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Association of Congenital Deficiency of Multiple Vitamin K–dependent Coagulation Factors and the Phenotype of the Warfarin Embryopathy: Clues to the Mechanism of Teratogenicity of Coumarin Derivatives

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SUMMARY

We have evaluated a boy who had excessive bleeding and bruising from birth and showed markedly prolonged prothrombin times, partially correctable by oral vitamin K administration. Additional laboratory studies demonstrated decreased activities of plasma factors II, VII, IX, and X; near normal levels of immunologically detected and calcium binding–independent prothrombin; undercarboxylation of prothrombin; excess circulating vitamin K epoxide; decreased excretion of carboxylated glutamic acid residues; and abnormal circulating osteocalcin. These results all are consistent with effects resulting from decreased posttranslational carboxylation secondary to an inborn deficiency of vitamin K epoxide reductase. This individual also had nasal hypoplasia, distal digital hypoplasia, and epiphyseal stippling on infant radiographs, all of which are virtually identical to features seen secondary to first-trimester exposure to coumarin derivatives. Therefore, by inference, the warfarin embryopathy is probably secondary to warfarin's primary pharmacologic effect (interference with vitamin K–dependent posttranslational carboxylation of glutamyl residues of various proteins) and may result from undercarboxylation of osteocalcin or other vitamin K–dependent bone proteins.

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INTRODUCTION

The potential teratogenicity of oral vitamin K–antagonist anticoagulants was first widely recognized in 1975 (Becker et al. 1975; Pettifor and Benson 1975; Shaul et al. 1975). Subsequently the major clinical features (Hall et al. 1980), critical period of intrauterine exposure (Pauli et al. 1976; Hall et al. 1980), and risk levels (Hall et al. 1980; Kort and Cassel 1981; O'Neill et al. 1982; Oakley 1983; Chong et al. 1984) for the warfarin embryopathy have been determined. Nonetheless, as with virtually all other human teratogens (Juchau 1981), the precise mechanism of teratogenicity of coumarin derivatives remains unknown. Warfarin inhibits the vitamin K–dependent synthesis of γ-carboxyglutamic acid in various proteins (Gallop et al. 1980; Suttie 1980). Although it has been postulated that the features of the warfarin embryopathy result from the primary pharmacologic effects of coumarin derivatives (Hauschka et al. 1978; Hall et al. 1980), neither direct nor indirect proof of this hypothesis has been previously obtained.

We have clinically and biochemically evaluated a boy who has an inborn deficiency of vitamin K–dependent coagulation factors and shows all the clinical and radiographic features of the warfarin embryopathy. This provides the first evidence that the warfarin embryopathy is, in fact, secondary to warfarin's primary pharmacologic effect.

CASE REPORT

This 7½-year-old boy was born following a pregnancy complicated only by renal colic in the eighth month. Intrauterine-exposure history included Bendectin® (doxylamine succinate + pyridoxine hydrochloride) in the first month and diphenhydramine and an unknown oral pain medication in the eighth month. There was no exposure to anticoagulants or antiepileptics. Delivery was vaginal at term; birth weight was 3.31 kg, and birth length 54.6 cm. The infant incurred an accidental 3-cm scalp laceration when an episiotomy was performed. Despite suturing, the wound continued to bleed subcutaneously. Because of abnormal coagulation measurements (see below) and clinical evidence of decreased intravascular blood volume, blood products including fresh red blood cells and fresh plasma were administered. Bleeding from the scalp laceration stopped and did not recur.

The infant was noted to bruise easily throughout infancy. However, only three significant bleeding episodes occurred: at 1 year of age a lip-frenulum laceration resulted in profuse bleeding that was difficult to control; at 3½ years a nosebleed was controlled only after ~30 min of direct pressure; and 4 wks following that episode uncontrollable epistaxis resulted in hospitalization and hematologic evaluation. Multiple ecchymoses, palatal petechiae, tarry stools, and mild anemia with depleted iron stores also were noted at that time.

Initial coagulation studies showed markedly prolonged prothrombin time and selective deficiency of factors VII, IX, and X (see table 1, which provides a chronologic summary of hematologic investigations). An inborn error of vita-

