Detection of Carriers for Factor IX (PTC) Deficiency

By Paul Didisheim and Robert L. E. Vandervoort

Factor IX deficiency (PTC deficiency, Christmas disease, hemophilia B) is a congenital, often familial, hemorrhagic disorder which was distinguished from classical hemophilia (AHG deficiency, factor VIII deficiency, hemophilia A) in 1952.¹³ Like the latter, it is usually inherited as a sex-linked recessive trait: only the male hemizygote for the abnormal gene is clinically affected, and the female heterozygote or carrier is asymptomatic. From a eugenic standpoint it would be of value to have a method which could distinguish carriers from noncarriers in factor IX deficiency kindreds. An occasional carrier has had a tendency to bruise or bleed somewhat more easily than do normal women, or has had an abnormality in some clotting test (serum prothrombin time, partial thromboplastin time, thromboplastin generation test, or plasma factor IX assay), suggesting that at least some carriers may have a partial deficiency of factor IX. The purpose of this paper is to present the results of clotting tests performed on 14 carriers for factor IX deficiency, and to describe a new assay for factor IX. By means of this assay, 11 of the 14 were found to have subnormal factor IX activity.

Methods and Materials

Coagulation methods used have been described previously⁴ except for the following:

1. Serum for the serum IX assay was prepared by collecting 2 ml of blood in a new 13 x 100 mm. Pyrex tube which contained three 3-mm. borosilicate beads (Propper Mfg. Co., Long Island City, N. Y.). The tube was capped with Parafilm, placed on an Aloe red cell pipette rotor for five minutes, then incubated at 37 C. for four hours. The serum was separated by centrifugation in the cold at 2000 g for 10 minutes, transferred to a siliconized tube, and used immediately for the assay. IX-deficient plasma was prepared as follows: Blood from a patient with severe congenital IX deficiency (coagulation time in glass tubes over an hour; plasma prothrombin time normal; serum defect and no adsorbed plasma defect in the thromboplastin generation test) was collected in 1:100 volume of 38 per cent trisodium citrate in lusteroid tubes, centrifuged at 2000 g for 30 minutes, and the plasma separated and stored in small aliquots in Parafilm-capped lusteroid tubes at -20 C. Just before the assay, a tube of such plasma was thawed and placed in an ice bath. The assay consists of adding the following in the order listed to a 12 x 75 mm. unscratched Pyrex tube at 37 C.: 0.1 ml. 0.025 M CaCl₂, 0.1 ml. of a 10 per cent dilution of the serum to be assayed, and 0.1 ml. IX-deficient plasma. An electric timer (Labchron, Labline Inc., Chicago) was started as the plasma was added. The tube was mixed gently once, then tilted 45° every 25 seconds until a clot was visible, and the clotting time was recorded. The test was performed in duplicate. The 10 per cent dilutions were prepared in barbital buffer⁵ in siliconized tubes 10 seconds before use.

2. The serum thromboplastin generation test (STGT) is a previously described method⁶ with minor modifications.⁴ A thromboplastin generation test⁷ was performed with a mixture

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Blood, Vol. 20, No. 2 (August), 1962
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of normal adsorbed plasma, a suspension of normal platelets, and the serum to be assayed. The test is thus a specific assay for IX if one assumes the X in the serum to be normal. This assumption is probably valid since all carriers had normal plasma and serum prothrombin times.

3. Capillary fragility was measured by the application of 20 cm. Hg of suction to an avascular area on the flexor surface of the forearm for three minutes with an Angiometer (Laboratoires de Physique Médicale, Paris). The number of petechiae which appear were counted. The range for 45 normal subjects was 0 to 50 petechiae.

Normal Subjects

Normal values for the serum prothrombin time (SPT), serum IX, STGT, and TGT were established by the study of the blood of 14 healthy women aged 20 to 64.

Obligatory Carriers

The carriers comprising this study are all “obligatory carriers,” i.e., either (a) mothers of more than one IX-deficient male, (b) mothers of more than one obligatory carrier, (c) mothers of at least one IX-deficient male and one obligatory carrier, (d) daughters of an IX-deficient male, or (e) mothers of a single IX-deficient male if one of the mother’s siblings is also an IX-deficient male or an obligatory carrier.

