Effect of Antiheparin Agents on Venous Thrombosis Following Vascular Injury

By William A. Mersereau, M.A.

The study of thrombosis, its prevention, and treatment is hampered by the fact that it is difficult to induce thrombosis satisfactorily in animals. Virchow believed that injury to the endothelial lining of blood vessels is one of the factors responsible for thrombus formation; however, it has been found that relatively large areas of endothelium can be destroyed without thrombosis occurring.1-3 These experiments would suggest that either the conditions necessary for thrombosis are not present (e.g., stasis or hypercoagulability of the blood) or that some factor is present in the animal's tissue which prevents thrombus formation. This latter possibility has been explored in the experiments here described.

The discovery of the anticoagulant heparin and its subsequent localization to tissues rich in mast cells have suggested the possibility that heparin is normally secreted by these cells to maintain the fluidity of the blood. Their histological arrangement along the blood vessels of most species also suggests such a function. McGovern,4 in studying injured endothelial surfaces, observed that the exudate found on the lesions stained metachromatically, and he suggested that this might be due to heparin derived from adventitial mast cells. In suitably stained specimens of injured veins of the rat, we have observed disrupted mast cells at the trauma zone. This suggested the possible local liberation of heparin to control the growth of the normal hemostatic platelet plug. To test this hypothesis, the antiheparin agents, hexadimethrine bromide and protamine sulfate, have been applied locally at the site of vascular injury or administered systemically.

Methods

RAT EXPERIMENTS

One hundred and seventy-nine female white rats (200 to 300 Gm.) were used in these studies. Under intraperitoneal pentobarbital (50 mg./Kg.) anesthesia, the abdomen was opened and the vena cava gently exposed at a point below the left renal vein. Then 0.2 ml. of the agent to be studied was applied and the vessel injured with the spring-loaded trauma apparatus (fig. 1). A standard injury was produced by placing the vein under the ball tip of this instrument and by rotating the handle through an arc of 180 degrees eight times. Following trauma, the area was swabbed, and an additional 0.3 ml. of the agent was applied before the incision was closed. The animal was left in a supine position for 30 minutes and was then perfused with dextrose and formalin, as previously described,2 and the vena cava removed for study.

The agents applied directly to the vein were protamine sulfate 1 per cent (Lilly), hexadimethrine bromide 1 per cent, "Polybrene" (Abbott), 1 per cent toluidine blue in saline, saturated ammonium sulfate solution, and 0.9, 1.0, and 3.0 per cent saline.

Twenty-three animals were used to determine the intravenous effect of hexadimethrine bromide, protamine sulfate, and toluidine blue. Doses ranged from 0.05 ml. to 0.5 ml. per rat and were administered five minutes before trauma. In 11 animals, hexadimethrine bromide or protamine sulfate was applied to the nontraumatized vena cava to determine their effect.

ANTICOAGULANTS

Dicumarol

Twenty rats received a single oral dose of Dicumarol (20 mg./100 Gm. body weight). Fifteen control animals received the methyl cellulose solution used to suspend the Dicumarol powder. Twenty-four and 48 hours after gavage, the animals were traumatized as described above and hexadimethrine bromide applied. One hour after trauma, a 1-ml. blood sample was taken in sodium oxalate and the Quick one-stage prothrombin time determined. The animal was perfused immediately after the blood sample was taken.
TABLE 1

