Preservation of Human Platelets with Prostaglandin E\textsubscript{1} during in Vitro Simulation of Cardiopulmonary Bypass

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SUMMARY Platelet abnormalities that occur during cardiopulmonary bypass may be produced in vitro by recirculating 500 ml of human blood at 37°C in a silicone rubber circuit (0.9 m\textsuperscript{2}) containing a spiral coil membrane oxygenator. Platelet counts fall to 20% of initial levels, plasma levels of low affinity platelet factor 4 (LA-PF\textsubscript{4}) rise from 0 to 66% of that in control platelet rich plasma, and sensitivity to aggregating agents diminishes. Inhibition of platelet function with prostaglandin E\textsubscript{1} (PGE\textsubscript{1}) prevents these alterations. In 14 trials (PGE\textsubscript{1} \leq 0.1 \textmu M), the thrombocyte count achieved a stable value of 88% within 1 hour. Plasma LA-PF\textsubscript{4} (PGE\textsubscript{1} \leq 0.3 \textmu M) rose to only 10% after 6 hours. Electron microscopy revealed intact platelet granules in recirculated platelets. Platelets incubated and recirculated with PGE\textsubscript{1} remained equally sensitive to epinephrine and ADP for 3 hours. At 6 hours, recirculated platelets in plasma (PGE\textsubscript{1} \geq 0.1 \textmu M) and gel-filtered platelets (PGE\textsubscript{1} < 0.3 \textmu M) became significantly less sensitive to epinephrine. At PGE\textsubscript{1} \geq 0.3 \textmu M, however, gel-filtered recirculated platelets responded normally to epinephrine even after 6 hours of recirculation. PGE\textsubscript{1} preserves platelet numbers, prevents contact-initiated release, and preserves platelet sensitivity to aggregating agents during in vitro extracorporeal bypass. Circ Res 44: 350-357, 1979

CARDIOPULMONARY bypass results in a 40-60% decrease in the circulating platelet count, prolongation of the bleeding time, deficient clot retraction, reduced platelet adhesion to glass beads, and decreased sensitivity of platelets to aggregating agents (McKenna et al., 1975). These changes can be reproduced in vitro (Hennessy et al., 1977) and probably are due to a combination of factors including surface adsorption (Kim et al., 1974) and alteration of proteins (Wright et al., 1962), platelet surface interactions (Lyman et al., 1968), and vortex formation in regions of non-laminar flow (Goldsmith, 1974). We tested the hypothesis that prostaglandin E\textsubscript{1} (PGE\textsubscript{1}) would prevent the loss and alteration of platelets during in vitro cardiopulmonary bypass by temporarily inhibiting platelet function during periods of extensive contact between blood and an artificial surface. PGE\textsubscript{1} was chosen in preference to other platelet inhibitors (Mielke, 1973; Rittenhouse, 1972) because the compound is biologically active at low concentrations (Emmons et al., 1967; Kinihough-Rathbone, 1970), is rapidly metabolized by the lung, and therefore, its effects potentially are reversible.

Methods

Perfusion System

Perfusion circuits with a surface area of 0.95 m\textsuperscript{2} were assembled from standard silicone rubber components and contained a spiral coil membrane oxygenator (Sci-Med Life Systems, Inc.). Circuits, priming procedures, and the conditions and rates of recirculation were identical to those described previously (Hennessy et al., 1977). Briefly, blood and gas compartments were flushed with 100% carbon dioxide for 15 minutes prior to priming. Oxygenators were primed by applying a vacuum to the lower gas port of the oxygenator, and blood was permitted to enter the device by gravity. Blood was recirculated by a precisely shimmmed, barely occlusive, calibrated roller pump at a rate equal to twice the circuit blood volume per minute. Five hundred milliliters of blood were drawn directly from each random donor who had abstained from all medications for 2 weeks. This blood was drawn directly into circuit reservoirs which contained 2500 U beef heparin (Upjohn Company), 1.65 g of glucose, and enough PGE\textsubscript{1} (courtesy of Dr. John Pike, Upjohn Company) to make final whole blood concentrations of 0.001 to 10 \textmu M. For studies on platelets, one control sample was obtained directly from the donors, as described previously, and a second control sample of 100 ml was removed from the circuit after 2 minutes of recirculation. Both control samples...
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and recirculated blood were maintained at 37°C throughout the experiment. Twenty-five to 35-ml samples were withdrawn from the circuit at 1, 3, and 6 hours for platelet studies. Similar samples were withdrawn at 2 minutes and at 2, 4, and 6 hours during trials in which gel-filtered platelets (GFP) were studied. Additional samples (1 ml) were withdrawn after 15 and 30 minutes, 2, 4, and 5 hours for platelet counts.

