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 K1 levels (an indicator of cofactor availability) and vitamin K1 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 K1 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 K1 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 K1; this suggests possible inefficiencies in hepatic reductase cycling.

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.1,2 Low levels of coagulation proteins in the neonate may be complicated by abnormalities of the vitamin K1-dependent hemostatic system. Vitamin K1 is the predominant form of the vitamin found in the fetus, although some vitamin K2 (menaquinone) has been detected as well.3 Despite adequate maternal vitamin K1 levels, newborns are deficient in the vitamin primarily because of inadequate placental transport.4 Newborns also may be unable efficiently to use available vitamin K1 because of immaturity of the hepatic vitamin K cycle and/or carboxylase mechanism.

The group of molecules, referred to collectively as vitamin K1-dependent proteins, is defined by the presence of the unique amino acid, gamma carboxy glutamic acid (Gla). The vitamin K1-dependent coagulation proteins consist of the procoagulants (factors II, VII, IX, and X) and the coagulation inhibitors (proteins C and S). These proteins serve as substrates for the posttranslational carboxylation of selected glutamic acid residues by a vitamin K1-dependent carboxylase.5 The hepatic vitamin K1-dependent gamma glutamyl carboxylase is associated with the rough endoplasmic reticulum and requires reduced vitamin K1, oxygen, and carbon dioxide.6 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 K1 epoxide reductase converts the epoxide to either vitamin K1 quinone or hydroquinone.7 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 K1-dependent proteins (proteins induced by vitamin K absence or PIVKA).8

Most studies of neonatal vitamin K1-dependent hemostasis have been conducted in term neonates. The focus of these studies has been the detection of PIVKA prothrombin9 with only a few limited studies of PIVKA factors VII, IX, X, and protein C.8,10 The reported incidence of PIVKA prothrombin ranges from 0% to 50% in term neonates.7 The wide range of estimates of incidence is primarily a result of differences in assay methodology and sensitivity.7 Investigation of PIVKA protein production in preterm neonates has been limited to studies using relatively insensitive assays and, with one exception, small study populations.7 These studies show that the incidence of PIVKA prothrombin is considerably smaller in preterm infants when compared with term infants,11,12 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.13 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 K1-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 ICH14-16 and recent studies have shown significant decreases in the incidence of ICH in infants.
administered antenatal vitamin K\textsuperscript{20,21} or fresh frozen plasma.\textsuperscript{22} However, another recent study found no effect of prophylactic vitamin K administration on ICH.\textsuperscript{23}

The presence of PIVKA proteins in neonates has been primarily attributed to inadequate availability of vitamin K in the neonate.\textsuperscript{16} All neonates have low vitamin K levels compared with adults,\textsuperscript{4} but not all infants produce PIVKA proteins.\textsuperscript{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.\textsuperscript{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 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 as an indication of cofactor availability and vitamin K epoxide levels as a measure of the efficiency of the hepatic vitamin K cycle.

Specimen acquisition. Umbilical cord blood samples were collected according to the procedure of Hathaway et al\textsuperscript{19} 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 3000g 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.\textsuperscript{26,27} We have recently described a murine monoclonal antibody (MoAb) designated H-1 that binds a subset of vitamin K-dependent coagulation proteins including protein C, prothrombin, and factors VII and X.\textsuperscript{19,28} The epitope recognized by H-1 was identified within the aminoterminal residues of the Gla-domain at a site containing the conserved sequence Phe-Leu-Glu-Glu, which includes the first two Gla-residues.\textsuperscript{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-1 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.\textsuperscript{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 with 1% BSA/TBS temperature. The H-1 antibody (provided by Dr Jean Amiral, Diagnostic Stago, Asnieres, France) conjugated with horseradish peroxidase was diluted in 0.1% BSA/TBS containing 10 mmol/L CaCl\textsubscript{2}. After washing the plate three times with wash buffer, the H-1 conjugate was added and the plate allowed to incubate 1 hour at room temperature. The plate was then washed five times and o-phenylenediamine substrate (0.4 mg/mL in 75 mmol/L citrate-phosphate buffer, pH 5.0, 0.012% H\textsubscript{2}O\textsubscript{2}) was added. After a 7-minute incubation in the dark, 50 µL of 4M H\textsubscript{2}SO\textsubscript{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-1 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 neonatal 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-1 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.\textsuperscript{25} Total protein C was measured with a previously described sandwich enzyme-linked immunosorbent assay that recognizes both fully carboxylated and undercarboxylated protein.\textsuperscript{25}

Quantitation 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-1 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 D2Hl plasma pool. Total protein C was determined by an amidolytic, venom-activated assay system (American Bioproducts, Parsippany, NJ) as previously described. Functional carboxylated protein C was measured by a calcium-dependent clotting assay (American Bioproducts) as previously described. 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 gln domain mutation.

