Effects of Heparin on Platelet Aggregation and Release and Thromboxane A_2 Production

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Heparin, when added to citrated platelet-rich plasma (PRP), caused potentiation of platelet aggregation and the release reaction induced by the aggregating agents adenosine diphosphate (ADP), arachidonic acid, collagen, and epinephrine. At low concentrations (4.7×10^{-5} M) arachidonic acid failed to cause aggregation of platelets in citrated PRP. However, in the presence of heparin, the same concentration of arachidonic acid caused aggregation. Examination of PRP for the presence of thromboxane A_2 (TxA_2) by use of a bioassay revealed that heparin also stimulated release of TxA_2. This finding indicated that platelets released more TxA_2 when they were challenged by low concentrations of arachidonic acid in the presence of heparin than in its absence. Platelets were labeled with ^{3}H-arachidonic acid and ^{14}C-serotonin, and attempts were made to determine whether heparin stimulated the platelet release reaction first with subsequent increased production of TxA_2, or alternatively, whether heparin stimulated TxA_2 production first with subsequent enhancement of the release reaction. In view of the demonstrated simultaneous release of ^{14}C-serotonin and ^{3}H-arachidonic acid metabolites, it appeared that either release of ^{14}C and ^{3}H occurs concurrently or, even if one of these two events is dependent on the other, both events take place in rapid succession. Timed sequential studies revealed that in the presence of arachidonic acid, the addition of heparin hastened the apparently simultaneous release of both ^{14}C and ^{3}H. (Am J Pathol 1981, 104:132–141)

THE DEVELOPMENT of thrombocytopenia following intravenous injection of heparin recently has been observed with increasing frequency,[1-3] but the etiology of this phenomenon is not clear. Heparin has been shown to affect certain platelet functions. Potentiation of platelet aggregation and the platelet release reaction has been observed,[4-6] although this remains a subject of controversy. Some investigators have observed no effect of heparin on platelet aggregation,[7] while in some publications heparin has been shown to inhibit aggregation.[8] The mechanisms by which heparin may alter platelet function have not been delineated to date.

The present study was undertaken to examine certain of the effects of heparin on platelets. Heparin potentiated platelet aggregation and the release reaction under the experimental conditions described in this study. Further, heparin was found to potentiate platelet aggregation and the release reaction induced by arachidonic acid. Since thromboxane A_2 (TxA_2) is a potent platelet-aggregating agent and possibly a major product of arachidonic acid metabolism in platelets, the question of whether heparin might act by stimulating production of thromboxane A_2 in platelets arose. Experiments were carried out to investigate this hypothesis.

Materials and Methods

Blood was drawn from adult human volunteers who denied receiving medication of any type for at least 10 days preceding venipuncture. ^{3}H-arachidonic acid (80 Ci/mmol) and ^{3}H-thromboxane B_2 (TxB_2, 150 Ci/mmol) were purchased from New England Nuclear (Boston, MA) and ^{14}C-5-hydroxytryptamine creatinine sulfate (^{14}C-serotonin, specific activity: 30–50 mCi/mmol) from Amersham (Chicago, Ill).

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Several different commercial preparations of heparin dissolved in 0.154 M NaCl (saline) were examined; however, the majority of experiments were carried out with heparin purchased from Sigma Chemical Company (porcine mucosa, St. Louis, Mo) or Upjohn (beef lung, Kalamazoo, Mich). In preliminary experiments, these preparations were chromatographed on a Sephadex G-15 column (2.5 × 40 cm) equilibrated with 0.15 M NaCl at 4 C. Various fractions emerging from the column were analyzed for their effect on platelets. Using anticoagulant property (U/ml) as the criterion, all fractions exhibited similar effects on platelets. Therefore, in all experiments reported in this article, the effect of commercially available heparin on platelets was studied. ADP, arachidonic acid, and l-epinephrine were obtained from Sigma Chemical Company. A collagen suspension was purchased from Hormon-Chemie, Munich, West Germany. 0.22 μ filters were purchased from Millipore Corporation, Boston. Goat anti-guinea pig gammaglobulin was obtained from Antibodies, Inc., Davis, California, and a guinea pig gammaglobulin preparation was purchased from Research Plus Labs, Penville, New Jersey. All other chemicals used were of analytical reagent grade.

PRP was obtained by centrifugation of blood anticoagulated with 0.108 M sodium citrate (one part anticoagulant to 8 parts whole blood) or heparin (5 U/ml whole blood) at 350*g* for 10 minutes at 23 C. Platelet-poor plasma (PPP) was prepared by centrifugation of PRP or the remaining erythrocytes at 1500*g* for 15 minutes and then passing of the supernatant through a 0.22-μ filter. The platelet count in PRP was adjusted to 400,000/μl with autologous PPP. PRP was stored in an atmosphere of 5% CO_2 and 95% air and used within 2 hours after venipuncture.

Platelet aggregation was performed following previously published methods[9] using a Payton Dual Channel Aggregation Module (Payton, Buffalo, NY). Heparin was dissolved in saline, and the freshly prepared solution was mixed with PRP 5 minutes before the addition of an aggregating agent. Unless indicated otherwise, platelet aggregation was followed for 5 minutes after the addition of an aggregating agent. The reaction mixtures contained 0.45 ml PRP, 0.025 ml heparin solution or saline, and 0.025 ml aggregating agent or saline.