There were three recirculation studies at each concentration of PGE, between 0.1 and 3 μM (0.1, 0.3, 1.0, 3.0) (12 trials). In addition, two studies at a PGE, concentration of 10 μM, four at 0.03 μM, three at 0.01 μM, and one at 0.001 μM also were carried out. Finally, for studies involving GFP, three recirculation trials at a PGE, concentration of 1 μM one at 0.3 μM, and three at 0.1 μM were performed. These studies were approved by the University of Pennsylvania Committee on Human Investigation and the National Institutes of Health. Written and verbal informed consent was obtained from each donor.

Studies on Platelets

Platelets were counted by phase microscopy (Brecher and Cronkite, 1950). Platelet rich plasma (PRP) was obtained and platelet aggregation studied as described by Hennessy (1977). On the basis of previous studies, we determined that the platelet release reaction is complete when epinephrine-induced aggregation exceeds 57% and ADP-induced aggregation exceeds 61%. Consequently, we defined complete second wave aggregation as more than 61% (ADP) and 57% (epinephrine) light transmission through PRP. The minimum dose required to produce complete second wave aggregation indicated platelet sensitivity to that aggregating agent. Maximal final concentrations tested were 50 μM of ADP and 250 μM of epinephrine in 0.5 ml PRP.

GFP were studied as described for PRP. Sepharose 2B columns were prepared by a modification (Colman and Schreiber, 1976) of the procedure described by Tangen (Tangen et al., 1971). Refrigerated Sepharose 2B that had been stored in 0.2% azide was suspended in distilled water (1:1 vol/vol), poured into a 30-ml plastic syringe, and allowed to settle. Columns were washed, at 23°C, with effluent buffer (1:4 vol/vol) which contained apyrase, 200 μg/ml (Levy-Toledano, 1972), (Sigma Chemical Co.). One liter of buffer (pH 7.35) contained 1 g of dextrose, 3.5 g of bovine serum albumin (Pentex), 0.2 g of KCl, 8 g of NaCl, 1 g of NaHCO3, and 50 g of Na2HPO4. Mixed with 20 ml of 0.1 M MgCl2. Fibrinogen (2.5 mg/ml GFP) was added to the aggregometer cuvette immediately prior to installation of aggregating agent.

Low Affinity Platelet Factor 4 Assay

We used the appearance of low affinity platelet factor 4 (LA-PF4) in plasma as a sensitive indicator of the occurrence of the platelet release reaction in our circuits. At least three proteins are responsible for platelet antiheparin activity: platelet factor 4 (PF4), LA-PF4, and β thromboglobulin. PF4 and LA-PF4 can be separated from the material released by stimulated platelets by affinity chromatography using heparin-agarose columns (Niewiarowski et al., 1977). Beta thromboglobulin and LA-PF4 migrate differently during acetate cellulose electrophoresis at pH 8.6. PF4 is eluted with 1.5 M NaCl from heparin-agarose columns. A number of laboratories recently characterized this factor, including its primary protein structure (Hermodson et al., 1977; Deuel et al., 1977). LA-PF4 is eluted from a heparin-agarose column at 0.5 M NaCl. Although specific antiheparin activity of PF4 is 5 to 7 times higher than that of LA-PF4, LA-PF4 accounts for about 30% of total platelet antiheparin activity (Niewiarowski, 1977). In our previous publications, the term PF4 was used to denote LA-PF4 before separation of these two factors was achieved by means of affinity chromatography.

Samples (5 ml) for LA-PF4 determinations were drawn into syringes containing 0.75 ml of acid citrate dextrose and immediately processed. PRP and platelet poor plasma (PPP - <1000 platelets/μl) were prepared as described previously. PRP and PPP then were frozen quickly and stored at —60°C until assayed.

LA-PF4 was measured in plasma by modified radial immunodiffusion with a monospecific antibody against purified LA-PF4 (Niewiarowski et al., 1976). Comparison of different batches of purified LA-PF4 antigen, homogeneous in SDS polyacrylamide gel electrophoresis showed some variations of the immunoprecipitation zones, depending upon the preparation used. For this reason, only one LA-PF4 preparation, lot DEAE/190c/7/Pfg, was used as a reference standard and the values were expressed in μg of the equivalent of this standard. LA-PF4 also was measured in platelets (intracellular content of LA-PF4) by freezing PRP and analyzing it after exposure to 20% Triton X-100 (20 μl/ml PRP) and subtraction of the LA-PF4 in PPP. Total platelet LA-PF4 was expressed as equivalents of μg LA-PF4/10⁶ platelets. The lowest concentration of LA-PF4 detectable by this method was 0.5 μg/ml and the addition of Triton X-100 to PPP did not significantly modify the results.

