THE MECHANISMS responsible for the maintenance of hemostasis on the level of terminal blood vessels are only partially understood. Although platelets have a major role in preventing or arresting hemorrhages from small vessels, clinical and experimental evidence suggests that other factors may also be involved. The role of blood coagulation products or components requires further elucidation, although attention has recently been focused on the deposition of fibrin on the endothelial surface as a means of increasing vascular resistance.\(^1\) The occurrence of spontaneous hemorrhages in the course of anticoagulant therapy is of interest in this respect.

The spontaneous onset of hematuria, epistaxis or other types of bleeding in patients receiving heparin or Dicumarol is similar to that in patients with hemorrhagic tendencies due to vascular impairment. Previous studies in animals have suggested a decreased vascular resistance following administration of the above anticoagulants.\(^2,3\) The bioassays employed, however, have not permitted a more extensive consideration of these effects.

Studies by Drinker\(^4\) on the mechanisms regulating lymph formation and composition have demonstrated that macromolecules as well as particulate material, including red blood cells, may appear in the lymph without morphologic change of the small blood vessels. Red blood cells are present in the lymph in small numbers under physiologic conditions. Studies of lymph composition have recently indicated that increased red cell diapedesis occurs in thrombocytopenic x-irradiated animals as a result of generalized endothelial damage.\(^5\) This phenomenon has been utilized in a sensitive bioassay for the quantitative evaluation of thrombocytopenic bleeding in animals.\(^6\)

The lymph duct cannulation technic and determination of red blood cell diapedesis in rats were employed in the present investigation for the assessment of vascular changes following administration of heparin.

**METHODS AND MATERIALS**

Normal Wistar rats,\(^*\) weighing 250–350 Gm., were used. The abdominal portion of the main lymph duct was cannulated, following Bollman's\(^7\) technic, under Nembutal (40 mg./Kg.) anesthesia. In order to assure multiple or continuous intravenous infusions, the external jugular vein was also exposed and cannulated with a PE-10 polyethylene cannula.\(\dagger\)

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*Charles River Laboratories, 1018 Beacon Street, Boston, Mass.

†Intramedic-Clay Adams, Inc., New York, N. Y.
Observation was usually initiated 12 to 15 hours later, allowing for recovery of the animal from the anesthesia and return to near-normal activity. By this time the red cell content of the lymph, which may increase spontaneously during the early hours following surgery, usually returned to normal. Continuous collection of the lymph was initiated at the beginning of the observation period. The total content of red blood cells in aliquots collected during 30 minutes in test tubes containing 0.1 ml 3.8 per cent Na citrate was determined. Heparin-sodium (Abbott Laboratories) was given intravenously either at 30-minute intervals or by continuous infusion. For the latter purpose the automatic compensator of the Tiselius electrophoresis (Elmer-Perkin) apparatus was adjusted to deliver 2 ml per hour and was connected to the cannula placed in the external jugular vein. Control animals were handled similarly and were given infusions of isotonic saline. The in vivo effects of equivalent doses of heparin on blood coagulation were assessed in normal non-cannulated rats. Blood samples were collected (in 0.1 M sodium oxalate, 1:9 volumes) from the abdominal aorta, using siliconized glassware and the two-syringe technic. The effects of single or multiple infusions of heparin were investigated by the study of three animals for each time interval. Changes of prothrombin consumption, clotting time of recalcified oxalated plasma, and thrombin time were studied, according to standard procedures.

**RESULTS**

Forty-six animals were studied. Heparin at various doses, levels, and rates of administration was given to 35 rats with lymph fistulas, while 11 cannulated rats were studied as saline controls.

As indicated in figure 1, the administration of high doses of heparin did not produce an immediate increase of the RBC content of the lymph, despite a concomitant profound disturbance of the coagulation mechanism (table 1). Continued administration of heparin, however, was eventually accompanied by a gradual increase of the output of red blood cells in the lymph. The first appearance of such changes usually occurred 5 to 8 hours following the beginning of heparin administration when the injections were given every 30 minutes. When continuous infusion was used, bleeding usually started within 1 to 3 hours.