The platelet release reaction was monitored by following previously published methods.[9] Briefly, platelets in PRP were incubated with ^{14}C-serotonin for 15 minutes at 37 C. In our hands, under these conditions uptake of ^{14}C-serotonin generally was greater than 85%. Incubation was continued until ^{14}C-serotonin incorporation in platelets had reached a maximum.[10] Platelets then were challenged by the addition of various aggregating agents (or saline in controls) under a variety of experimental conditions, and the amount of ^{14}C-serotonin released by these platelets into the surrounding medium was quantitated by scintillation counting after removal of platelets by centrifugation.[11]

TxA_2 concentration was determined either by the use of a bioassay or by the determination of the concentration of its stable metabolite, TxB_2, by use of a radioimmunoassay.

Bioassay

The ability of TxA_2 to contract segments of rabbit aorta was used as a measure of TxA_2 concentration.[12,13] Briefly, a segment of rabbit aorta was cut spirally and mounted as is shown in Figure 1. Upon addition of PRP, contraction of rabbit aorta in the presence of TxA_2 was recorded and, as reported earlier,[12,13] under appropriate experimental conditions, the degree of contraction was a function of the amount of TxA_2 present in the solution bathing the aortic spiral. This method was helpful in the determination of the relative concentrations of TxA_2.

Radioimmunoassay of TxB_2

A carbodiimide conjugate of TxB_2 was prepared following the method described by Jubiz et al.[14] The radioimmunoassay utilized an antibody developed in guinea pigs to the carbodiimide conjugate of TxB_2. In the TxB_2 assay, 350 μl of 0.05 M phosphate buffer (pH 7.4) containing 0.01% guinea pig gammaglobulin was incubated overnight at 4 C with a mixture of 50 μl of labeled TxB_2 (5 pg, 1300 cpm, ^{3}H-TxB_2), 50 μl of either sample or standard, and 50 μl of the diluted antibody. Then 25 μl of goat anti-guinea pig gammaglobulin was added, and the tubes were allowed to incubate for 3 hours at 23 C. The tubes subsequently were centrifuged for 20 minutes at 2000*g*, and 350 μl of the supernatant were examined for the presence of ^{3}H. This procedure permitted the detection of as little as 0.2 ng TxB_2/ml.

Double Label of Platelets

Washed platelets were obtained by the following method. Blood was collected using acid-citrate-dextrose (ACD) as anticoagulant.[15] Erythrocytes were sedimented at 350*g* for 10 minutes, and the PRP was siphoned out carefully without disturbing the buffy coat. PRP obtained in this manner then was subjected to centrifugation at 1000*g* for 10 minutes. Sedimented platelets were resuspended in a pH 6.5 ACD buffer that contained one part ACD to 9 parts modified

Ringer solution.[16] These platelets subsequently were sedimented at 1000*g* for 10 minutes, and the pellet was resuspended in 2 ml of saline, incubated at 37 C for 5 minutes, and then diluted with 8 ml of Ca^{2+}- and Mg^{2+}-free Tyrode's solution. To this washed platelet suspension, 0.1 μCi of ^{14}C-serotonin and 0.3 μCi of ^{3}H-arachidonic acid were added, and the mixture was incubated for 90 minutes at 30 C. At the end of this incubation period, an aliquot was removed and examined for incorporation of ^{14}C and ^{3}H into the platelets. In most experiments, uptake of ^{14}C-serotonin was greater than 85%, and uptake of ^{3}H-arachidonic acid varied between 70% and 90%. These results were determined as follows. After adjusting the pH of the suspension to 6.5 with ACD buffer, platelets were sedimented at 1000*g* for 10 minutes, and the supernatant was discarded. Sedimented platelets were resuspended in 2 ml of saline, incubated at 37 C for 5 minutes, and then diluted with 8 ml of Tyrode's solution or citrated PPP. Platelet preparations obtained by following the above procedure contained less than 0.5% leukocytes when examined by phase contrast microscopy. Because of the small number of contaminating leukocytes, no attempt was made to differentiate their subpopulations. The amounts of ^{3}H and ^{14}C in the same platelet suspension were determined in a Beckman LS100 three-channel scintillation counter (Beckman, Irvine, Calif.).

Results

Effects of Heparin on Platelet Aggregation and the Platelet Release Reaction

Platelet Aggregation

When PRP was prepared from blood drawn with heparin as the anticoagulant, the aggregation of the platelets in the heparinized PRP was significantly different from that of platelets in citrated PRP, even though both blood samples were obtained sequentially from the same donor. Platelets in heparinized PRP reacted more strongly to the presence of added ADP than did platelets in citrated PRP (Figure 2). It was observed also that the platelet count in heparinized PRP varied considerably, and in order to render comparisons more meaningful, the citrated PRP had to be diluted with citrated PPP to obtain a platelet count identical to that in heparinized PRP. When citrated PRP was studied in both the presence and absence of heparin, even more distinct differences were discernible. Platelet aggregation induced by either ADP or arachidonic acid was increased markedly by the presence of heparin in citrated PRP (Figure 3).

