Factor IX in Intravascular and Extravascular Blood Coagulation

By F. J. W. Lewis and F. Nour-Eldin

Previous observations have shown that factor IX (plasma thromboplastin component or Christmas factor) in serum differs from that in plasma. Bergsagel suggested that a change, possibly an activation of the factor, took place in the presence of calcium and AHG during the initial stages of coagulation. The fact that following blood coagulation, the activity of factor IX increases to more than fourfold led Quick to suggest the possibility of the occurrence of a clinical condition in which the basic defect is a failure to activate, rather than a deficiency of, factor IX. The laboratory findings in the case reported by Benney and Lewis seemed to support this and drew attention to the lack of information regarding factor IX in the body circulation and the mechanism of its activation. On the other hand, Nour-Eldin and Wilkinson demonstrated that, in patients with Christmas disease, the intravenous administration of normal serum was without value, while plasma (with lower in vitro activity) was effective in improving the generation of plasma thromboplastin. This interpretation of activity with regard to factor IX must, therefore, be approached with considerable caution.

The present work demonstrates that factor IX in the circulating blood exists in a form different from that recognized in vitro. The change from intravascular to extravascular factor IX is shown to be brought about by an agent or a process which, for simplicity only, has been termed factor IX evolver (FNE). The influence of certain factors on the action of this “evolver” is also studied.

Materials and Methods

Cadaveric blood: The distal end of the common iliac vein was tied and the proximal end of this vein or the lower part of the inferior vena cava was cut. Using a 15-ml. pipette with a wide opening, a blood sample was obtained and delivered into a universal container. Serum was separated after centrifugation at 3,000 rpm for 15 minutes.

Glassware and needles: All pipettes (inside and outside surfaces) and glass containers were rendered water repellent by the application of dimethyl-dichlorosilane (DDS). After draining the excess of the latter, the glassware was continuously washed in tap water for at least 72 hours and then in distilled water before being dried. Unfortunately this corrosive reagent was not suitable for preparing needles and plastic tubes. These were coated with silicone 550 (Hopkins and Williams Ltd.).

Blood-clotting tests: These were carried out essentially as described by Nour-Eldin and Wilkinson. The method used to estimate factor IX consisted of incubating at 37 C. the solution tested with equal volumes of Christmas disease serum (1:10), normal Al(OH)_3-treated plasma (1:5), platelet-substitute suspension, imidazole buffer pH 7.3 and 0.025 M CaCl_2. After 6 minutes, 0.1 ml. of this mixture was added together with 0.1 ml. 0.025 M CaCl_2 to 0.1 ml. normal citrate plasma (substrate) at 37 C., and the clotting-time, was recorded.

From the Department of Pathology, Southmead Hospital, Bristol, England.
Table 1.—Details of Blood Specimens

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Hours after death</th>
<th>Males</th>
<th>Hours after death</th>
</tr>
</thead>
<tbody>
<tr>
<td>52</td>
<td>8</td>
<td>61</td>
<td>16½</td>
</tr>
<tr>
<td>64</td>
<td>16½</td>
<td>59</td>
<td>17½</td>
</tr>
<tr>
<td>61</td>
<td>24</td>
<td>28</td>
<td>22*</td>
</tr>
<tr>
<td>80</td>
<td>24</td>
<td>61</td>
<td>33</td>
</tr>
<tr>
<td>73</td>
<td>26</td>
<td>76</td>
<td>52</td>
</tr>
<tr>
<td>76</td>
<td>41</td>
<td>85</td>
<td>65f</td>
</tr>
<tr>
<td>85</td>
<td>41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>47</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*few filaments of fibrin formed in the serum.
|Slight hemolysis was observed.

Platelet-substitute suspension: Inosithin phospholipid (Associated Concentrates, Inc.) was utilized in a concentration which, in the thromboplastin generation test with normal reagents, gave a substrate clotting-time of 10 seconds.

Clotting of plasma: This was carried out at 37°C, without introducing a high dilution factor by the addition of 7 ml. M CaCl₂ to each 100 ml. plasma from acid-citrate-dextrose blood.

Some of the investigations carried out in the present work, however, required specially devised experiments. Accordingly, it appeared preferable to detail these later in the appropriate sections.