TABLE 1

CHRONOLOGY OF COAGULATION STUDIES AND TREATMENT IN THE PROBAND

AGE AND DATE	TREATMENT	FACTOR ACTIVITIES (%)								PROTHROMBIN TIME (s; %)	PARTIAL THROMBOPLASTIN TIME (s)	OTHER
		II	V	VII	VIII	IX	X	XI	XII			
1 Day:												
6/13/77	...									180	112	
6/13/77	Post transfusion of fresh plasma and red cells									22.6; 32	43.6	
3 Years, 6 mo:												
12/10/80	...		115	6	190	6	5	140		48.8; <10	73.8	Hemoglobin 7.1; microcytic indices; bleeding time 5.5 min; reticulocyte count 2.8%; iron/total iron-binding capacity 66/360; liver function tests normal
12/11/80	...									39.5; <10		
12/13/80	After two oral daily doses of 5 mg vitamin K									16.4; 39		
12/18/80	Continued vitamin K									16.8; 45		
3 Years, 8 mo:												
2/13/81	Continued vitamin K					14	24			14.9; 41		Hemoglobin 14.1
2/19/81	Oral vitamin K discontinued for 5 days									21.2; 29	41.2	
6 Years, 2 mo:												
8/18/83	Continued vitamin K									14.8; 39	32.4	
7 Years, 6 mo:												
10/25/84	Continued vitamin K	31	128	36	120	58	40	115	110	14.9;	27.9	

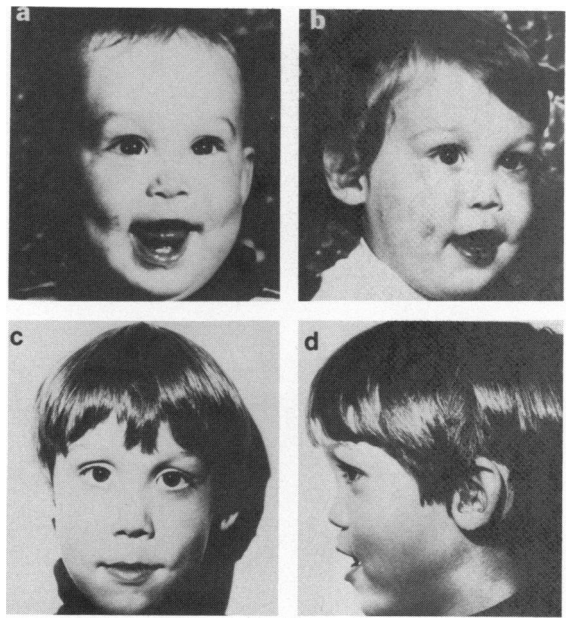

FIG. 1.—Sequential facial photographs at 10 mo (*a*), 23 mo (*b*), and 7½ years (*c*, *d*). Note persistent nasal hypoplasia.

min K utilization was assumed and oral vitamin K therapy (5 mg/day) was initiated. Therapy resulted in improvement of coagulation measurements within 48 h and in resolution of all symptoms except persistent mild bruisability. A single trial discontinuation of vitamin K supplementation showed lengthening of the prothrombin time within 5 days of cessation of the vitamin K supplementation (table 1). Never has there been any clinical or laboratory evidence for malabsorption or liver dysfunction (Blanchard et al. 1981).

In addition to these hematologic problems, reported physical features on newborn examination included a small nose (fig. 1) and "stubby" fingers. Cytogenetic studies were normal. No other clinical problems were recognized until age 6½ years, when a 30–40 dB bilateral conductive hearing loss, thought to be secondary to ossicular-chain fixation, was found. Growth had been normal throughout childhood. The child was referred for genetic evaluation at age 7½ years because of concerns about implications of his dysmorphic features. Positive physical findings were limited to the face and hands. He showed marked flattening of the nasal base and the impression of telecanthus (inner canthal distance = 2.8 cm; outer canthal distance = 7.8 cm). The nose was very small, with anteverted nares and mild septation between the alae nasi and nasal tip (figs. 1*c*, 1*d*), the philtrum being long and mildly prominent and the palate intact but narrow and deep. The upper limbs showed mild hyperextensibility of both elbows and significant brachydactyly secondary to distal digital hypoplasia (fig. 2*c*).