In 15 consecutive normal individuals who donated blood for these studies, each ml (after adding triton) of PRP (3.0 ± 0.8 × 10⁸ platelets) contained 32 ± 2 (SEM) μg LA-PF4. In these 15 individuals, when LA-PF4 was expressed per 10⁶ platelets, 88 ± 8 μg of LA-PF4 was obtained, which agrees with results previously reported with this standard and antibody. No LA-PF4 (<0.5 μg/ml) could be detected in control PRP. In these studies, release of LA-PF4 into plasma is expressed as a percent of LA-PF4 contained in 1 ml of PRP (32 μg), and intracellular concentrations of LA-PF4 are ex-
pressed as a percent of LA-PF4 contained in 10^9 control platelets (88 μg).

Morphology

Blood samples for transmission electron microscopy were obtained after 2 minutes, 1 hour, 3 hours, and 6 hours of recirculation. PRP was prepared and added to an equal volume of 0.1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3. Fixed platelets were then collected by centrifugation at 1500 rpm for 10 minutes. Platelet pellets were overlayed with 3% glutaraldehyde in 0.1 M cacodylate, pH 7.4, and allowed to harden at room temperature for 30 minutes. Platelets were then postfixed in 1% OsO₄ in 0.1 M collidine buffer (pH 7.3) for 1 hour at 4°C. Blocks were washed in buffer, stained en bloc for 1 hour at room temperature with 0.5% uranyl acetate in collidine buffer (initial pH 6.1), dehydrated in a graded ethanol series, and embedded in Epon-araldite. Sections were briefly stained with lead citrate and examined in a Hitachi EMU-11F electron microscope.

Statistical Analysis

At each sampling time, with concentrations of PGE₁ ranging from 0.1 to 10 μM, mean platelet counts, plasma LA-PF4, and platelet LA-PF4 were compared by analysis of variance. Since no differences were found at any sample time, results from trials with PGE₁ concentrations 0.1 μM or greater were combined (n = 14) and compared by analysis of variance to results obtained for 0.01 μM (n = 3) and 0.03 μM (n = 4) concentrations of PGE₁. Results from trials with PGE₁ ≥ 0.1 μM also were compared by analysis of variance to those obtained when blood was recirculated without PGE₁ (n = 6). The data from recirculation trials without PGE₁ were generated in our laboratory under conditions identical to those now presented.

Results

Platelet Counts

In 14 trials at concentrations of PGE₁ ranging from 0.1 to 10 μM, the thrombocyte count, expressed as a percent of the initial whole blood platelet count, fell within 15 minutes to 78% and rose within 1 hour to a stable value of 87% (Fig. 1). This represented significant (P < 0.01) preservation of platelet numbers, at all sampling times, when compared by analysis of variance to results obtained without PGE₁ (Fig. 1). The mean platelet counts in four trials at 0.03 μM PGE₁ ranged from 72 ± 5% at 15 minutes to 82 ± 3% at 6 hours. Although lower at each sampling time than the mean platelet counts obtained for higher concentrations of PGE₁, the differences were not significant (P > 0.05). In three trials at 0.01 μM PGE₁, mean platelet counts at each sampling time were depressed to 40–50% of initial counts; this value is significantly lower (P < 0.01) than those obtained at higher concentrations of PGE₁ despite large standard deviations. At 0.001 μM PGE₁, platelet counts were similar to those previously observed when PGE₁ was not added (Hennessy et al., 1977).

Platelet Release

In 18 trials at concentrations of PGE₁ ranging from 0.03 to 10 μM, no LA-PF4 antigen was detected in plasma after 1 hour of recirculation, and only 10% of that noted in control PRP was detected after 6 hours of recirculation (Fig. 2). Percent release of LA-PF4 was not significantly different from that found in PGE₁-inhibited, incubated blood which had recirculated for only 2 minutes, but was signifi-
cantly different \((P < 0.01)\) at each sampling time from that noted in blood recirculated without PGEl (Fig. 2). In three trials, with a low concentration of PGEl \((0.01 \mu M)\), percent release of LA-PF4 plasma increased within 2 minutes to 7% and continued to rise with time to 28% by 6 hours. Percent release of LA-PF4 at this concentration of PGEl was significantly higher \((P < 0.01)\) at each sampling time greater than 2 minutes than that noted with higher concentrations of prostaglandin (Fig. 2). At concentrations of PGEl ranging from 0.03 to 10 \(\mu M\), the intracellular content of LA-PF4 remained stable, decreasing by only 12% after 6 hours (Fig. 3). In contrast, without PGEl, intracellular levels of LA-PF4 decreased to 21% of that noted in control platelets (Fig. 3) \((P < 0.01)\).