The Effect of Antiheparin Agents on Venous Thrombosis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rat</th>
<th>Rabbit</th>
<th>Dog</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical Polybrene without trauma</td>
<td>0/6</td>
<td>0/6</td>
<td></td>
</tr>
<tr>
<td>Topical protamine sulfate without trauma</td>
<td>0/6</td>
<td>0/6</td>
<td></td>
</tr>
<tr>
<td>Topical 0.9, 1.0, and 3.0% NaCl solutions with trauma</td>
<td>0/20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Topical saturated (NH₂)₂ SO₄ solution with trauma</td>
<td>0/5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Topical toluidine blue 1% in saline with trauma</td>
<td>0/7</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>Control (nothing topical) with trauma</td>
<td>0/25</td>
<td>0/12</td>
<td>2/19</td>
</tr>
<tr>
<td>Topical Polybrene 1% with trauma</td>
<td>19/19</td>
<td>7/7</td>
<td>19/19</td>
</tr>
<tr>
<td>Topical protamine sulfate 1% with trauma</td>
<td>13/13</td>
<td>6/6</td>
<td>2/6</td>
</tr>
<tr>
<td>Intravenous protamine sulfate 1% with trauma</td>
<td>0/5</td>
<td>0/7</td>
<td>0/6</td>
</tr>
<tr>
<td>Intravenous Polybrene 1% with trauma</td>
<td>2/9</td>
<td>8/9</td>
<td>2/10</td>
</tr>
<tr>
<td>Intravenous toluidine blue 1% in saline with trauma</td>
<td>0/6</td>
<td>1/7</td>
<td></td>
</tr>
<tr>
<td>Intravenous 0.9% saline with trauma</td>
<td>0/12</td>
<td>2/16</td>
<td></td>
</tr>
<tr>
<td>Control (prothrombin time 20 to 25&quot;), topical Polybrene with trauma</td>
<td>15/15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dicumarol (prothrombin time 40 to 100&quot;), topical Polybrene with trauma</td>
<td>0/20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (clotting time 3 to 9&quot;), topical Polybrene with trauma</td>
<td>11/11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150 units heparin (clotting time 7 to 50&quot;), topical Polybrene with trauma</td>
<td>3/5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>250 units heparin (clotting time 50&quot; to ∞), topical Polybrene with trauma</td>
<td>0/6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 units heparin (clotting time ∞), topical Polybrene with trauma</td>
<td>0/7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Incidence of thrombosis is reported as the ratio of number thrombosed to the number of animals studied.

†Hexadimethrine bromide (Abbott).

Heparin

Eighteen rats were given beef heparin intravenously prior to trauma and topical hexadimethrine bromide. One hour after trauma, a blood sample was taken for clotting time determination and the animal perfused.

RABBIT EXPERIMENTS

Eighty-six albino rabbits (2 to 3 Kg.) were used in these experiments. Under pentobarbital (25 mg./Kg.)-ether anesthesia, a shorter section of inferior vena cava was freed and a wooden tongue depressor (1 cm. wide) passed under the vessel. The vein was then collapsed and rubbed with the blunt handle of a scalpel. The vessel was then dilated and 2 ml. of the agent applied topically. Two hours after trauma, the animals were killed with intravenous pentobarbital and immediately perfused with 5 per cent dextrose via the femoral vein. Forty animals received hexadimethrine bromide, protamine sulfate, or toluidine blue (1 ml./Kg.) intravenously five minutes before trauma.

DOG EXPERIMENTS

Preliminary experiments using the tongue-depressor method, as described in the rabbit experiments, demonstrated that if the femoral veins of the dog were severely injured, so that they oozed on distention, thrombosis would occur without the application of hexadimethrine bromide. It was then necessary to construct a trauma apparatus (fig. 2) for the dog experiments, so that a reproducible amount of injury not producing thrombosis in controls could be inflicted.

This apparatus applied the same scraping...
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FIGURE 1
Apparatus used to traumatize the vena cava of the rat.

FIGURE 2
Apparatus used to traumatize the femoral veins of the dog. The rubber tube indicates the position of the vein under the scraping edge. The upper arm bearing the scraping edge is slotted for side-to-side movement under the spring and pivots on the bolt at the right.

Trauma to the vein as did the scalpel handle used in the rabbit experiment; however, the pressure of the scraping edge was controlled by a spring. It was found that each dog could serve as its own control in the topical hexadimethrine bromide experiment. Both femoral veins were traumatized, and a coin was tossed to determine which vein would receive the antithrombin agent. The animals were kept under pentobarbital anesthesia for two hours following trauma, and then the veins were examined. In the intravenous antithrombin agent study, the femoral veins of two or more dogs were traumatized. Hexadimethrine bromide was applied topically to one vein of each dog, so that we could observe (a) whether or not the trauma was adequate, and (b) whether or not the coagulation system of the dog was affected by the intravenous agent. The control dog not receiving the

FIGURE 3
Lesions produced by trauma apparatus figure 1. Lines of Zahn are seen at right angles to the long axis of the vessel. Unstained X 10.

FIGURE 4
Mast leucocytes on the damaged intima of the rabbits' vena cava. Toluidine blue X 1200.
antiheparin agent was selected by a coin toss, and the experimental dog was then given the intravenous solution (0.5 or 1 ml/Kg.).

**HISTOLOGY**

Longitudinal paraffin sections were prepared from the thrombosed veins of the three species. The sections were stained with hematoxylin-eosin or Mallory’s phosphotungstic acid hematoxylin (P.T.A.H.). The nonthrombosed veins were pinned to a cork board and the endothelial surface stained with aqueous toluidine blue, quickly rinsed in tap water, dehydrated in air, and then mounted in permount.

**Results**

The following is discernible from table 1:

1. The topical application of hexadimethrine bromide or protamine sulfate to uninjured veins of the rat failed to induce thrombosis.