Radiographs taken in the first day of life showed increased irregularity and

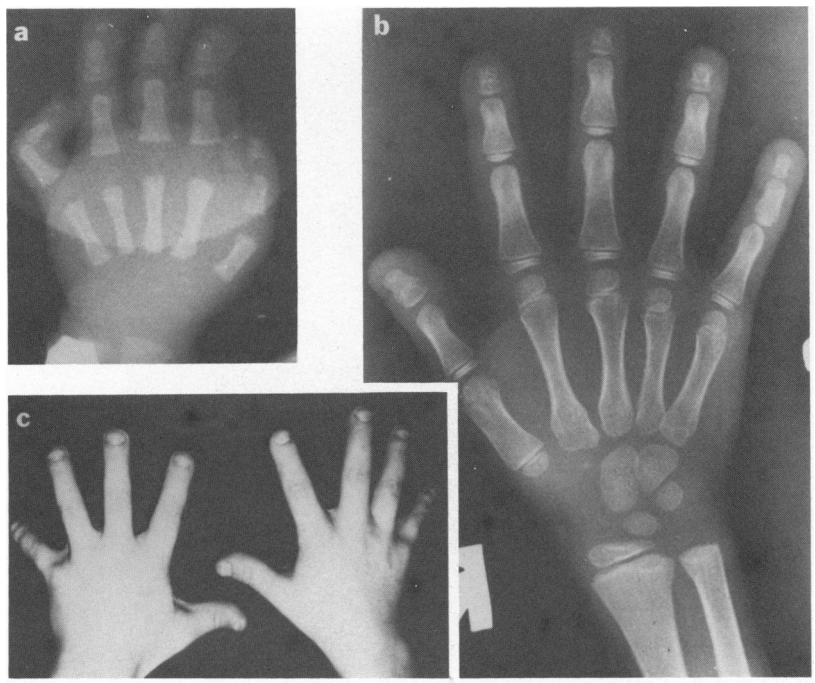

FIG. 2.—*a*, Hand radiograph in the first day of life, showing decreased development of the distal phalanges. *b*, Hand radiograph at 7½ years, showing persistence of distal digital hypoplasia. *c*, Photograph of hands at 7½ years showing generalized brachydactyly, which parallels the radiographic features.

mild stippling in the perilumbar and perisacral region (fig. 3)—a feature not recognized at the time. No other areas of stippling were present. Hand X-rays showed marked hypoplasia of the distal phalanges of all fingers (fig. 2*a*). At 7½ years all radiographic evidence for irregular calcification had disappeared, and calcaneal irregularity was absent (Sheffield et al. 1976); but the distal phalanges remained quite hypoplastic (fig. 2*b*). Incidental findings included mildly delayed bone age and spina bifida occulta at L-5.

SPECIAL LABORATORY STUDIES: METHODS

Prothrombin Determinations

Prothrombin levels were estimated by means of correction of the standard prothrombin time, done by adding dilutions of standard or proband plasma to plasma congenitally deficient in prothrombin. In addition, prothrombin and abnormal (des-γ-carboxy) prothrombin levels in plasma were determined before and after barium-salt ($BaCl_2$) adsorption, with use of a chromogenic substrate as described by Shah et al. (1984). Electroimmunoassay of prothrombin in patient and control plasma was performed by using polyclonal antiprothrombin (Calbiochem) and methods described elsewhere (Laurell 1972); ethylenediaminetetraacetate was present during the assay. Normal and abnormal

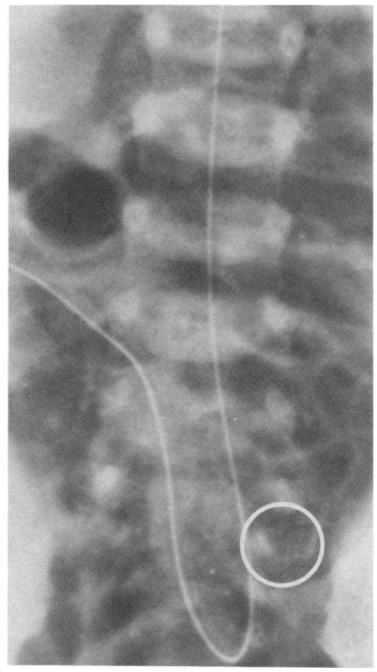

FIG. 3.—Neonatal radiograph showing the lumbar and sacral spine. An umbilical catheter has been placed. Multiple areas of perivertebral stippling are present. One such area is enclosed within the white circle. Stippling is more evident on the original radiographs than on this photographic reproduction.

prothrombin antigen levels were measured by Dr. B. C. Furie (Tufts New England Medical Center, Boston, MA) with use of a competition radioimmunoassay; the mode of preparation of the specific antibody populations used in these assays has been described elsewhere (Blanchard et al. 1981, 1983).

Determination of Plasma Vitamin K and Vitamin K Epoxide Levels

Circulating phylloquinone and phylloquinone epoxide were determined by means of the method of Ueno and Suttie (1983), as modified to use an analytical column of DuPont Zorbax ODS (25 cm × 4.6 mm) eluted at a flow rate of 1.0 ml/min with ethanol/water (96.5/3.5) and monitored with a Waters model 450 variable-wavelength ultraviolet detector.

Carboxylated Glutamic Acid Excretion

Aliquots of urine were prepared protein free by means of precipitation with 20% sulfosalicylic acid, and the supernatants were diluted in 0.2 M sodium citrate, pH 2.2, for measurement of free carboxylated glutamic acid by means of amino acid analysis employing a Beckmann 121-M automatic amino acid analyzer (Gundberg et al. 1984). Total urinary carboxylated glutamic acid was measured after hydrolysis of aliquots of urine in 2 M KOH according to methods described elsewhere (Gundberg et al. 1984).

