, with a 0.4-mm gap between the two surfaces. A silicone rubber disk, 10 mm in diameter and of the same thickness as the channel gap, was placed in the middle of the chamber to vary the local shear rate. The chamber itself is a machined piece of polymethylmethacrylate (Plexiglass). The lower flat surface of the channel is formed by the deck of the chamber, while a glass slide (75 x 38 mm) constitutes the upper surface. It is held onto the chamber by means of a perimeter vacuum pump. The glass slides that serve as the test surfaces were coated with one of the proteins listed before. Inflow and outflow ports are at either end of the chamber. A thermistor was placed in an additional port opposite the inlet port and attached to a telethermometer (model 43 Ta, Yellow Springs Instruments Co, Yellow Springs, OH). This enabled continuous monitoring of the temperature of the PRP perfusing the flow chamber throughout the experiment.

We used a flow chamber with a centrally located obstacle having a circular cylindrical shape because this allows us to obtain measurements of platelet adhesion over a range of different wall shear rates by simply altering the x- and y-coordinates central to our place of observation with the microscope objective while keeping all other aspects of the flow constant. The maximum wall shear stress that the flow experiences anywhere in the flow chamber is close to four times what prevails at the site of our observations. The streamlines of the flow that experience this maximum shear rate do not pass through the place of our observations. We do not report here any measurements at other x, y locations that would have other shear rates. Another advantage of the use of the obstacle is that at the end of the experiment we can scan the entire slide for the relative distribution of platelet adhesion, which varies with local shear rate and therefore with location on the surface. Very occasionally one can encounter a donor with an abnormal pattern of adhesion that may cause one to exclude such a donor from the study. We did not encounter such a donor in this investigation.

**Experimental procedure.** The flow chamber was manually filled with lactated Ringer’s solution, pH 7.2 (approximate mEq/L: sodium, 130; potassium, 4; calcium, 3; chloride, 109; lactate, 28), containing 4 U heparin/mL. This ensured that no air bubbles were present in either the chamber or the inflow line and eliminated the air-blood interface. A 35-mL plastic syringe containing PRP was connected by silicone rubber tubing of 1/8-inch internal diameter to the inlet port. Syringe and inflow tube leading to the chamber were surrounded by jackets that were perfused with water at 37°C. The prefilled chamber with the glass surface secured was inverted and mounted on the stage of a Nikon Diaphot inverted microscope that was equipped with a Hoffman Modulation Contrast System (40× objective, 0.55 numerical aperture). This optical system provided an almost three-dimensional image of cells and thus facilitated recognition of platelets. PRP was pumped through the chamber at a flow rate of 0.58 mL/min by a syringe infusion pump (model 940, Harvard Apparatus Co, Inc, Millis, MA). PRP that had circulated through the flow chamber was drained by gravity through the outflow line into a waste container (Fig 1).

To record the adhesion process, a Nikon FE 35-mm camera was mounted on the microscope. The microscope was focused on the inside glass surface of the chamber at a location that had previously been etched onto the glass surface after chamber assembly (Fig 1A). This observation site was downstream from the disk and therefore passed an area of 71,000 μm², which was approximately 1/8,000 of the total chamber area available for adhesion. The shear rate in this area ranged from 20 to 25 s⁻¹. Shear rates were calculated according to methods described by Batchelor for Hele-Shaw cells, ie, flow channels made from parallel walls and partly occupied by obstacles in the form of cylinders with generators perpendicular to the walls. The wall shear rate can be calculated readily at any position x, y in the chamber except for the narrow regions within one gap width from the rectangular side walls and the side of the cylindrical obstacle. Our observation point is outside these narrow regions, and therefore, edge effects do not affect the wall shear rate determination. The Reynold’s number, a dimensionless flow parameter, was calculated to be 0.6 for our flow chamber system.