Total prothrombin was measured by a one-stage Echis carinatus assay by a modification of the method of Franz et al. 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 a one-stage calcium-dependent factor assay as previously described. 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-1 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 K, and K1 epoxide measurements.** Vitamin K1(25) (2-methyl-3-phenyl-4,6-naphthoquinone) was purchased from a commercial source (Sigma). The internal standard K1(25), a synthetic analogue of vitamin K1, was a gift (Hoffman-La Roche & Co, Basel, Switzerland). Vitamin K1(25) 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 published method. 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 [K1(25)] in methanol, 2 vol of ethanol, and 0.25 vol 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, Phillipsburg, 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 510 reciprocating pump and an 860 VAX-based data station with ExpertEase 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-ODS (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/min. 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 zinc 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 K1 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 χ2 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 was 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 K1 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.

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</th>
<th>PIVKA Prothrombin</th>
<th>PIVKA Protein C</th>
</tr>
</thead>
<tbody>
<tr>
<td>(wks)</td>
<td>Absent</td>
<td>Present</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 = .0001).

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.
NEONATAL VITAMIN K-DEPENDENT HEMOSTASIS

Many fell just above the lower end of the detectable range. Consequently, we elected to analyze the relationship between the presence or absence of PIVKA protein and the presence or absence of measurable vitamin K₁. The results of this analysis are presented in Tables 4 and 5. No significant relationship was observed.

DISCUSSION

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. Protein C has a considerably shorter half-life than prothrombin (4 v 60 hours) and thus may be a more sensitive indicator of changes in the vitamin K-dependent coagulation 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 system. We considered the possibility that the increased glycosylation observed in neonatal coagulation proteins 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. Furthermore, the H-11 epitope is 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 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 possibility 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 Glu, 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 = .0001). We also considered the possibility that the increased glycosylation observed in neonatal coagulation proteins 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 plasma systems. Furthermore, the H-11 epitope is 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.

Table 2. Vitamin K₁ in Term and Preterm Infants

<table>
<thead>
<tr>
<th>Vitamin K₁</th>
<th>Median (range)</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin K₁</td>
<td>0.00 (0-0.088)</td>
<td>0.009 (0.02)</td>
</tr>
<tr>
<td>Vitamin K₁0</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 K₁ and K₁ Epoxide to Gestational Age

<table>
<thead>
<tr>
<th>Gestational Age (wks)</th>
<th>Vitamin K₁</th>
<th>Vitamin K₁ Epoxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>&lt;34</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>34-37.9</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>38-41.9</td>
<td>69</td>
<td>57</td>
</tr>
<tr>
<td>≤42</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

For vitamin K₁: Pearson’s χ² test; χ² = 4.25, P = .24. For vitamin K₁ epoxide: Pearson’s χ² test; χ² = 3.19, P = .363.

Table 4. Relationship of PIVKA Protein C to Vitamin K₁

<table>
<thead>
<tr>
<th>Vitamin K₁</th>
<th>PIVKA Protein C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>48</td>
</tr>
<tr>
<td>Present</td>
<td>42</td>
</tr>
</tbody>
</table>

Pearson’s χ² test; χ² = 0.048, 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. 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.

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

Vitamin K₁ metabolism in neonates was evaluated. We observed extremely low levels of vitamin K₁ 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. Low neonatal vitamin K₁ levels have been attributed to poor placental transport of the vitamin. The presence of high levels of vitamin K₁, epoxide relative to total vitamin K₁ levels (Table 2) suggests an inefficient cycling of vitamin K₁ by the hepatic vitamin K reductase(s) in some neonates. Efficient 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.

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 K₁ and K₁ 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 K₁ 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 K₁ and K₁ 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 K₁ with no clear relationship between vitamin K₁ levels and production of PIVKA proteins. The relatively high levels of vitamin K₁ epoxide as a proportion of total vitamin K₁ 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

13. Pieterse-de Bruyn ALJM, van der Straaten PM, van der Linden IK, van Tilburg NH: Coagulation factors in the premature infant born after about 32 weeks of gestation. Biol Neonate 47:9, 1985
Vitamin K1 metabolism and the production of des-carboxy prothrombin and protein C in the term and premature neonate

EG Bovill, RF Soll, M Lynch, F Bhushan, M Landesman, M Freije, W Church, T McAuliffe, K Davidson and J Sadowski