Studies in Intravascular Coagulation

IV. The Effect of Heparin and Dicumarol on Serum-Induced Venous Thrombosis

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A method has been previously described whereby the infusion of canine serum and serum fractions rich in convertin, but essentially devoid of thromboplastin, prothrombin, ac-globulin and thrombin, initiates venous thrombosis in dogs at sites of partial or complete venous stasis far removed from the site of infusion. It has been suggested that the pathogenesis of venous thrombosis in various clinical states may be referable to local stasis combined with a transient increase in circulating convertin activity. The present study demonstrates that heparin is superior to dicumarol in inhibiting the evolution of such serum-induced intravascular thrombi in dogs.

In a previous study the infusion of heterologous, canine serum or serum fractions rich in convertin (SPCA) was shown to initiate venous thrombosis in dogs at sites of partial or complete stasis far removed from the site of infusion. It was suggested, as a working hypothesis, that the pathogenesis of venous thrombosis in various clinical states might be referable to local venous stasis combined with a transient increase in circulating convertin activity.

To gain further insight into the clot-promoting effect of serum and the efficacy of anticoagulant drugs, the role of Dicumarol and heparin in blocking the evolution of serum-induced venous thrombosis was studied in dogs.

Methods and Material

Intravascular thrombosis can be routinely induced in untreated animals by a technique previously described. Briefly, in dogs under sodium pentobarbital anesthesia, a segment of jugular vein 3 to 6 cm. in length was freed from its surrounding structures and its tributaries ligated. Thirty cc. of a barium sulphate eluate of serum* rich in convertin but essentially devoid of thromboplastin, prothrombin and thrombin was then infused into a distant antecubital vein. One minute later, after the infused serum eluate had been dispersed throughout the circulation, the previously freed jugular segment was gently isolated with 27 mm. sraffine clamps. A large red clot was routinely formed in the isolated vein segment within 60 seconds after clamping. Similar clots were readily induced with 90 cc. of canine serum but not with canine plasma.

Convertin is one of the factors necessary for normal coagulation in man, and is found in appreciable amounts in serum stored for 24 hours at 4 C. Current knowledge does not permit definition of SPCA as one or more distinct substances. For the present, it can be described as an entity which, though distinct from ac-globulin, is also essential for the physiologic conversion of prothrombin. A precursor of convertin, proconvertin (pro-SPCA), is present in plasma. During coagulation proconvertin is converted to convertin. The in vitro tests used in this study, however, do not distinguish between the inactive and active forms but measure the total amount of convertin complex present in a given sample. The failure of plasma to induce clotting in the isolated venous segment sug-

* This fraction was obtained by adding barium sulphate to serum, shaking the mixture, decanting the supernatant liquid, washing the barium sulphate with acetate buffer, eluting the convertin SPCA with sodium citrate and dialyzing this eluate against tap water.
TABLE 1.—Overall Prothrombic Activity, Convertin (SPCA) and Prothrombin Concentrations in Thirteen Dogs 72 to 96 hours after receiving 3.8 to 4.5 mg. Dicumarol per Kg.

<table>
<thead>
<tr>
<th>Dog</th>
<th>Overall one-stage prothrombic activity (% of Normal)</th>
<th>Convertin (% of Control)</th>
<th>Two-stage prothrombin concentration (% of Control)</th>
<th>One-stage prothrombin concentration (% of Control)</th>
<th>Clot in Vein</th>
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Serum-induced thrombosis occurred in all but one dog; in this animal the coagulation defect was so severe that the clotting time had been tripled.

Data suggests, however, that the effects observed are not attributable to proconvertin but rather to its activated form, convertin.

In the experiments to be described, established methods were used to determine the overall one-stage plasma prothrombic activity, prothrombin, ac-globulin, convertin concentrations and clotting times.

**Data**

Dicumarol was administered to 13 dogs in a single oral dose ranging from 3.6 to 4.5 mg. per Kg. Following reduction of the overall one stage prothrombin and convertin activities to 1 per cent or less of the control values (table 1), 30 cc. of the serum eluate was injected through an antecubital vein, and a previously-freed jugular vein segment then isolated in the usual manner.* Despite the profound, induced clotting defect and hemorrhagic diathesis before and during surgery, thrombus formation, indistinguishable from that found in untreated animals, was observed in all but one dog; in this animal the coagulation defect was so severe that the clotting time had been trebled. In these dogs, toxic from Dicumarol, the ac-globulin concentration was normal, and appreciable amounts of plasma prothrombin were present. Thus the marked reduction in the one-stage activity reflected primarily the absence from the circulation of the convertin complex.

The effect of heparin was similarly studied in 10 dogs. After control clotting times were obtained, the animals were given 0.23 mg. of heparin per Kg. intravenously. At varying intervals after the injection, 30 cc. of the serum eluate was injected through an antecubital vein, and a previously freeced vein segment was then isolated in the standard manner. At the moment of each vein isolation, the clotting time was determined. Thirty cc. aliquots of the serum eluate were injected at 15 to 30 minute intervals,* followed in each instance by the isolation of a previously freed vein segment until a clot was obtained. As indicated in figure 1, so long as the clotting time of the animals exceeded twice the control value, the serum eluate never induced intravascular clotting. Even below this level some interference with thrombus formation could still be

* Ten minutes after the infusion of the serum eluate its clot promoting activity is dissipated.
demonstrated in some animals by the finding of only small, fragmented clots. Compared with the extensive operative bleeding in the dogs receiving Dicumarol, the relative lack of hemorrhage during surgery in the animals receiving heparin was noteworthy.

DISCUSSION

When 30 cc. of a serum eluate rich in convertin is infused into an antecubital vein of a normal anesthetized dog, no detectable increase in the convertin content of jugular vein plasma or serum can be demonstrated during or following the infusion. The failure to detect a rise in the convertin activity of jugular vein plasma or serum may be explained by the large dilution of the infused material in its passage through the circulation from the antecubital to the jugular vein. In any event, an amount of convertin, not measurable by present techniques and infused into animals themselves essentially devoid of convertin, induces extensive intravascular coagulation. On the other hand, the infusion of the same amount of convertin in animals with a normal level of convertin, in which heparin administration has only doubled the clotting time, does not induce clotting.

There is additional experimental evidence to support the view that heparin has greater anticoagulant properties than does Dicumarol. In contrast to the efficacy of heparin, we have demonstrated that severe Dicumarol-induced hypoprothrombinemia and convertin deficiency does not retard spontaneous fibrin deposition in an isolated segment of vein,15 and does not effectively block clot production by trypsin,12 thrombin10 or thromboplastin.10 We believe the significance of this cumulative experience lies in the observation that substances such as Dicumarol, which depress that part of the coagulation sequence concerned with thrombin conversion may not provide as optimal an anticoagulant effect as heparin-like compounds which block fibrin deposition through other mechanisms.13-16 These studies also suggest that heparin may have, in addition to its other properties, a specific anticoagulant effect.

It is not unlikely that the pathogenesis of intravascular thrombosis may be different in different clinical states. It has been suggested that convertin may play a key role in thromboembolism.17,18 Several investigators have in fact demonstrated a temporary rise in the convertin complex of human pregnant and postpartum plasma.19-22 In an earlier report from this laboratory,1 direct experimental evidence showed that serum fractions rich in convertin may, in dogs, initiate venous thrombosis at sites of partial or complete stasis. The experiments described herein indicate that if convertin is a responsible initiating agent in vascular thrombosis in man, heparin, in contrast to Dicumarol, may provide more effective protection against the induction or propagation of such clots.

SUMMARY

Convertin (SPCA) may play a key role in initiating venous thrombosis in some clinical states. In dogs, heparin is superior to Dicumarol in blocking the evolution of intravascular thrombi induced by canine serum fractions rich in convertin.

SUMMARIO IN INTERLINGUA

Convertina (accelerator del conversion de prothrombina seral) ha possibilemente un function decisive in le initiation de thrombosis venose in certe statos clinic. In canes, heparin es superior a Dicumarol como agent blocaor del evolution de thrombos intravascular inducites per fractiones de serum canin ric in convertina.

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