Discontinuation of the administration of heparin at this time did not affect the subsequent course of events. The output of red blood cells continued to increase and high levels were present in all animals for the length of observation (usually 24 to 48 hours). Parallel control animals showed only minor changes of the RBC content in their lymph (fig. 2). It should be noted that normal clotting mechanism was observed in a group of similarly treated animals within less than 15 hours following the last dose of heparin. The changes described above occurred in animals given one or more mg. of heparin per hour. When 1.0 mg./hr. was given, bleeding in the lymph was observed in 12 of 15 animals. Two animals given 0.4 mg./hr. showed transient increase of the RBC in their lymph which cleared completely 12 hours following cessation of administration of heparin (table 2).

Administration of protamine following cessation of the infusion of heparin did not decrease the RBC output. Since accurate determination of residual heparin to indicate the dosage of protamine required was not practical, continuous infusion of 2 mg. protamine per hour was given. At no time was the lymph RBC output in those animals reduced. In fact, considerable increase of
Fig. 1.—Effect of intravenous administration of heparin on the output of red blood cells in the lymph of a normal rat. Arrows indicate the infusion of 0.5 mg. of heparin-sodium. Each test tube contains the lymph collected during 30 minutes. Tube A16 shows lymph obtained 13 hours following sample A15.
Thrombin time 16
Recalcification time 82
Serum pro-thrombin time 98

Fig. 2.—Changes of the output of red blood cells in the lymph of normal rats given heparin (0.5 mg. at 30-minute intervals) or 0.85 per cent NaCl (0.5 ml. every 30 minutes).

Table 1.—Effects of Repeated Administration of Heparin on Coagulation in Rats

<table>
<thead>
<tr>
<th>Test</th>
<th>Clotting Time Prior to Heparin (Seconds)</th>
<th>Clotting Time after Heparin (Seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin time</td>
<td>16</td>
<td>1 hr.†</td>
</tr>
<tr>
<td>Recalcification time</td>
<td>82</td>
<td>2 hrs.</td>
</tr>
<tr>
<td>Serum pro-thrombin time</td>
<td>98</td>
<td>3 hrs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 hrs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 hrs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 hrs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 hrs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 hrs.</td>
</tr>
</tbody>
</table>

*A group of normal rats was given 0.5 mg. heparin every 30 minutes for 6 hours. Determinations were made at hourly intervals after cessation of heparin infusions. Individual rats were used for each hourly determination. Duplicates were obtained from two separate animals.
†Number of hours following administration of heparin.
‡Prolongation due to presence of large amount of heparin in serum.

Both lymph volume and RBC output was observed on several occasions. Post-mortem examination carried out on 10 animals, sacrificed 12 hours following cessation of the infusion of heparin, and while still exhibiting highly abnormal output of RBC in the lymph, failed to reveal gross anatomical changes. Petechial and ecchymotic changes on the intestinal and mesenteric surfaces such as are found in bleeding thrombocytopenic (x-irradiated) rats were not observed. Histologic examination of the intestine and liver, however, indicated a diffuse increase of free red cells throughout the tissues.

Studies of platelet levels during and following the administration of heparin were carried out in 10 of the experimental animals with lymph fistulas. Comparison with platelet counts in 10 control (saline-treated) rats indicated statistically significant changes in only two of the heparin-treated animals (fig. 3). The degree of bleeding observed did not appear to correlate with...
Table 2.—Effect of Heparin on Spontaneous Bleeding in the Lymph of Normal Rats

