Background—Heparin and direct thrombin inhibitors, such as hirudin, have limitations in the treatment of acute coronary syndromes. Heparin does not inactivate fibrin-bound thrombin, whereas hirudin fails to block thrombin generation. In contrast, Vasoflux is a novel anticoagulant that inactivates fibrin-bound thrombin and attenuates factor Xa generation.

Methods and Results—Vasoflux is prepared by depolymerization of heparin, restricting molecular size to between 3000 and 8000 Da, and reducing antithrombin affinity by periodate oxidation. Vasoflux catalyzes fibrin-bound thrombin inactivation by heparin cofactor II (HCII) and inhibits factor IXa activation of factor X independently of antithrombin and HCII. Compared with other anticoagulants in a thrombogenic extracorporeal circuit, Vasoflux maintains filter patency at concentrations that produce an activated clotting time (ACT) of 220 seconds. In contrast, to maintain filter patency, heparin, low-molecular-weight heparin (LMWH), and hirudin require concentrations that produced an ACT of 720, 415, and 1500 seconds, respectively, whereas dermatan sulfate was ineffective at concentrations that produced an ACT of 360 seconds.

Conclusions—Vasoflux is more effective than heparin and LMWH because it inactivates fibrin-bound thrombin and is superior to hirudin and dermatan sulfate because it also blocks factor Xa generation. (Circulation. 1999;99:682-689.)

Key Words: anticoagulants ■ heparin ■ coagulation
Methods

Materials
Unfractionated heparin from porcine intestinal mucosa (grade II; 166.9 USP units/mg) and Atroxin (the thrombin-like venom from Bothrops atrox) were purchased from Sigma Chemical Co. Human factor X, factor IXa, α-thrombin, and plasminogen-free fibrinogen were obtained from Enzyme Research Laboratories. Heparin fractions of defined molecular weight (3000 and 5000 Da) were isolated from unfractionated heparin by gel filtration on a TSK G2000 SWX gel column (30 cm × 7.8 mm, Supelco) as previously described. The LMWH used in these studies was enoxaparin (Rhône-Poulenc Rorer). Anti thrombin and HCII, isolated from human plasma by affinity chromatography as described, migrated as single bands on SDS-PAGE analysis with apparent molecular weights of 58 000 and 70 000, respectively. When titrated against thrombin by the method of Olson et al., both inhibitors completely inactivated thrombin when inhibitor and enzyme were added in equimolar amounts. Phosphatidylcholine (75%) / phosphatidylserine (25%) vesicles were prepared as described.

Effect of Fibrin on Rates of Thrombin Inactivation by Antithrombin and HCII
To examine the effect of fibrin on the rates of thrombin inactivation by antithrombin or HCII, fibrin clots were formed in wells of a 96-well plate containing hydrophilic membranes (Millipore) by clotted 6 μmol/L fibrinogen (suspended in 20 μL of 20 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl (TBS)) with 10 μL each of α-thrombin and CaCl₂ (final concentrations, 10 mmol/L and 1 mmol/L, respectively). After incubation for 30 minutes at 23°C, 40 μL of TBS containing 400 mmol/L HCl or antithrombin was added to consecutive wells at 5-minute intervals. Buffer alone was added to control clots. Five minutes after the last addition, 200 μL TBS containing 200 μmol/L of thrombin-directed substrate, tosyl-Gly-Pro-Arg-p-nitroanilide (Sigma), was added. After incubation for 60 minutes at 23°C, 75 μL of clot supernatant was removed and added to the wells of a 96-well plate prefilled with 75 μL of 0.2N acetic acid. Residual thrombin activity, determined by measuring absorbance at 405 nm, was plotted as a function of time. The slope of this line yielded the first-order rate constant. The second-order rate constant was calculated by dividing this value by the inhibitor concentration. Second-order rate constants for thrombin inhibition by antithrombin or HCII in the presence of fibrin were compared with those obtained in its absence. As controls, the rates of factor Xa inhibition in the absence and presence of fibrin were measured in a similar fashion except that fibrinogen was clotted with Atroxin in place of fibrin and the factor Xa-directed chromogenic substrate, methoxy carbonyl-d-norleucyl-Gly-Arg-p-nitroanilide (Boehringer Mannheim Canada), was substituted for the thrombin substrate.