All carriers were questioned with regard to bleeding tendency; one (#8, table 1) had a positive history. She had excessive bleeding with each of 18 dental extractions as well as following tonsillectomy and hysterectomy, although not following appendectomy. She has bruised easily all her life, and her menstrual periods lasted an average of 10 days. She had four episodes of melena between 1952 and 1957; a “stomach ulcer” was demonstrated by gastrointestinal series during that time, but has since healed. She has had no hemarthroses, hematuria, or frequent epistaxes.

Routine coagulation tests were performed on all carriers. Bleeding times were slightly prolonged (9, 10, 10 min.) in three. Platelet counts, tourniquet tests, capillary fragility, clot retraction, clotting times in glass and silicone, and plasma prothrombin times were all within normal limits.

RESULTS

Results of the serum prothrombin times (SPT), serum thromboplastin generation tests (STGT), thromboplastin generation tests (TGT), and serum IX assays are presented in tables 1 and 2. The SPT values of the carriers did not differ significantly from those of the normal subjects. The STGT and TGT data presented are the shortest clotting times achieved during the entire generating period. With the STGT, 5 of the 14 carriers had abnormally prolonged clotting times, i.e. longer than the mean plus 2 standard deviations of the clotting times of the normal subjects. With the TGT, 6 of the 11 carriers studied had abnormally prolonged clotting times. With the serum IX assay, 11 of the 14 carriers had abnormally prolonged clotting times.

DISCUSSION

Five of 14 carriers had an abnormally slow STGT, whereas 6 of 11 had an abnormally slow TGT. The latter test may therefore be somewhat more sensi-
Table 1.—Values for Serum Prothrombin Time (SPT), Serum Thromboplastin Generation Test (STGT), Thromboplastin Generation Test (TGT), and Serum Factor IX Assay in 14 Obligatory Carriers for Factor IX Deficiency

<table>
<thead>
<tr>
<th>Obligatory carrier</th>
<th>Kindred</th>
<th>SPT in sec.</th>
<th>STGT in sec.</th>
<th>TGT in sec.</th>
<th>Serum IX assay in sec.</th>
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<tr>
<td>1</td>
<td>1 1</td>
<td>30.8</td>
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<td>12.2</td>
<td>12.3</td>
<td>237</td>
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<tr>
<td>6</td>
<td>3 1</td>
<td>37.2</td>
<td>13.1</td>
<td>—</td>
<td>312</td>
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<td>15.3</td>
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<tr>
<td>9</td>
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<td>11.6</td>
<td>13.9</td>
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<td>50.5</td>
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<tr>
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<td>350</td>
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<tr>
<td>14</td>
<td>6 1</td>
<td>26.9</td>
<td>11.7</td>
<td>—</td>
<td>175</td>
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</tbody>
</table>

Table 2.—Summary of Results of SPT, STGT, TGT, and Serum Factor IX Assay in Normal Subjects and in Obligatory Carriers for Factor IX Deficiency

<table>
<thead>
<tr>
<th>Assay</th>
<th>Normal subjects mean ± S.D. in sec.</th>
<th>Obligatory carriers mean ± S.D. in sec.</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
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<td>SPT</td>
<td>36.1 ± 6.7</td>
<td>34.9 ± 6.8</td>
<td>0.44</td>
<td>0.7 &gt; P &gt; 0.6</td>
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<td>STGT</td>
<td>12.1 ± 0.7</td>
<td>13.2 ± 1.7</td>
<td>2.21</td>
<td>0.05 &gt; P &gt; 0.02</td>
</tr>
<tr>
<td>TGT</td>
<td>12.1 ± 0.7</td>
<td>13.6 ± 2.3</td>
<td>2.65</td>
<td>0.02 &gt; P &gt; 0.01</td>
</tr>
<tr>
<td>Serum IX</td>
<td>203 ± 30</td>
<td>321 ± 78</td>
<td>5.12</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Active to mild deficiencies of IX than the STGT, although it is a highly nonspecific test, being sensitive to fluctuations in any of the clotting factors known to be involved in the formation of blood thromboplastin: Hageman factor (XII), plasma thromboplastin antecedent (XI), V, VIII, X, and platelet factor 3, as well as IX. The STGT is somewhat more specific, but is sensitive to both IX and X, and may be partly sensitive to XI and XII as well, since these latter two factors are supplied by the test serum as well as by the normal adsorbed plasma. In addition, two other more recently described clotting activities, prephase accelerator (PPA) and labile serum factor, may also influence both the TGT and the STGT.

A possible reason why the TGT may be somewhat more sensitive than the STGT to mild deficiencies of IX may be the presence in the STGT of normal platelets, which are likely to be contaminated by traces of IX adsorbed onto their surface and therefore to result in more rapid clotting times. For these reasons of nonspecificity and of contamination with traces of IX, neither the TGT nor the STGT appears on theoretical grounds to be the ideal assay system for IX.

Some authors attach significance not only to the shortest clotting time attained during a TGT, but also to the generation rate, i.e., time taken for the shortest clotting time to be attained. Our data were analyzed in this regard. Generation rates of those carriers with normal shortest clotting times in the
STGT or TGT were not significantly slower than generation rates of the normal subjects. Thus, in our experience the shortest clotting time attained was the significant value, and the generation rate was of no additional value, in detecting the carrier state for IX deficiency by the STGT or TGT. These two tests might be made more sensitive if the sera were assayed at higher dilutions than the 10 per cent used here; however no data were collected on this point.

By the use of the serum IX assay, 11 of 14 carriers of IX deficiency, or 79 per cent, had significantly prolonged clotting times, i.e., reduced IX activity. This suggests that the routine use of this assay in suspected carriers of IX deficiency should make it possible to detect most of them. The test is not perfect however; a certain proportion of carriers, estimated from this study at about 20 per cent, would be expected to have normal results.

In contrast to our finding that 79 per cent of carriers have abnormal results by the serum IX assay, analysis of published reports on carriers of IX deficiency reveals that 22 of 66, or 33 per cent, had abnormal clotting tests. Two possible reasons for this difference are presented below.

1. Some published studies have been based, in part or entirely, on the SPT, which is less sensitive than the STGT, TGT, or serum IX assay in detecting mild deficiencies of IX. This is seen in table 2; none of the carriers in the present study had an abnormal SPT. Our data confirm those of Aggeler and his colleagues on the relative lack of sensitivity of the SPT in detecting minor clotting defects in patients with mild forms of hemophilia and related disorders.

2. Although the nature of the foreign surface in contact with blood has been known for 100 years to influence the rate of clotting, only recently have investigators taken this variable carefully into account in the design of their clotting factor assays. Glass accelerates coagulation by activating Hageman factor, which then interacts with plasma thromboplastin antecendent to form activation product (AP). These reactions do not require the presence of calcium, hence they begin as soon as plasma or serum is placed in a glass tube. Thus the amount and duration of contact of blood with glass profoundly affect the clotting time in tests of the first stages of clotting, such as the STGT, TGT, and serum IX assay. Our rigid control of these variables may have contributed to the increased sensitivity of our method.

**Summary**

1. A new assay for factor IX (PTC) is described.
2. With the use of this assay, 11 of 14 obligatory carriers of factor IX deficiency were found to have subnormal factor IX activity.
3. One of these carriers has a mild hemorrhagic history.

**Summario in Interlingua**

1. Es describite un nove essayage pro le determination de factor IX (componente de thromboplastina del plasma).
2. Per medio de iste metodo il eseva trovate que 11 inter 14 obligatori...
portatores de deficientia de factor IX habeva activitates subnormal de factor IX.

3. Un de iste portatores habeva antecedentes de leve hemorrhagias.

ACKNOWLEDGMENTS

The authors are greatly indebted to Miss Dorothy Bunting for her valuable technical assistance. Miss Lora Alired compiled the normal data for the negative pressure capillary fragility test.

REFERENCES


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