FACTOR IX COMPLEX

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FACTOR IX CONCENTRATES

In 1950, Aggeler et al. [2] and Biggs et al. [11] independently described a bleeding diathesis, indistinguishable from classic hemophilia on clinical and genetic grounds, but clearly a separate biochemical entity. These basic reports characterized some properties of the defective coagulant and convincingly demonstrated that, like prothrombin, this coagulation factor, now called Factor IX, was adsorbable on $Al(OH)_3$ or $BaSO_4$. Thus, the initial reports indicated a simple method for the concentration and purification of the Factor IX.

Further investigation revealed that Factor IX, like prothrombin, factor VII and factor X, was sensitive to the action of vitamin K. Other similarities included their adsorbability on $BaSO_4$ or $Al(OH)_3$ and the decreased blood levels seen in a variety of acquired diseases and following anticoagulant therapy using vitamin K antagonists.

The development of factor IX concentrates (Factor IX Complex)* was stimulated by the thought that one might be able to more satisfactorily handle a wide variety of these clinical states characterized by subnormal levels of the vitamin K dependent factors. Patients with hemophilia B (3,000 in the U.S.A. [74]) are but a small proportion of patients with defective hemostasis associated with subnormal levels of the vitamin K dependent coagulant.

Current clinical data support the efficacy of the Factor IX concentrates for congenital deficiencies. Data supporting its efficacy in the acquired states characterized by low levels of the "Prothrombin Complex" are less convincing. As will be discussed below, there are substantial concerns about the side effects and adverse reactions reported subsequent to Factor IX complex infusion.

BIOCHEMISTRY AND PHYSIOLOGY OF THE VITAMIN K DEPENDENT FACTORS

The active components in Factor IX concentrates include prothrombin, Factor VII and Factor X, as well as Factor IX. These four coagulants are

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*These preparations have as many names as the hero in a Russian novel. The official generic name in the U.S. is Factor IX Complex (Human). Other widely used names include prothrombin complex concentrates, prothrombin concentrates, Factor IX concentrates, PPSB.

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characterized by being proenzymes of active serine proteases, sensitive to the action of the coumarin drugs and synthesized in the liver. The active enzymes are characterized by being inhibited by the plasma protein anti-thrombin III (AT-III) in a reaction dramatically speeded up by the presence of heparin (AT-III is not the only plasma inhibitor of these enzymes).

The effect of vitamin K has recently been elucidated in an elegant series of studies. Stenflo and Ganrot [73] demonstrated the presence of "normal" protein structure in an inactive prothrombin isolated from plasma from a dicumarolized cow; Suttie's group [55] demonstrated that one could generate a completely normal thrombin from such a prothrombin and followed this with studies showing the region affected by these drugs was the NH_2-terminal portion of prothrombin. Studies of Stenflo et al. [72] and Magnusson and co-workers [46] then identified a hitherto undescribed amino acid, γ-carboxyglu-tamic acid. Further studies [56] showed that the primary protein structures of Factors II, VII, IX and X are completely synthesized and subsequently, in a vitamin K dependent reaction, the glutamic acid residues in the NH_2-terminal region are further carboxylated.

The γ-carboxyglutamate residues give certain unique properties to these proteins. The γ-carboxyglutamate regions are strong binders of divalent ca-tions. This function is important since the activation steps, or the steps at which they activate other proenzymes, are strongly calcium-dependent. Secondly, the presence of the γ-carboxyglutamate residues are responsible for their ability to be adsorbed on inorganic precipitates such as calcium phosphate, $Al(OH)_3$ and $BaSO_4$. Finally, these groups are extremely acidic, so that on ion exchange chromatography, there is little separation of prothrom-bin, Factor VII, IX and X. Thus, the salient physical and chemical features of these procoagulants are defined by the presence of the γ-carboxygluta-mate residues.

All of the vitamin K dependent zymogens are activated by limited proteoly-sis. With the exception of thrombin, the active enzymes have to interact with macromolecular cofactors for significant expression of their coagulant activity. Figure 1 shows the cleavages made to generate the active protease. The activation of prothrombin and Factor IX requires two cleavages, the ulti-mate result of which is a 2-chain protein which has lost a peptide segment. Factor X_a, at least as currently isolated, is a 2-chain molecule which is acti-vated by a single clip, resulting in loss of a small peptide, and Factor VII is clipped to a 2-chain active protein without the loss of a peptide.