HCII-Mediated Inhibition of Fibrin-Bound Thrombin by Unfractionated Heparin or LMWH Fractions
We compared unfractionated heparin with 5000- and 3000-Da heparin fractions in terms of their ability to catalyze fibrin-bound thrombin inactivation by HCII. Varying concentrations of the different heparin preparations, diluted in TBS, were added in 25-μL aliquots to wells of a 96-well plate containing 500 nmol/L HCII, 9 mmol/L fibrinogen, 2 mmol/L CaCl₂, and 200 μmol/L tosyl-Gly-Pro-Arg-p-nitroanilide in 50 μL of TBS. Fibrinogen was clotted by addition of 125 ng Atroxin and 4 mmol/L α-thrombin, both suspended in 25 μL of TBS. A molar excess of fibrinogen relative to thrombin was used to ensure that most of the thrombin was fibrin-bound. After a 90-second delay to allow fibrinogen clotting, residual thrombin activity, determined by subtracting absorbance at 490 nm from that at 405 nm, was plotted as a function of time. The slope of this line yielded the rate of thrombin inhibition, which was then expressed as a percentage of the rate measured in the absence of glycosaminoglycans.

Preparation of Vasoflux
Unfractionated heparin was depolymerized with nitrous acid to generate an LMWH fraction with a mean molecular weight of 5000. Unfractionated heparin, dissolved in distilled water, was mixed with sodium nitrite in a ratio of 33 to 1 (wt/wt). Concentrated HCl was added to lower the pH to 3.0. After 2 hours of incubation at 23°C, the pH was increased to 6.5 by addition of 5% NaOH. The resultant LMWH was then subjected to ultrafiltration with a 3000-Da-cutoff cellulose membrane fitted to an ultrafiltration unit (Millipore) and lyophilized. To reduce antithrombin affinity, the heparin fraction was oxidized with sodium periodate. Lyophilized LMWH was dissolved in 25 mmol/L sodium phosphate buffer, pH 7.0. Sodium periodate, dissolved in distilled water, was added to 20 mmol/L. After incubation in the dark for 24 hours at 23°C, 50% NaOH was added to raise the pH to 7.4, and the sample was again subjected to ultrafiltration with a 3000-Da-cutoff membrane. Aldehydes formed during the oxidation reaction were reduced with sodium borohydride to yield primary alcohols. After a final ultrafiltration step, the material was lyophilized.

Fluorescence Labeling of HCII
To label HCII with anilinonaphthalene-6-sulfonic acid (ANS), 10 μmol/L protein was dialyzed against 20 mmol/L HEPES, 100 mmol/L NaCl, 1 mmol/L EDTA, pH 7.0, for 18 hours at 4°C. After the protein had been recovered, 5 μL of a 10 mmol/L solution of 2,14-(iodoacetamide)anilino)naphthalene-6-sulfonic acid (Molecular Probes, Inc), dissolved in dimethyl sulfoxide, was added, and the reaction mixture was incubated for 60 minutes at 4°C in the dark. Unreacted fluorocephore was removed by dialysis against 500 mL of TBS at 4°C with 4 changes of buffer over 18 hours. After dialysis, the sample was recovered, and protein concentration was determined by measurement of absorbance at 280 nm with an extinction coefficient of 1.1 (mg/mL)⁻¹ · cm⁻¹ and correction for light scattering and ANS absorbance (ε₅₆₀ = 26) as described. In the presence of heparin, ANS-labeled HCII complexed thrombin to the same extent as unlabeled protein, as assessed by SDS-PAGE (data not shown). The heparin-catalyzed rate of thrombin inhibition by ANS-labeled HCII was similar to that of unlabeled HCII [second-order rate constants of 2.0 × 10⁸ and 3.7 × 10⁸ (mol/L)⁻¹ · min⁻¹, respectively].

Affinities of Glycosaminoglycans for Antithrombin, HCII, and Factor IXa
To measure the affinities of Vasoflux, LMWH, and unfractionated heparin for antithrombin or factor IXa, a 1 × 1-cm quartz cuvette containing 100 nmol/L antithrombin or factor IXa in 2 mL TBS was excited at 280 nm (6-nm slit width), and intrinsic fluorescence was continuously monitored in time drive at 340 nm (6-nm slit width) with a Perkin-Elmer LS50B luminescence spectrometer. The contents of the cuvette were stirred with a micro stir-bar and maintained at 25°C with a recirculating water bath. Intrinsin fluorescence intensity was measured before (I₀) and after (I) addition of 5 to 10 μL of 10-ng/mL solutions of the various glycosaminoglycans. Titrations were continued until there was no change in I. After the experiment, I values were read from the time drive profile, and I/I₀ values were calculated and plotted versus glycosaminoglycan concentration. The data were analyzed as described below.