Platelet Aggregation

During incubation, donor platelets gradually became slightly less sensitive to epinephrine but continued to respond within the normal range (Fig. 4). Platelets recirculated for 2 minutes and then incubated (PGEl \(\geq 0.1 \mu M\)) initially were insensitive to epinephrine but tended to become more sensitive to epinephrine with time (Fig. 4). The duration and extent of PGEl inhibition was inversely related to the initial concentration of PGEl. For the first 3 hours of recirculation, the sensitivity of platelets recirculated with PGEl \(\geq 0.1 \mu M\) became more sensitive with time, suggesting gradual loss of PGEl-induced inhibition. The results differed from those obtained when platelets were recirculated without PGEl, in which instance they remained insensitive to epinephrine for the duration of the experiment (Fig. 4). At 6 hours, however, platelets from blood recirculated with PGEl (all concentrations) became much less sensitive to epinephrine than platelets recirculated for only 2 minutes at all concentrations of PGEl tested (Fig. 4). Finally, at 0.03 \(\mu M\) PGEl, all dose response curves (not shown) were abnormal.

To determine whether this decrease in sensitivity of recirculated platelets, noted at 6 hours, was due to the development of an intrinsic functional platelet defect or the appearance of a plasma factor other than PGEl, we evaluated GFP. The response of recirculated platelets to epinephrine after gel filtration remained similar to that of incubated controls at PGEl concentrations of 0.1, 0.3, and 1 \(\mu M\) for up to 4 hours. At 6 hours, however, platelets recirculated with 0.1 \(\mu M\) PGEl were 250-fold less sensitive after gel filtration than platelets recirculated with higher doses of PGEl (0.3 and 1 \(\mu M\)) (Fig. 5). Indeed, platelets recirculated with the higher doses of PGEl continued to respond to normal doses of epinephrine even after 6 hours of recirculation (Fig. 5).

ADP responsiveness tended to be a less sensitive indicator of functional injury to platelets than epinephrine responsiveness. At all sample times, at PGEl concentrations \(\geq 0.1 \mu M\), platelets recirculated for 2 minutes and incubated, and platelets recirculated for the entire 6 hours, remained much less sensitive to ADP than incubated donor platelets, indicating persistence of the PGEl effect (Fig. 6). Furthermore, platelets from blood incubated and recirculated with PGEl \(\geq 0.1 \mu M\) and platelets from blood recirculated without PGEl were similar in their response to ADP at all sample times. Platelets from blood incubated with 0.03 \(\mu M\) PGEl did become more sensitive with time, and these results were qualitatively similar to those obtained with epinephrine.

**Figure 3** The intracellular concentration of LA-PF4 is expressed as a percent of that contained in 10⁶ platelets. ○ = PGEl \(\geq 0.03 \mu M\), \(n = 18\); ○..○.○ = no PGEl, reproduced from data published by Hennessy et al. (1977), \(n = 6\).

**Figure 4** Mean concentrations of epinephrine required to produce complete second wave aggregation. Each point represents the mean of three determinations. Log dose epinephrine is plotted against time. The results from nine experiments at whole blood concentrations of PGEl = 0.1, 0.3, and 1.0 \(\mu M\) are presented. ○ = whole blood drawn directly from donor and incubated without PGEl; ■ = whole blood recirculated for 2 minutes of recirculation and incubated without PGEl; ○—○ = whole blood recirculated for 6 hours with PGEl; ○—○ = whole blood recirculated for 6 hours without PGEl, reproduced from Hennessy et al. (1977), \(n = 6\).
FIGURE 5 Mean concentrations of epinephrine required to produce complete second wave aggregation in GFP are depicted. Log dose of epinephrine is plotted against time. •—• = whole blood drawn directly from donor and incubated without PGE₁ (n = 7); •••• = whole blood withdrawn from the circuit after 2 minutes of recirculation and incubated for 6 hours, with PGE₁ ≥ 0.1 μM (n = 7); ○ ... ○ = whole blood recirculated for 6 hours with PGE₁ ≥ 0.3 μM (n = 4); △ ... △ = whole blood recirculated for 6 hours with PGE₁ = 0.1 μM (n = 3).