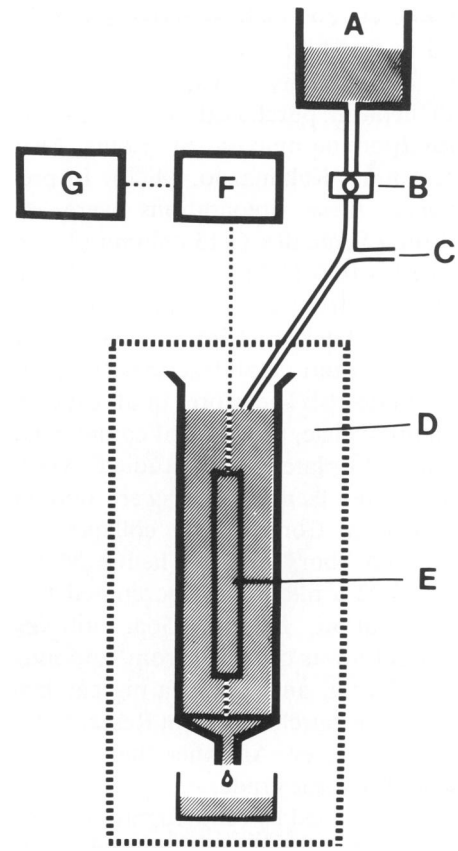

Figure 1—Experimental procedure used to determine the contraction of a spiral segment of rabbit aorta following the method described by Piper and Vane.[13] *A*—Reservoir containing Krebs solution with a number of blocking agents.[13] *B*—Peristaltic pump. *C*—Entry port for test solutions. Test preparations contained 450 μl PRP (or PPP as control), 25 μl of heparin solution or saline, and 25 μl of aggregating agent or buffer. After platelets were challenged by an aggregating agent and the reaction was monitored in the aggregometer for a predetermined time, 200 μl of this reaction mixture were added to the aortic spiral preparation through the entry port. The effects of the reaction mixture on the aorta then were recorded for the next 30 minutes. *D*—Chamber maintained at 37 C, 95% air, 5% CO_2. *E*—The spiral segment of rabbit aorta mounted on a transducer. *F*—Transducer. *G*—Recorder.

Heparin also potentiated aggregation induced by collagen or epinephrine, but distinct differences were demonstrable only at low (1–2 U/ml) heparin concentrations (Figures 4 and 5). Higher concentrations of heparin appeared to inhibit collagen- or epinephrine-induced aggregation. Similar results were obtained with heparin of either porcine mucosa or beef lung origin.

Release Reaction

The presence of heparin in test mixtures produced a marked increase in the release of ^{14}C-serotonin in addition to enhancement of aggregation when platelets were challenged by the addition of ADP (Figure 6). At lower heparin concentrations (1–5 U/ml),

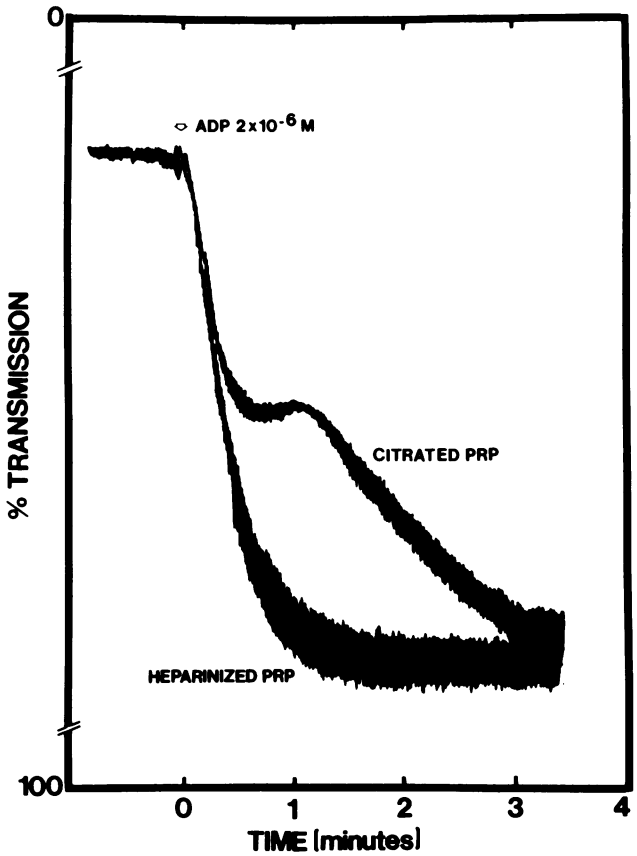

Figure 2—Effects of 2 × 10^{-6} M ADP on platelet aggregation in citrated PRP and in heparinized PRP. A representative experiment based on studies performed with blood from 26 donors.

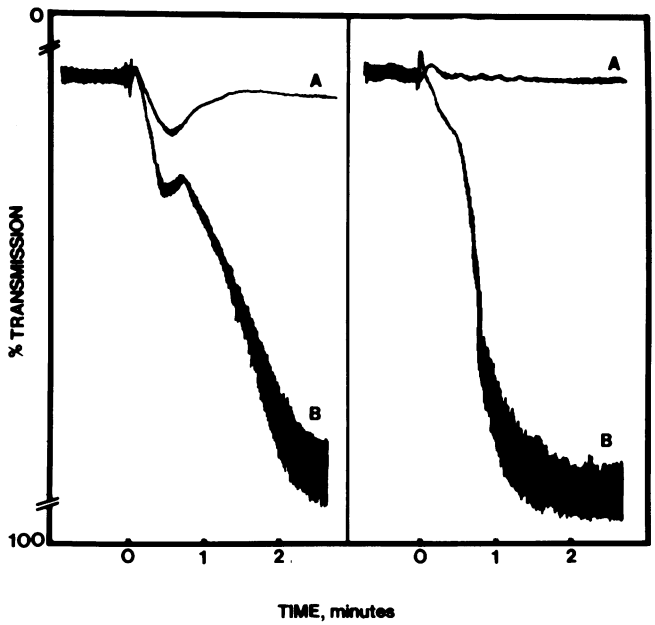

Figure 3—Effects of ADP (1 × 10^{-6} M, *left panel*) or arachidonic acid (5 × 10^{-5} M, *right panel*) on platelet aggregation in citrated PRP in the absence (*A*) or presence (*B*) of 5 U heparin/ml. This is a representative experiment based on a minimum of 30 separate experiments. To 450 μl PRP, 25 μl of heparin or saline were added, and after 5 minutes' incubation at 37 C, 25 μl of aggregating agent were added and resultant changes in transmittance recorded.