Osteocalcin Determinations

Serum osteocalcin (the γ-carboxyglutamic acid [Gla]–containing protein first identified in mineralized tissue has been called osteocalcin or bone Gla protein [BGP] by various investigators) was measured at several dilutions from ½ to 1/20 by means of radioimmunoassay, according to methods described in detail elsewhere (Gundberg et al. 1984). Ten-milliliter aliquots of citrated plasma from the proband and from normal controls (a pool from three 8-year-old males) were chromatographed over Sephadex G-100 eluting with 0.05 M NH_4HCO_3 (Price and Nishimoto 1980; Gundberg et al. 1984). Although the optical density profiles of control and proband plasmas were identical, immunoreactive osteocalcin did not elute in a single major fraction in the proband's sample: in the normal profile, 105 ng of the applied 112 ng of osteocalcin eluted in the region of standard osteocalcin, whereas only 39% (141 ng) of the applied osteocalcin (390 ng) in the proband sample eluted in this expected region. The remaining 60% of immunoreactive osteocalcin eluted in several additional fractions between the void volume and the region for standard osteocalcin. Therefore, each of these four immunoreactive G-100 fractions that had coeluted with high-molecular-weight serum proteins was rechromatographed on a second G-100 column equilibrated and eluted with 6 M guanidine–0.01 M Tris, pH 7.6. All fractions then eluted in the position of standard osteocalcin. Following dialysis and lyophilization, each immunoreactive osteocalcin was further purified and identified by means of reverse-phase high-performance liquid chromatography (HPLC) in a C18 column (Waters, Woburn, MA) with 0.1% TFA–10% acetonitrile as the mobile phase eluting over a gradient to 90% acetonitrile.

SPECIAL LABORATORY STUDIES: RESULTS

Prothrombin Determinations

As shown in table 2, a standard prothrombin-activity assay, an assay with chromogenic substrate following physiological activation, and direct radioimmunoassay of normally carboxylated prothrombin all demonstrate that the proband maintained only ~30%–40% of normal levels of functional prothrombin despite chronic oral vitamin K administration. Higher levels were obtained by immunochemical assay and by chromogenic assay of generated thrombin following *E. carinatus* venom activation. This difference suggests the presence of circulating abnormal (des-γ-carboxy) prothrombin. This interpretation is supported by the persistence of a high concentration of *E. carinatus* venom–activatable prothrombin following $BaCl_2$ adsorption and is confirmed by radioimmunoassay demonstration of excess des-γ-carboxy prothrombin.

Measurements of prothrombin species in plasmas of both parents of the proband were within normal ranges.

Plasma Vitamin K and Vitamin K Epoxide Levels

As shown in table 3, consistent with the daily oral administration of vitamin K, the proband's plasma vitamin K level was elevated ~10–20 times above the normal range, whereas his parents exhibited normal values. Vitamin K epoxide

TABLE 2

SUMMARY OF FACTOR II (Prothrombin) DETERMINATIONS IN THE PROBAND

METHOD	ACTIVITY OR SUBSTANCE MEASURED	PROTHROMBIN LEVEL	
		Proband	Normal
Prothrombin time on congenitally deficient substrate plasma	Functional activity of prothrombin	31%	80%–120%
X_A, V, plasma and Ca^{++} activation	Functional activity of prothrombin	32 μg/ml	113–143 μg/ml
X_A, V, plasma and Ca^{++} activation after $BaCl_2$ precipitation	Activity of prothrombin after removal of calcium-binding prothrombin fraction	ND	ND
E. carinatus venom activation	Total prothrombin	87 μg/ml	129–155 μg/ml
E. carinatus venom activation after $BaCl_2$ precipitation	Nonfunctional (des-γ-carboxylated) prothrombin	30 μg/ml	8 μg/ml
Electroimmunoassay	Antigenic levels of prothrombin	54%	80%–120%
Prothrombin radioimmunoassay	Antigenic levels of carboxylated prothrombin	39 μg/ml	98 μg/ml
Abnormal prothrombin radioimmunoassay	Antigenic levels of des-γ-carboxylated prothrombin	43, 48 μg/ml	ND

NOTE.—ND = not detectable.

TABLE 3

PLASMA VITAMIN K AND PLASMA VITAMIN K EPOXIDE CONCENTRATIONS

SUBJECT	CONCENTRATION OF (ng/ml)	
	Vitamin K	Vitamin K 2,3 epoxide
Normal subjects	1–5	ND
Proband	42	50
Father of proband	3.7	ND
Mother of proband	2.7	ND
Control	1.8	ND
Control + vitamin K[a]	86	ND
Control + vitamin K + warfarin[a]	22	61

NOTE.—ND = not detectable.