The affinity of Vasoflux, LMWH, and unfractionated heparin for ANS-labeled HCII were determined similarly. A 1 × 1-cm quartz cuvette containing 50 mmol/L ANS-labeled HCII in 2 mL TBS was excited at 328 nm (10-nm slit width) and continuously monitored in time drive at 437 nm (10-nm slit width). I was measured before and after the addition of 5- to 10-μL aliquots of 60-μg/mL solutions of the various glycosaminoglycans. Intensity values read from time-drive profiles were corrected for the I obtained when equivalent concentrations of each glycosaminoglycan were titrated in the absence of ANS-labeled HCII. I/I₀ values were calculated and plotted versus the glycosaminoglycan concentration. Binding parameters were calculated by nonlinear regression of the binding isotherm equation using Tble Curve (Jandel) to solve for α, the maximum
Vasoflux, a New Anticoagulant

At 20-second intervals, 20 mmol/L CaCl₂ and various glycosaminoglycans. The effect of the addition of CaCl₂ and various glycosaminoglycans on factor XIa–initiated clotting reactions could thus be determined. For all clotting assays, an ST4 coagulation analyzer (Diagnostica Stago) was used.

Coagulation Assays
Activated clotting times (ACTs) were measured with a Hemochron whole-blood coagulation system (International Technologic Corp). Activated partial thromboplastin times (aPTTs) were measured with Thromboseril (Ortho), a commercial reagent containing rabbit brain phospholipid extract with a micronized silica activator. Dilute prothrombin times were determined with Thromborel (Behringwerke AG), a human plasma–derived thromboplastin with an international sensitivity index of 1.16, diluted to obtain a baseline prothrombin time of 60 seconds. With this Thromborel concentration, the prothrombin time of factor IX–deficient plasma was identical to that in factor IX–sufficient plasma, consistent with factor IX–independent activation of factor X by the factor VIIIa/tissue factor complex. Thrombin clotting times were performed by diluting 50 µL of plasma in 150 µL of calcium-containing Seeger’s buffer and initiating clotting by addition of 5 U thrombin diluted in 50 µL of 10 mmol/L HEPES, pH 6.8, and 150 mmol/L NaCl. Clotting reagents were added to plasma in the absence or presence of Vasoflux, LMWH (4 to 125 µg/mL), and unfractionated heparin (0.1 to 0.5 U/mL). A modified aPTT also was performed, for which reagents were added to plasma in the absence or presence of Vasoflux, LMWH (4 to 125 µg/mL), or unfractionated heparin (0.1 to 125 µg/mL), hirudin (0.5 to 2.0 µg/mL), or dermatan sulfate (1.0 to 4.0 mg/mL) was added to 50-ml aliquots of blood incubated at 37°C in a recirculating water bath. With a Master-flex roller pump system (Cole-Parmer Instrument Co) and R3603 Tygon tubing (ID, 3/16 in; OD, 5/32 in; and wall, 9/64 in; Fisher Scientific Co), blood was pumped through a 40-µm blood transfusion filter (Pall Biomedical Inc). Pressure in the filter was monitored with a pressure transducer (Sorenson TransPac; Abbott Critical Care Systems) and Hewlett-Packard model 78205 D pressure monitor attached proximal to the filter via a connector and 3-way stopcocks. Anticoagulated blood was circulated through the system for 5 minutes before recalcification by addition of 800 µL of 1 mol/L CaCl₂. After an additional 5 minutes, 3 mL of blood was withdrawn for ACT determination. Over the subsequent 90-minute period, pressure across the filter was monitored continuously. At the end of this interval, or when flow across the filter was impaired, the experiment was terminated and consumption of 125I-labeled fibrinogen was determined by expressing residual blood radioactivity as a percentage of original radioactivity.

Statistical Analyses
Where appropriate, means and 95% CIs were calculated. Significance of differences was determined by 2-way ANOVA or linear regression analysis. A value of P<0.05 was considered statistically significant.

Results
Effect of Fibrin on Rates of Thrombin Inhibition by Antithrombin and HCII
The rate of thrombin inhibition by HCII in the presence and absence of fibrin is similar [second-order rate constants of 3.2×10⁻⁴ (mol/L)⁻¹·min⁻¹, respectively]. In contrast, fibrin reduces the rate of thrombin inactivation by antithrombin 9-fold [from a second-order rate constant of 4.0×10⁻⁴ to 4.4×10⁻⁵ (mol/L)⁻¹·min⁻¹]. This is unlikely to reflect rate-limited diffusion of antithrombin into the fibrin clot, because the rate of factor Xa inhibition by antithrombin is unaffected by fibrin [second-order rate constants of 1.5×10⁻⁴ and 1.4×10⁻⁴ (mol/L)⁻¹·min⁻¹, respectively] and the molecular weights of antithrombin and HCII are similar.

Inhibition of Fibrin-Bound Thrombin by Heparin Fractions of Various Molecular Weights
At concentrations of ≥30 µg/mL (Figure 1), a 5000-Da heparin fraction inhibits fibrin-bound thrombin to almost the same extent as equivalent concentrations of unfractionated heparin (P=NS by 2-way ANOVA), whereas a 3000-Da heparin fraction has significantly lower activity (P<0.001).

We prepared a size-restricted 5000-Da heparin fraction by first depolymerizing unfractionated heparin with nitrous acid. On gel filtration analysis, its molecular weight ranged from 1000 to 8000 Da (mean, 5000 Da). The nitrous acid concen-
tration was chosen to produce sufficient depolymerization such that, by size-exclusion chromatography, <3% of the material was $\geq 8000$ Da. Heparin chains $<3000$ Da were removed by ultrafiltration.

**Effect of Periodate Oxidation Followed by Borohydride Reduction on the Affinity of a Size-Restricted Heparin Fraction for Antithrombin and HCII**

We chemically modified the size-restricted LMWH fraction to reduce its affinity for antithrombin 1500-fold (from a $K_d$ value of 24.7 nmol/L to 43.7 μmol/L) so as to target the agent to HCII and allow its use in high concentrations. Such concentrations are needed for inactivation of fibrin-bound thrombin because Vasoflux has low affinity for HCII: $K_d=60.5$ μmol/L (Figure 2), an affinity similar to those of LMWH and dermatan sulfate ($K_d$ values of 62.6 and 71 μmol/L, respectively), but lower than that of unfractionated heparin ($K_d=13.1$ μmol/L).

**Effect of Vasoflux on Coagulation Assays**

Vasoflux has minimal effects on the thrombin clotting time (Figure 3) in control plasma because it has low affinity for antithrombin, and the heparin chains are too short to bridge HCII to thrombin. At clinically relevant concentrations (Table 1), LMWH prolongs the thrombin clotting time to a greater extent than Vasoflux because, unlike Vasoflux, its pentasaccharide sequence is intact. Consequently, longer heparin chains within the preparation bridge antithrombin to thrombin. This concept is supported by the observation that like Vasoflux, LMWH also has a minimal effect on the thrombin clotting time in antithrombin-depleted plasma. Clinically relevant doses of unfractionated heparin (concentrations up to 6 μg/mL, or 1.0 U/mL) have the most marked effects on the thrombin clotting time (Figure 3). This reflects the capacity of longer heparin chains to bridge antithrombin and HCII to thrombin because unfractionated heparin still prolongs the thrombin clotting time in antithrombin-depleted plasma, albeit to a lesser extent.

Vasoflux has no effect on the dilute prothrombin time (Figure 4). In contrast, LMWH produces concentration-dependent prolongation of the dilute prothrombin time. This reflects its ability to catalyze factor Xa and thrombin inactivation by antithrombin, because LMWH has no effect on the dilute prothrombin time in antithrombin-depleted plasma.
TABLE 1. Comparison of Therapeutic Concentrations, and Doses Needed to Achieve These Concentrations, for Heparin, LMWH, Vasoflux, Hirudin, and Dermatan Sulfate

<table>
<thead>
<tr>
<th>Anticoagulant</th>
<th>Therapeutic Concentration, μg/mL</th>
<th>Dose to Achieve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin</td>
<td>1.2–3.0</td>
<td>0.10–0.25</td>
</tr>
<tr>
<td>LMWH</td>
<td>5–15</td>
<td>0.06–0.18</td>
</tr>
<tr>
<td>Vasoflux*</td>
<td>40–80</td>
<td>4–8</td>
</tr>
<tr>
<td>Hirudin†</td>
<td>0.5–1.0</td>
<td>0.1–0.2</td>
</tr>
<tr>
<td>Dermatan sulfate‡</td>
<td>3–8</td>
<td>0.12–0.36</td>
</tr>
</tbody>
</table>

*Therapeutic concentrations of Vasoflux have yet to be established in humans. Vasoflux concentrations ≥40 μg/mL are effective in vitro and in rabbit arterial thrombosis models.‡ Hirudin doses of 0.1 to 0.2 mg · kg⁻¹ · h⁻¹ produce a 2- to 3-fold prolongation of the APTT.‡ Dermatan sulfate doses of 2.9 or 8.6 mg · kg⁻¹ · h⁻¹ (which produced plasma levels of 3 to 8 μg/mL) were significantly better than placebo at reducing postoperative deep vein thrombosis in hip surgery patients.

Figure 4. Effects of Vasoflux and LMWH on dilute prothrombin time (Δ), aPTT (○), and modified aPTT (■) ratios. Points represent means of 2 experiments, each done in duplicate; bars reflect 95% CI. Linear regression analysis shows significant (P<0.001) differences among slopes of all lines.

Figure 5. Comparison of anti–factor Xa activity of gravimetrically equivalent concentrations of LMWH (□) and Vasoflux (●). Points represent means of 2 experiments, each done in duplicate; bars reflect 95% CI. Linear regression analysis shows significant (P<0.001) differences between slopes of lines.

The observation that Vasoflux prolongs the aPTT but not the prothrombin time suggests that it targets the intrinsic pathway, a concept supported by its lack of anti–factor Xa activity and its minimal effect on the thrombin clotting time. We therefore performed a modified aPTT in which micronized silica and phospholipid are incubated in plasma for 5 minutes before recalcification in the absence or presence of Vasoflux. Preincubation results in activation of the contact system and generation of factor Xa, which triggers clotting on addition of calcium. When Vasoflux is added after preincubation, it has less effect on the aPTT than when it is present throughout (Figure 4). This suggests that a small part of its effect is to inhibit contact activation. LMWH has almost the same effect on the aPTT, regardless of when it is added (Figure 4), consistent with the concept that it prolongs the aPTT by inhibiting factor Xa and thrombin.

Effect of Vasoflux on Factor Xa Generation

To explore the antithrombin- and HCII-independent mechanism by which Vasoflux prolongs the aPTT, we examined its capacity to inhibit factor IXa–mediated factor Xa generation in plasma immunodepleted of antithrombin and prothrombin. Vasoflux blocks factor Xa generation (Figure 8), whereas clinically relevant concentrations of unfractionated heparin (0.5 or 1.0 U/mL; ie, 3 to 6 μg/mL) or LMWH (5 to 10 μg/mL; data not shown) have only minimal effects on factor Xa generation in the absence of antithrombin. The sites of
Vasoflux action are compared with those of unfractionated heparin and LMWH in Figure 9.

**Affinity of Vasoflux for Factor IX/IXa**

On the basis of intrinsic fluorescence measurements, Vasoflux binds to factor IX and factor IXa with $K_d$ values of 8.2 and 7.2 $\mu$mol/L, respectively (data not shown), values similar to those for unfractionated heparin ($K_d$ values of 4.2 and 4.0 $\mu$mol/L, respectively) and LMWH ($K_d$ values of 5.9 and 6.4 $\mu$mol/L, respectively).

**Comparison of Vasoflux With Other Anticoagulants in an Extracorporeal Circuit**

When human blood is circulated through a thrombogenic 40-$\mu$m blood filter (Figure 10), unfractionated heparin and LMWH must be used in concentrations of 2.0 and 8.0 U/mL, respectively, to maintain filter patency and prevent fibrinogen consumption, concentrations that produce a starting ACT of 720 and 415 seconds, respectively (Table 2). Vasoflux also prolongs the time to filter failure and prevents fibrinogen consumption, and at 125 $\mu$g/mL, a concentration that produces a starting ACT of 220 seconds. Vasoflux maintains filter patency and reduces fibrinogen consumption to background levels.