The various interactions of these coagulants have been well worked out in purified systems; however, the significant interactions in plasma or whole blood may be somewhat different. Factor X is activated by either IX_a or VII_a. However, neither of these enzymes seems to be biologically effective in the absence of, respectively, Factor VIII (antihemophilic factor) or tissue factor. There is evidence supporting the concept that in the presence of tissue factor, Factor VII is activated without proteolytic cleavage. The Factor VII cleavage by X_a or XII_a results in a substantial increase in activity. The X_a in turn, in the presence of Factor V, rapidly cleaves off the NH_2-terminal region of prothrombin and splits the remaining protein within a disulfide bond to form the clot-forming enzyme, thrombin.
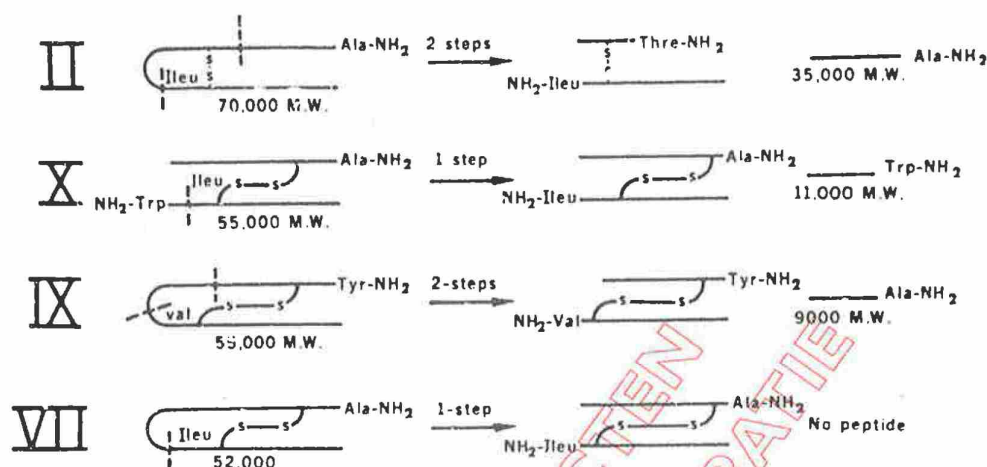
FIG. 1. Bonds cleaved for activating vitamin K dependent coagulants.

Once formed, there must be a mechanism for limitation of these enzyme actions. Much attention has been given to the role of the plasma inhibitors, with particular interest in the role of antithrombin III and α_2-macroglobulin. Thrombin in plasma is rapidly inactivated, and, from available evidence, α_2-macroglobulin and AT-III may have important roles [65]. Antithrombin III seems uniquely involved in the inactivation of Factor Xa [78]. Activated Factor IX and VII seem to be much more stable in the presence of the plasma inhibitors, although, in the presence of heparin, there is rapid inactivation of Factor IXa [62]. Thus, it would seem that other mechanisms of controlling the activity of Factors IXa and VIIa must be available. The rapid feedback destruction of Factor VIII by thrombin is a likely mechanism for the control of the intrinsic system [5]. No such mechanism is known to be operative in the extrinsic system.

THERAPEUTIC PREPARATIONS OF VITAMIN K DEPENDENT CLOTTING FACTORS

With the advent of blood banking, all hemophilias were treated with whole blood or plasma, with partial success. Following the identification of the different forms of hemophilia and knowledge of the characteristics of the proteins involved, it was clear that hemophilia A and hemophilia B would be treatable by different plasma fractions.

Experimental Factor IX concentrates were originally produced by adsorption on $BaSO_4$ [50]. These preparations, which were associated with many reactions, were never widely utilized.