Results

Since the experiments carried out are inter-related, the results are presented with minimal comment, full interpretations and conclusions being reserved for the discussion.

Factor IX in cadaveric blood: Sera separated from intravascular clots obtained from the bodies of 14 individuals, with no history of hemorrhagic diathesis or an anomaly likely to affect the blood-clotting factors, were examined. The age distribution and the time of obtaining the blood after death are given in Table 1. Factor IX activity was absent in all cases. It is unlikely that this was due to deterioration in the activity of factor IX, which is known to be stable. Furthermore, specimens of blood kept in vitro at temperatures and for periods comparable to those in the cadaver contained high concentrations of this factor. Nor does it seem to be due to postmortem autolysis since other factors, as mentioned later, were still present in an active state and, with the exception of one specimen, there was no hemolysis. When examined for prothrombin activity by the two-stage method, cadaveric serum showed a residual amount of prothrombin equivalent to 0–1 per cent of that in normal plasma, indicating a good consumption of this factor. Full formation of active plasma thromboplastin must, therefore, have occurred intravascularly. This led to the conclusion that factor IX had either been consumed during the process of coagulation in the blood vessels of the cadaver, or—less likely—that it was in a form completely undetectable by existing methods.

Storage of serum in cadaveric vessels: The lower end of the inferior vena cava as well as its tributaries were tied. The vessel was then carefully removed. After emptying the blood, the interior of the vessel was washed with sterile
0.9 per cent NaCl. Having drained the latter, the vessel was suspended in 0.9 per cent NaCl in a sterile container. Extravascularly-clotted blood was introduced aseptically in the blood vessel through the opened upper end. Storage was then carried out for 2 hours at 37 C., 3 hours at room temperature, and thereafter at 4 C. Specimens removed after 24 and 48 hours were examined for factor IX activity and compared with those kept in glass tubes. No appreciable reduction in factor IX activity was found, indicating no destruction or lysis of this factor inside the cadaveric blood vessels.

Simulation of intravascular conditions: The behavior of factor IX using non-wettable surfaces was studied. The apparatus used for collecting blood in these investigations consisted of a wide-bore intravenous needle connected to a 6 cm. plastic tube (intravenous needle supplied with the disposable recipient set manufactured by Fenwall Laboratories, Inc., U.S.A., or Capon Heaton & Co., Birmingham, England). After being treated with silicone 550 it was replaced with the needle downwards in the glass container and resterilized before being used for withdrawing venous blood. Plasma or serum obtained in this way and collected directly into DDS-treated tubes will be referred to hereafter as DDS-plasma and DDS-serum.

Experiment 1—To study factor IX when normal blood clots in tubes with non-wettable surfaces: polystyrene (lustroid), polypropylene (Nalgene), glass coated with silicone 550 and glass treated with dimethyl-dichlorosilane (DDS). This information was important in selecting the container for separating plasma from venous blood in the next experiment. The results presented in table 2, show that in only the tubes siliconed with DDS was the amount of serum factor IX appreciably reduced. Accordingly, a DDS-treated centrifuge tube was chosen for preparing the Al(OH)₃-treated DDS-plasma by the following method.

Experiment 2—To investigate the adsorption on Al(OH)₃ of intravascular factor IX. Here, the immediate mixing of blood with 3.8 per cent sodium citrate and Al(OH)₃ suspension in siliconed apparatus, was aimed at. In order to achieve this, the container cf the needle was filled with sterile sodium citrate solution as shown in figure 1. The siliconed needle with the attached plastic tube was then carefully removed without allowing the citrate solution to

### Table 2.—Effect of Different Surfaces on Evolvement of Factor IX in Serum

<table>
<thead>
<tr>
<th>Tube used for blood-clotting</th>
<th>Substrate clotting-time (sec.)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lusteroid (polystyrene)</td>
<td>11 ½</td>
</tr>
<tr>
<td>Nalgene (polypropylene)</td>
<td>14</td>
</tr>
<tr>
<td>Glass coated with silicone 550</td>
<td>12 ½</td>
</tr>
<tr>
<td>Glass treated with dimethyl-dichlorosilane</td>
<td>28</td>
</tr>
<tr>
<td>Untreated glass</td>
<td>10</td>
</tr>
<tr>
<td>Control (factor-IX-deficient serum in glass)</td>
<td>37</td>
</tr>
</tbody>
</table>