[a] 10 mg of vitamin K was administered orally to the control 2–4 h prior to obtaining plasma samples. Warfarin was administered 28 h (10 mg) and 4 h (5 mg) prior to obtaining the plasma sample.

is undetectable in normal subjects but was elevated in the proband's plasma as well as in that of a healthy subject to whom vitamin K and warfarin were administered orally as a positive control. Chromatograms of the relevant plasma samples are shown in figure 4. A second sample of the proband's plasma obtained 8 mo after the first showed 37 ng of vitamin K/ml and 3.9 ng of vitamin K epoxide/ml. The first sample was obtained 3 h after his daily oral vitamin K, whereas the latter was obtained ~8.5 h after vitamin K administration. The plasma vitamin K:plasma vitamin K epoxide ratio varies greatly with time following vitamin administration to a warfarin-treated patient (Bechtold et al. 1983); it has not been possible to follow these changes in the proband in more detail.

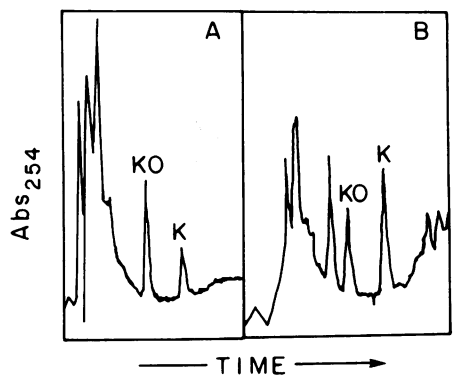

FIG. 4.—HPLC separation of plasma vitamin K. Chromatogram of plasma from an adult control subject given vitamin K and warfarin (*A*) and proband plasma (*B*). K = vitamin K; and KO = vitamin K 2,3 epoxide.

TABLE 4

URINARY CARBOXYLATED GLUTAMIC ACID (Gla) EXCRETION AND PLASMA OSTEOCALCIN DETERMINATIONS

Subject	Urinary Gla (μg/g creatinine)	Plasma Osteocalcin (ng/ml)
Age-matched control run simultaneously with proband samples	62.8	15.0
	56.0	18.7
Proband	13.2	47.5
	17.5	39.1
Mother of proband	...	6.7
Father of proband	...	4.9
Normal adults		6–10

Carboxylated Glutamic Acid Excretion

The proband consistently excreted approximately one-third as much carboxylated glutamic acid as did age-matched controls (table 4). This excretion of free carboxylated glutamic acid (Shah et al. 1978; Gundberg et al. 1984) reflects turnover of proteins from all tissues that contain this modified amino acid, including ~80% of that derived from liver-synthesized vitamin K–dependent proteins and ~10% of that obtained in bone-tissue turnover (Gundberg et al. 1983*b*). Decreased carboxylated glutamic acid excretion despite oral administration of vitamin K is expected if there is a generalized defect of posttranslational modification of glutamyl residues that is not completely correctable by the vitamin K.

Osteocalcin Determinations

Plasma osteocalcin levels were elevated approximately threefold compared with levels in normal 8-year-old males (table 4). Although it was not possible to directly measure carboxylated glutamic acid levels in the proband's circulating osteocalcin, chromatographic behavior suggested the presence of two populations of osteocalcin molecules in the proband's samples but not in age-matched controls (fig. 5). The first purification step on G-100 resulted in a single osteocalcin fraction from control plasma that was well separated from albumin and other serum proteins, whereas similar methods resulted in numerous immunoreactive fractions in samples of the proband. Indeed, only 40% of the applied osteocalcin from the proband's plasma eluted in the standard position. Under dissociating conditions all the immunoreactive osteocalcin fractions eluted in the expected position. The multiple osteocalcin fractions likely resulted from an aggregation of a population of osteocalcin molecules with other plasma proteins. HPLC reverse-phase chromatography of each of the immunoreactive osteocalcin fractions showed two types of osteocalcin molecules (fig. 5). All control-plasma osteocalcin eluted as a single peak at 14 min. The proband's osteocalcin consistently eluted at two peaks—14 and 18 min. Isoelectric focusing (data not shown) also indicated that a second species of