Photographs of the experimental run were taken at 30-second intervals. Previous experiments by Richardson et al⁴ and Kane⁵ using videomicroscopy have shown that at times events do occur in less than 30 seconds. However, these studies also demonstrated that a 30-second observation interval captures more than 80% of the events. Technical considerations prompted us to choose 30-second intervals as an acceptable compromise. A shutter speed of ¼ second allowed stationary platelets on the surface to remain in focus while moving platelets were blurred. Each run lasted 13 minutes. A typical example of the appearance of adherent platelets on the adhesive surface is shown in Fig 2.

**Data analysis.** Computer-aided analysis was performed on the series of developed photomicrographs resulting from each experiment (black-and-white negatives of Tri-X Pan 400). Individual negatives were projected onto a Summagraphics microgrid digitizing tablet by a 35-mm film projector. When using this method, the projected pictures of platelets measured on average 3 mm in diameter, which is greater than the resolution limit of the digitizing tablet. A crosshair within the transparent window of the cursor enabled us to precisely identify the location of each platelet. Hence, platelets that had adhered side by side were registered as having distinct and separate coordinates. Using a Digital Equipment Corporation VT 100 terminal connected to a VAX II computer system made possible a site-by-site temporal and spatial analysis. A modified computer program written in BASIC by Kane⁵ provided the following information for each discrete time period: (a) occupied sites, ie, the total number of sites currently occupied by platelets; (b) fresh adhesion sites, ie, the number of currently occupied sites that were not occupied during the immediately preceding (30-second)
cumulative many of the sites were described earlier were washed once in S mL of PRP was incorporated to be found

assessed. Repeated measurement of the adhesion test were assessed. Repeated measurement of

of the blood was good. The coefficient of variation on repeating the assay twice within three hours after collecting the blood, isolating PRP, and ranged from 2.0% to 8.3%.

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adhesion than did any of the other three adhesive surfaces. The lowest adhesion rates were recorded with noncoated glass slides. A number of albumin-coated slides were included as negative controls. The number of platelets that adhered to this surface at any one time in the area of observation was ≤2. The number of cumulative sites over the entire period of observation was 10 ± 2 (mean ± SD; four experiments). There was also very pronounced variability in the rate of adhesion of platelets from different individuals to the various adhesive surfaces. The large SE of the mean adhesion rates reflect this individual variability. For this reason statistics were computed by using individuals as their own controls instead of comparing group means. The reuse-of-sites index, on the other hand, showed far less variation among individuals at baseline levels (Table 1).

A typical relationship between time and accumulation of sites for a set of adhesion assays is shown in Fig 4. After an initial lag time, the cumulative number of sites rises steadily throughout the experimental run. Because the adhesion rate was best represented by a linear regression curve, we deleted the first three points in our experiments and computed the slope on the basis of the remaining observation points. The initial time lag in platelet adhesion may be an important phenomenon. For the purpose of using a consistent adhesion rate parameter, however, the first two minutes were ignored.

Platelets have a distinct tendency to reoccupy sites that had been previously vacated by other platelets. Thus, a large number of platelets occupied sites (an average of 44.5% for all four adhesive surfaces) that had been previously vacated by other platelets. Thus, a large number of platelets occupied sites (an average of 44.5% for all four adhesive surfaces) that had been visited twice, three times, or up to eight times before. The reuse-of-sites data for baseline conditions showed a strong exponential relationship between the number of sites occupied and the number of times of occupation (Fig 5).

Two experiments were performed with PRP obtained from patients with severe and moderately severe type I von Willebrand disease. Baseline platelet adhesion to fibrinogen-coated glass slides was studied at baseline (circles), after 2 weeks of 200 IU vitamin E (triangles), and after an additional 2 weeks of 400 IU vitamin E supplementation (squares). The data depicted are representative of those obtained during a typical adhesion assay.

Fig 3. Adhesion trend lines for all four adhesive surfaces examined in this study. Mean adhesion rates (cumulative platelet number x min⁻¹) ± SEM are shown for baseline and vitamin E-supplemented periods. Differences between baseline and 200 and 400 IU were significant at P < .01 for fibrinogen and glass and between baseline and 400 IU for collagen I. The remaining differences in adhesion rate between baseline and vitamin E-supplemented levels were significant at the P < .05 level.