*The serum tested (diluted 1:10) was incubated at 37 C. with equal volumes of Al(OH)₃-treated normal citrate plasma (1:5), platelet-substitute suspension, Christmas disease serum (1:10), imidazole buffer pH 7.3 and 0.025 M CaCl₂. After 6 minutes, 0.1 ml. of this mixture was added together with 0.1 ml. of 0.025 M CaCl₂ to 0.1 normal citrate plasma (substrate) at 37 C., and the clotting-time recorded.
escape. The needle was quickly inserted into a large vein in the cubital fossa of a normal individual. Blood mixed with 0.4 ml. citrate solution in the needle and plastic tube was directly received in a DDS-treated centrifuge tube (125 x 11 mm.) containing 0.1 ml. sodium citrate solution and 0.5 ml. Al(OH)$_3$ suspension, to a final volume of 5 ml. Experience showed that this amount should not be exceeded on any occasion.

After mixing by rotating the tube between the palms (no inversion of tube) and incubation at 37 C. for 2 minutes, Al(OH)$_3$ treated DDS-plasma was separated by centrifugation. Throughout the procedure the temperature was kept at 37 C. by pre-warming the solutions and centrifuge tube and surrounding the latter with water at 37 C. during centrifugation. In order to ensure that Al(OH)$_3$ had been properly applied, the one-stage prothrombin time of the plasma obtained was checked; this should be between 1½–4 minutes. Plasma was then tested for factor IX activity. The results illustrated in table 3 show that in spite of treatment with Al(OH)$_3$, Factor IX activity in DDS-plasma is still present. This was equivalent to a concentration of 12–20 per cent of normal.

It must be emphasized that accurate comparison between the Al(OH)$_3$-treated DDS-plasma and normal plasma (which had to be tested unadsorbed to avoid removal of factor IX therein) is impossible. The formation of some
FACTOR IX IN BLOOD COAGULATION

Table 3.—Factor IX Content of DDS-Plasma

<table>
<thead>
<tr>
<th>Reagent tested</th>
<th>Donor (normal individual)</th>
<th>Substrate clotting-time (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffered saline* (factor IX deficient control)</td>
<td>—</td>
<td>39</td>
</tr>
<tr>
<td>Al (OH)₃-treated DDS-plasma 1:5</td>
<td>D. Q.</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>S. B.</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>G. M.</td>
<td>22</td>
</tr>
<tr>
<td>Glass contact plasma 1:5</td>
<td>D. Q.</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>S. B.</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>G. M.</td>
<td>11</td>
</tr>
</tbody>
</table>

*Buffered saline consisted of equal volumes of 0.9 per cent NaCl and imidazole buffer pH 7.3.

†The reagent mentioned was mixed at 37 C. with equal volumes of Al(OH)₃-treated plasma (1:5), Christmas disease serum (1:10), platelet-substitute suspension, imidazole buffer pH 7.3 and 0.025 M CaCl₂. The activity of plasma thromboplastin formed was tested after 6 minutes by adding 0.1 ml. of the mixture together with 0.1 ml. 0.025 M CaCl₂ to 0.1 ml. normal citrate plasma (substrate) at 37 C., and the clotting-time recorded.

thrombin in the mixtures containing serial dilutions of normal glass-contact plasma gives relatively higher results. The real concentration of factor IX left in DDS-plasma after treatment with Al(OH)₃ is, therefore, slightly higher than the estimated 12–20 per cent of normal.

Stability of factor IX: Glass-contact plasma stored at 4 C., showed a gradual deterioration in the activity of factor IX, reaching, after 14 days, 10–15 per cent of its original value, after which it remained constant. DDS-plasma after treatment with Al(OH)₃ contained a comparable residual factor IX activity equivalent to 12–20 per cent of that in normal glass-contact plasma. This activity did not deteriorate after storage for the same period under the same conditions. There was a difference between these two apparently stable fractions or forms of factor IX in that it could be masked or removed by Al(OH)₃ from normal stored plasma in contrast to the DDS-plasma, which even after storage and retreatment with Al(OH)₃ remained active.