The first plasma concentrate used extensively for the treatment of hemophilia B was prepared by Didisheim et al. [18] and baptized PPSB (*Pro*thrombin, *P*roconvertin, *S*tuart Factor, Antihemophilic *B* Factor). To produce this fraction, it was obligatory that plasma be drawn using either ion exchange resins or ethylene diamine tetraacetic acid (EDTA) as the calcium chelating anticoagulants. This plasma was then adsorbed with tricalcium

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phosphate, the calcium phosphate precipitate eluted with citrate, and dried after further fractionation with ethanol. While this material was a very satis-factory therapeutic product, the necessity for using plasma with special anti-coagulants was a major drawback. Two other strategies are currently used for the production of Factor IX concentrates. In the first method, citrate plasma is fractionated by the cold ethanol or ether procedures and the fractions con-taining Factor IX are adsorbed with $CaPO_4$ [7,27]. In the second method, plasma or the supernatant from Cohn Fraction I is treated with an ion ex-changer such as DEAE-cellulose [15,19,34,36,54,76]. With appropriate condi-tions, the vitamin K dependent factors are adsorbed and the nonadsorbed proteins are used for the standard fractionation products. The adsorbed vita-min K dependent factors are eluted from the ion exchangers with appropri-ate buffers, filtered and dried. It has also been proposed to use $Al(OH)_3$ as an adsorbant for small scale Factor IX preparations [6,15].

The most common production method utilized in the world today is purifi-cation by DEAE adsorption of either whole plasma or the supernatant after removal of the Antihemophilic Factor by cryoprecipitation [14,19,34,35,54, 76]. The adsorbed vitamin K dependent factors are eluted with phosphate and/or citrate or ammonium bicarbonate buffers. The use of the latter vola-tile salt leaves a salt-free protein after lyophilization, which can be reconsti-tuted in any desired media. When citrate or phosphate are used for elution, the eluate can be lyophilized directly if the salt concentration is low enough, or, if not, the eluate must be further treated by fractionation or dialysis to remove the excess salts.

The final DEAE products vary somewhat in degree of purification, salt content and extraneous proteins. All DEAE preparations have relatively low levels of Factor VII and about equal levels of Factors II and X, as compared with Factor IX (Table 1). The presence of the other vitamin K dependent fac-tors must be presumed (Protein S) and their effect on the recipients is totally unknown.

The production of Factor IX concentrates utilizing $CaPO_4$ adsorption is also still in use. The degree of purification may be a little higher than with DEAE preparations, a property possibly derived from the ethanol fractiona-tion that is part of those procedures either before or after the adsorption step. In contrast to the DEAE preparations, the level of Factor VII seems higher than that of Factor IX. The levels of Factors II and X, while variable, tend to be lower than that of Factor IX.

TABLE 1. Relative Concentrations of Factors II, VII, and X in Factor IX Concentrates

Reference	Adsorbent	$\dfrac{II}{IX}$	$\dfrac{VII}{IX}$	$\dfrac{X}{IX}$
[19]	DEAE	0.7	0.07	0.85
[18]	$CaPO_4$	1.7	2.1	1.5
[7]	$CaPO_4$ (Either Fraction)	1.5	1.2	.35
	"Alcohol"	1.1	2.6	1.3
[6]	$Al(OH)_3$	0.77	2.2	0.6

Factor IX concentrates made by Al(OH)₃ adsorption have been described from England and the Netherlands [6,15]. It is not clear whether these are in clinical use and, if so, to what extent. One unique property of these preparations is their rather high content of Antithrombin-III. The vitamin K dependent clotting factors are present in proportion to their plasma content.

The Factor IX concentrates have in common their content of Factor IX. The content of the other vitamin K dependent factors varies, and the clinician using these materials for indications other than Factor IX deficiency should be aware of the approximate content of these factors in any preparation being used. These concentrates also vary in whether they contain heparin, and if they do, the level can vary from 1-10 u/ml.

RECOVERY AND HALF-LIFE OF FACTOR IX

There are three pieces of information necessary for successful replacement therapy. These are *in vivo* recovery, the turnover rate and the minimum level to prevent hemorrhage.