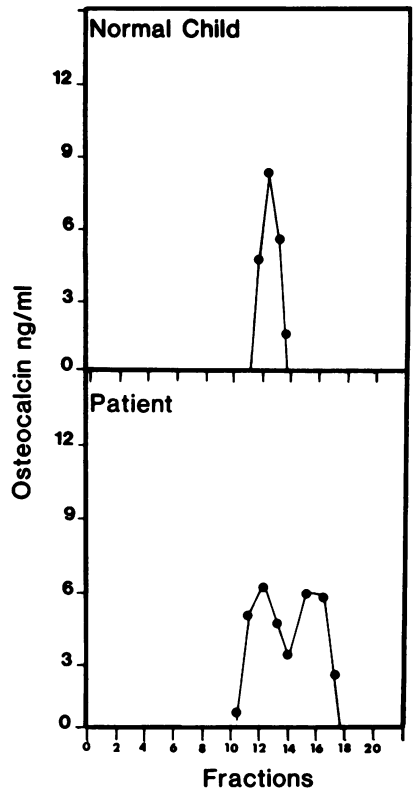

FIG. 5.—Reverse-phase HPLC chromatography of G-100-purified osteocalcin. Each fraction was assayed for osteocalcin by means of radioimmunoassay. *Upper panel,* age-matched control; *lower panel,* proband.

circulating osteocalcin was present in the proband, a finding consistent with the presence of osteocalcin that is des-γ-carboxylated (Delmas et al. 1984).

DISCUSSION

Biochemical Basis for the Vitamin K–dependent Coagulopathy

Vitamin K is a necessary cofactor for γ-carboxylation of glutamyl residues of a variety of precursor proteins, including plasma coagulation factors II, VII, IX, and X; protein C; and protein S (Suttie 1980). This vitamin K–dependent posttranslational modification creates calcium-dependent phospholipid-binding sites, which are necessary for enzymatic activity of these proteins (Suttie 1980). Synthesis of the biologically active form of these proteins is inhibited by the oral anticoagulant warfarin and other coumarin derivatives (Davie and Fujikawa 1975; Suttie 1983).

Coagulation-factor measurements in the patient indicate a selective deficiency of vitamin K–dependent factors (table 1). Furthermore, nonphysiologic assays demonstrate the presence of significant amounts of des-γ-carboxy prothrombin (table 2). Failure of the amount of abnormal (*E. carinatus* activation

after $BaCl_2$ adsorption, as described above) and normal prothrombin to equal total prothrombin (*E. carinatus* activation of untreated plasma) suggests that much of the prothrombin that binds to $BaCl_2$ is also undercarboxylated to some extent. Electroimmunoassay with a polyclonal antibody detects both normal and undercarboxylated prothrombin; these total antigenic levels of prothrombin (table 2) are decreased less than levels estimated by means of functional assays. Direct assay of antigenic levels of carboxylated and des-γ-carboxylated prothrombins (table 2) confirms the presence of both of these species in the proband. The presence of both normal and undercarboxylated prothrombin in plasma is completely analogous to that seen in individuals therapeutically treated with coumarin derivatives (Ganrot and Nilehn 1968). Since the patient exhibited no sign of liver disease (Blanchard et al. 1981) or any evidence of malabsorption, these data are consistent with an enzymatic abnormality of vitamin K utilization. This abnormality could be partially overcome by administration of oral vitamin K.

γ-Carboxylated glutamic acid is not metabolized (Shah et al. 1978) and is excreted as the free amino acid (Levy and Lian 1979), and its levels of excretion are closely correlated to warfarin anticoagulation status (Levy and Lian 1979). Decreased excretion of γ-carboxy glutamic acid (table 4) further supports the hypothesis that these functional abnormalities result from undercarboxylation of vitamin K–dependent proteins (as was previously described, by Gallop et al. [1980], in a similarly affected patient).

Previously, few individuals with combined coagulation deficiencies similar to those described here have been reported. McMillan and Roberts (1966) and Chung et al. (1979) evaluated a female with more severe functional abnormalities of all of the vitamin K–dependent coagulation factors. Immunologic and nonphysiologic measures indicated the presence of abnormal, dysfunctional proteins. This individual also showed partial correction following administration of vitamin K and recurrence of clinical symptoms following discontinuation of therapy. An 8-wk-old female with similar severe deficiencies who was partially responsive to vitamin K administration was reported by Fischer and Zweymüller (1966), and Johnson et al. (1980) described a male with modest combined functional deficiencies and normal immunologic and calcium-independent levels of coagulation factors who showed no biochemical response to oral vitamin K administration. Siblings with modest functional abnormalities of vitamin K–dependent factors and in whom complete correction was obtained using oral vitamin K were reported by Goldsmith et al. (1982). Finally, Newcomb et al. (1956) described an adult who probably also had combined vitamin K–dependent coagulopathy. The individual described in the present paper is, therefore, the seventh reported case of vitamin K–dependent multiple-factor deficiency—or familial multiple-factor deficiency type III (Soff and Levin 1981). Recurrence in siblings (Goldsmith et al. 1982) and normal evaluations in parents of affected individuals suggest that these deficiencies may result from dysfunction of an autosomal recessive gene or genes. In none of the previously reported patients have phenotypic abnormalities similar to those described here been noted.