Fig 4. Cumulative number of adhesion sites over a 13-minute period. Platelet adhesion to fibrinogen-coated glass slides was studied at baseline (circles), after 2 weeks of 200 IU vitamin E (triangles), and after an additional 2 weeks of 400 IU vitamin E supplementation (squares). The data depicted are representative of those obtained during a typical adhesion assay.

Fig 5. Graphic representation of the reuse of sites plotted against the number of times a specific adhesion site was occupied during an adhesion assay. The data shown in the figure were obtained with platelets before any dietary vitamin E supplementation was begun. The regression coefficient was >0.95.

Table 1. Reuse-of-Sites Index Before and After Dietary Supplementation

<table>
<thead>
<tr>
<th>Adhesive Surface</th>
<th>Baseline</th>
<th>200 IU, Mean ± SD, n = 6</th>
<th>400 IU, Mean ± SD, n = 6</th>
</tr>
</thead>
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<tr>
<td>Fibrinogen</td>
<td>-1.0 ± 0.1</td>
<td>-1.3 ± 0.2</td>
<td>-1.5 ± 0.4</td>
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<tr>
<td>Collagen I</td>
<td>-0.7 ± 0.2</td>
<td>-1.1 ± 0.7</td>
<td>-1.5 ± 0.7</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>-1.3 ± 0.3</td>
<td>-1.6 ± 0.4</td>
<td>-1.0 ± 0.3</td>
</tr>
<tr>
<td>Glass</td>
<td>-0.9 ± 0.2</td>
<td>-1.2 ± 0.7</td>
<td>-2.0 ± 0.7</td>
</tr>
</tbody>
</table>

Summary of the reuse-of-sites indices at baseline and after two different levels of dietary vitamin E supplementation for four different adhesive surfaces. The more negative the index, the smaller the number of times a particular adhesion site was occupied. For further clarification of this index, see Fig 5.
Willebrand's disease. Both patients exhibited a severe reduction in the rate of platelet adhesion to collagen that was found to be decreased by 92% in the severely affected patient and 74% in the one with less severe disease. Adhesion to fibronectin and fibrinogen was also reduced although not quite to the degree as that of collagen. There was a decrease of 56% and 37% with fibrinogen and 49% and 30%, respectively, for the severe and less severe patient with fibronectin as the adhesive surface.

Vitamin E supplementation of the diet produced a striking decrease of the rate of adhesion to all four adhesive surfaces (Fig 3). This decrease was statistically significant at both levels of vitamin E supplementation. The baseline value for each individual was compared with the value after each supplementation level by using paired Student's t tests. The adhesion rate trend lines illustrate a consistent decrease in platelet adhesion that is independent of the type of adhesive surface studied as the dose of a-tocopherol increases. The magnitude of reduction in the adhesion rate, however, was found to be dependent on the adhesive surface. Collagen I exhibited the largest decrease at both the 200-IU level (84% decline) and at the 400-IU level (88% decline). Adhesion to fibronectin decreased by 82%, whereas adhesion to uncoated glass slides declined by 77%. All of these declines were statistically significant. The average decrease in adhesion after 2 weeks of 200 IU vitamin E supplementation was 75%, whereas at the 400-IU dose vitamin E reduced platelet adhesion to the four surfaces by an average of 82%.

Vitamin E supplementation also decreased the release of sites index (Table 1). The difference between baseline and experimental values, however, did not reach statistical significance for any of the adhesive surfaces studied. Although the number of times a particular site gets reoccupied decreases after vitamin E supplementation, this decrease may be due to the overall reduction in adhesion events. Since the reduction is so steep, the number of times sites are reoccupied 3, 4, and 5 times increases too small for meaningful statistical analyses of differences in slope. For this reason, we determined the ratio of the cumulative number of sites occupied once to the cumulative number of sites occupied twice (0,02/0,01) (Table 2). Because these ratios result from greater numbers of sites, more reliance can be placed on them. Fibrinogen and collagen showed increased ratios of 0,02/0,01 in platelets from vitamin E-supplemented individuals. Fibronectin and glass, on the other hand, did not produce a consistent change in such ratios. Therefore, the effect of vitamin E on the rate of second occupations of sites is very dependent on the adhesive surface itself.