After infusion of most plasma proteins there is a rapid intravascular equilibration and a blood sample taken at 5 minutes after infusion will show a rise in concentration of the given protein to a level accounting for essentially all of the infused protein. Infused Factor IX deviates from this behavior in that only 30-50% of the activity can usually be detected in the hemophilia B recipient's plasma immediately after infusion [3,6,7,9,19,79]. Aggeler et al. [3] reported that one could find quantitative recovery of the Factor IX after plasma infusion, while Biggs et al. [9] found that 30-50% recovery was found for plasma Factor IX and for Factor IX concentrates. Further, their studies illustrated that some characteristic of the patient determined the *in vivo* recovery and not the type of product infused. Zauber and Levin [79] have recently published new data confirming the findings of Biggs. That this is not simply a problem of assay technic is ruled out since low *in vivo* recovery is seen with a variety of different assays. Accepting the data as valid, the conclusion is that there is a rapidly saturable "compartment," separate from the fluid phase of plasma. While most reported recovery experiments have been done in hemophilia B patients, it may be that this "compartment" would be saturated in some cases of acquired disease or in normals. The reports available on the *in vivo* recovery of Factor IX in such acquired disease do not, however, show a consistent pattern of increased *in vivo* recovery.

Thus, there is a large body of evidence supporting low *in vivo* recovery of Factor IX in hemophilia B patients and perhaps in patients on long-term anticoagulant therapy and with liver disease. The concept of an unsaturated "compartment" is a tenable model to explain the poor *in vivo* recovery. It would, however, seem wise to be aware of the possibility that in certain states this pool would be saturated, with consequently greater recovery in the plasma compartment.

Despite the structural similarity of the Factor II, VII and X molecules to factor IX, there is no such inexplicable low *in vivo* recovery of these other coagulants whether infused as crude concentrates into deficient patients or as purified isotopically labeled components [10,45,59,61,66,70].

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HALF-LIFE STUDIES OF FACTOR IX AND OTHER COMPONENTS

All studies of the turnover of Factor IX in recipients have been done by following the decay of biologic activity in the patients with Factor IX deficiency. The analysis of these decay curves has usually assumed that there is a two-component logarithmic disappearance of Factor IX, and the time constants for each of these have been estimated by appropriate curve-fitting technics. All the reports suffer from the same two practical problems: sufficient number of samples and the inherent imprecision of the assay. Other models can be suggested for the analysis of the data, but until these problems are solved, there seems little need to assume a more complex model. Another, even more pragmatic, method of analysis is to calculate the time of disappearance of 50% of the infused dose. While lacking theoretical sophistication, this will give the clinically relevant information [79].

The analysis of the two-component logarithmic decay shows good agreement among the published data, with a half-life for the second phase of 24 hours. The faster first-phase decay is more difficult to measure precisely but is of the order of 5 hours. The assumption is that the first phase represents equilibration of the plasma and extravascular spaces. No direct experiment validates this assumption, although Factor X has been detected in the lymph [61].

It has been proposed that a major portion of the decay of coagulants is due to consumption during an ongoing coagulation process and that half-lives during bleeding episodes might be shorter than during a nonbleeding time interval. Current evidence shows that there is in fact no difference in turnover in these two situations [79].

Comparison of the three types of products used for replacement therapy in hemophilia B, i.e., plasma, $CaPO_4$ and DEAE concentrates, shows similar decay rates for the infused Factor IX [3,4,9,27,33,36,45,51,62,79]. The presence of small amounts of heparin does not affect the Factor IX catabolism.

Even for the treatment of Christmas disease it is important to be cognizant of the intravascular half-life of the other factors found in the Factor IX concentrates, since they will accumulate to supernormal levels during prolonged therapy.

Factor VII has the shortest half-life (5 hours) of all the vitamin K dependent factors. This half-life has been found not only by the decay of Factor VII after infusion [48] but also by decay of Factor VII after administration of dicumarol [45]. Since the half-life is so short, Factor VII will build up significantly less than the other components infused with Factor IX. This short half-life is also extremely important for the replacement therapy in Factor VII deficiency. Marder and Shulman [48] found that without replacement therapy every day, a severe Factor VII deficient patient would have abnormal bleeding, even at bed rest.

The 30-50 hour reported half-life of Factor X [10,59,61] is somewhat longer than that of Factor IX. Consequently, during the treatment of Factor IX deficiency, supranormal levels of Factor X will be found. Prothrombin has an even more prolonged half-life. While native prothrombin has a half-life of almost 4 days [10,45,51,66,70], at least some of the prothrombin present in

Factor IX concentrates is in a degraded form which might have a significantly shorter half-life. Theoretical considerations would indicate that prolonged infusion to a factor IX deficient patient would result in a level of 5-6 times normal. Biggs and Denson [10] have reported levels as high as 5 times normal in such patients.