Site of Enzymatic Deficiency

Two distinct enzymatic deficiencies would most likely cause the generalized undercarboxylation of vitamin K–dependent proteins that was seen both in the individual described in the present paper and in the six individuals previously reported. The vitamin K–dependent carboxylase could possess an abnormal binding site, so that higher tissue concentrations of vitamin K would be required for maximum activity (Suttie 1985). Alternatively, the defect could result from an inability to recycle the product of the carboxylase, vitamin K 2,3-epoxide, to the active coenzyme form, the hydroquinone. This reduction is dependent on the warfarin-inhibitable vitamin K epoxide reductase and vitamin K reductase of liver microsomes (Fasco et al. 1982). When maintained on large doses of oral vitamin K, this patient showed the expected excesses of circulating vitamin K (table 3). The presence of increased amounts of vitamin K epoxide (fig. 4) is consistent with decreased activity of vitamin K epoxide reductase, would be unexpected if the protein carboxylase were dysfunctional, and is analogous to that seen following administration of warfarin (Bechtold et al. 1983). Decreased excretion of carboxylated glutamic acid (table 4) is also analogous to that seen after coumarin-derivative administration (Levy and Lian 1979).

Osteocalcin Investigations

Osteocalcin is synthesized in osteoblasts and accumulates in bone in proportion to hydroxyapatite content (Nishimoto and Price 1980; Lian et al. 1982). A fraction of bone osteocalcin circulates (Price and Nishimoto 1980; Gundberg et al. 1983*a;* Melick et al. 1985). The elevation of circulating-osteocalcin levels in the proband (table 4) is similar to that demonstrated following warfarin administration (Price and Williamson 1981) and could reflect either decreased carboxylation—and resultant decreased affinity of the osteocalcin for binding to hydroxyapatite—or secondary increased synthesis (Gundberg et al. 1983*a,* 1983*b;* Riggs et al. 1984). Although it was not possible to directly measure carboxylated glutamic acid in the proband's circulating osteocalcin, the presence of two populations of osteocalcin (fig. 5) with features consistent with des-γ-carboxylation suggests that this individual has a generalized, tissue-nonspecific defect in vitamin K–dependent carboxylation.

Phenotypic Features and Relationship to the Warfarin Embryopathy

Features in the boy described in the present paper include irregular ossification (i.e., stippling, present on newborn radiographs but disappearing with maturation), nasal hypoplasia, distal digital hypoplasia, and mild conductive hearing loss. The first two characteristics are uniformly present in individuals with the warfarin embryopathy whereas the others are more variable but consistent with this disorder (Hall et al. 1980). Comparison of features in the proband and those in the warfarin embryopathy is shown in table 5. Although it is conceivable that this child has both a mild form of chondrodysplasia punctata (Sheffield et al. 1976) and vitamin K–dependent coagulopathy by chance, a

TABLE 5

COMPARISON OF CLINICAL FEATURES OF THE WARFARIN EMBRYOPATHY AND THE DESCRIBED PROBAND

Feature	Warfarin Embryopathy	Proband
Warfarin exposure	+	−
Nasal hypoplasia	+ + +	+ +
Stippled epiphyses	+ + +	+
Brachydactyly	+	+
Distal digital hypoplasia	+	+
Conductive hearing loss	+/−	+
Coagulopathy	−	+ +

NOTE.—Data are subjective assessments; plus sign(s) denotes presence and severity of feature, and minus sign denotes absence of feature.

more parsimonious and reasonable explanation is that all these abnormalities are explicable on the basis of the demonstrated enzymatic defect.

Vitamin K, Bone Development, and the Postulated Biochemical Basis for the Warfarin Embryopathy

Since its initial description, the warfarin embryopathy has been assumed to result from some primary pharmacologic action of coumarin derivatives (Becker et al. 1975; Pettifor and Benson 1975). Initially, the phenotypic features were postulated to result from hemorrhage secondary to inhibition of fetal coagulation factors (Becker et al. 1975). However, with demonstration of a critical period of exposure at 6–9 wk gestation (Hall et al. 1980) and since vitamin K–dependent coagulation proteins do not appear until 12–14 wk gestation (Bleyer et al. 1971), the warfarin embryopathy either must result from inhibition of some other vitamin K–dependent protein(s) or is secondary to some action completely unrelated to the pharmacologic action of warfarin. Recognition of other vitamin K–dependent, γ-carboxy glutamyl residue–containing, warfarin-inhibitable proteins in bone (Hauschka et al. 1978) has provided information upon which alternative models have been built.