The high-pressure liquid chromatographic quantification of the a-tocopherol content of platelets and plasma at different levels of vitamin E supplementation is summarized in Table 3. Considerable variability was found among individuals before supplementation was begun as well as at the different dose levels of vitamin E. Nevertheless, all individuals experienced a progressive increase in plasma and platelet a-tocopherol levels when the dietary intake of vitamin E was raised (Table 3). Evaluation of platelet a-tocopherol in relation to the rate of adhesion to each surface is shown in Fig 6. Even though the slopes of the lines plotted on a logarithmic scale are quite different for each individual, increased loading of platelets with a-tocopherol produced a progressive decrease in platelet adhesion. In rare instances, the adhesion rate actually showed a slight increase as the vitamin E dose increased from 200 to 400 IU. This was found in <10% of the measurements. It must be stressed that this increase in the rate of adhesion that was occasionally registered was small and remained well below the baseline values. To compare individuals, we normalized our results for two arbitrary a-tocopherol levels and interpolated the corresponding adhesion rates. Thus, rates corresponding to 360 ng a-tocopherol/10^9 platelets were always higher than those at 500 ng/10^9 platelets (Table 4). There were no apparent correlations between adhesion rates and sex or age of the individuals. Correlation of plasma levels of a-tocopherol with adhesion rates was very similar to that of platelet a-tocopherol individual correlations not shown).

The appearance of platelets deposited on the adhesive surfaces was also investigated by scanning electron microscopy (Fig 7). Under baseline conditions and before supplementation of the diet with vitamin E, platelets exhibited extensive pseudopodium formation when adherent to the surface. Vitamin E administration resulted in a reduction of pseudopodia at 200 IU/d and a virtual disappearance at 400 IU/d. All of the different coatings tested in our experiments produced similar results.

**DISCUSSION**

The assessment of platelet adhesion by a reproducible assay that mimics in vivo conditions of flow has been used in this study. The flow chamber originally designed by Hele-Shaw and subsequently modified by Richardson et al for the evaluation of platelet adhesiveness provides a convenient method for the in vivo assessment of platelet adhesiveness and the ex vivo determination of pharmacologic and nutritional factors affecting this platelet function. The simple design and operation of this flow chamber makes it easy to set up the system. A particularly attractive feature of this
Fig 6. Relation of adhesion rate (cumulative number of platelet x min⁻¹) to α-tocopherol content of platelets (ng/10⁶ platelets). Results obtained with fibrinogen as an adhesive surface are shown in panel A, with fibronectin in B, collagen in C, and with glass in D. Data are shown for five of the six individuals tested. One subject in the study was omitted because his baseline value of platelet α-tocopherol was >3 SD from the mean of the other five individuals. Age and sex of the subjects are indicated in the inset.
adhesion assay is the ability to make time-resolved observations that can be evaluated by computerized methods and give a permanent record of the adhesion process. A further advantage is the ability to make observations on different adhesive surfaces. The reproducibility of the assay is good, provided adhesion measurements are done within a three-hour period after the collection of blood.

For these studies we chose fibrinogen, fibronectin, collagen I, and glass as adhesive surfaces. The plain glass surface of course becomes coated by plasma proteins within a short period of time after starting the perfusion of the chamber. Our choices were determined by the ready availability of pure proteins and the relevance of these proteins to platelet adhesion. The individual variation of platelet adhesion to the different adhesive surfaces was quite high upon repeated testing, while those who had a low adhesion rate continued to have such when tested again.