While the above information should in fact be sufficient to predict the effect of a given Factor IX infusion, the individual patient may respond in different ways, and it would seem advisable, where practicable, to follow blood levels at least at the start of treatment.

HEMOSTATIC LEVELS OF THE VITAMIN K DEPENDENT FACTORS

As with classic hemophilia, the clinical severity of Factor IX deficiency varies considerably. In general, a level of less than 5% of normal will give rise to spontaneous hemorrhage, while levels greater than 20% will lead to satisfactory hemostasis, even in the face of trauma or surgery. The hemostatic status of the congenital hypoprothrombinemias and dysprothrombinemias shows a similar pattern, with spontaneous hemorrhage occurring with prothrombin levels less than 5% and minor hemostatic problems appearing up to 25% of normal levels [67]. These levels are probably sufficient in Factor VII and Factor X deficiency, although the published data are insufficient for a critical evaluation. While it has been reported that 40% levels are necessary for hemostasis in a kindred with an abnormal form of Factor X, there have been no hemorrhagic deaths in patients with levels of 10%. The only "disease"-related death was due to transfusion-associated hepatitis [28].

It is of interest to note that multiple deficiencies do not seem to have a cumulative effect. McMillan and Roberts [49] have reported a patient who, despite levels of ~15% for all vitamin K dependent factors, had, at most, a very mild hemostatic defect.

CLINICAL USE OF FACTOR IX CONCENTRATES

A wide variety of clinical states associated with decreases in the vitamin K dependent proenzymes has been considered as possibly benefiting by replacement therapy with Factor IX concentrates. These indications for treating with Factor IX complex are comprised of both congenital deficiencies and acquired deficiencies.

The congenital deficiencies are usually deficiencies or dysproteinemias of a single coagulant. In contrast to the congenital deficiencies, the acquired deficiencies are almost always of more than one element of the prothrombin complex. The exceptions here are certain types of amyloid which selectively and essentially quantitatively remove Factor X from plasma [52]. Table 2 lists a variety of the conditions in which Factor IX concentrates have been used for replacement therapy.

Replacement Therapy in Congenital Deficiency States

Of all the conditions in which these concentrates have been used, hemo-

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TABLE 2. Proposed Uses for Factor IX Complex

A. Congenital Deficiencies	B. Acquired Deficiency States
Prothrombin	Liver Disease
Factor VII	Neonatal Hemorrhagic States
Factor IX	Postoperative (Cardiac Bypass)
Factor X	Reversal of Coumarin Drug Effect
	Bypass of Factor VIII Inhibitors

philia B (Christmas disease) is the one for which the most clinical data exist clearly demonstrating efficacy. Despite all the unknowns concerning the *in vivo* fate of Factor IX, it is well established that treatment with 25-50 u/Kg, 1-2x per day yields a normal hemostatic state in even the most severe hemophilia B patients.

Since deficiency states of the other vitamin K dependent factors are extremely rare, the data concerning replacement therapy is very limited. Factor X deficiency is the second most common pure deficiency state. Because of the long half-life of Factor X, plasma is a reasonably effective and safe form of replacement therapy, although Factor IX concentrates have been used [28,59]. Since the concentrates are not standardized for Factor X activity, it is important that laboratory control be available. In emergency situations where such information is unavailable, one can (a) call the manufacturers for any information they have, or (b) assume that Factor X is present at the same level as Factor IX. In fact, the primary decision to be made with these patients must be the relative advantage of the concentrate vs. plasma for Factor X replacement.

Factor VII deficiency is even rarer than Factor X deficiency and thus treatment data are again fragmentary. The review of Marder and Shulman [48] depicts the differing degrees of severity seen with these patients. Replacement therapy in this patient group can be useful, but because of the extremely short half-life of Factor VII (5 hours), repetitive infusions must be given. These in turn lead to buildup of the other coagulant factors infused to supernormal levels. One Factor VII deficiency patient had multiple episodes of thromboembolism after intensive concentrate therapy [26]. It would thus seem best to treat with discretion in these cases and to utilize the concentrate with highest ratio of Factor VII to Factor IX. It has been proposed to produce a special Factor VII rich product for such patients, but at this time no clinical information is available [20].

