Congenital Factor VII Deficiency with Normal Stuart Activity: Clinical, Genetic and Experimental Observations

By Henry G. Kupper, Bertram L. Hanna and Dixie R. Kinne

UNTIL RECENTLY it was believed that the speed of conversion of prothrombin into thrombin depended upon two factors, quite distinct and easy to differentiate. One, labile on storage and disappearing during coagulation, is called appropriately the labile factor or, because of its chemical nature, accelerator globulin. It is also known as factor V since in historical order of discovery it was preceded by four other factors; thromboplastin, calcium, prothrombin and fibrinogen. The other accelerator is stable on storage and its activity does not decrease after blood has clotted. This is known as stable factor and, because of its persistence in serum, as serum prothrombin conversion accelerator (SPCA) or, in order of discovery, as factor VII.

A deficiency of factor VII may result from dicumarol therapy or liver cell damage, always with a concurrent decrease of other coagulation factors. Congenital deficiency of factor VII is generally an isolated defect, with all other coagulation factors normal.

Over 38 cases of bleeding believed to result from congenital factor VII deficiency have been reported. Clinical symptoms observed in these cases include spontaneous epistaxis, deep subcutaneous hematomata, gastrointestinal and genitourinary tract bleeding and hemarthroses without the permanent limitation of motion frequently seen in hemophilia. The occurrence of many cases with family histories of bleeding and of families with several affected members led early to the conclusion that congenital deficiency of factor VII is an inherited disorder.

As the accuracy of laboratory diagnostic methods improved, a well defined pattern of findings emerged to characterize this condition. The findings included a marked prolongation of the one-stage (Quick) prothrombin time contrasted with a normal or nearly normal clotting time, clot retraction and bleeding time in most patients. The prothrombin concentration determined by the two-stage method was uniformly normal. Differing results were reported, however, for several tests. Prothrombin consumption was found to be normal in eight and abnormal in 13 of 21 cases in which it was reported. The thromboplastin generation test, performed in 16 cases, was normal in six and abnormal in 10. Viper venom, found to have thromboplastic activity equal to tissue thromboplastin and capable of substituting for factor VII, was included in the one-stage prothrombin time test mixtures of six...
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patients. This test gave normal results in four and abnormal results in two cases.\textsuperscript{14} It became obvious that these patients were in reality a heterogeneous group, having a bleeding tendency resulting from deficiency of at least two different clotting factors.

Hougie et al.\textsuperscript{14} published in 1957 a re-evaluation of a case previously reported as factor VII deficiency.\textsuperscript{23} They found this patient deficient in a factor which differs from factor VII, although possessing many similar qualities. It was found that plasma from their patient and from the factor VII-deficient patient of Alexander et al.\textsuperscript{2} were mutually corrective when tested together in the prothrombin time test. Hougie and his co-workers called the deficient factor the Stuart clotting factor, after the surname of the patient, and showed it to be identical to the factor lacking in a patient previously diagnosed as having "hypoprothrombinemia,"\textsuperscript{5} later believed to be deficient in factor VII.\textsuperscript{1,18} It has also been demonstrated by cross matching that the patients of Owren\textsuperscript{26} and Alexander\textsuperscript{2} are deficient in factor VII.\textsuperscript{3,9,14} It is now clear that the results of prothrombin consumption and thromboplastin generation tests are normal in factor VII deficiency but faulty in Stuart factor deficiency. The addition of viper venom corrects the prolonged one-stage prothrombin time in factor VII deficiency but not that of Stuart Factor deficiency.\textsuperscript{13} Factor VII decreases rapidly on therapy with dicumarol whereas Stuart factor persists longer. Genetic studies have indicated that Stuart clotting factor deficiency is inherited as a highly penetrant, incompletely recessive autosomal trait.\textsuperscript{11}

Frick and Hagen\textsuperscript{7} suggested that factor VII deficiency is inherited as an autosomal dominant trait. Re-examination shows their patient to be deficient in Stuart factor.\textsuperscript{9} Quick et al.,\textsuperscript{28} observing mild subclinical deficiencies in the families of several factor VII-deficient patients, concluded that this deficiency is inherited as an incompletely recessive trait. One of the families studied was that previously reported by Frick and Hagen. It is clear that this genetic hypothesis is based upon study of a mixed group including both factor VII- and Stuart factor-deficient patients, since mutual correction of prothrombin times was found among some of these patients.

The present paper reports clinical and experimental observations obtained from the study of a girl normal with respect to Stuart factor but having a deficiency of factor VII, together with a study of three generations of her family.

\section*{Material and Methods}

Laboratory examinations were performed during the years 1955, 1956 and 1957 on the patient and members of her family. Blood for examination was drawn in all cases by the two syringe technic, with the use of siliconized syringes and needles. Nine volumes of blood were added to either one volume of 3.8 per cent sodium citrate or to 1.34 per cent sodium oxalate, depending on the requirements of each method, and centrifuged for 10 minutes at 2000 rpm. The plasma was immediately separated and frozen whenever studies were not performed at once. Serum was separated from blood placed in uncoated glass tubes and kept for two hours at 37 C. Blood taken in the field was handled in the same manner and transported in a car for several hours. Blood from the patient and from 31 relatives was obtained in our laboratory. Twelve family members living
in a rural community were examined in their homes. Histories of nine individuals in
two family groups living in southern Mississippi were taken, and laboratory examinations
were made by physicians in the area. Blood was shipped to us by air express, together
with samples of normal blood collected and handled in the same manner, which served
as controls. Data were considered reliable when the controls were within the limits of
normal.

Factor VII was prepared from normal serum\(^6\) as was plasma thromboplastin component
(PTC) fraction.\(^5\) Serum from the original Stuart factor deficiency patient was generously
supplied by Dr. Cecil Hougie. Russell's viper venom cephalin reagent was prepared by
the method of Hjort et al.\(^12\) Cohn Fraction I, consisting of fibrinogen and antihemophilic
globulin, was obtained from the Michigan State Laboratory, Lansing, through the courtesy
of Dr. H. D. Anderson. Difco thromboplastin was used throughout the study.

The prothrombin of all family members was assayed by the method of Hjort\(^12\) and
that of the patient by the two-stage methods of Johnson and Seegers\(^16\) and Wagner et al.\(^34\)
Thromboplastin generation tests were performed according to Biggs and Douglas.\(^4\)
Prothrombin conversion was measured by the method of Frick and Hagen\(^7\) which
(quoting the authors) "is used to indicate the changes in prothrombin concentration
against time as measured by the modified two-stage determination of prothrombin in
plasma." The prothrombin conversion ratio was determined by the method of Wolf,\(^37\)
which utilizes the technic of the thrombin generation test.\(^57\) We have assayed the
influence of factor VII prepared from normal serum\(^6\) on the prothrombin conversion ratio
of patients' plasma, rather than that of brain thromboplastin as used by Wolf\(^37\) and
Owren,\(^25\) which measures both factors VII and Stuart, and a specific Stuart factor assay
by the method of Sisc.\(^31\)

**Case History**

The patient, C. R., is a four year old girl, the only child of a family of Scotch-
English ancestry. She was born with huge bilateral cephalhematoma which were
aspirated by the delivering obstetrician. In spite of frequent upper respiratory infections,
her development has been essentially normal. At the age of 3 months, she developed
an acute diffuse abdominal pain which disappeared in several days without treatment.
At the age of one year her left foot became swollen and red. This lesion cleared in
about 10 days, leaving a limp for which corrective shoes were ordered. The limp disap-
peared after several months. During this period petechiae and ecchymoses were observed
on the extremities and trunk. Two months later her left knee became swollen, hot, red and
tender. This improved gradually without therapy. Easy bruising had been noticed from
the time she began to walk. At the age of two years her right calf became swollen, hot,
red and tender, and finally a large area of discoloration appeared, characteristic of a deep
hematoma.

The patient continues to have deep ecchymoses on the extremities and trunk, oc-
casional swelling of knees and ankles and spontaneous bleeding from the gums. Treat-
ment has included ice packs, transfusions with 100 ml. of stored plasma and with a
preparation of ACC-76, which will be discussed. Cortisone preparations to counteract
vascular bleeding and wide-spectrum antibiotics to combat the upper respiratory infection
have been given by her pediatrician. The patient was referred to us for evaluation of
the safety of a planned tonsillectomy, which has not been performed to date.

**Results**

Studies on the patient's blood indicated a mild degree of anemia with
occasional leukocytosis, apparently related to throat infection.

The results of determinations of coagulation factors are given in table 1.
Prothrombin was determined by the method of Hjort\(^12\) as 90 per cent of
normal and by the Johnson and Seegers method\(^16\) as low as 18.6 and 24 units.
The latter method employs the addition of barium carbonate-absorbed beef
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<table>
<thead>
<tr>
<th>Test</th>
<th>Normal Value</th>
<th>Patient Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding Time</td>
<td>1–3 min.</td>
<td>7–8 min.</td>
</tr>
<tr>
<td>Capillary Fragility</td>
<td>negative</td>
<td>mildly positive</td>
</tr>
<tr>
<td>Platelet Count</td>
<td>180–350,000/cu.mm.</td>
<td>283,000/cu.mm.</td>
</tr>
<tr>
<td>Clotting Time</td>
<td>5–12 min.</td>
<td>10–12 min.</td>
</tr>
<tr>
<td>Clot Retraction</td>
<td>good in 1 hr.</td>
<td>good in 1 hr.</td>
</tr>
<tr>
<td>Prothrombin (Quick)</td>
<td>60–100%</td>
<td>8, 12.5, 11.7%</td>
</tr>
<tr>
<td></td>
<td>12–14 sec.</td>
<td>50, 35, 38, 57 sec.</td>
</tr>
<tr>
<td>AHF Assay</td>
<td>68–165%</td>
<td>210%</td>
</tr>
<tr>
<td>PTC Assay</td>
<td>60–140%</td>
<td>87%</td>
</tr>
<tr>
<td>Prothrombin</td>
<td>a. stypven method</td>
<td>80–120% 12</td>
</tr>
<tr>
<td></td>
<td>b. 2-stage method</td>
<td>230–300 units34</td>
</tr>
<tr>
<td>Factor V</td>
<td>70–100%</td>
<td>86, 100%</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>200–400 mg.%</td>
<td>381 mg.%</td>
</tr>
<tr>
<td>Stuart Factor in plasma*</td>
<td>15–18 sec.</td>
<td>16–18 sec.</td>
</tr>
<tr>
<td>Factor VII</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. SPCA</td>
<td>80–160%</td>
<td>24%</td>
</tr>
<tr>
<td>b. Owren’s</td>
<td>70–120%</td>
<td>25, 18, 23, 16, 11.9%</td>
</tr>
<tr>
<td>Thromboplastin Generation Test</td>
<td>100% in 6 min.</td>
<td>100% in 6 min.</td>
</tr>
</tbody>
</table>

* Determined in 1958 and 1959.

serum diluted 1:30. With the method of Wagner et al.,34 using beef serum diluted 1:10, the prothrombin concentration was estimated as well within the normal limits.

Deficiencies of factors participating in the formation of thromboplastin and the presence of a circulating anticoagulant were ruled out by normal results obtained with the thromboplastin generation test. Antithrombin and antithromboplastin levels were normal. The partial thromboplastin time was slightly elevated, varying from 176 to 197 seconds. Bleeding time was slightly prolonged and capillary fragility was increased. Clotting time was at the upper limit of normal and clot retraction was good in one hour. All clotting factors were found to be within the normal range except factor VII, which was found to have a level varying from 16 per cent to 25 per cent when measured by the method of Owren23 and to be 24 per cent when measured on one occasion by the method of deVries et al.33 During two recent bleeding periods the patient has been found to have factor VII levels of 11 per cent and 9 per cent. The thromboplastin generation tests, with the use of normal alumina plasma, platelets, substrate and sera from our patient and from patients having PTC and Stuart factor deficiencies are summarized in table 2. The results indicate that the thromboplastin generation of the patient’s serum is equal to that of a normal individual. The sera of Stuart factor and PTC-deficient patients show faulty thromboplastin generation, but both are corrected by addition of equal parts of either normal serum or serum from our patient. Subsequent tests have shown that the faulty thromboplastin generation of Stuart-deficient serum may be corrected by the addition of 1:5 dilutions of either our patient’s or normal serum. The
results indicate that both our patient and the PTC-deficient patient are normal with respect to Stuart factor, and that our patient and the Stuart-deficient patient are normal with respect to PTC.

Although in both factor VII and Stuart factor deficiency the total amount of prothrombin is normal, the evolution of thrombin from prothrombin is delayed. Studies of prothrombin conversion of our patient's plasma and of her plasma together with added clotting components and with normal
FIG. 2.—Prothrombin conversion of Stuart plasma, plasma from a dicumarolized patient and the influence of Stuart plasma and Cohn Fraction I on the prothrombin conversion of patient’s plasma.

FIG. 3.—The effect of addition of factor VII on prothrombin conversion of normal and patient’s plasma.
plasma are shown in figure 1. The patient's plasma reached the peak of prothrombin conversion activity after 30 minutes of incubation whereas normal plasma reached its peak in 3 to 5 minutes. The patient's plasma, stored in glass at –20 C. for seven months, reached its peak activity in 18 minutes, although the factor VII level was equally low in both samples. It has been previously observed that prolonged exposure to glass tends to increase factor VII activity.29 The addition of an equal amount of normal serum as well as of factor VII or PTC containing fractions of normal serum results in normal conversion of the patient's prothrombin into thrombin. Similar results were obtained by Goldstein and Alexander.8 The PTC fraction derived from normal serum by the method of White et al.15 is found to have factor VII activity.

Figure 2 shows the effect of the addition of Cohn fraction I and Stuart deficient plasma to the test mixture. The addition of Cohn fraction I in 1 Gm. per cent concentration did not influence the prothrombin conversion of the patient's plasma although this concentration was capable of fully correcting the thromboplastin generation of a severe, classic hemophilic. Plasma from a dicumarolized patient and from the Stuart-deficient patient showed a prolonged prothrombin conversion. The addition of an equal amount of Stuart factor-deficient plasma to that of our patient resulted in normal prothrombin conversion. The addition of 10 volumes of the patient's plasma or of normal plasma had equal corrective effects on the prothrombin conversion of Stuart-deficient plasma.

The prothrombin conversion ratio compares the amount of thrombin generated after recalcification of plasma with the amount generated after the addition of a substance believed to be deficient. Results obtained with normal plasma and plasma from our patient are shown in figure 3. The addition of 0.1 ml. of a factor VII fraction to normal plasma 11½ minutes after recalcification did not change the rate of thrombin generation, which had reached its peak 5 to 6 minutes after the beginning of the test. The rate of thrombin generation of the patient's plasma increased sharply when factor VII fraction was added, suggesting that deficiency of this clotting factor was responsible for the faulty generation of thrombin in this plasma.

All the above results indicate that the bleeding in our patient does not result from a deficiency of Stuart clotting factor, which is present in normal concentration, but from a deficiency of factor VII.

Effect of treatment with ACC-76.—Because of the stability of factor VII, bleeding resulting from deficiency of this factor may be controlled with stored blood, plasma and even serum transfusions. Patients stop bleeding following transfusion with blood or blood products, but the hemostatic effect is of short duration. The blood level of factor VII returns to the pre-transfusion level within a few hours. It may become difficult to transfuse factor VII-deficient patients, especially small children, at frequent intervals with amounts of blood or plasma sufficient for effective hemostasis without overloading the patient's circulation.

When our patient was hospitalized in 1957 with spontaneous bleeding from the gums, a preparation called ACC-76, obtained from Behringwerke in
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Table 3.—Changes* in Certain Coagulation Tests Following Intravenous Injection of 1 Ampule of ACC-76 in 100 ml. of Saline

<table>
<thead>
<tr>
<th></th>
<th>Bleeding Time (min.)</th>
<th>Quick 1-stage Proth. (Sec.) (%)</th>
<th>Factor VII (Owren) (Sec.) (%)</th>
<th>Partial Thromboplastin Time (sec.)</th>
<th>Factor V (%)</th>
<th>Prothrombin (Hjort et al.) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Injection</td>
<td>6½</td>
<td>37</td>
<td>11</td>
<td>83</td>
<td>14.5</td>
<td>191</td>
</tr>
<tr>
<td>15 min.</td>
<td>20</td>
<td>33</td>
<td>60</td>
<td>26</td>
<td>167</td>
<td></td>
</tr>
<tr>
<td>1 hr.</td>
<td>26</td>
<td>20</td>
<td>59</td>
<td>26.5</td>
<td>157</td>
<td></td>
</tr>
<tr>
<td>2 hrs.</td>
<td>22</td>
<td>18</td>
<td>68</td>
<td>20</td>
<td>176</td>
<td></td>
</tr>
<tr>
<td>4 hrs.</td>
<td>32</td>
<td>14</td>
<td>73</td>
<td>18</td>
<td>197</td>
<td></td>
</tr>
</tbody>
</table>

*Normal values are given in table 1.

Marburg-Lahn, Germany, was employed in an attempt to control bleeding. ACC-76 has been noted by Koch et al.18 to have a beneficial effect when used in the treatment of factor VII-deficient patients. This fraction of human plasma is supplied in ampules containing 100 mg. of lyophilized material with a 10 ml. ampule of sterile saline. The preparation contains materials with factor VII and Stuart factor activities. One ampule is said to be equivalent in factor VII activity to 400 ml. of blood. From preliminary calculations, based on the response of our patient's blood levels of factor VII and from its evaluation in vitro, the factor VII activity is estimated to be roughly 150 times greater than that of normal plasma. Because of untoward effects of rapid infusion, one ampule of ACC-76 was diluted to 100 ml. with saline and administered to the patient by slow intravenous drip with a transfusion set. Bleeding from the gums stopped immediately after the beginning of injection. Certain changes in coagulation efficiency were followed after treatment and are given in table 3. The injection of ACC-76 did not shorten the prolonged bleeding time. Factor V and the two-stage prothrombin time were normal initially and were not followed. Quick's one-stage prothrombin and factor VII, respectively 11 and 14.5 per cent during this bleeding episode, increased immediately and then began to slowly decrease, but did not

Fig. 4.—Pedigree of family exhibiting a deficiency of factor VII.
return to preinjection levels within the four hour period of observation.
Similar changes in the coagulation factors were obtained by Goldstein and
Alexander after transfusions with 250 ml. of blood and after 100 ml. of serum.
Partial thromboplastin time was shortened following the injection, but re-
turned to its pretransfusion level within four hours.

Family study.—All living members of both the paternal and maternal
branches of the family were investigated except for one child, six months
of age, and the wife of a maternal grand uncle, whose whereabouts is
unknown. Information about additional family members was obtained by
interview of the older relatives. The pedigree is given in figure 4. The
paternal grandmother and several of her children consider themselves easy
bruisers. The father, a paternal aunt (III-9) and her two children (IV-7 and
IV-8) present histories of spontaneous epistaxis. Cousins on the maternal
side gave histories of spontaneous nose bleeding (III-23) and easy bruising
(III-26). The father, mother, two paternal aunts (III-3 and III-9) and the
paternal grandmother (II-2) exhibit slight increase in capillary fragility.
There is no history of bleeding in any distant or deceased relative. There
is no evidence of consanguinity in the family.

All investigated family members were found to have prothrombin levels
(Hjort et al. 12) within the normal range and to have normal prothrombin
conversion. The faulty thromboplastin generation of serum from a Stuart
factor-deficient patient was corrected by the addition of a 1:5 dilution of
serum from the patient, her father, mother, paternal grandmother and
maternal first cousin (III-14). It may be concluded that the deficiency
segregating in this family is not Stuart factor deficiency but a deficiency of
factor VII.

The factor VII concentrations of the patient and of 51 relatives were
determined by the method of Owren. The percentage concentrations of
all family members found to have levels lower than the mean of a control
sample are given in table 4. Family members having levels falling more
than two standard deviations beyond the control mean are listed as carriers
of an allele determining factor VII deficiency. The probability is less than
0.025 that any one of these individuals belongs to the normal group. Other
relatives having apparently normal factor VII levels are classed as carriers
because their children have significantly reduced levels.