stimulation of the release reaction was directly proportional to the amount of heparin in the reaction mixture. This stimulation of the release reaction by heparin was more marked in citrated PRP to which heparin was added than in PRP obtained from blood drawn directly into heparin (5 U/ml, final concentration). For these latter studies, the release reaction in heparinized PRP was compared with that in citrated PRP or citrated PRP plus heparin obtained from the same donor (data not shown).

Effects of Heparin on TxA_2 Production by Platelets

Experiments in which platelet aggregation and the release reaction induced by arachidonic acid were shown to be enhanced distinctly by the presence of heparin (Figure 3) suggested that the mechanism whereby heparin induced such changes might involve arachidonic acid metabolites. In the light of the several studies demonstrating the conversion of aracidonic acid to TxA_2 in platelets, this aggregating agent was selected for further study.

TxA_2 Levels in Platelets Challenged by Various Aggregating Agents

TxA_2 Levels in Platelets Challenged by Arachidonate

Observations presented in Figure 3 indicated that subthreshold concentrations of arachidonic acid that failed to cause aggregation of platelets in citrated PRP produced aggregation in the presence of heparin, suggesting the possibility that increased formation of certain arachidonic acid metabolites might be responsible for stimulation of platelet aggregation under these conditions. Since TxA_2 is possibly a major metabolite of arachidonic acid in platelets, experiments were designed to detect any increased production of TxA_2 by platelets in the presence of heparin and arachidonic acid. Using the experimental design described in Figure 1, we examined the rabbit aorta contracting property of platelet-derived TxA_2. As is shown in Figure 7, addition of 6×10^{-5} M arachidonic acid to citrated PRP produced negligible contraction when an aortic segment was exposed to an aliquot of this reaction mixture. However, under the same experimental conditions, the presence of 10 U of heparin/ml of PRP resulted in a significant increase in the contraction of the aortic strip upon exposure to this reaction mixture, suggesting the presence of elevated levels of TxA_2. The presence of

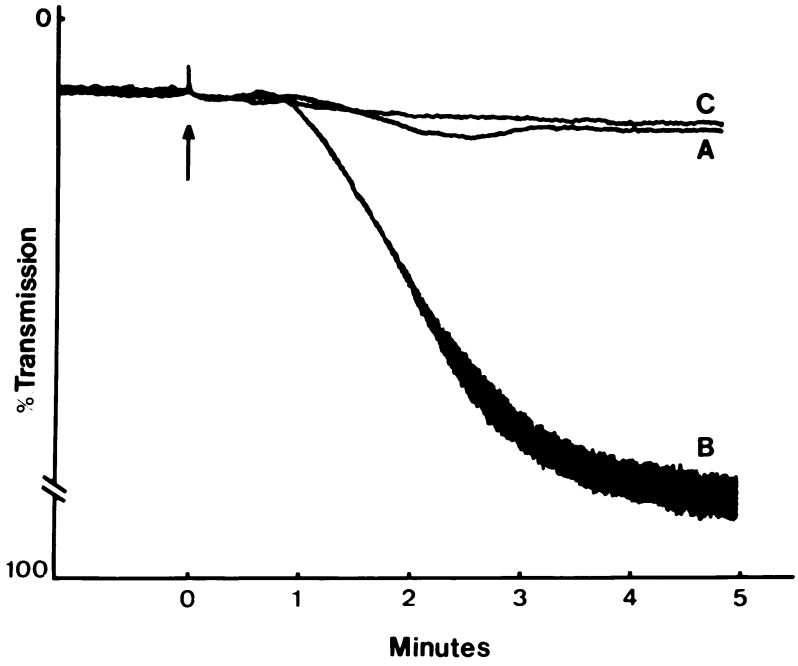

Figure 4—Effects of 0.25 μg of collagen on platelet aggregation in the presence of saline (*A*); 1.2 U heparin/ml (*B*); and 18 U heparin/ml (*C*). This is a representative experiment from a total of 30. The actual amount of collagen added to PRP that caused no platelet aggregation varied from donor to donor (range 0.1–0.75 μg), but the potentiating effect of heparin was noted consistently.

heparin in PPP (10 U/ml) failed to cause contraction of the aortic segment under similar experimental conditions. Although addition of increasing concentrations of arachidonic acid to citrated PRP caused an increasing degree of contraction of the aortic segment in both the presence and the absence of heparin, contraction of the aorta in the presence of heparin was always greater than in the absence of this anticoagulant until a concentration of 3.5×10^{-4} M arachidonic acid was reached. When the concentra-