Serious congenital defects of prothrombin, like Factor VII and X, are rare. While many such patients seem to have a very mild bleeding disorder, others need frequent treatment [67]. Since the half-life of prothrombin is long, as compared with the other vitamin K dependent coagulants, one could anticipate relatively little buildup of other coagulants. No thrombotic disorders have been reported in such patients after treatment with Factor IX concentrates. The decision to use concentrates, as opposed to plasma, should be carefully weighed in terms of the risk of hepatitis (see below).

The one patient reported with defective vitamin K utilization has not needed any plasma coagulant replacement therapy. Clinically, the patient has responded well to large doses of vitamin K [16,49] and, despite low laboratory levels of coagulant activity (<18%), has adequate hemostasis.

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Acquired Deficiencies of Vitamin K Dependent Coagulants

As soon as quantitative measurements of coagulation components began to be made it was apparent that in a wide variety of pathologic states there were large transient shifts in all of the coagulation parameters being measured. With the advent of concentrates for replacement therapy there was an immediate intuitive reaction to use these preparations in lieu of plasma for replacement therapy to raise the coagulant levels toward normal [69,71]. However, despite restoration of normal levels of Factors II, VII, IX and X, there can be prolongation of the thromboplastin time due to abnormalities in other coagulation factors (e.g. Factor V) [63]. For these concentrates to have an effect, even in theory, it must be clear that the hemorrhagic state is one due solely to low levels of a vitamin K dependent proenzyme(s), and that there is neither substantial decrease of other coagulants nor the presence of inhibitors such as the fibrin/fibrinogen degradation products (FDP).

Liver Disease

The bleeding disorders seen associated with liver disease stem from a variety of defects. Whether Factor IX concentrates are useful in any of these situations must be considered unproven. The early observations of a long "prothrombin time" were interpreted to be caused by decreased hepatic production of prothrombin. For this reason, Factor IX concentrates were proposed to be potentially useful as a "hemostyptic" agent in acute fulminant hepatitis, pre-, intra- and post-surgery, in patients with cirrhosis and prior to liver biopsy in the face of a prolonged prothrombin time. With the advent of other quantitative coagulation tests it became clear that there could be disseminated intravascular coagulation (DIC) in acute, subacute and chronic liver disease. Both the decrease in Factor V and the presence of FDP's in DIC could account for the prolonged prothrombin time, and this would not be corrected by Factor IX concentrates.

The use of Factor IX concentrates in acute hepatitis has been without any benefit and most probably has had a significant detrimental effect. Guillin and co-workers [32], studying hepatic failure associated with acute viral hepatitis, found evidence that a "compensated" DIC became aggravated following the infusion of Factor IX concentrates. Gazzard et al. [25] treated patients with acute liver failure (3 viral hepatitis, 6 toxic hepatitis) with Factor IX concentrate infusion. Five of these patients received the concentrate alone, while four received heparin concomitantly. Those patients receiving only concentrates showed marked deterioration after treatment and the predominant cause of death was hemorrhage. When heparin was administered concurrently with the Factor IX concentrates, the hemostatic state of the patients did not seem to deteriorate. The authors concluded that in acute hepatic necrosis, the use of Factor IX concentrates cannot be recommended. Other authors using other concentrates have also seen the deterioration of patients with acute hepatic disease after Factor IX concentrates [63,69]. The pathogenesis of this extremely acute DIC is not known but could be related to decreased AT-III in the presence of high levels of infused coagulants such as prothrombin.

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Another proposed use of Factor IX concentrates has been during surgical intervention in patients with hepatic cirrhosis. The result of such infusions seems less catastrophic than in patients with acute hepatic disease, and while many reports have stated that there is "effectiveness" in this situation, the evaluation of effectiveness was based on the rise in blood levels which are certainly more substantial than in acute hepatic failure. Breen and Tullis [14] found it hemostatically effective, while Sandler et al. [63] found little clinical benefit in that surgical patients with raised levels of the vitamin K dependent factors persistently bled from surgical wounds. These patients can develop either DIC or a fibrinolytic state after infusion of Factor IX concentrates, even those containing heparin [35,53].