To maintain filter patency and to prevent fibrinogen consumption, 8 $\mu$g/mL hirudin is required, a concentration that produces an unmeasurable ACT. Dermatan sulfate is ineffective, even at concentrations that produce a starting ACT of 360 seconds. These observations indicate that inactivation of free and fibrin-bound thrombin is insufficient to prevent clotting in this thrombogenic circuit.

When a neutralizing antibody against HCII is added (Table 3), the Vasoflux concentration needed to maintain filter patency is increased from 125 to 360 $\mu$g/mL, although a concentration of 300 $\mu$g/mL is partially effective. However, even with these high Vasoflux concentrations, fibrinogen consumption is not totally blocked. These findings indicate that the HCII-dependent and -independent activities of Vasoflux are both important determinants of its antithrombotic effect.

**Discussion**

Vasoflux, a derivative of heparin, is a new antithrombotic agent with novel properties. Whereas heparin and LMWH...
exert their anticoagulant activity by catalyzing the inactivation of factor Xa and thrombin by antithrombin. 

Vasoflux, a New Anticoagulant

TABLE 2. Effectiveness of Vasoflux, Heparin, LMWH, Hirudin, or Dermatan Sulfate in an Extracorporeal Circuit

<table>
<thead>
<tr>
<th>Addition</th>
<th>Starting ACT, s</th>
<th>Time to Filter Failure, min</th>
<th>Fibrinogen Consumption, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasoflux, μg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>200</td>
<td>18</td>
<td>85</td>
</tr>
<tr>
<td>125</td>
<td>220</td>
<td>&gt;90</td>
<td>10</td>
</tr>
<tr>
<td>175</td>
<td>240</td>
<td>&gt;90</td>
<td>10</td>
</tr>
<tr>
<td>Heparin, U/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>225</td>
<td>12</td>
<td>&gt;90</td>
</tr>
<tr>
<td>1.0</td>
<td>300</td>
<td>28</td>
<td>&gt;90</td>
</tr>
<tr>
<td>1.5</td>
<td>480</td>
<td>50</td>
<td>78</td>
</tr>
<tr>
<td>2.0</td>
<td>720</td>
<td>&gt;90</td>
<td>31</td>
</tr>
<tr>
<td>LMWH, U/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>200</td>
<td>22</td>
<td>&gt;90</td>
</tr>
<tr>
<td>4.0</td>
<td>254</td>
<td>40</td>
<td>&gt;90</td>
</tr>
<tr>
<td>6.0</td>
<td>402</td>
<td>45</td>
<td>86</td>
</tr>
<tr>
<td>8.0</td>
<td>415</td>
<td>&gt;90</td>
<td>24</td>
</tr>
<tr>
<td>Hirudin, μg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>300</td>
<td>30</td>
<td>&gt;90</td>
</tr>
<tr>
<td>4</td>
<td>480</td>
<td>63</td>
<td>&gt;90</td>
</tr>
<tr>
<td>6</td>
<td>600</td>
<td>75</td>
<td>&gt;90</td>
</tr>
<tr>
<td>8</td>
<td>&gt;1500</td>
<td>&gt;90</td>
<td>10</td>
</tr>
<tr>
<td>Dermatan sulfate, μg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>280</td>
<td>12</td>
<td>&gt;90</td>
</tr>
<tr>
<td>2000</td>
<td>320</td>
<td>20</td>
<td>89</td>
</tr>
<tr>
<td>4000</td>
<td>360</td>
<td>30</td>
<td>80</td>
</tr>
</tbody>
</table>

The observation that Vasoflux prolongs the aPTT yet has no effect on the dilute prothrombin time is consistent with its inhibitory effect on factor Xa generation via the intrinsic pathway, an activity that is largely independent of both antithrombin and HCII. Vasoflux influences several steps in the intrinsic pathway of coagulation. It binds to factor IX/IXa, a finding consistent with previous reports that factor IXa is has a heparin-binding site. The interaction of Vasoflux with factor IXa may impair the catalytic activity of the enzyme, thereby explaining why Vasoflux blocks factor Xa generation by factor IXa. This concept is supported by studies in buffer systems that showed that heparin and LMWH reduce the procoagulant activity of factor IXa.

The limitations of hirudin and dermatan sulfate in the extracorporeal circuit suggest that antithrombotic drugs that target only thrombin are problematic in situations in which high concentrations of thrombin are generated. Although heparin and LMWH inactivate factor Xa and thrombin, they are ineffective against clot-bound thrombin when given in clinically acceptable doses. Vasoflux is superior to dermatan sulfate and hirudin because it has low affinity for antithrombin.