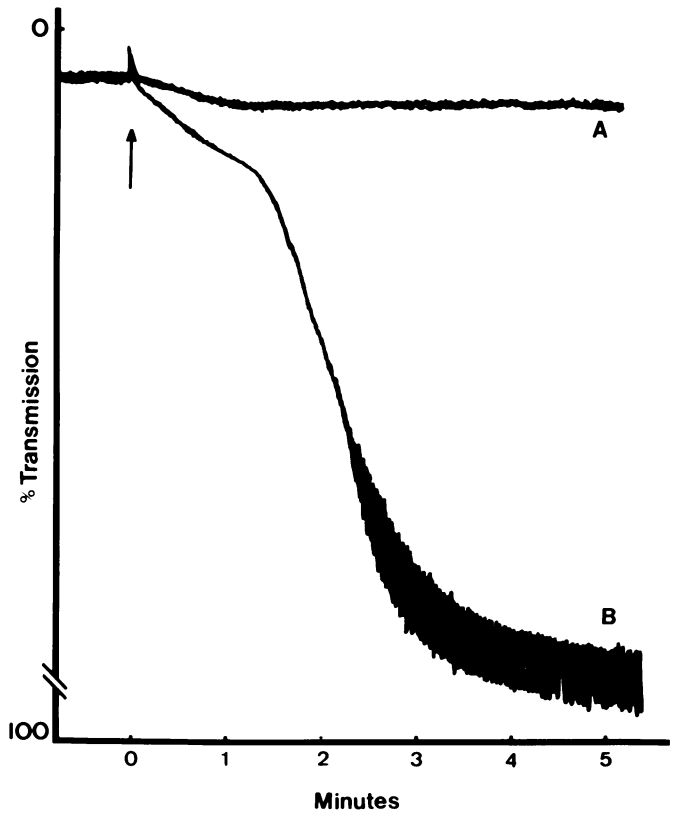

Figure 5—Effects of 2.5×10^{-7} M epinephrine on platelet aggregation in the presence of saline (*A*) and 1.2 U of heparin/ml (*B*). Although the maximal concentration of epinephrine that caused no aggregation varied from donor to donor (1×10^{-7} M to 5.5×10^{-7} M), the potentiating effect of low concentrations of heparin was noted consistently. This is a representative experiment from a total of 30.

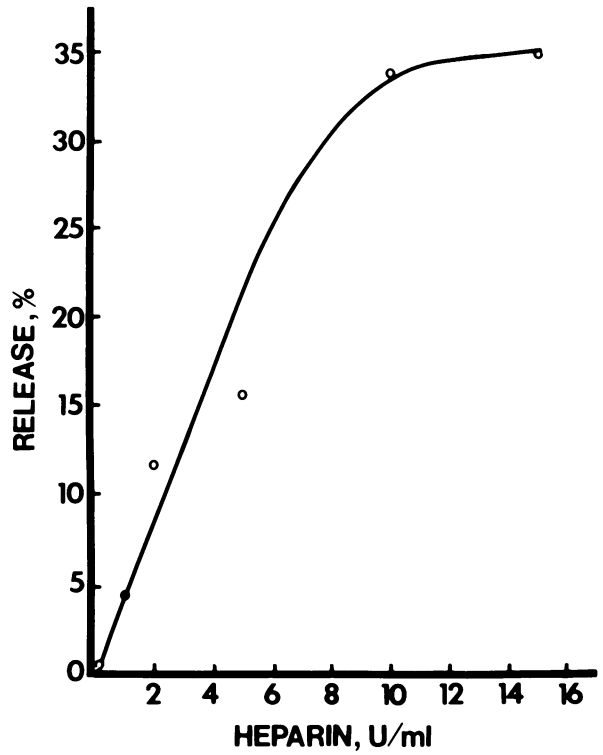

Figure 6—Effect of heparin concentration on release from platelets of ^{14}C-serotonin induced by 5×10^{-7} M ADP. Each point is the mean of 3 experiments carried out on 3 separate occasions with blood obtained from the same donor. Although actual ^{14}C-serotonin released from platelets in the presence of heparin varied considerably from donor to donor, increasing concentrations of heparin always resulted in increased release of radioactivity. In the absence of heparin, addition of 5×10^{-7} M ADP caused less than 5% release of ^{14}C-serotonin in 17 separate experiments and less than 2% release in 29 experiments.

tion of arachidonic acid was increased above 3.5×10^{-4} M, aortic contraction remained at a constant level. At a concentration of 4.7×10^{-4} M arachidonic acid, no significant difference was evident in the contraction of the aortic segment in either the presence or absence of heparin.

In a parallel study, the effects of a fixed concentration of arachidonic acid (4.7×10^{-5} M) were examined in the presence of varying concentrations of heparin. The results are presented in Figure 8. After 5 minutes incubation of citrated PRP with varying concentrations of heparin, arachidonic acid was added, and the reaction was followed in the aggregometer for exactly 60 seconds. At the end of this time, the reaction mixture was analyzed for the presence of aortic-segment-contracting activity. It was observed that the addition of 4.7×10^{-5} M arachidonic acid to nonheparinized, citrated PRP caused neither appreciable platelet aggregation nor contraction of rabbit aorta. In the presence of 4.7×10^{-5} M arachidonic acid and 0.125 units of heparin/ml, slight enhancement of platelet aggregation was discernible, and the reaction mixture showed the presence of minimal aorta-contracting activity. Both platelet aggregation and aorta-contracting activity increased in parallel with increasing amounts of heparin, clearly suggesting a dose-dependent relationship.

In order to confirm whether contraction of the aortic segment truly reflected the action of TxA_2 in test mixtures, a radioimmunoassay for TxB_2, a stable metabolite of TxA_2, was employed also. Observations presented in Table 1 demonstrate that the presence of heparin in PRP resulted in elevated levels of TxB_2. It was not possible for us to use the radioimmunoassay to study the effects of arachidonic acid, because in our hands the presence of this fatty acid in the reaction mixture in large amounts interfered with the assay.