2. The topical application of hypo- and hypertonic saline solutions, saturated ammonium sulfate, or toluidine blue to injured veins of the rat did not bring about thrombosis.

3. Control veins of the rat, rabbit, or dog, which were traumatized but to which nothing was applied topically, were rarely thrombosed.

4. The topical application of hexadimethrine bromide to the injured veins of the rat, rabbit, or dog resulted in severe occluding thrombosis in every case. Topical protamine sulfate induced thrombosis in the rat and rabbit but was found to be relatively ineffective in the dog.

5. The intravenous administration of protamine sulfate failed to influence the control picture in all three species. Intravenous hexadimethrine bromide was effective in inducing thrombosis in the traumatized veins of the rabbit. It was observed, however, that two other lots of hexadimethrine bromide failed to induce thrombosis in this species. Intravenous toluidine blue was ineffective in those species tested.

6. The elevation of the rats’ one-stage prothrombin time, from a normal of 20 to 25 seconds to 40 to 100 seconds with Dicumarol, blocked the hexadimethrine bromide-induced thrombus.

7. The elevation of the clotting time with heparin of the rat’s blood from a normal of 3 to 9 minutes to 50 minutes or higher, blocked formation of the hexadimethrine bromide-induced thrombus.

**HISTOLOGICAL FINDINGS**

The endothelial lesions of control animals were covered to varying degrees with red cells and platelets forming the lines of Zahn (fig. 3). Only in the rabbit did toluidine-blue staining demonstrate large numbers of basophils.
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FIGURE 7
Edge of a trabecula (left) demonstrating platelets. Red blood cells are seen at the right. Wright's stain X 1200.

Grossly, the thrombi induced by the antiheparin agents were composed of a red and white occluding mass attached to the site of endothelial injury, a red or mixed proximal free floating head, and a distal red tail (fig. 5).

Longitudinal paraffin sections of the thrombi demonstrated that the thrombus had formed as the result of the formation of platelet trabeculae at the site of injury (figs. 6 and 7). Mallory's phosphotungstic acid hematoxylin stain demonstrated fine fibrin fibrils radiating from the platelet trabeculae (fig. 8).

Discussion

The dramatic action of antiheparin agents in inducing thrombosis in injured veins of the rat, rabbit, and dog could be explained in at least three ways: (1) by the precipitation of blood proteins, (2) by increasing platelet stickiness, or (3) by blocking anticoagulants liberated at the site of vascular injury. The failure of saturated ammonium sulfate and hypotonic and hypertonic saline to induce thrombosis with rat, as well as the thrombotic action of intravenous hexadimethrine bromide in the rabbit, suggest that protein precipitation is not responsible for these thrombi. The intravenous administration of these agents would be expected to exert a more marked effect on platelet stickiness than its topical application; however, the topical administration yielded the best results in all species. It is possible, however, that the intravenous administration of these agents may have had an anticoagulant effect and so masked their effect on platelet stickiness. The observation was made, however, that while intravenous protamine failed to induce thrombosis in one injured femoral vein, the topical application of hexadimethrine bromide to the other femoral vein of the same dog induced thrombosis; hence, platelet stickiness and coagulability appeared to be normal.

The rabbit is unique in that its mast cells are found only in the blood. It is possible that this may explain the observation that hexadimethrine bromide was effective in inducing thrombosis when administered intravenously. The accumulation of large numbers...
of mast leucocytes on the endothelial lesion of this species suggests that they may have a role in limiting the growth of the platelet fibrin mass by liberating heparin locally.

The prevention of the hexadimethrine bromide-induced thrombus in the rat by Dicumarol and heparin supports the histological evidence that fibrinogenesis is essential for the formation of the thrombus.

The histological characteristics of the hexadimethrine bromide-induced thrombus, its adherence to the vessel wall, and its ease of production make it a suitable subject for the evaluation of fibrinolytic agents.6

Summary

The effect of the antiheparin agents hexadimethrine bromide and protamine sulfate on venous thrombosis have been studied in the rat, rabbit, and dog. Untreated traumatized veins were not thrombosed. Traumatized veins treated with these agents topically, or in some species intravenously, thrombosed. The thrombi so produced consisted of an occluding mass of platelet trabeculae at the site of vascular injury, a red and white proximal tail, and a distal stasis clot. The hexadimethrine bromide-induced thrombus in the rat is prevented by pretreatment with Dicumarol or heparin. It is suggested that the antiheparin agents bring about thrombosis of the injured veins by neutralizing an anticoagulant liberated locally.

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References

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