Effects of clotting on plasma with low Factor IX activity: This varied according to the method used. When plasma stored for 19–28 days was clotted, the resulting serum was unexpectedly rich in factor IX (fig. 2), with an activity 60–70 per cent of that in serum of fresh unstored plasma.

When DDS-plasma, however, was clotted in glass tubes, no factor IX activity could be detected in the serum even after storage for 14 days. There was, nevertheless, a most interesting phenomenon: when, to the stored DDS-plasma was added plasma from a patient receiving phenindione (Dindevan) and containing low (< 5 per cent of normal) factor IX activity (table 4), factor IX was evolved in the serum with an activity 60–70 per cent of that in normal serum from fresh plasma.

Effect of dilution: The appearance of factor IX in the serum formed from expired plasma was, at first, puzzling since a clot is usually formed in the incubation mixture of the thromboplastin generation test in which diluted
Fig. 2.—In vitro factor IX activities of fresh and stored plasma and serum. 

P = plasma; S = Serum; D = difference between plasma and serum content.

(1:5–1:80) tested plasma is included. It was, therefore, decided to test the concentration of factor IX in sera resulting from the clotting of diluted expired plasma. The results indicated that a dilution of 1:5 or more before adding calcium is detrimental to the subsequent evolution of factor IX; negligible amounts of factor IX were present in the serum of a plasma diluted 1:20. This would explain the absence of factor IX activity in the incubation mixture where the final dilution of the tested plasma is at least 1:30.

Contact with glass: At the commencement of this work, all efforts were made to avoid bringing intravascular serum into contact with glass surfaces. However, it was later found that subsequent contact of this serum with glass at 37 °C for periods of 5–24 hours did not affect the concentration of blood-clotting factors therein. Similarly, there was no alteration in the activity of the small amount of factor IX in DDS serum treated in this way.

Presence of other factors in intravascular serum: The shortening of the one-stage prothrombin time of plasma from patients receiving Dindevan showed that factors VII and X (Stuart-Prower factor) are present (table 5). Also found was a platelet-like activity similar to that described in normal serum20 and human saliva, milk and amniotic fluid7, i.e., it can replace platelets in the thrombin generation test but not in the plasma thromboplastin generation test.
Table 4.—Evolution of Factor IX in DDS-Plasma

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Source of serum</th>
<th>Source of phenindione-plasma†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(equal volumes)*</td>
<td>I. W.† substrate clotting-time (sec.)†</td>
</tr>
<tr>
<td>A</td>
<td>Phenindione-plasma &amp; Al (OH)₃-treated normal plasma</td>
<td>33 30</td>
</tr>
<tr>
<td>B</td>
<td>Al (OH)₃-treated DDS-plasma &amp; Al (OH)₃-treated normal plasma</td>
<td>38 37½</td>
</tr>
<tr>
<td>C</td>
<td>Al (OH)₃-treated DDS-plasma &amp; Al (OH)₃-treated phenindione-plasma</td>
<td>40 39</td>
</tr>
<tr>
<td>D</td>
<td>Al (OH)₃-treated DDS-plasma &amp; phenindione-plasma</td>
<td>12½ 13½</td>
</tr>
<tr>
<td>E</td>
<td>Normal plasma &amp; phenindione-plasma</td>
<td>11½ 12</td>
</tr>
</tbody>
</table>

*To avoid a high degree of dilution, 1 ml. of plasma mixture was clotted by the addition of 0.05 ml. M CaCl₂ and 0.05 ml. platelet-substitute suspension.
†Patients receiving phenindione with one-stage prothrombin time of 64 seconds (I. W.) and 66 seconds (M. T.); normal being 12 seconds.
†The resulting serum in each experiment, diluted 1:5 was incubated at 37 C., with equal volumes of normal Al (OH)₃-treated plasma (1:5), Christmas disease serum (1:10), platelet-substitute suspension, imidazole buffer pH 7.3 and 0.025 M CaCl₂. After 6 minutes, the activity of plasma thromboplastin was tested by adding 0.1 ml. of this mixture together with 0.1 ml. 0.025 M CaCl₂ to 0.1 ml. citrate plasma (substrate) at 37 C., and the clotting-time recorded.