A third proposed indication for Factor IX concentrates in liver disease is prophylaxis prior to liver biopsy, in the presence of a prolonged prothrombin time. Again data clearly assessing beneficial effect of this are lacking. Liver biopsy has a bleeding complication rate of about one for every 500 biopsies [68]. No data exist demonstrating (a) higher bleeding rates with prolonged thromboplastin time, or (b) prevention of bleeding by Factor IX concentrate prophylaxis. Green et al. [31] reported on 11 cases so treated without any problem; however, it would take several thousand cases to show any effect in preventing post-biopsy hemorrhage. While no DIC has been reported in these patients, the crucial point contraindicating the use of Factor IX concentrates is the high risk of hepatitis (see below).

Over the years, a great deal of Factor IX concentrate has been infused into patients deficient in the vitamin K dependent factors as a consequence of liver disease. At this time, there is substantial evidence for the induction of adverse reactions. Thus, it would seem that the use of Factor IX concentrates in the treatment of hemorrhage associated with liver disease should be used with very great care, if at all.

Neonatal Hemorrhagic States

Two newborn hemorrhagic states are associated with deficiencies in the vitamin K dependent factors, hemorrhagic disease of the newborn due to vitamin K deficiency, and hemorrhage in low birth weight premature infants. Factor IX concentrates have been used successfully in the former. However, in this case it is not clear whether there are benefits to be derived from the use of concentrates as opposed to plasma. The routine intrapartum use of vitamin K seems to have abolished this syndrome from the obstetrical scene.

The use of Factor IX concentrates in low birth weight infants has been investigated in a controlled study. Routine administration of Factor IX concentrates not only failed to improve survival, but resulted in a higher incidence of intraventricular hemorrhage [77].

Treatment of Patients after Extracorporeal Circulation

The routine use of Factor IX concentrates after heart surgery has come to light in several reports on hepatitis following such use [23,41]. No data establish that it is efficacious for any complication of surgery.

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Reversal of the Effect of Coumarin Drugs and Acquired Vitamin K Deficiency

Patients who have been anticoagulated with the vitamin K antagonists may need prompt reversal. The use of Factor IX concentrates in this situation has several advantages [75]. First there is a predictable and instantaneous reversal of the anticoagulant effect, and secondly the patient may be maintained on his standard dosage of anticoagulant without the problems of re-equilibration. The dosage used is in the range of 15 u/Kg [63,75]. While the efficacy of Factor IX concentrates is clear, it must be remembered that the recipients have a very high probability of acquiring hepatitis.

It has also been proposed to use Factor IX concentrates for treatment of vitamin K deficiency secondary to other conditions such as gastroenteritis [29]. The authors' data do not indicate any advantage over vitamin K therapy and further showed 2 of 8 patients having substantial decreases in platelet counts.

Factor IX Concentrates as Bypassers of Factor VIII Inhibitors

Breen and Tullis [14] reported that infusion of an experimental Factor IX concentrate during surgery to a patient with hemophilia A resulted in the cessation of bleeding. These authors hypothesized that an activated coagulant such as XI_a might be responsible for bypassing the Factor VIII dependent step in coagulation. This study was followed by that of Fekete et al. [24], wherein an "activated" Factor IX was utilized for treatment of patients with inhibitors to Factor VIII.

A series of patients treated in a variety of clinics with either regular [1,44] or "activated" Factor IX concentrates have, at least transiently, developed improved hemostasis. These patients in general reveal a shortened although not normal PTT after treatment. This shortening usually persists for several hours. The dosage reported varies (50-100 u/Kg) but is usually much higher than for replacement therapy in Factor IX deficient patients.

While the current reports are encouraging, unequivocal definition of efficacy should be obtained from a prospective blinded study currently underway. If this study shows efficacy, there will be a major unanswered question—what is the active component(s)? Until this is clearly established, it will be impossible to standardize the efficacy of the concentrate for this use. This spectre has already arisen. The regular Factor IX concentrates which were reported to be effective several years ago [1] are not considered effective currently for "bypassing" Factor VIII inhibitors.

The above highlights the fact that the current state of basic knowledge does not allow us to designate an active coagulant with the appropriate characteristics. Thrombin and Factor Xa would both act independently of Factor VIII, but their survival *in vitro* is of such short duration that they would be detectable only for a few minutes. Activated Factor IX is present in these preparations, varying from 20 ng/ml in the regular preparations to 2000 ng/ml in the activated preparations [40]. Not only would the available data suggest that Factor IXa would not survive in the circulation, but in the absence of functional Factor VIII there is no mechanism for the activation of significant amounts of Factor X. Activated Factor VII might have the ability

to persist in plasma and possibly in the circulation for a long time [64]. In the absence of tissue factor it is questionable whether there would be sufficient Factor X activating capability to have a hemostatic effect. On the other hand, at the point where hemostasis is needed there may be sufficient tissue factor available to be effective.