Evaluation of platelet adhesion in relation to time showed a distinct initial delay phase that under baseline conditions was two minutes. During this interval few platelets adhere to the adhesive surface. This initial time lag was noticed by previous investigators who used this flow chamber. Although it is probably not the dominant factor, there is some competitive adsorption of plasma proteins, especially albumin, gamma globulin, and fibrinogen, to form a proteinaceous layer on the surface. Also, there may be a lower concentration of platelets near the wall at early times because the priming solution is displaced first near the center of the flow channel and more slowly near the walls. Until the surface is coated, the conditions result in an initially low adhesion rate. Another explanation for the delay phases is that the adhesion of platelets and the accompanying release of their granular contents leaves a concentration of agonists in the immediate surroundings of such platelets that may induce shape change in other platelets that stray into their vicinity. Diffusion of agonists from the adherent platelets should be relatively little disturbed by the flow because the flow close to the adhesive surface would be almost stationary.

It is very interesting that adhesion sites that had been occupied once by platelets are often reoccupied by newly arriving platelets. Close to 50% of the platelets adhere to sites previously occupied. This high reoccupation rate of previously vacated sites suggests that the platelets that had resided there before have left some “traces” of their presence, possibly portions of their membrane or parts of pseudo-

<table>
<thead>
<tr>
<th>Subject</th>
<th>360*</th>
<th>500</th>
<th>360</th>
<th>500</th>
<th>360</th>
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<td>J.N.</td>
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<td>2.1</td>
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<td>3.4</td>
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<td>7.0</td>
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<td>2.0</td>
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<tr>
<td>K.M.</td>
<td>46.5</td>
<td>16.0</td>
<td>7.6</td>
<td>3.8</td>
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<td>10.7</td>
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</tr>
<tr>
<td>A.A.</td>
<td>5.3</td>
<td>3.0</td>
<td>3.5</td>
<td>2.3</td>
<td>11.0</td>
<td>7.6</td>
<td>5.1</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Data for two of the individuals tested are not shown because their baseline values of platelet α-tocopherol were >360 ng/10⁹ platelets.

*α-Tocopherol, 360 or 500 ng/10⁹ platelets.

**Fig 7.** Scanning electron microscopic examination of platelets adherent to the fibronectin-coated upper deck of the flow chamber. Control platelets obtained before dietary vitamin E supplementation (A), platelets after 2 weeks of 200 IU vitamin E/d (B), and platelets after 400 IU vitamin E/d for 2 weeks (C) (A: original magnification x 12,000; B: original magnification x 10,000; C: original magnification x 8,000).
podia at those sites. One can speculate that newly arriving platelets will be more likely to adhere to such sites than to a random area of the adhesive surfaces. Especially the glycoprotein(s) involved in the adhesion process, eg, glycoprotein Ib\textsuperscript{13,14} could be a possible candidate for such a trace-leaving process. An alternative explanation could be the existence of “hot spots,” very reactive sites of the adhesive surface.

It should be pointed out that the shear rates at which adhesion was measured (20 to 25 s\textsuperscript{-1}) do not correspond to those normally present in coronary or other major arteries (200 to 800 s\textsuperscript{-1}). Because the primary purpose of our studies was to obtain time-resolved measurements of platelet adhesion under conditions where arrival and departure of platelets on the adhesive surface could be observed, the use of whole blood that would have greatly increased platelet transport to the adhesive surface had to be excluded. Only the terminal deposition of platelets can be evaluated in the latter case. High shear rates enhance the frequency of platelet arrival at the surface,\textsuperscript{15-17} but the process of removal of adherent platelets also appears to be enhanced, which results in fact in a net decrease in adhesion.\textsuperscript{2,18} When observations were made in areas of the flow chamber where higher shear rates (100 to 120 s\textsuperscript{-1}) exist, ie, the narrow channel adjacent to the central obstruction in the chamber, lower adhesion rates were actually found than at our site of observation. There are also technical difficulties in trying to simulate high shear rate conditions in the flow chamber we used. Although the chamber can withstand shear rates of 600 to 800 s\textsuperscript{-1}, extremely large volumes of PRP would be required to perform an experiment.