Osteocalcin is a protein with these properties that is found in both embryonic and adult bone (Hauschka et al. 1976; Price et al. 1976). By extrapolation from animal studies (Hauschka and Reid 1978), osteocalcin would be expected to appear by 6–8 wk gestation in man—timing that would be consistent with the demonstrated critical period for production of the warfarin embryopathy (Hall et al. 1980). As for other vitamin K–dependent proteins, posttranslational modification allows calcium binding; therefore, it is conceivable that osteocalcin is critical in control of early calcium deposition in developing bone (Hauschka et al. 1978). Since the warfarin embryopathy is principally characterized by abnormalities of calcium deposition (stippled epiphyses) and bone development (nasal hypoplasia and distal digital hypoplasia), these teratogenic effects may result from warfarin's pharmacologic inhibition of posttranslational carboxylation of osteocalcin. If this mechanism is true, then *if* there were an

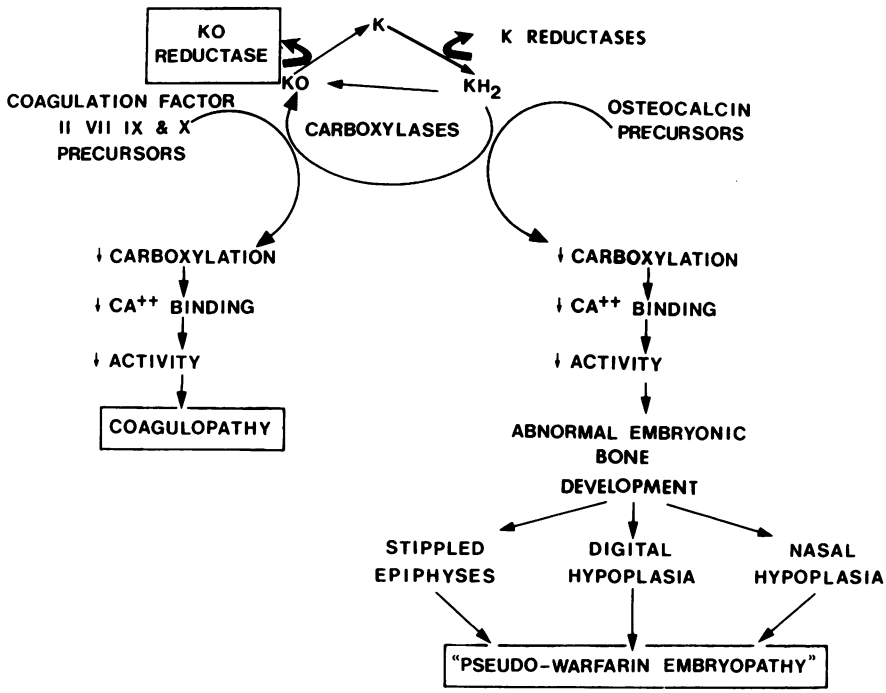

FIG. 6.—Diagram of model used to explain the combination of features present in proband. K = vitamin K, KO = vitamin K 2,3 epoxide.

inborn error of metabolism that resulted in inhibition of all vitamin K–dependent function, it should result in a "genocopy" of the warfarin embryopathy (as well as in abnormalities of the vitamin K–dependent coagulation factors; fig. 6). The combined coagulopathy of the patient described in the present paper is almost certainly secondary to undercarboxylation of vitamin K–dependent factors. It appears to be secondary to an inborn deficiency of vitamin K epoxide reductase. This inborn error of metabolism also has produced a virtual copy of the warfarin embryopathy. Therefore, by inference, the warfarin embryopathy is almost certainly secondary to warfarin's primary pharmacologic effect—i.e., inhibition of vitamin K epoxide reductase. Furthermore, the demonstration of undercarboxylation of osteocalcin *suggests* that the phenotypic effects in this boy (as well as those affected by the warfarin embryopathy) may result from intrauterine abnormalities of carboxylation of osteocalcin. Of course, we cannot rule out involvement of other vitamin K–dependent bone proteins, such as matrix Gla protein (Price et al. 1983). It is not surprising, particularly given that fetal vitamin K levels may be substantially less than maternal levels (Shearer et al. 1982; Greer et al., in press), that unsupplemented maternal levels of vitamin K would not be sufficient to overcome the embryonic enzymopathy. The apparent lack of similar embryopathic-like clinical features in previously reported cases of vitamin K–dependent co-

agulopathy could simply reflect lack of recognition of the relatively subtle clinical and radiologic characteristics. Alternatively, in some individuals the coagulopathy might result from a tissue-specific enzyme dysfunction that does not affect carboxylation of bone proteins.

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