Table 5.—Effect of Intravascular Serum on One-Stage Prothrombin Time

<table>
<thead>
<tr>
<th>Plasma*</th>
<th>Original Brain extract +10% serum</th>
<th>Stypven + lecithin +10% serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>29</td>
<td>20</td>
</tr>
<tr>
<td>B</td>
<td>42</td>
<td>20</td>
</tr>
<tr>
<td>C</td>
<td>23½</td>
<td>19</td>
</tr>
<tr>
<td>D</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>E</td>
<td>37</td>
<td>23</td>
</tr>
<tr>
<td>F</td>
<td>46</td>
<td>25</td>
</tr>
<tr>
<td>Normal</td>
<td>11</td>
<td>—</td>
</tr>
</tbody>
</table>

*Obtained from patients receiving phenindione.

DISCUSSION

From these experimental data it would seem that when blood clots intravascularly without breach or contact with water-wettable surfaces, Factor IX does not appear in the serum; in other words it is consumed. In order to reproduce as nearly as possible conditions existing in circulating blood, Al(OH)₃-treated DDS-plasma was collected as described above. That we had at least partially succeeded was shown by the fact that there was a residual 20 per cent factor IX activity in the plasma of this reagent in spite of the Al(OH)₃, and that only after the prior addition of glass-contact factor IX deficient phenindione plasma to the DDS-plasma was factor IX evolved in the serum. On storing DDS-plasma for 14 days, factor IX activity remained almost constant even after retreatment with Al(OH)₃—the serum still remaining free of such activity. This is an important observation since the activity of
factor IX can be completely removed by Al(OH)₃ from glass-contact plasma, including the fraction detectable after storage. In contrast, when fresh glass-contact plasma clots in vitro, factor IX is not consumed; moreover it appears in the serum with an activity apparently increased fourfold. If this plasma is stored for 14 days, the activity falls to less than 20 per cent of its original plasma value, after which it remains constant. When this is now clotted, factor IX appears in the serum with an activity that is apparently increased fifteenfold. This may be due to one of the following:

(a) Increased efficiency of the mechanism whereby factor IX changes, with an enhancement of activity, to the form detectable in serum. Without considerable supporting evidence, this is difficult to accept.

(b) The development of an inhibitor active in plasma but not in serum. Had this been so, one would have expected dilution to have increased the apparently evolvable factor IX, whereas it is, in fact, diminished. This detrimental effect of plasma dilution on the evolvement of factor IX in serum, together with the accelerating effect of prothrombin on the early stages in the formation of plasma thromboplastin, however, may suggest that factor IX in serum is a derivative of prothrombin or is affected by prothrombin concentration in plasma. Two of our observations provide evidence against this. The first is the ability of phenindione plasma (table 4), with a one-stage prothrombin time of 64 and 66 seconds, to evolve high concentrations of factor IX from Al(OH)₃-treated DDS-plasma. The second is the failure to demonstrate factor IX activity in serum after intravascular coagulation, in spite of the normal prothrombin concentration therein.

(c) The presence of a portion of factor IX in a form less detectable by existing techniques.

If we postulate that existing laboratory techniques are able to detect factor IX in serum, but only poorly in plasma—and that the apparent fourfold enhancement of activity as demonstrated in serum is merely a reflection of this—then, using the serum factor IX activity from fresh glass-contact plasma as the standard, it would seem that glass-contact blood loses approximately 25 per cent of its factor IX after storage (fig. 2) and that intravascular blood loses the same proportion, possibly the same fraction during the collection of DDS-plasma. This would suggest that there is a labile fraction of factor IX that is also vulnerable to the action of Al(OH)₃; that factor IX has more than one form—one resistant to Al(OH)₃ existing only in normal intravascular circulating blood; that this takes part in the purely intrinsic generation of thromboplastin and that during intravascular clotting it is consumed.

When, however, there is a vascular breach or contact with water-wettable surfaces, a mechanism comes immediately into action whereby factor IX becomes vulnerable to Al(OH)₃ and its consumption is prevented. Bearing in mind that factor IX is generally recognized as a serum factor, we have named this mechanism factor IX evolver (FNE) since factor IX appears in the serum as a result of its activity.