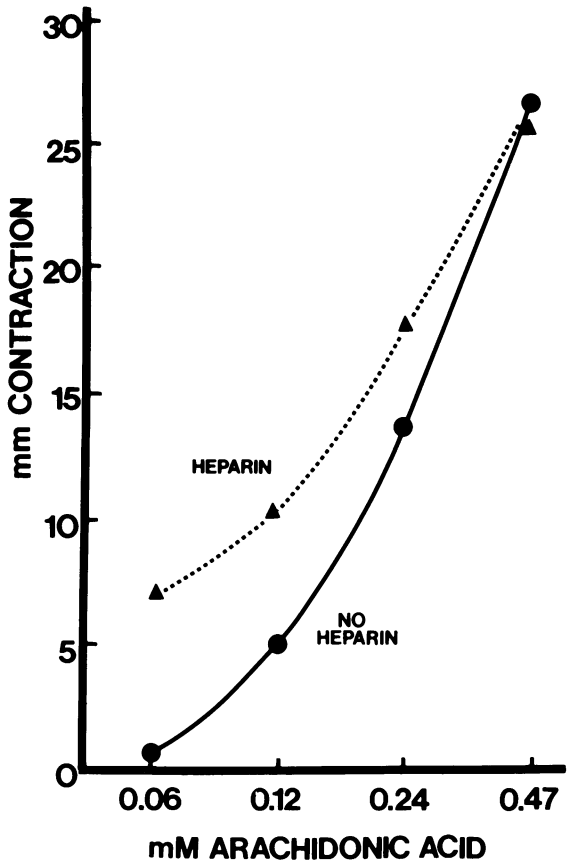

Figure 7—Contraction of rabbit aorta induced by a mixture of arachidonic acid and citrated PRP containing either heparin (10 U/ml) or buffer. After incubation of PRP with heparin or buffer for 5 minutes at 37 C, different concentrations of arachidonic acid were added. After continuous stirring of the reaction mixtures for 60 seconds, the rabbit aorta spirals were exposed to the test solutions as described in Figure 1.

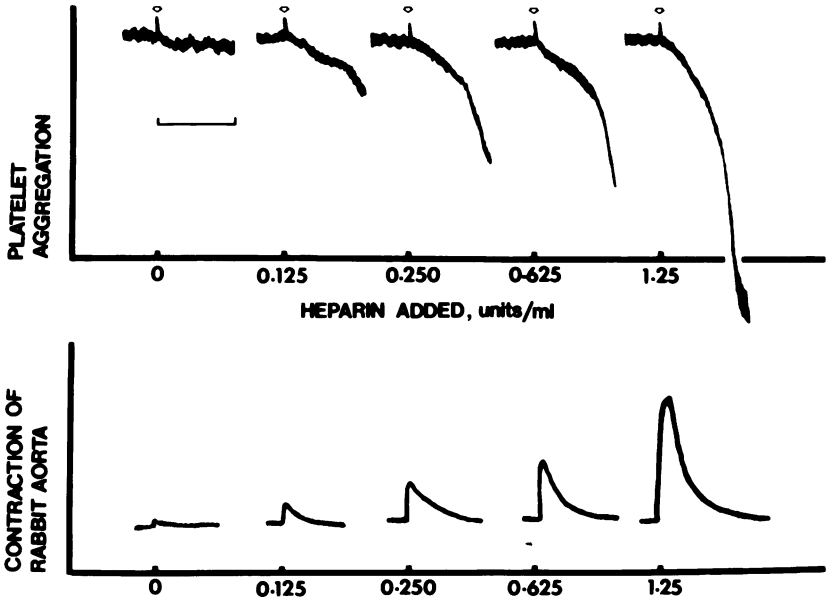

Figure 8—Effects of varying the concentration of heparin on the aggregation of platelets (*top panel*) and on the rabbit aorta contracting activity from platelets (*lower panel*) in the presence of 4.7×10^{-5} M arachidonic acid. After incubation of citrated PRP with heparin (or saline) for 5 minutes at 37 C, arachidonic acid was added, and the aggregation of platelets was monitored for exactly 60 seconds. At the end of this time, 200 μl of the reaction mixture were removed and used for determination of aortic segment contracting activity. This is a representative experiment based on 4 separate experiments.

TxA_2 Levels in Platelets Challenged by Collagen

Platelets challenged by collagen produced a greater contraction of aorta in the presence of heparin than in its absence only at low heparin concentrations (1 U/ml). In contrast, at higher heparin concentrations (10–20 U/ml) there was inhibition of the collagen-induced platelet aggregation, the release reaction, and TxA_2 production (data not shown).

TxA_2 Levels in Platelets Challenged by Epinephrine

Platelets challenged by epinephrine in the presence of heparin (2 U/ml) showed only a slightly increased contraction of aorta. Figure 9 illustrates that in control experiments in the absence of heparin, production of TxA_2 by platelets in citrated PRP as detected by the rabbit aorta contraction assay peaked at about 120 seconds after the platelets were challenged with 2×10^{-7} M epinephrine. In a similar experiment, the presence of heparin caused no increase in TxA_2 levels, as judged on the basis of contraction of aortic segments, but maximal contraction was now achieved at 60 seconds after platelets were challenged with epinephrine. Moreover, although the reaction was not studied beyond 180 seconds following the addition of epinephrine, the trend of the data indicates that TxA_2 levels in controls decreased at a faster rate than did those in reaction mixtures in which heparin was present.