Platelet Morphology

Most platelets recirculated without PGE₁ showed some form of morphological alteration. After 6 hours of recirculation, platelets demonstrated varying degrees of organelle breakdown including complete loss of organelles and tubular structure (Fig. 7A). Many of these platelets were found in large aggregates, and virtually all platelets had lost their discoid shape.

In contrast, platelets recirculated with PGE₁ (Fig. 7B) retained an appearance similar to that of incubated control platelets with preservation of essentially normal organelle composition and distribution. Most platelets, however, had undergone changes in shape and exhibited pseudopodia. Recirculated platelets also appeared to have more dilation of the surface-connected tubule system and more vacuole formation than did incubated platelets. Only rarely did platelets recirculated with PGE₁ demonstrate complete loss of intracellular granules, and no platelet microaggregates were observed.

Discussion

When human blood is recirculated in vitro through a membrane oxygenator, there is an 80% drop in the circulating platelet count within 2 minutes, a progressive rise in the plasma levels of the platelet-specific protein LA-PF4, and a persistent loss of platelet sensitivity to aggregating agents (Hennessy et al., 1977). This study demonstrates that reversible inhibition of platelet function with PGE₁ during contact with the nonbiological surface prevents these adverse changes in platelets in this in vitro model of extracorporeal circulation.

Prostaglandin E₁ is a potent inhibitor of platelet shape change induced by ADP. It also prevents aggregation and release induced by ADP, epinephrine, antigen-antibody coated surface, collagen, and thrombin (Emmons et al., 1967; Kinlough-Rathbone et al., 1970; Ball et al., 1970). The onset of inhibition is immediate, and PGE₁ does not interfere with other coagulation factors (Karim, 1972). PGE₁ is rapidly metabolized in the pulmonary circulation (Golub et al., 1975) and thus presents the possibility that its effects on platelets might quickly be reversed. This hypothesis is supported by the observation that removal of the plasmatic environment from platelets by washing (Karim, 1972) or gel filtration (Fig. 5) also reverses PGE₁ inhibition.

Since PGE₁ is rapidly metabolized in the lung, it should be removed rapidly from the circulation following restoration of pulmonary blood flow at the conclusion of bypass, leaving normal functioning platelets (Becker et al., 1974).

PGE₁ covalently bound to surfaces has been shown to reduce platelet adherence (Grode et al., 1974), and Stibbe (1974) has demonstrated that PGE₁ can reduce platelet aggregate formation and the thrombocytopenia that would otherwise occur in a circuit containing a bubble oxygenator. PGE₁ prevents most of the decrease in thrombocyte count in our model of extracorporeal circulation. The temporary decline to 78% of initial levels may represent the transient formation of platelet aggregates and/or reversible adherence to the foreign surface (Hennessy et al., 1977).

LA-PF4 was used as a sensitive indicator of contact-initiated release of granule contents into plasma. Measurement of LA-PF4 by radial immunodiffusion is not sufficiently sensitive to determine the extracellular level of this antigen in PPP. As
FIGURE 7  A: Platelets recirculated for 6 hours without PGE₁. The majority of platelets have lost their granules. Small clumps of platelets are evident. B: Platelets recirculated for 6 hours in the presence of PGE₁. Although most platelets have undergone some degree of shape change, most have retained their granule morphological integrity. 12,000×.
determined by radioimmunoassay, the level of platelet-specific proteins in PPP prepared under special precautions is below 30 ng/ml (Ludlam et al., 1976; Levine et al., 1977). However, radial immunodiffusion has adequate sensitivity to measure loss of LA-PF4 during incubation of PRP at room temperature and during platelet release reaction. It has been observed previously that platelets incubated in the plasma medium at 37°C for 6 hours continuously lose LA-PF4, and the level of this antigen in plasma increases up to 10% of that contained in control PRP. This loss of LA-PF4 is temperature independent and probably unrelated to the platelet release reaction. It is only partially inhibited by PGE1 (Niewiarowski et al., 1977). Our inability to detect the presence of LA-PF4 in plasma after 1 hour of recirculation with PGE1, and its increase in plasma to only 10% of that in control PRP after 6 hours, suggests that only minimal release was induced by recirculation. The absence of platelet LA-PF4 release suggests that platelet granules remain functionally intact. This conclusion was corroborated by the electron microscopic observations that morphological integrity of the granules was preserved as well.

Aggregation studies on platelets in plasma indicated that, after 6 hours of recirculation, platelets became significantly less sensitive to epinephrine at all concentrations of PGE1. This loss of sensitivity in recirculated platelets occurred despite a continued increase in sensitivity in control samples incubated with PGE1 (Fig. 4). However, both PGE1- incubated and PGE1-recirculated platelets, after removal of their plasma environment by gel filtration, responded normally to epinephrine at all sampling times when protected by adequate concentrations of PGE1 (Fig. 5). Therefore, with PGE1 $\geq 0.3 \mu M$, the decline in sensitivity observed in recirculated platelets in plasma at 6 hours must be due to the appearance of a plasma factor other than PGE1 and contrasts with the intrinsic functional defect observed with PGE1 = 0.1 $\mu M$. ADP accumulation in plasma is known to decrease platelet reactivity (Holme et al., 1975) and could account for both the need to add apyrase to the effluent buffer or our gel filtration system (Levy-Toledano et al., 1972) and the decline in platelet sensitivity noted at 6 hours in recirculated platelets otherwise completely protected by 1.0 $\mu M$ PGE1.

Preservation of platelet number and function is related to the concentration of PGE1 employed. At a concentration of PGE1 of 0.3 $\mu M$ or greater, recirculation does not change platelet response to aggregating agents or the release of LA-PF4. At this concentration, only a slight (11%) decrease in the circulating thrombocyte count occurs. PGE1 at 0.1 $\mu M$ allowed a decrease in platelet sensitivity to aggregating agents after 6 hours of recirculation but prevented other changes. PGE1 (0.05 $\mu M$) prevented release of LA-PF4 but failed to completely protect function and loss of circulating platelets. Although 0.01 $\mu M$ PGE1 incompletely prevented platelet loss, this concentration partially prevented platelet release of LA-PF4.

The response of circulating platelets to different concentrations of PGE1 is consistent with observations which show that higher doses of PGE1 are required to prevent shape change than to prevent aggregation (Kinlough-Rathborne et al., 1970). These findings are consistent with the established relationship that links platelet function to metabolic energy. Because more ATP is required for release II, less for release I and primary aggregation, and least for adhesion and shape change, the inhibitor cyclic AMP is more effective against the release reaction and less effective against shape change. Platelet inhibition with PGE1 is associated with an increase in intracellular levels of cyclic AMP. Therefore, lower concentrations of PGE1 would be required to prevent the release reaction (LA-PF4) than to prevent platelet aggregation, and the lowest concentration would be required to prevent change in the numbers of platelets which probably depends on preventing platelet adhesion.

The gradual decrease of PGE1 inhibition in incubated blood mentioned above might result from the activity of plasma enzymes which are capable of metabolizing PGE1 to inactive products (McDonald-Gibson et al., 1972). The possibility exists, however, that the concentration of a physiological prostaglandin antagonist, such as Ca2+, might increase with time in blood. Since this alteration in PGE1 effect coincides in time with the intrinsic functional defect observed in GFP (PGE1 = 0.1 $\mu M$, Fig. 5), platelet damage must result either from too low an initial concentration with the gradual appearance of a previously acquired platelet injury or from a gradual loss of inhibition by PGE1 and subsequent vulnerability of the platelet to the nonbiological surface. In either case, continuous infusion of PGE1 might circumvent this problem and allow for reduction in the effective concentration.

In our studies, ADP responsiveness tended to be a less sensitive indicator of functional injury to platelets than epinephrine-induced aggregation despite the observation that these agents may modulate similar pathways for platelet activation (Charo et al., 1977). Nevertheless, ADP but not epinephrine induces platelet shape change, and epinephrine but not ADP inhibits adenyl cyclase in broken cell preparations (Zieve et al., 1969). The two agents also differ quantitatively in their ability to inhibit PGE1-stimulated formation of cyclic AMP (Haaslam et al., 1971). Indeed, others have reported similar findings in patients who have undergone open cardiac surgery (McKenna et al., 1975).

The mechanism by which endothelial cells prevent platelet adhesion in vivo seems to be by synthesis and local release of a prostaglandin, prostaglandin I2 (Gorman et al., 1977). It is possible that adding PGE1 to the venous reservoir provides for silicone rubber circuits what endothelial cells pro-
vide in vivo, a reversible platelet inhibitor which prevents adverse platelet-surface interactions by stimulating platelet adenylate cyclase.

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