FNE survives storage for at least 28 days at 4°C and is still present in freeze-dried pooled plasma. It can be completely inactivated by Al(OH)₃—as shown in the in vitro clotting of DDS-plasma in which no factor IX appears in the
FACTOR IX IN BLOOD COAGULATION

serum unless an outside source of FNE is previously added. This dependence on an outside source lends support to the conception of FNE as an entity. FNE also appears to be inhibited but not destroyed by citrate. It is, therefore, not Hageman factor.$^5,12$

What is the function of FNE? The evidence suggests that it comes into action as part of the defense mechanism that substitutes for the slow intrinsic generation of thromboplastin—the rapid extrinsic clotting system in which the evolvement of serum factor IX plays an important part. It is impossible from our present observations to attribute to FNE any earlier role whereby an Al(OH)$_3$ resistant precursor is activated to an Al(OH)$_3$ adsorbable intermediate form of factor IX as a prerequisite to the generation of thromboplastin—the serum factor representing the final product. In fact, we have no evidence that the factor IX taking part in intravascular clotting is vulnerable to Al(OH)$_3$, or that any part of it is labile. On the other hand, we have evidence that such clotting can take place in the apparent absence of FNE. It is conceivable, therefore, that FNE only comes into action on glass-contact or vascular trauma and converts intravascular factor IX into extravascular factor IX—the properties of which are that:

(a) it is adsorbable by Al(OH)$_3$;
(b) a portion, but not all is labile;
(c) it is not consumed during clotting;
(d) it is active as a plasma factor in the treatment of Christmas disease, but is therapeutically inert in serum.$^6,9$

It is possible that FNE is itself vulnerable to other agents and may even be partially so to a gross imbalance of circulating clotting factors associated with the initial stages of normal clotting.$^1$ This is being further investigated.

It is remarkable that, in spite of a reduction in at least four known blood-clotting factors, viz., prothrombin and factors VII, IX and X, the blood-clotting time in glass tubes of patients receiving coumarin compounds remains within normal limits and that only some have prolonged clotting-times in siliconed tubes.$^4$ It is possible that this, as well as the limited benefit of anticoagulant therapy,$^3$ bears some relations to the failure of these drugs to affect FNE. This is also being further investigated.

SUMMARY

1. Sera separated from intravascular clots obtained postmortem showed no factor IX activity.
2. Evidence is presented suggesting that there are two forms of factor IX, intravascular and extravascular, and that there is an agent, named for convenience factor IX evolver (FNE) responsible for converting the former into the latter with the evolvement of factor IX in the serum.
4. The significance of these experiments and some related problems are discussed.

SUMMARIO IN INTERLINGUA

1. Seros separate ab coagulos intravascular obtenite post morte monstrava nulle activitate de factor IX.
2. Evidentia es presentate que suggere le existentia de duo formas de factor IX, i.e., formas intravascular e extravascular, e de un agente—al qual nos applica le designation evolutor do factor IX—que es responsabile pro le conversion del forma intravascular ad in le forma extravascular in le evolution de factor IX in le sero.

3. Es discutite le signification de iste experimentos, insimul con vane problemas affin.

ACKNOWLEDGMENTS

The authors wish to thank members of the Department for their help, including the donation of their blood on several occasions; Dr. T. F. Draisey and Dr. A. B. Raper for some of the material used; and Mr. W. G. Sweet for help in preparing the figures. We would also like to acknowledge the Ethel Showering Research Fund for defraying some of the expenses.

REFERENCES


F. J. W. Lewis, M.B., Ch.B., Consultant Pathologist, Lecturer in Haematology & Clinical Pathology, University of Bristol, Bristol, England.

F. Nour-Eldin, M.B., Ph.D., Clinical Pathologist, Registrar in Pathology, Southmead Hospital, Bristol, England.
Factor IX in Intravascular and Extravascular Blood Coagulation

F. J. W. LEWIS and F. NOUR-ELDIN

Updated information and services can be found at:
http://www.bloodjournal.org/content/20/1/41.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml