This study investigated the incidences of undercarboxylated (protein induced by vitamin K absence: PIVKA) prothrombin and protein C in 496 neonates across a wide range of gestational ages. These findings are related to vitamin K levels (an indicator of cofactor availability) and vitamin K, epoxide levels (a measure of the efficiency of the hepatic vitamin K cycle). PIVKA protein C was present in at least trace amounts in 27% of infants, whereas, PIVKA prothrombin was present in 7% of infants. PIVKA prothrombin and protein C were present at high plasma concentrations in 2% to 3% of term and preterm neonates and both PIVKA protein C and prothrombin increased with gestational age. Despite elevated plasma concentrations of PIVKA protein C and diminished levels of normally carboxylated protein C, clinical thrombosis was not observed. The mean (±SD) vitamin K, level in the study population was 0.009 ± 0.02 nmol/L (adult reference interval: 0.3 to 2.6 nmol/L) with no clear relationship between vitamin K, levels and production of PIVKA protein C or prothrombin. By comparison with adults, the epoxide form of the vitamin comprised an abnormally high proportion of total vitamin K,; this suggests possible inefficiencies in hepatic reductase cycling.

© 1993 by The American Society of Hematology.

The Report Committee on Hemostasis and Thrombosis in the Newborn, 1988. Vitamin K, Metabolism and the Production of Des-Carboxy Prothrombin and Protein C in the Term and Premature Neonate


The neonatal hemostatic system is characterized by low concentrations of many of the plasma coagulation proteins. The concentration of coagulation proteins increases in proportion to gestational age, reaching 30% to 50% of adult levels in full-term infants. Low levels of coagulation proteins in the neonate may be complicated by abnormalities of the vitamin K-dependent hemostatic system. Vitamin K, is the predominant form of the vitamin found in the fetus, although some vitamin K, (menaquinone) has been detected as well. Despite adequate maternal vitamin K, levels, newborns are deficient in the vitamin primarily because of inadequate placental transport. Newborns also may be unable efficiently to use available vitamin K, because of immaturity of the hepatic vitamin K cycle and/or carboxylase mechanism.

The group of molecules, referred to collectively as vitamin K-dependent proteins, is defined by the presence of the unique amino acid, gamma carboxy glutamic acid (Gla). The vitamin K-dependent coagulation proteins consist of the procoagulants (factors II, VII, IX, and X) and the anticoagulants (proteins C and S). These proteins serve as substrates for the posttranslational carboxylation of selected glutamic acid residues by a vitamin K-dependent carboxylase. The hepatic vitamin K-dependent gamma glutamyl carboxylase is associated with the rough endoplasmic reticulum and requires reduced vitamin K, oxygen, and carbon dioxide. Reduced vitamin K is derived from the quinone form of the vitamin through enzymatic reduction to the hydroquinone form. During carboxylation, the reduced hydroquinone form of vitamin K is converted to the stable 2,3 epoxide form. To regenerate active cofactor for the carboxylase, the enzyme vitamin K epoxide reductase converts the epoxide to either vitamin K quinone or hydroquinone. The carboxylation process can be inhibited by 4-hydroxycoumarin type anticoagulants and is incomplete in vitamin K deficiency, leading to the production of undercarboxylated vitamin K-dependent proteins (proteins induced by vitamin K absence or PIVKA).

Most studies of neonatal vitamin K-dependent hemostasis have been conducted in term neonates. The focus of these studies has been the detection of PIVKA prothrombin with only a few limited studies of PIVKA factors VII, IX, X, and protein C. The reported incidence of PIVKA prothrombin ranges from 0% to 3% in term neonates. The wide range of estimates of incidence is primarily a result of differences in assay methodology and sensitivity. Investigation of PIVKA protein production in preterm neonates has been limited to studies using relatively insensitive assays and, with one exception, small study populations. These studies show that the incidence of PIVKA prothrombin is considerably smaller in preterm infants when compared with term infants, suggesting that events occurring near term play a role in the expression of PIVKA proteins. The long half-life of prothrombin (60 hours) is a potential limitation of studies of both term and preterm neonates. Shorter half-life proteins such as protein C (6 to 8 hours) and factor VII (5 to 7 hours) may be more sensitive indicators of changes in vitamin K metabolism during gestation. Evaluation of the anticoagulant protein C would also improve our understanding of the control of neonatal coagulation.