Nevertheless, we believe that our studies are of clinical relevance. Recent investigations by Davies and Thomas\textsuperscript{19,20} have demonstrated that a majority of patients with coronary thrombosis sustain the thrombotic event at a rupture site of an atherosclerotic plaque. Platelets were found in the cavities of these rupture sites, with the evolving thrombus growing out into the lumen of the blood vessel. Flow rates at such sites are very low, and equally low are the shear rates.

Platelet adhesivity was decreased in the patients with von Willebrand’s disease that we were able to test. Although we cannot make a general statement about the behavior of such platelets, having studied only two patients with this disease, our findings differ somewhat from those reported by Turitto et al\textsuperscript{21} who observed decreased platelet adherence but only at high shear rates (\textgtrapprox 800 s\textsuperscript{-1}). We do not know the reason for this difference, but the respective methods of adhesion measurement are quite dissimilar: not only the shear rates but also the suspension media (whole blood v PRP) are different.

Supplementation of the diet with vitamin E produced a striking reduction in platelet adhesiveness. It was interesting to note that all six individuals studied showed a decrease not only after 400 IU but also after 200 IU vitamin E daily for 2 weeks. Especially remarkable and sharp was the reduction in platelet adhesivity after 200 IU (up to 80% from the original baseline value). The further decline in adhesivity upon increasing vitamin E to 400 IU was relatively modest in comparison. Although our study did not include a placebo group, we tested platelet adhesion in several of the subjects after the vitamin E supplementation had ended. All individuals showed a complete return to their presupplementation adhesion rates.

The reutilization of once-occupied sites also changed after vitamin E supplementation. The $0_1/0_2$ ratio relates the number of second occupations to the number of primary occupations. In this way a change in the rate of recollection is scaled to any change seen in the rate of primary adhesion, $0_1$. By omitting consideration of $0_1$, $0_2$, etc, the effects from small counts are eliminated. A decrease in the recollection of sites is shown by an increase in this ratio. Judging by the $0_1/0_2$ ratios (Table 2), only collagen I and fibrinogen demonstrated reduced recollection of sites that had been vacated by platelets. This would suggest a reduced amount of platelet “traces” left by the first occupants of specific adhesion sites. The results were not as clear for glass and fibronectin.

Even though the individual levels of vitamin E in platelets were quite variable, all individuals showed a very marked and progressive increase in platelet tocopherol levels upon increasing the dietary supplementation of vitamin E (Table 3). Although the baseline levels of platelet $\alpha$-tocopherol showed no distinct relation to the adhesiveness of the platelets, supplementary vitamin E did. This finding suggests that a high platelet tocopherol level by itself does not produce any inhibition of platelet adhesiveness. Only $\alpha$-tocopherol ingested in excess of the amount taken in with the individual’s “normal” diet will affect platelet adhesivity. Dietary abundance of $\alpha$-tocopherol is closely related to the content of polyunsaturated fatty acids.\textsuperscript{22} We believe that this balance has to be tilted in favor of $\alpha$-tocopherol to observe the inhibitory action of platelet adhesivity.

The dose-related reduction of pseudopodium formation in $\alpha$-tocopherol–enriched platelets may be responsible for the observed decrease in platelet adhesivity. The reason for this phenomenon is not clear at this time, but a causal relation between the $\alpha$-tocopherol supplementation and the inhibition of pseudopodium formation appears to exist.

We believe that our studies give convincing evidence of the antiadhesive properties of vitamin E supplementation of the diet. It was especially gratifying that “reasonable” doses of vitamin E that had no untoward side effects were able to accomplish such reduction. Even though vitamin E has not shown to be an effective antiaggregating agent when tested ex vivo,\textsuperscript{1} from our data it would appear that it could be an effective antiadhesive agent in vivo.

ACKNOWLEDGMENT
We wish to thank Georg Panol of Brown University for his technical assistance and design of the flow chamber system.

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Alpha-tocopherol, an effective inhibitor of platelet adhesion

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