Table 1—Radioimmunoassay of TxB_2 in Citrated PRP or Citrated PRP Containing Heparin (5 U/ml) After Platelets were Challenged by an Aggregating Agent*

Aggregating agent		TxB_2 (ng/ml)	
Type	Concentration	No heparin†	Heparin (5 U/ml)‡
None	—	3.0	2.0
ADP	7×10^{-7} M	4.0	16.5
Epinephrine	1×10^{-6} M	18.5	40.5
Collagen	3 μg/ml	107.5	122.5

* Data from a single experiment representative of 3 separate experiments.

† Reflects the concentration of TxB_2 when platelets in citrated PRP were challenged by addition of either saline (control) or an aggregating agent.

‡ Citrated PRP to which heparin (5 U/ml) was added 5 minutes prior to the addition of saline or an aggregating agent.

Relationship Between Platelet Aggregation, the Release Reaction, and TxA_2 Production

Experiments were carried out in an effort to establish possible relationships between aggregation, the release reaction, and arachidonic acid metabolism. The question of whether the release reaction in platelets preceded increased production of TxA_2, or whether the reverse occurred, was probed. Platelets labeled with both ^{14}C-serotonin and ^{3}H-arachidonic acid were used in these studies. Observations presented in Table 2 show a slight gradual increase in the release of ^{14}C and ^{3}H. Under identical experimental conditions, addition of heparin in the reaction mixture caused enhancement of both ^{14}C and ^{3}H release. It should be noted, however, that even though heparin caused an increase in the release of ^{14}C and ^{3}H, both isotopes appeared to have been released simultaneously. Similar temporal observations were made

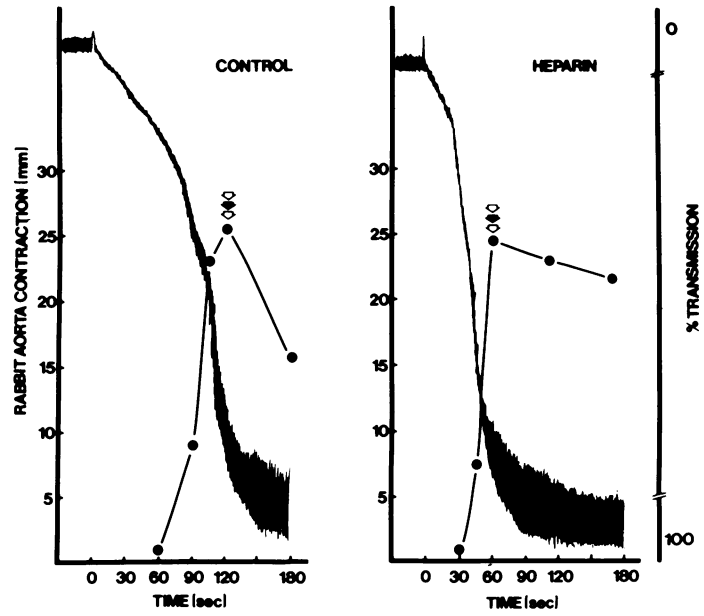

Figure 9—Time course of the aggregation of platelets in citrated PRP and the appearance of aortic segment contracting activity when platelets were challenged by 2×10^{-7} M epinephrine. In the control (*left panel*), maximum rabbit aorta contracting activity was observed in the reaction mixture 120 minutes after the addition of epinephrine. In the presence of heparin (*right panel*), maximum activity was noticed after only 60 seconds. Maximum contraction (O——O) of rabbit aorta was nearly identical in both experiments.

concerning aggregation and aorta-contracting activity when platelets were challenged by epinephrine in the presence or absence of heparin (Figure 9).

Discussion

Development of thrombocytopenia in some patients following intravenous administration of heparin has been reported frequently.[1-3,17,18] The reasons this occurs only in certain patients is not known, although at least in some cases delayed-onset thrombocytopenia appears to have an immunologic basis.[1,19] It is surprising that relatively little attention has been paid to the thrombocytopenic effects of this agent, while the anticoagulant properties of heparin have been the subject of extensive investigation. In order for our studies to be comparable with clinical observations, experiments were carried out with that form of heparin used for intravenous administration in humans.

The observations reported here clearly demonstrate enhancement of platelet aggregation and of the release reaction by heparin *in vitro*. These findings confirm observations published by others.[4-6] Enhancement by heparin is more pronounced when this anticoagulant is added to citrated PRP than when heparin is present in noncitrated PRP. These findings suggest that calcium or citrate, or both, may be involved in producing some part of the enhancement of aggregation and the release reaction found in the presence of heparin.

Stimulation of platelet aggregation, of the release reaction, and of contraction of aortic segments was observed in tests with a variety of aggregating agents in the present studies. However, differences in TxA_2 levels were documented quantitatively only under certain of these test conditions. Attention was focused in the present studies on the effects of the plate-

Table 2—Effects of Heparin on ADP-Induced Release of ^{3}H and ^{14}C From Platelets Previously Labeled With ^{3}H-Arachidonic Acid and ^{14}C-Serotonin*

Time (seconds)	Control (no heparin) (cpm)		Tests (heparin, 10 U/ml) (cpm)	
	^{3}H†	^{14}C†	^{3}H†	^{14}C†
0	206	280	316	244
15	218	321	227	229
30	209	318	382	335
60	215	362	416	588
95	233	411	624	912
120	248	437	1043	1769
180	281	458	1105	1855

* Due to large variation in the uptake and release of radioactivity in different experiments, representative data from 1 out of 5 different experiments is presented. Platelets labeled with ^{14}C-serotonin and ^{3}H-arachidonic acid were resuspended in citrated plasma and incubated at 37 C for 15 minutes. Prior to the experiment, heparin (10 U/ml) (experimental) or saline (control) was added, and after an additional 15 minutes' incubation, platelets were challenged by 5×10^{-6} M ADP. At a predetermined time, an aliquot of this mixture was transferred to a microcentrifuge tube and centrifuged at 5000*g* for 15 seconds. The supernatant (200 μl) was removed rapidly, and the presence of ^{3}H and ^{14}C was determined by scintillation counting. Each value is based on 10-minute counts.

† The presence of ^{14}C in the supernatant suggests the release of serotonin from platelet granules. The presence of ^{3}H in the supernatant could be due to the dissociation of previously incorporated ^{3}H-arachidonic acid or the release from platelets of arachidonic acid metabolites.

cpm = counts per minute.

let-aggregating agent arachidonic acid, since TxA_2 has been shown to be a metabolite of this fatty acid in platelets.[20] Observations reported in Figure 7 suggest that heparin potentiates the production of TxA_2 by platelets and that this potentiation is more easily detected at lower arachidonic acid concentrations. This is due possibly to the fact that a significant difference in TxA_2 levels can be observed only at lower concentrations of arachidonic acid. As the concentration of arachidonic acid is increased, differences in the TxA_2 levels in the presence or absence of heparin become smaller, because the higher concentrations of added arachidonic acid apparently are able to induce similar effects whether or not heparin is present.

A concentration of arachidonic acid that failed to produce any effect on platelet aggregation in citrated PRP was selected in order to explore further the effects of this agent on platelets. Platelets in citrated plasma were challenged by this concentration (4.7×10^{-5} M) of arachidonic acid in the presence of varying concentrations of heparin. Figure 8 illustrates that, under these conditions, heparin influenced platelet aggregation and TxA_2 production similarly and in a dose-dependent manner. These and other observations reported here establish the potentiating effect of heparin on platelet aggregation, the release reaction, and TxA_2 production. However, these observations also raise the question of whether heparin causes a direct stimulation of the release reaction, which in turn may be responsible for increased production of TxA_2, or whether heparin in fact stimulates the production of TxA_2, which in turn enhances the release reaction.

Experiments designed to explore the mechanism whereby heparin affects platelet function failed to provide conclusive answers. When platelets labeled with both ^{14}C-serotonin and ^{3}H-arachidonic acid were challenged by ADP, the presence of heparin in the reaction mixture resulted in enhancement of the release reaction (Table 2). Although TxA_2 concentration was not determined in the experiments summarized in Table 2, on the basis of observations reported in Figure 8, it appears reasonable to suggest that the presence of ^{3}H in the platelet supernatant was due to arachidonic acid metabolites. These ^{3}H-arachidonic acid metabolites and ^{14}C-serotonin either were released simultaneously into the surrounding medium, or the time gap involved between occurrence of one phenomenon and its impact on the other was so short that our experimental approach could not detect these small differences.

It was demonstrated clearly, however, that the presence of heparin in the reaction mixture hastened the release of both ^{14}C and ^{3}H when platelets were challenged by ADP (Table 2). Similar observations were made when platelets were challenged by epinephrine. Observations summarized in Figure 9 indicate that maximum contraction of rabbit aorta was nearly identical in both the presence and the absence of heparin, possibly due to the fact that the highest TxA_2 concentration in the presence or the absence of heparin may have been greater than the concentration of TxA_2 required for maximal contraction. TxA_2 levels in excess of this maximal concentration would not be recorded in the bioassay used in this study. Data presented in Table 1 support this argument. Although heparin potentiated epinephrine-induced TxA_2 production, TxA_2 levels in the absence of heparin were considerably elevated and possibly sufficient to cause maximal contraction of rabbit aorta. Data presented in Figure 9 also suggest that the presence of heparin hastened the production of TxA_2, shown by the finding that in the absence of heparin, maximal TxA_2 concentration was reached in 120 seconds after exposing platelets to epinephrine, compared with only 60 seconds in the presence of heparin. Moreover, TxA_2 levels remained elevated for a longer interval in the presence of heparin than in its absence. Thus, in light of the data presented in this manuscript, it appears reasonable to conclude that platelet aggregation, the release reaction, and TxA_2 production induced by several different aggregating agents are accelerated and are more pronounced generally in the presence of heparin than in its absence.

The mechanism whereby heparin potentiates platelet aggregation, the release reaction, and TxA_2 production remains to be elucidated. One may speculate that heparin stimulates platelet phospholipase A_2 analogous to its effect on lipoprotein lipase.[21] Stimulation of phospholipase A_2 in the presence of heparin would cause the comparatively greater release of arachidonic acid that could then be metabolized by platelets to generate TxA_2.[22]

The increased production of TxA_2 by platelets in the presence of heparin may be of importance for patients whose blood is heparinized prior to extracorporeal circulation. As blood passes through an extracorporeal circulatory system, some platelets may undergo the release reaction due to platelet adhesion or the effects of shear stresses. Thus the presence in blood of low concentrations of products released from platelets may become a significant stimulus for thromboembolism if heparin is present simultaneously.

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