Hemorrhagic disease of the newborn remains a significant clinical problem in those parts of the world where vitamin K prophylaxis at birth is not available. Of greater importance in North America are hemostatic challenges associated with prematurity. Premature infants experience disseminated intravascular coagulation (DIC) in a variety of clinical settings and abnormalities of their vitamin K-dependent hemostatic system diminish their limited hemostatic reserve. Intracranial hemorrhage (ICH) complicates up to 35% of births of infants with birth weights less than 1,500 g. Coagulation defects have been reported in infants with ICH and recent studies have shown significant decreases in the incidence of ICH in infants.
administered antenatal vitamin K\(^{20,21}\) or fresh frozen plasma.\(^{22}\) However, another recent study found no effect of prophylactic vitamin K administration on ICH.\(^{23}\)

The presence of PIVKA proteins in neonates has been primarily attributed to inadequate availability of vitamin K in the neonate.\(^{18}\) All neonates have low vitamin K levels compared with adults,\(^{4}\) but not all infants produce PIVKA proteins.\(^{7}\) There have been no reports evaluating the hepatic vitamin K cycle or carboxylase mechanism in human neonates despite evidence for immaturity of these systems in fetal animal models.\(^{24}\) This study reports the incidence of PIVKA prothrombin and protein C across a wide range of gestational ages. These findings are related to vitamin K levels as an indicator of cofactor availability and vitamin K\(_{\text{epoxide}}\) levels as a measure of the efficiency of the hepatic vitamin K cycle. Total prothrombin and protein C levels across the range of gestational ages were also measured as an indication of substrate presentation to the hepatic vitamin K-dependent gamma glutamyl carboxylase.

**MATERIALS AND METHODS**

**Patient population.** This study was reviewed and approved by the Institutional Review Board of the University of Vermont. Clinical data were collected from neonates and their mothers and were stored at 4°C. The plasma was then separated, aliquoted, snap frozen in dry ice/acetone, and stored at -70°C until analysis.

**Specimen acquisition.** Umbilical cord blood samples were collected according to the procedure of Hathaway et al\(^{25}\) into a liquid anticoagulant to achieve a final concentration of 10 U/mL heparin and 10 mmol/L sodium citrate. The samples were immediately transferred to the laboratory and centrifuged at 3,000 g for 15 minutes at 4°C. The plasma was then separated, aliquoted, snap frozen in dry ice/acetone, and stored at -70°C until analysis.

**PIVKA and total prothrombin and protein C immunoassays.** PIVKA protein C and prothrombin were measured by modification of previously described methods.\(^{26,27}\) We have recently described a rabbit polyclonal antibody (MoAb) designated H-1 that binds a subset of vitamin K\(_{\text{dependent}}\) coagulation proteins including protein C, prothrombin, and factors VII and X.\(^{28}\) The epitope recognized by H-11 was identified within the aminoterminal residues of the Gladomain at a site containing the conserved sequence Phe-Leu-Glu-Glu, which includes the first two Gla-residues.\(^{28}\) We have shown that in the presence of physiologic calcium concentrations this epitope is exposed in descarboxy protein C and prothrombin but hidden, and thus unavailable for H-1 antibody binding, in fully carboxylated protein C and prothrombin. This latter observation is the basis for the PIVKA protein C and prothrombin assays described below.

The capture antibodies are important components of the PIVKA protein C and prothrombin assays. Neither the polyclonal antiprotein C nor the antihuman protein C MoAbs interfere with the binding of antibody H-11 with its epitope. Preliminary evidence suggests that the epitope recognized by antibody HPC-2 is in the heavy chain of protein C but the exact peptide site has not been localized.

Plasma pools from 25 to 35 normal individuals and individuals who had been on stable warfarin therapy for at least 1 month were prepared as previously described.\(^{26,29}\) One warfarin plasma pool was used to construct a standard curve and another warfarin plasma pool and a normal plasma pool were run as controls. These pools were used for both the PIVKA protein C and prothrombin assays described below.

Antiprotein C MoAb (HPC-2) was diluted in 25 mmol/L bicarbonate buffer, pH 9.5, to a concentration of 30 μg/mL and 96-well Nunc-Immuno Plates (Roskilde, Denmark) were coated with 100 μL/well overnight at 4°C. After coating, the plates were washed and blocked with 2% bovine serum albumin (BSA) in 20 mmol/L Tris, 0.15 mmol/L NaCl (TBS) pH 7.4, and stored at -20°C until needed. The neonatal and standard plasmas were diluted 1:2 to 1:32 in 0.1% BSA/TBS. The normal and warfarin plasma controls were run at a 1:8 dilution. On the day of assay, plates were thawed 1.5 hours at room temperature. The thawed plates were washed with TBS, 0.1% Tween-20, and 100 μL of appropriately diluted plasma was added to each well. The plate was then incubated 1.5 hours at room temperature. The H-11 antibody (provided by Dr Jean Amiral, Diagnostica Stago, Asnières, France) conjugated with horseradish peroxidase was diluted in 0.1% BSA/TBS containing 10 mmol/L CaCl\(_2\). After washing the plate three times with wash buffer, the H-11 conjugate was added and the plate allowed to incubate 1 hour at room temperature. The plate was then washed five times and o-phenylene-diamine substrate (0.4 mg/mL in 75 mmol/L citrate-phosphate buffer, pH 5.0, 0.012% H\(_2\)O\(_2\)) was added. After a 7-minute incubation in the dark, 50 μL of 4M H\(_2\)SO\(_4\) was added to each well to stop the color development. The plate was then read at 490 nm on a BioTek Microplate Reader (Biotek, Inc, Burlington, VT). The interassay coefficient of variation (CV) was 4% (n = 19). The dynamic range of the assay extended from 3% to 50% of the PIVKA protein C present in the warfarin standard plasma pool. Patient samples were quantitated against the warfarin standard plasma pool and reported in arbitrary units (AU/mL where one AU is equivalent to the PIVKA protein C present in 1 mL of the standard plasma pool.

For the H-11 prothrombin immunoassay, plates were coated with a rabbit polyclonal antiprothrombin fragment pre-1 antibody at a concentration of 10 μg/mL and blocked with 1% BSA/TBS. The neonate plasmas, standards, and controls were diluted in 0.1% BSA/TBS, 0.1% Tween-20. The standard curve was constructed from 1:32 to 1:1,024 dilutions of plasma. The conjugate addition and developing steps that followed were similar to the H-11 protein C assay. The interassay CV was 13% (n = 17). The dynamic range of the assay extended from 0.09% to 1.5% of the PIVKA prothrombin present in the warfarin standard plasma pool. Patient samples were quantitated against the warfarin standard plasma pool and reported in AU/mL where one AU is equivalent to the PIVKA prothrombin present in 1 mL of the standard plasma pool.

Total prothrombin was measured using a previously described competitive radioimmunoassay that recognizes both carboxylated and undercarboxylated prothrombin.\(^{30}\) Total protein C was measured with a previously described sandwich enzyme-linked immunosorbent assay that recognizes both fully carboxylated and undercarboxylated protein.\(^{30}\)

**Quantification of PIVKA protein C and prothrombin in the warfarin standard plasma pool.** The following strategy was used to quantitate the amount of descarboxy protein C and prothrombin in the warfarin standard plasma pool used in the H-11 assay described above. Protein C and prothrombin were each measured in the warfarin standard plasma pool by two different assays, one of which measured total protein and the other functional carboxylated protein. The same calibrator was used in both assays for protein C and prothrombin. The amount of descarboxy-protein was determined from the difference between the total protein assay and the functional carboxylated protein assay.

A frozen pool (n = 32; males = females) of normal plasma designated D2H1 (George King Biomedical Inc, Kansas City, KS) was used as a calibrator for all assays described below. The D2H1 plasma
pool was assayed for total prothrombin and protein C using the immunoassays described in the preceding section. The protein C concentration was 3.7 μg/mL and the prothrombin concentration was 86 μg/mL.

Total and functional carboxy-protein were measured by the following assay systems calibrated with the D2H1 plasma pool. Total protein C was determined by an amidolytic, venom-activated assay system (American Bioproducts, Parsippany, NJ) as previously described.27 Functional carboxylated protein C was measured by a calcium-dependent clotting assay (American Bioproducts) as previously described.27 We have shown recently that the difference between these two assays gives a quantitative measure of undercarboxylated protein C in an investigation of a thrombophilic family with a protein C gla domain mutation.27

Total prothrombin was measured by a one-stage Echis carinatus assay by a modification of the method of Franz et al.30 The prothrombin converting enzyme in the E. carinatus venom activates prothrombin to thrombin in the absence of phospholipid, calcium ions, or factor V. In brief, calibration and patient plasmas were initially diluted in veronal buffer (neat to 1:8), then further diluted 1:3 in 0.4% bovine fibrinogen and incubated for 2 minutes at 37°C. Following the incubation step 50 μL (1.5 mg/mL) E. carinatus venom (Sigma, St Louis, MO) was added and the clotting time noted from the point of venom addition. Total prothrombin in the warfarin pool plasma standard was then measured from the calibration curve described above. The E. carinatus assay was performed on an ST-4 automated coagulation instrument (American Bioproducts Inc). Functional carboxylated prothrombin was measured by one-stage calcium-dependent factor assay as previously described.31 These four assays were performed in the special coagulation laboratory of the Medical Center Hospital of Vermont (under direction of E.G.B.) where they are run routinely. The four assays have interassay CVs of approximately 8%.

Thus, the concentration (μg/mL) of descarboxy-protein C and prothrombin were calculated as follows: (nonfunctional descarboxy-protein) = (total protein) – (functional carboxy-protein). Using this calculation the following conversions can be made from the AUs used in the H-11 assay system: 1 AU/mL of protein C is equivalent to 1.6 μg/mL nonfunctional descarboxy-protein C and 1 AU/mL of prothrombin is equivalent to 25 μg/mL nonfunctional descarboxy-prothrombin.

Vitamin K₁ and K₂ epoxide measurements. Vitamin K₁(25) (2-methyl-3-phytyl-1,4-naphthaquinone) was purchased from a commercial source (Sigma). The internal standard K₂(25), a synthetic analogue of vitamin K₁, was a gift (Hoffman-La Roche & Co Basel, Switzerland). Vitamin K₁,2,3-epoxide was synthesized from vitamin K₁. Standard solutions were prepared in high performance liquid chromatography (HPLC) grade methanol and the compounds characterized by spectroscopic (UV) and HPLC analyses before use.

Vitamin K₁ concentrations in the samples were determined using a modification of a previously published method.32 Plasma samples were pipetted into a 20 × 125-mm disposable borosilicate glass screw top culture tube (using teflon-lined screw caps) and 2.2 pmol of the internal standard [K₂(25) in methanol], 2 mol of ethanol, and 0.25 mol of water were added. The mixture was vortexed for 15 seconds followed by the addition of 6 plasma volumes of hexane. The resulting mixture was shaken vigorously for 2 minutes followed by centrifugation at 3,500g (4°C) for 5 minutes. The hexane layer was removed after centrifugation and evaporated to dryness. The resulting lipid residue was further extracted on silica (3 mL SPE silica column; J.T. Baker, Inc, Philippsburg, NJ) followed by extraction by reverse-phase partition (3 mL C₁₈ column, J.T. Baker, Inc). The partially purified extract from the reverse-phase partition was dissolved in 0.01 mL of methylene chloride followed by the prompt addition of 0.09 mL of methanol containing 10 mmol/L zinc chloride, 10 mmol/L acetic acid, and 5 mmol/L sodium acetate (1 L MeOH: 5 mL aqueous solution).

Sample injection (50 μL) was accomplished using a model 231-401 automated sample injector (Gilson Medical Electronics, Inc, Middleton, WI) fitted with a Rheodyne 7010 injection valve and a 50 μL loop. The chromatography system consisted of a model 310 reciprocating pump and an 860 VAX-based data station with Expert-Ease software for integration and quantitation (Waters Assoc, Milford, MA). Separation was achieved using a narrow-bore analytical column (250 mm × 2.1 ID) packed with Hypersil-BDS (Keystone Scientific, Bellefonte, PA) (5 μm) and a gradient flow rate. The initial flow rate was 0.25 mL/min, and at 16 minutes the flow rate was increased to 0.5 mL/min for 13 minutes before returning to 0.25 mL/minute. The total run time was 30 minutes. The mobile phase was composed of methylene chloride-methanol (10:90, vol/vol) and to each liter was added 5.0 mL of an aqueous solution containing 2 mol/L zinc chloride, 2 mol/L glacial acetic acid, and 1 mol/L sodium acetate. The analytes were detected by measuring the fluorescence of their hydroquinone derivatives formed by solid-phase postcolumn reduction on zinc³ using a Spectroflow 980 fluorescence detector (Kratos Analytical, Ramsey, NJ). Detection was performed at an excitation wavelength of 244 nm and emission monitored at 418 nm using a longpass cutoff filter.

Statistical methods. With levels of vitamin K₁, vitamin K₂ epoxide, PIVKA prothrombin, and PIVKA protein C often decreasing below the detectable range of currently available assays among infants with gestational age from 25 to 42 weeks and still other levels frequently just above the detectable range, statistical analyses were limited to an examination in terms of their presence or absence. The Pearson X² test was used in testing for a relationship between the concentration of these coagulation proteins as well as their relationship with gestational age. Differences in total prothrombin and total protein C between preterm infants (<38 weeks gestation) and term infants were tested using the Wilcoxon rank sum test.

RESULTS

Demographic and clinical description of study population. A total of 496 term and preterm infants were entered into this study. Of this group, 448 met our prospectively defined criteria for healthy neonates as outlined above. The study comprised of 279 term and 169 preterm infants. Of the infants who met our study criteria, 397 had samples sufficient to perform both prothrombin and protein C assays, and 133 term and 26 preterm infants had adequate samples to perform vitamin K₁ and K₂ epoxide assays. The population characteristics included: median gestational age of 38.9 weeks (range = 22.5 to 43); median birthweight of 3,175 g (range = 685 to 4,790); sex distribution 45% male and 55% female; modes of delivery were 41% cesarean section and 59% vaginal.

Total and PIVKA prothrombin and protein C. The proportions of neonates with PIVKA prothrombin and protein C as a function of gestational age are shown in Table 1. The expression of PIVKA protein C increased significantly (P = .0001) with increasing gestational age. There was a trend to increased expression of PIVKA prothrombin with increasing gestational age that did not reach statistical significance. There was also a higher proportion of infants with PIVKA protein C than with PIVKA prothrombin at all gestational ages. No independent effect of mode of delivery was noted. The increased incidence of PIVKA protein C with increasing gestational age might be explained by increased substrate
Table 1. Relationship of PIVKA Prothrombin and Protein C to Gestational Age

<table>
<thead>
<tr>
<th>Gestational Age (wks)</th>
<th>PIVKA Prothrombin</th>
<th>PIVKA Protein C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>&lt;34</td>
<td>72</td>
<td>4</td>
</tr>
<tr>
<td>34-38</td>
<td>83</td>
<td>5</td>
</tr>
<tr>
<td>≥38</td>
<td>210</td>
<td>23</td>
</tr>
</tbody>
</table>

PIVKA prothrombin: Pearson’s χ² test; χ² = 2.51, P = .29. PIVKA protein C: Pearson’s χ² test; χ² = 63.84, P = .0001.

presentation to the carboxylase. To address this possibility, we measured total protein C and prothrombin levels from 30 to 42 weeks gestation in a randomly selected subpopulation (n = 38) of our study participants (Figs 1 and 2). There is a clear increase in total prothrombin and protein C levels over this period of time. If one compares the levels of total prothrombin and protein C between infants ≥38 weeks with those <38 weeks, the difference is highly significant (P = .001).

The frequency distribution of PIVKA protein C and prothrombin concentration for term and preterm infants are presented in Figs 3 and 4, respectively. The majority of the infants had relatively low levels, or no detectable PIVKA prothrombin or protein C. Seven percent of neonates had at least trace amounts of PIVKA prothrombin and 27% of neonates had trace or greater amounts of PIVKA protein C. Figure 3 shows that about 3% of term neonates had protein C levels >0.4 AU/mL, approximately equivalent to >0.64 μg/mL PIVKA protein C with a few infants having plasma concentrations of PIVKA protein C equivalent to >1 μg/mL.

Vitamin K₁ and K₁ epoxide levels. Vitamin K₁ levels were measured in 133 term and 26 preterm neonates. Sample volume constraints restricted the number of samples available for vitamin K₁ assays that require 1 mL of plasma for each determination of either vitamin K₁ or K₁ epoxide. Forty percent of the neonatal samples had undetectable levels of vitamin K₁. The mean, median, and range of values for vitamin K₁, vitamin K₁ epoxide, and total vitamin K for term and preterm infants are shown in Table 2. For comparison, the adult reference interval for vitamin K₁ is 0.3 to 2.6 nmol/L.

The mean vitamin K₁ epoxide level as a proportion of the mean total vitamin K₁ level was considerably higher than observed in adult plasma samples analyzed during the same time period (n = 255 adults, mean ratios of vitamin K₁ epoxide to total vitamin K₁ = 0.065).

Vitamin K₁ and vitamin K₁ epoxide levels are presented as a function of gestational age in Table 3. No significant relationships with gestational age were noted.

Relationship between vitamin K₁ and PIVKA protein C and prothrombin. The levels of vitamin K₁ were very low.
The status of the neonatal vitamin K-dependent hemostatic system across a range of gestational ages was assessed by measurement of PIVKA protein C and prothrombin levels. Previous investigators have focused their attention almost entirely on PIVKA prothrombin, have used a variety of different methods, and reported a wide range of results. differing specificity and sensitivity of methods seem to explain this wide range of reported results.7 Protein C has a considerably shorter half-life than prothrombin (4 v 60 hours)33 and thus may be a more sensitive indicator of changes in the vitamin K cycle and hepatic vitamin K-dependent gamma glutamyl carboxylase function. Protein C is also a major inhibitor of the hemostatic system and, together with prothrombin, yields information with respect to both the procoagulant and anticoagulant pathways of hemostasis.

Using a unique MoAb to PIVKA prothrombin and protein C, we observed elevated levels of the PIVKA form of both proteins that increased with gestational age. PIVKA protein C was seen more frequently than PIVKA prothrombin across the range of gestational ages (Table 1). This difference may result from the more sensitive response (shorter half-life) of protein C to changes in the hepatic vitamin K-dependent carboxylase system. We considered the hypothesis that increased production of vitamin K-dependent precursor proteins late in the gestational period might overload the vitamin K-dependent gamma glutamyl carboxylase and decrease the conversion of glutamic acid residues to Gla, resulting in increased PIVKA expression. Measurement of total prothrombin and protein C levels across the range of gestational age seems to support this hypothesis with a significantly higher level of both proteins present in infants >38 weeks of age compared with those <38 weeks of age (P = .001). We also considered the possibility that the increased glycosylation observed in neonatal coagulation proteins24,35 might affect reactivity of our antibody. However, whereas it is difficult to exclude an effect of glycosylation, this seems unlikely because the reactivity of the H-11 antibody has been clearly shown to be a function of the state of carboxylation in purified and plasma systems.26*28 Furthermore, the H-11 epitope is fully contained within a discrete dodecapeptide arising within the first 12 residues of prothrombin and there are no known glycosylation sites within or near this epitope.

Over one third of healthy term neonates had some evidence of PIVKA protein C. In the majority of infants, the concentration of PIVKA protein C was very low. However, Fig 3 shows that about 3% of term infants had protein C levels >0.4 AU/mL approximately equivalent to >0.64 µg/mL PIVKA protein C with a few infants having PIVKA protein C levels equivalent to >1 µg/mL. The mean total protein C level in term neonates was 1.05 µg/mL compared with an adult mean of 3.1 µg/mL. Therefore, the infants with >0.4 AU/mL PIVKA protein C had considerably diminished plasma concentrations of normally carboxylated (and thus

### Table 2. Vitamin K<sub>1</sub> in Term and Preterm Infants

<table>
<thead>
<tr>
<th>Vitamin K&lt;sub&gt;1&lt;/sub&gt;</th>
<th>Median (range)</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Term (n=233)</td>
<td>0.00 (0-0.088)</td>
<td>0.009 (0.02)</td>
</tr>
<tr>
<td>Preterm (n=164)</td>
<td>0.00 (0-0.034)</td>
<td>0.003 (0.01)</td>
</tr>
<tr>
<td>Total K</td>
<td>0.0075 (0-0.095)</td>
<td>0.012 (0.02)</td>
</tr>
</tbody>
</table>

Number of infants in study was 159.

### Table 3. Relationship of Vitamin K<sub>1</sub> and K<sub>1</sub> Epoxide to Gestational Age

<table>
<thead>
<tr>
<th>Gestational Age (wks)</th>
<th>Vitamin K&lt;sub&gt;1&lt;/sub&gt; Absent</th>
<th>Vitamin K&lt;sub&gt;1&lt;/sub&gt; Epoxide Absent</th>
<th>Vitamin K&lt;sub&gt;1&lt;/sub&gt; Absent</th>
<th>Vitamin K&lt;sub&gt;1&lt;/sub&gt; Epoxide Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;34</td>
<td>2</td>
<td>2</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>34-37.9</td>
<td>12</td>
<td>11</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>38-41.9</td>
<td>69</td>
<td>57</td>
<td>97</td>
<td>29</td>
</tr>
<tr>
<td>≤42</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

For vitamin K<sub>1</sub>: Pearson's χ<sup>2</sup> test; x<sup>2</sup> = 4.25, P = .24. For vitamin K<sub>1</sub> epoxide: Pearson's χ<sup>2</sup> test; x<sup>2</sup> = 3.19, P = .363.

### Table 4. Relationship of PIVKA Protein C to Vitamin K<sub>1</sub>

<table>
<thead>
<tr>
<th>PIVKA Protein C</th>
<th>Vitamin K&lt;sub&gt;1&lt;/sub&gt; Absent</th>
<th>Vitamin K&lt;sub&gt;1&lt;/sub&gt; Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>48</td>
<td>38</td>
</tr>
<tr>
<td>Present</td>
<td>42</td>
<td>31</td>
</tr>
</tbody>
</table>

Pearson's χ<sup>2</sup> test; x<sup>2</sup> = 0.04, P = .83.