The optimal effectiveness of Vasoflux in a heparin-resistant extracorporeal circuit is dependent on catalysis of HCII because addition of a neutralizing antibody against HCII reduces its activity. The residual activity probably reflects inhibition of contact activation and factor IXa activation of factor X. Further support for the concept that Vasoflux has multiple mechanisms of action comes from the data with dermatan sulfate; even when used in concentrations that produce a higher ACT than Vasoflux, dermatan sulfate is ineffective. Dermatan sulfate has been reported to inactivate fibrin-bound thrombin and fluid-phase thrombin equally well, whereas Vasoflux inhibits fibrin-bound thrombin but has little activity against fluid-phase thrombin. The observation that Vasoflux is effective in the extracorporeal circuit, whereas dermatan sulfate is not, indicates that efficacy in this heparin-resistant system requires more than just thrombin inhibition. This concept is supported by the observation that hirudin is effective only when used in concentrations that produce an unmeasurable ACT, most likely because large amounts of thrombin are generated in this system.

The limitations of hirudin and dermatan sulfate in the extracorporeal circuit suggest that antithrombotic drugs that target only thrombin are problematic in situations in which high concentrations of thrombin are generated. Although heparin and LMWH inactivate factor Xa and thrombin, they are ineffective against clot-bound thrombin when given in clinically acceptable doses. Vasoflux is superior to dermatan sulfate and hirudin because it not only inhibits fibrin-bound

TABLE 3. Effect of an Inhibitory IgG Against HCII (+) Compared With Control IgG (−) on the Effectiveness of Vasoflux in an Extracorporeal Circuit

<table>
<thead>
<tr>
<th>Vasoflux Concentration, μg/mL</th>
<th>Starting ACT, s</th>
<th>Time to Filter Failure, min</th>
<th>Fibrinogen Consumption, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>138</td>
<td>5</td>
<td>&gt;90</td>
</tr>
<tr>
<td>240</td>
<td>296</td>
<td>246</td>
<td>10</td>
</tr>
<tr>
<td>300</td>
<td>334</td>
<td>279</td>
<td>10</td>
</tr>
<tr>
<td>360</td>
<td>357</td>
<td>300</td>
<td>10</td>
</tr>
</tbody>
</table>

Downloaded from http://ircp.ahajournals.org/ by guest on April 5, 2017
thrombin but also blocks factor Xa generation. Vasoflux is better than heparin and LMWH because it inactivates fibrin-bound thrombin. With minimal effects on fluid-phase thrombin and little anti-factor Xa activity, Vasoflux is less likely than heparin or LMWH to interfere with the burst of thrombin generated when blood is exposed to high concentrations of tissue factor found in the adventitia of severed vessels. Because Vasoflux inhibits fibrin-bound thrombin to a greater extent than fluid-phase thrombin, Vasoflux is likely to cause less bleeding than hirudin, a concept supported by preliminary studies comparing the relative efficacy and safety of these agents in a rabbit arterial thrombosis model.26 Vasoflux is currently being compared with heparin as an adjunct to streptokinase in patients with acute myocardial infarction, with 90-minute infarct-related artery patency used as the efficacy end point. Once the effective dose of Vasoflux is established, a large phase III trial will be needed to determine whether Vasoflux will improve clinical outcome when used as an adjunct to thrombolytic therapy.

Acknowledgments
This work was supported in part by a grant from the Medical Research Council of Canada. Dr Frederburgh is the recipient of a Fellowship Award from the Heart and Stroke Foundation of Canada, and Dr Weitz is a Career Investigator of the Heart and Stroke Foundation of Ontario. The authors are indebted to Drs M. Nesheim and C. Esmon for many discussions, Dr P. Shaklee at Scientific Protein Laboratories, Waunakee, Wis, for producing Vasoflux, and S. Crnic for secretarial assistance.

References
Vasoflux, a New Anticoagulant With a Novel Mechanism of Action
Jeffrey I. Weitz, Edward Young, Marilyn Johnston, Alan R. Stafford, James C. Fredenburgh and Jack Hirsh

*Circulation*. 1999;99:682-689
doi: 10.1161/01.CIR.99.5.682

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/99/5/682

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation* is online at:
http://circ.ahajournals.org//subscriptions/