ADVERSE EFFECTS OF FACTOR IX CONCENTRATES

Hepatitis

While the occurrence of hepatitis following the infusion of unheated blood products is predictable, the reported incidence of icteric hepatitis with Factor IX concentrates is surprisingly high [13,23,41,63] in those patient populations with low exposure to blood products. While commercial products made in the U.S.A. have been most frequently involved in the published reports of hepatitis, it would seem that this is in fact a universal problem. An international survey of sera from hemophilia B patients revealed that the overwhelming majority of such patients treated with Factor IX concentrates have serologic markers indicating prior infection with hepatitis B [37]. Presumably, the high level of immunity induced by frequent transfusion at an early age minimizes the evidence of viral hepatitis in this particular population.

In patients without prior exposure to blood products there is a dramatic appearance of icteric hepatitis. Infusion of Factor IX concentrates in such a population results in an incidence of icteric hepatitis of greater than 60%. This far exceeds the incidence with any other blood fraction. It must be noted that these incidences were reported prior to the routine screening of donor plasmas for hepatitis B antigen by current methods. However, it is established that many infectious units of plasma have an antigen content well below the level detectable by any measurement available, and it must be presumed that all lots of Factor IX concentrates are still contaminated with hepatitis B virus. The acute hepatitis cases associated with Factor IX concentrate infusion have, where tested, been positive for the hepatitis B antigen. It is likely, however, that the other viruses associated with blood product-borne hepatitis will, in turn, be found in Factor IX concentrates [38,39].

Thrombohemorrhagic Complications with Factor IX Complex

While the active enzymes in Factor IX concentrates are primarily in the zymogen form, some small but finite quantity of these are always present as active enzymes with the concomitant possibility of inducing a thrombotic state in the recipient. Hultin [40] has identified that commercial concentrates contain about 10 ng/ml of Factor IXa and Xa. Kasper [42] found that almost half of the postsurgical Christmas disease patients treated with Factor IX concentrates had evidence of some thrombohemorrhagic disorder. Prior to and subsequent to the report of Kasper there were other single case reports [12,26,47].

Analysis of the reports of abnormal coagulation reveals that in the congenitally deficient patients this complication occurs usually following surgical

procedures. The time after the start of treatment is extremely variable, with some problems being apparent immediately and others showing up after several days of intensive treatment. There is some feeling that the chronic liver disease so often present in the hemophiliac may play a role in some of these complications. As noted earlier, infusion of Factor IX concentrates in both acute and chronic liver disease can result in dramatic and sometime catastrophic results.

The origin of this intravascular coagulation is unknown, but laboratory investigation reveals that Factor IX concentrates can shorten the clotting time of plasma *in vitro* and cause venous thrombosis *in vivo* [43]. Both of these actions can be modified by the use of inhibitors of activated coagulants. *In vivo* studies show acute drops in the level of platelets and Factor VIII and increased FDP after infusion with some Factor IX preparations [22,60]. There is further evidence of abnormal intravascular coagulation by the presence of fibrinopeptide release after Factor IX infusion [58].

Acute allergic types of reactions have also been reported after infusion of Factor IX concentrates [21].

CONCLUSIONS

Factor IX concentrates produced by a variety of different methods have been successfully used in the treatment of congenital Factor IX deficiency. While there are clearly risks associated with the use of these products, the risk/benefit ratio is favorable for the treatment of the severe Factor IX deficient patient. Moderately severe congenital deficiencies may be better handled with plasma replacement, where possible.

The usefulness of Factor IX concentrates for the treatment of acquired deficiencies is severely compromised by the occurrence of two serious and not infrequent side effects—viral hepatitis and thrombohemorrhagic disorders. No efficacy exists to support the use of Factor IX concentrates in patients with liver disease. Those concentrates which are associated with lower risk of thrombosis and hepatitis might be used with caution in some acquired deficiency states such as reversal of the effect of dicumarol.

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