Number of infants in study was 159.
functional) protein C, similar in the more severely affected infants to plasma concentrations of protein C observed in homozygous protein C deficiency.\textsuperscript{36} Despite low levels of normal protein C and the challenge of birth among the more severely affected term neonates, clinical thrombotic complications were not observed. These findings seem to support the view that protein C deficiency may be a risk factor for thrombosis but is not sufficient alone to cause thrombotic disease.\textsuperscript{37}

Preterm neonates have a higher incidence than term neonates of thrombotic disease including arterial thrombosis and DIC.\textsuperscript{38} Intraventricular hemorrhage, common in preterm neonates, is thought by some to follow a thrombotic initiating event.\textsuperscript{39,40} It will require prospective studies to determine what role the presence of PIVKA protein C might play in these multifactorial diseases.

Vitamin K, metabolism in neonates was evaluated. We observed extremely low levels of vitamin K, in both term and preterm neonates. These findings are similar to previous studies of term neonates with little or no previously published data available for preterm neonates.\textsuperscript{13} Low neonatal vitamin K, levels have been attributed to poor placental transport of the vitamin.\textsuperscript{4} The presence of high levels of vitamin K, epoxide relative to total vitamin K, levels (Table 2) suggests an inefficient cycling of vitamin K, by the hepatic vitamin K reductase(s) in some neonates. Inefficient cycling of the vitamin would further reduce the concentration of available cofactors for the carboxylase and intensify the deficiency. This hypothesis is supported by the observation of immaturity of other neonatal hepatic enzyme systems.\textsuperscript{41}

Levels of PIVKA protein C and prothrombin were significantly correlated in spite of their observed difference in incidence (Spearman’s rank \( r = .33, P = .0001 \)). This supports the supposition that related mechanisms underlie the production of these two PIVKA proteins. However, vitamin K, and K, epoxide levels did not correlate with the level of PIVKA protein C or prothrombin. This observation may be explained by the fact that 40% of infants had vitamin K, levels below the limit of detection of our assay, that many of the measured values were near the detection limit of the assay, and that we were unable to measure vitamin K, and K, epoxide levels in all the infants.

If the vitamin K assay had a lower detection limit, thus incorporating more infants into the detectable range, a relationship might be observed. However, the relationship between cord blood levels of vitamin K and neonatal hepatic stores of the vitamin is unknown. It may well be that there is a more complex relationship between the postabsorptive state in the mother and placental transport with transient peak vitamin K levels in the fetus not well represented by the cord levels at birth.

In conclusion, both PIVKA prothrombin and protein C are present in considerable concentration in a small but significant proportion of term and preterm neonates. The clinical effect of these PIVKA proteins remains to be determined. The lack of significant clinical disease among affected term neonates in spite of low levels of normal protein C and prothrombin in a small but significant proportion of them, supports the hypothesis that the etiology of thrombotic disease is multifactorial. All of the infants had very low levels of vitamin K, with no clear relationship between vitamin K, levels and production of PIVKA proteins. The relatively high levels of vitamin K, epoxide as a proportion of total vitamin K, may reflect inefficiency of the hepatic reductase cycling of the vitamin.

**ACKNOWLEDGMENT**

The authors express their gratitude for the excellent secretarial and textual review provided by Laurie Sabens.

**REFERENCES**


**Table 5. Relationship of PIVKA Prothrombin to Vitamin K,**

<table>
<thead>
<tr>
<th>Vitamin K,</th>
<th>Absent</th>
<th>Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>80</td>
<td>6</td>
</tr>
<tr>
<td>Present</td>
<td>63</td>
<td>10</td>
</tr>
</tbody>
</table>

Pearson’s \( x^2 \) test: \( x^2 = 1.97, P = .16 \).

Number of infants in study was 159.