<table>
<thead>
<tr>
<th>Total Dose Rate</th>
<th>Rate</th>
<th>No. of</th>
<th>No. of</th>
<th>Total Dose Rate</th>
<th>Rate</th>
<th>No. of</th>
<th>No. of</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg. Heparin*</td>
<td>mg./hr.</td>
<td>Animals</td>
<td>Bleeding Animals</td>
<td>mg. Heparin*</td>
<td>mg./hr.</td>
<td>Exp.</td>
<td>Animals</td>
</tr>
<tr>
<td>1.35</td>
<td>0.3</td>
<td>1</td>
<td>0</td>
<td>4.4</td>
<td>0.4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>3.5</td>
<td>1.0</td>
<td>1</td>
<td>1</td>
<td>6.2</td>
<td>1.0</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>13.5</td>
<td>2.0</td>
<td>5</td>
<td>5</td>
<td>12</td>
<td>2.0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>16.4</td>
<td>4.0</td>
<td>4</td>
<td>4</td>
<td>32</td>
<td>4.0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td></td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

*Indicates amount given every 30 minutes or concentration per ml. in continuous infusion (2 ml. per hour).

†Bleeding lasted only 90 minutes, and then cleared spontaneously.

the changes of the platelet counts. In addition, bleeding in the lymph persisted after platelet counts returned to normal.

**DISCUSSION**

The red blood cell content of the lymph in normal rats increased following administration of large and repeated doses of heparin. The degree of bleeding in the lymph was comparable to that observed in thrombocytopenic, x-irradiated animals. The bleeding did not parallel the impairment of the coagulation mechanism induced with heparin. Individual variations of resistance to heparin were observed in the time and in the total dose of heparin required for the institution of gradually increasing bleeding. Return of the coagulation to normal, following cessation of the infusions of heparin, did not reduce the bleeding during the subsequent 24 to 48 hours. Although transient decrease of the
counts of circulating platelets were observed in some animals, the variations of platelet levels were not statistically significant. The doses of heparin used were high, in comparison to those required for anticoagulation in dogs or humans. The coagulation studies carried out, however, did not indicate an excessive inhibition of blood coagulation, thus suggesting a greater tolerance for heparin in rats.

The delayed appearance of bleeding into the lymph and its continuation after coagulation returned to normal may suggest certain dissociation of the anticoagulant and the hemorrhage-inducing effects of heparin. The role of coagulation products or components in the maintenance of vascular integrity should not, however, be minimized on this basis, since the delay as well as the continuation of bleeding could be due to a time-consuming damaging or repair process. A direct interaction or association of heparin with vascular wall structures with resultant changes of their properties could also be considered. Such change could range from a modification of the electrical charge of the endothelial lining to a severe enzymatic and metabolic disturbance of the vessel wall cellular components.

The phenomenon reported above is of interest in view of the clinical use of heparin. The hemorrhagic tendency complicating prolonged extracorporeal circulation is a major limitation for the wider use of artificial blood dialysis or oxygenation. Similar procedures almost invariably involve the use of large doses of heparin. Should heparin affect vascular integrity, effective and complete neutralization of its anticoagulant effects with agents such as protamine at the end of the therapeutic procedure may not be sufficient to prevent or reverse the bleeding tendency. The total amount of heparin to which the vessel structures or the blood have been exposed, as well as the duration of this exposure, may warrant consideration. The lymph duct cannulation technic and observations on lymph composition may be helpful in studies of other anticoagulants as an additional parameter reflecting vascular changes.

**Summary**

The administration of large and repeated doses of heparin to normal rats resulted in increased output of red blood cells into the lymph, comparable to that associated with x-ray induced thrombocytopenia. A considerable lapse of time was observed between the inhibition of blood coagulation and the increase of vascular permeability to red blood cells. The latter was not decreased by return of the blood clotting to normal.

**Summario in Interlingua**

Le administration de grande e repetite doses de heparina a rattos normal resultava in un augmento del liberation de erythrocytos ad in le lympha, simile a illo associate con thrombocytopenia inducite per radios X. Esseva notate un considerabile intervallo de tempore inter le inhibition del coagulation de sanguine e le augmento del permeabilitate vascular pro erythrocytos. Iste ultime non esseva reducite per le retourno del coagulation sanguinee al stato normal.
REFERENCES


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Blood Coagulation and Vascular Integrity: Effects of Heparin

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