No children with factor VII deficiency have been born to normal parents
in this family. Eight children, five exhibiting a mild factor VII deficiency,
have been born from four marriages in which one partner is mildly deficient
and the other is normal. The one child with a severe deficiency was born
to parents both of whom are mildly deficient. The observed pattern in this
family may be explained by postulating that factor VII deficiency is a
dominant autosomal trait. The allele determining the deficiency must, how-
ever, be extremely variable in its expression, leading to factor VII levels
from approximately 20 per cent to near normal in different generations of
this family. Because of the lack of uniformity in the degree of reduction within
sibships such variation cannot result from a single secondary genic modifier.
The pattern observed strongly suggests that factor VII reduction is independ-
FACTOR VII DEFICIENCY WITH NORMAL STUART ACTIVITY

Table 4.—Factor VII Levels of 23 Members from 3 Generations of a Family Deficient in Factor VII but Normal with Respect to Stuart Factor

<table>
<thead>
<tr>
<th>Pedigree number</th>
<th>Relationship to propositus</th>
<th>Observed Factor VII % of normal mean Average</th>
<th>Probable genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV-12</td>
<td>Propositus</td>
<td>18 25 23 24 16 21.1</td>
<td>Hemozgyous normal</td>
</tr>
<tr>
<td>III-13</td>
<td>Father</td>
<td>75 71 80 75.3</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>III-14</td>
<td>Mother</td>
<td>81 75 86 80.7</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>III-2</td>
<td>Paternal aunt</td>
<td>64</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>III-7</td>
<td>Paternal aunt</td>
<td>91</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>III-9</td>
<td>Paternal aunt</td>
<td>91 94 92.5</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>III-15</td>
<td>Maternal aunt</td>
<td>82 86 84</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>II-2</td>
<td>Paternal grandmother</td>
<td>86</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>II-7</td>
<td>Maternal granduncle</td>
<td>70</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>IV-6</td>
<td>Paternal 1st cousin</td>
<td>71</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>IV-7</td>
<td>Paternal 1st cousin</td>
<td>76</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>IV-8</td>
<td>Paternal 1st cousin</td>
<td>72 56</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>IV-13</td>
<td>Maternal 1st cousin</td>
<td>64 71</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>IV-14</td>
<td>Maternal 1st cousin</td>
<td>64</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>III-20</td>
<td>1st cousin once remvd</td>
<td>56</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>III-21</td>
<td>1st cousin once remvd</td>
<td>91</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>III-23</td>
<td>1st cousin once remvd</td>
<td>80</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>III-24</td>
<td>1st cousin once remvd</td>
<td>91</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>III-27</td>
<td>1st cousin once remvd</td>
<td>94</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>IV-10</td>
<td>Paternal 1st cousin</td>
<td>91</td>
<td>Homozygous normal</td>
</tr>
<tr>
<td>IV-3</td>
<td>Paternal 1st cousin</td>
<td>91</td>
<td>Homozygous normal</td>
</tr>
<tr>
<td>III-8</td>
<td>Uncle by marriage (unrelated)</td>
<td>91</td>
<td>Homozygous normal</td>
</tr>
<tr>
<td>III-12</td>
<td>Uncle by marriage (unrelated)</td>
<td>94</td>
<td>Homozygous normal</td>
</tr>
</tbody>
</table>

ent of sex and is correlated with gene dose, an individual heterozygous at this gene locus having a less severely reduced level than one homozygous for the deficiency-producing allele.

This study tends to confirm the hypothesis of Quick et al.28 that factor VII deficiency is inherited as an incompletely recessive autosomal characteristic. The hypothesis is validated by the demonstration that Stuart factor deficiency, known to have an identical mode of inheritance, does not segregate in this family.

Carrier diagnosis.—Several family members who must carry the factor VII deficiency allele have levels sufficiently high on the percentage scale that in the absence of a family study they would be assumed normal. Others, without progeny, cannot at present be categorically classed as normal or as carriers. It is possible, however, to estimate the probability that an individual may belong to the normal population. To make this estimate it is necessary to compare the distribution of factor VII levels among carriers with that among normal individuals. Lewis et al.24 determined the factor VII levels in 92 presumably normal individuals by Owren's method and found levels ranging from 149 to 64 per cent of the control sample mean. It is not possible to reconstruct from these data the distribution of levels.
in the normal population. A total of 119 normal individuals have been studied in this laboratory. Because of differences in the methods of sample collection, information from various sources has been analyzed separately. Blood samples were taken by 17 second year medical students as the first exercise in the technic of venipuncture, with the use of a single syringe. Duplicate samples were taken two weeks later. Each of these 34 samples was assayed in duplicate to estimate the reliability of the assay procedure. The remaining blood samples were taken by an experienced technologist (D. R. K.) by the two-syringe method, with the use of siliconized syringes and needles. The levels of these 102 individuals are taken as representative of the normal population. Twenty-six individuals were tested at least twice; these data have been analyzed to estimate the relative variation within individuals. The results of these analyses, recorded as clotting time in seconds, are given in the first five columns of table 5.

Owren's method gives relatively reproducible results as indicated by a small coefficient of variation for duplicate samples of blood (2.0 per cent). Column 2 shows that individuals vary considerably with respect to clotting factor levels; the average variation is not significantly less than the variation among individuals in the population \( F_{(25,26)} = 1.402; \) probability 0.05. The effect of sampling technic can be seen by comparing columns 1 and 2. The relative variation within individuals is twice as great for the students inexperienced in the technic of venipuncture (11.8 per cent) as for persons sampled by the two-syringe method by a trained technician (5.9 per cent). The mean clotting time of the students differs from that of the normal sample in column 5 \( t=2.14; \) Pr<0.05 and even more from that of the sample in column 2. The observed higher clotting factor level of the students may

<table>
<thead>
<tr>
<th>(1) Normal Students</th>
<th>(2) Normal Population</th>
<th>(3) Normal Males</th>
<th>(4) Normal Females</th>
<th>(5) Normal Population</th>
<th>(6) Normal Factor VII-Reduced Family Members</th>
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<tr>
<td>N=17</td>
<td>N=26</td>
<td>N=29</td>
<td>N=23</td>
<td>N=102</td>
<td>N=37</td>
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<tr>
<td>Variation of</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>assay method</td>
<td>.2463</td>
<td>--</td>
<td></td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Within individual</td>
<td>8.1175</td>
<td>2.5169</td>
<td>--</td>
<td>--</td>
<td>--</td>
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<tr>
<td>variation</td>
<td></td>
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<tr>
<td>Variation among</td>
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<td>3.5269</td>
<td>6.7356</td>
<td>3.3928</td>
<td>5.9955</td>
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<tr>
<td>Mean (seconds)</td>
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<td>±.241</td>
<td>±.482</td>
<td>±.384</td>
<td>±.242</td>
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<tr>
<td>Standard deviation</td>
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<td>1.736</td>
<td>2.595</td>
<td>1.842</td>
<td>2.449</td>
</tr>
<tr>
<td>(seconds)</td>
<td>2.955</td>
<td>3.04</td>
<td>3.25</td>
<td>3.25</td>
<td>3.25</td>
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Comparison of Mean Values

<table>
<thead>
<tr>
<th></th>
<th>t</th>
<th>P</th>
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<tr>
<td>1 and 5</td>
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<td>5 and 6</td>
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<td>5 and 7</td>
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<td>&lt;.001</td>
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<td>6 and 7</td>
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<tr>
<td>3 and 4</td>
<td>3.04</td>
<td>&lt;.01</td>
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FACTOR VII DEFICIENCY WITH NORMAL STUART ACTIVITY

result from the introduction of tissue juices into the syringe during blood sampling or from differences in sample structure. The sample in column 2 contains 19 females (73 per cent), whereas the first contains only males. Samples of 29 males and 23 females from the control population were compared to determine whether a sex difference in clotting factor levels may exist. The statistics for these samples are given in columns 3 and 4 of table 5. The mean clotting time of the males is significantly lower than that of the females ($t=3.04; Pr<0.01$). It should be noted that a sex difference is not observed among the normal members of the factor VII-deficient family; the average clotting time of 19 males was 24.8 seconds and of 15 females 24.7 seconds.

The control sample of 102 individuals has an average clotting time of $25.54 \pm 0.024$ seconds and a standard deviation of 2.45 seconds. The probability is less than 1 in 20 that anyone with an Owren's time of greater than 29.56 seconds (approximately 78 per cent normal) and less than 1 in 100 that anyone with a time greater than 31.23 seconds (approximately 69 per cent) belongs to the normal group.

Two samples from the factor VII-deficient family were compared with each other and with the normal population sample. The first family group includes all individuals except the patient having results of the Owren's test more than two standard deviations beyond the normal population mean and all of their ancestry in which, if factor VII deficiency is inherited as an incompletely recessive trait, the deficiency allele must occur. Thus II-2, III-7 and III-9 are included because their respective children have clotting times which deviate significantly from the normal mean. This group includes 14 individuals. The remaining 37 family members are considered normal, though some may actually be carriers. The mean clotting times and the standard deviations of these family groups are given in table 5 and the distributions are compared in figures 5 and 6.

Owren's test uses as substrate asbestos-filtered beef plasma, which retains most of its prothrombin but is free of factor VII. Such plasma is also low in Stuart factor. Abnormal results with this test may thus indicate a deficiency of factor VII, of Stuart factor, or both. Ideally, the plasma from our patient, lacking factor VII but with normal Stuart factor, should be used for factor VII assays; because of the age of the patient and the amount of plasma which would be required this was not attempted at this time. The distribution of test values obtained for the normal population is a joint distribution determined by the levels of at least two clotting factors in members of the sample. But since Stuart factor deficiency does not segregate in the family of our patient, it may be assumed that the distribution of Stuart factor levels in both the normal and factor VII-deficient groups in the family is about the same as that in the population. Thus, the only difference between groups which is subject to comparison is that resulting from factor VII level.

The mean value of the normal family members does not differ significantly from that of the control sample ($t=1.71; Pr>0.05$). The mean of the carrier members differs significantly from that of the normal family members ($t=8.52;
Distribution of Owren's test results in the population and in a Factor VII deficient family

![Histograms showing the observed distributions of Owren's test results in a normal control sample in homozygous normal and heterozygous factor VII-deficient family members.](image)

**Fig. 5.**—Histograms showing the observed distributions of Owren's test results in a normal control sample in homozygous normal and heterozygous factor VII-deficient family members.

![Fitted normal curves illustrating the distributions of Owren's test results in a normal control sample and in normal and heterozygous factor VII-deficient family members.](image)

**Fig. 6.**—Fitted normal curves illustrating the distributions of Owren's test results in a normal control sample and in normal and heterozygous factor VII-deficient family members. Distribution of Owren's test results in (1) population, (2) clinically normal factor VII-deficient family members and (3) the remaining normal family members.

Pr < 0.01). Despite the considerable overlap in factor VII concentrations in the family groups, the two may be considered distinct. The coefficients of variation, measuring the relative variation in the normal and carrier family groups (8.0 and 8.2 per cent, respectively) are not significantly different (t = 0.13; Pr > 0.80).

**DISCUSSION**

In the opinion of the majority of investigators factor VII is formed in the liver cells, although others believe that it may originate in the reticuloendothelial system. One hypothesis, based on experimental work with liver mitochondria, is that factor VII is produced in the general circulation during so-called "latent coagulation," when prothrombin is changed into thrombin, and is carried into the liver where it is, with the help of vitamin K, converted to prothrombin. 21
Factor VII has a unique position in the mechanism of blood coagulation and hemostasis. Its main role appears as a co-thromboplastin, a factor without which tissue thromboplastin is unable to initiate clotting of blood at the normal rate. This effect is seen in the abnormal results obtained in tests utilizing tissue thromboplastin, such as the one-stage prothrombin and partial thromboplastin tests. The latter test reflects abnormalities connected with thromboplastin generation and should not be influenced by deficiencies of factors participating in the later stages of coagulation. It is very useful and reliable for rapid screening for deficiencies of thromboplastin generation. It appears, however, that a marked deficiency of factor VII may cause a prolongation of clotting time not only with the “full” thromboplastin used in Quick’s test but also with the “partial,” reextracted brain thromboplastin used in this test. Essentially normal results are obtained with other tests not utilizing tissue thromboplastin. The two-stage prothrombin test utilizing very dilute beef serum appears to be unreliable with marked deficiency of factor VII.

Factor VII activity appears to be required for proper hemostasis. It may combine with tissue thromboplastin entering the circulation through a damaged endothelial lining of the blood vessels, and initiate conversion of prothrombin into thrombin. A marked decrease in factor VII appears to lead to faulty conversion of prothrombin in vivo as well as in vitro. Members of the investigated family heterozygous for factor VII deficiency with factor VII levels of 56 to 64 per cent present no clinical symptoms, show normal results in tests employing tissue thromboplastin and have normal rates of prothrombin conversion. Only when factor VII is low, at levels of 16 to 25 per cent or less, is the deficiency reflected in vivo by a bleeding tendency. During bleeding periods factor VII levels were found lower than any observed when the patient did not bleed. This may indicate either that a decrease below the level characteristic for this individual leads to bleeding or that the small amount of factor VII present is depleted by even a moderate blood loss.

The patient has consistently shown a prolonged bleeding time, abnormal capillary fragility, and has had spontaneous petechiae in addition to the ecchymoses typically occurring in factor VII-deficient patients. This may well indicate vascular impairment. The experimental results of Witte suggest that vascular impairment may be related to abnormalities of the blood-clotting mechanism. Both direct and indirect acting anticoagulants were found to induce seepage of fluorescin-tagged plasma protein through the capillary walls of the mesentery of rats. In our patient, correction of the factor VII deficiency with ACC-76 or blood transfusion does not appear to effect the vascular deficiency. The occurrence of increased capillary fragility in both the paternal and maternal ancestry suggests that this patient may be affected with a vascular deficiency which is inherited independently of factor VII deficiency.

The level of circulating clotting factors in an individual appears to be variable, and different persons may have different average levels. The hereditary make-up of an individual appears to determine whether his level will
vary about a high or a low mean concentration. The effect of one gene having a major effect on the determination of factor VII level is shown in this family. The great variation in concentration observed in the normal population suggests that more than two alleles may occur at this factor VII locus, with different gene combinations producing different "normal" levels. The occurrence of multiple alleles at this locus would explain both the population variation and the apparent variable penetrance of the deficiency allele. The great variation within individuals suggests, however, that nongenic factors may be largely responsible for the observed variation. There is some evidence suggesting that the sex of an individual may be a factor affecting his mean clotting factor level.

The factors which lead to variation about an individual's average value are unknown. Similar variation found in groups of normal and carrier members of the family suggests that the same factors may effect day to day variation in both groups.

The mode of inheritance of factor VII deficiency is similar to that of Stuart factor deficiency and of inherited factor V deficiency. It has been suggested that the frequencies of the genes producing these latter deficiencies may be estimated from determination of the frequency of carriers in the population. Such frequency studies of factor VII appear to be impossible, for carrier determination may be extremely difficult. Because of the intra-individual variation in factor VII levels, a carrier can be identified with certainty only by repeated testing and from the study of relatives.

The immediate therapeutic effect of an injection of ACC-76 appears to result from the high factor VII activity of this preparation. Koch has observed that ACC-76 appears to have a long-term effect, decreasing the bleeding potential of the patient for several weeks. He uses it as a preventive measure, and has found that three injections of ACC-76 given to one of his patients with mild factor VII deficiency within a period of six months prevented the occurrence of bleeding, although it was quite frequent previously. This effect was observed despite the return of blood factor VII levels to preinjection values four hours after therapy. The use of ACC-76 as a preventive was unsuccessful in our case. Injections of two ampules of ACC-76 every two weeks for a period of two months did not decrease the frequency of spontaneous ecchymoses.

Vitamin K therapy is considered by many to be of little value in factor VII deficiency because it fails to influence the level of factor VII in the blood. This view is opposed by Koch et al., who observed an increase following injections of 300 mg. of vitamin K, but no increase after injection of 100 mg. An improvement in the clinical condition of patients on this therapy was also noted. These workers postulate that vitamin K may lead to an increase in tissue factor VII, which, when massive doses of K are given, may spill over into the blood.

**Summary**

The case of a four year old white girl with a bleeding tendency characterized by the spontaneous occurrence of petechiae, ecchymoses and hemarthroses is presented. Laboratory studies indicate a deficiency of factor
FACTOR VII DEFICIENCY WITH NORMAL STUART ACTIVITY

VII, with normal levels of all other clotting components, and a vascular deficiency possibly related to frequent upper respiratory infection.

Serum from the patient and from four close relatives having reduced levels of factor VII is shown to correct the abnormal thromboplastin generation of Stuart factor-deficient serum. The rate of conversion of prothrombin into thrombin is markedly delayed in the patient's plasma but essentially normal in that of other family members. The patient's prothrombin conversion is corrected by the addition of normal or Stuart-deficient plasma. The partial thromboplastin time of the patient is somewhat prolonged.

It is concluded that factor VII deficiency is inherited in this family as an incompletely recessive autosomal characteristic. The penetrance of the deficiency allele in the heterozygous individual appears to be variable. Analyses of the distributions of clotting factor levels in the family and in samples from the normal population indicate that an individual may vary greatly in his clotting factor levels, the average variation being nearly as great as the variation among individuals. The magnitude of the variation among siblings suggests that the penetrance of the factor VII deficiency allele is affected by extrinsic factors and that the observed population variation does not result from the occurrence of multiple alleles. It is suggested that sex may be a factor determining clotting factor levels.

The bleeding of the patient is controlled by transfusion of plasma and by injection of a plasma preparation called ACC-76 (Behringwerke, Germany), the latter followed by a transient elevation of blood factor VII.

SUMMARIO IN INTERLINGUA

Es presentate le caso de un puera de racia blanc de quatro annos de etate, con tendentia sanguinatori characterisate per le occurantia spontanee de petechias, ecchymoses, e hemarthroses. Studios laboratorial indica un deficientia de factor VII, con nivellos normal de omne le altere componentes de coagulacion, e un deficientia vascular possibilemente relationate a frequente infecciones supero-respiratori.

Esseva demonstrate que sero ab le patiente e ab quatro immediate consanguineos de illa (in que reduceite nivellos de factor VII esseva constatate) possede le capacitate de corriger le anormalitate del generation de thromboplastina in seros a deficientia de factor Stuart. Le conversion de prothrombina in thrombina es marcatemente relentate in le plasma del patiente, sed illo es essentialmente normal in le plasma de altere membros de mesme familia. Le conversion de prothrombina in le plasma del patiente pote esser corrigite per le addition de plasma normal o de plasma deficientie in factor Stuart. Le tempore de thromboplastina partial in le patiente es levemente prolongate.

Es concludite que deficientia de factor VII es geneticamente transmititite in iste familia como un incompletamente recessive caracteristica autosomal. Le penetrantia del allelo de deficientia in le individuo heterozygotic es apparentemente variabile. Analises del distribution de nivellos del factorres de coagulacion in iste familia e in exemplos del population normal indica que un individuo pote manifestar un grande variabilitate in le nivellos de su factorres de coagulacion. De facto, le valor medie de iste variabilitate es quasi tanto extreme como illo del variabilitate intra gruppos de individuos. Le magnitude
del variation de iste valor ab un fraterno al altere suggere que le penetrantia del allelo pro deficientia de factor VII es influentiate per factores extrinsec e que le variationes observe in gruppos del population general non resulta del occurrentia de allelos multiple. Es exprimite le opinion que le sexo es possibilemente un factor que determina le nivellos del factores de coagulation.

ACKNOWLEDGMENTS

We are indebted to Dr. B. Kendig for referring of the patient, to Dr. Cecil Hougie for providing Stuart-deficient plasma and serum for use in cross matching, and to Dr. J. C. Longest, State College, Dr. R. F. Ellis, Starkville, Dr. J. G. Dees, Jackson and Dr. E. A. Trudeau, Biloxi, Miss., for examining various family members and for obtaining histories.

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Congenital Factor VII Deficiency with Normal Stuart Activity: Clinical, Genetic and Experimental Observations

HENRY G. KUPFER, BERTRAM L. HANNA and DIXIE R. KINNE

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