# Disturbances of blood clotting mechanism induced by antimetabolites of vitamin K

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**H. Frhr. von Kress**

und

**K.-U. Blum**


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# Disturbances of Blood Clotting Mechanism Induced by Antimetabolites of Vitamin K

H. C. Hemker, J. van der Meer, R. Hodge and E. A. Loeliger

Laboratory for Coagulation and Cardiovascular Biochemistry,
Dept. of Internal Medicine, University Hospital, Leyden

There is ample evidence that the coumarol and phenylandion drugs act by competition with vitamin K (28). Intake of these drugs thus leads to a relative vitamin K deficiency.

It is therefore impossible to discuss the mode of action of these drugs without discussing the mode of action of vitamin K. As the foregoing review of Pennock (46) already covers this subject some of the information presented in this communication is already familiar to you. I hope to demonstrate that the biochemistry of human pathology leads to essentially the same conclusions as does the general biochemistry reviewed by Pennock. I think his presentation and the present one will be supplementary because the postulate on the site of action of a vitamin K antagonist that will be presented here is essentially a postulate on the mode of action of vitamin K.

Because the methodology in coagulation biochemistry is still a cause of many conflicting reports, we will have to include a heading materials and methods. After that we will discuss the behaviour of the clotting factors after intake of coumarol congeners. Then we will report on the blood coagulation inhibiting protein present in vitamin K deficiency, and discuss its fundamental and clinical importance. Finally we will show how the coagulation factors react to the administration of vitamin K during Marcoumar intake.

## Materials and Methods

The studies reported here have mostly been carried out on blood samples of not severely ill, ambulant patients that were receiving anticoagulant treatment by administration of Marcoumar (phenprocoumon; 3-(1-phenylpropyl)-4-hydroxycoumarin) or of volunteers that received the same drug. Only where indicated below, plasma from patients with an absolute vitamin K deficiency or other important concomitant illness was used.

The methodology of coagulation factor determination is most important in this kind of investigations. All coagulation factors have been estimated in specific one stage tests. The reaction mixture consisted of: *0.1 ml reagent*, i.e. a plasma containing all coagulant factors in approximately normal amounts except the factor that had to be determined; *0.1 ml thromboplastin; 0.1 ml*

*sample; 0.1 ml $CaCl_2$ 25 mM.* The clotting time obtained with a sample of unknown clotting-factor content in such a system was related to the coagulation-factor concentration in that sample by comparison with a standard curve obtained with dilutions of normal plasma. The reagents and thromboplastins were: *Factor II assay:* Artificially factor II deficient plasma prepared according to LOELIGER (26) or congenitally factor II deficient plasma; thromboplastin according to OWREN and AAS (43). *Factor VII and X assay:* Reagent prepared according to BACHMAN (3); thromboplastin according to OWREN and AAS. Factor V assay: reagent according to KAHN (21); thromboplastin according to OWREN and AAS. *Factor VII or X assay:* congenitally factor VII *or* X deficient plasma as a reagent; thromboplastin according to OWREN and AAS. *Factor X assay:* reagent as in factor VII and X assay; thromboplastin: phospholipid suspension according to MILSTONE (40) containing RUSSELLS viper venom/ml. *Factor IX assay:* according to VELTKAMP (56). *Thrombotest assay:* (44, 45) 0.25 ml thrombotest reagent (NYEGAARD) 0.05 ml plasma sample. The standard error of the results varied between 3 and 5%.

## Reaction of the Blood Clotting Factors to Administration of Coumarol Congeners

Vitamin K is mandatory for the biosynthesis of the coagulation factors II (9, 52), VII (23, 43, 48), IX (39) and X (22), intake of coumarol congeners leads to a reduction or termination of the synthesis of these factors. Severe reduction of the level of these factors in the plasma causes a hemorrhagic diathesis. The degree of hemorrhagic diathesis depends upon the level of the clotting factors. Just as in hemophilia it can be qualified as mild (5–25%) moderate (1–5%) or severe (0–1%).

It has been proven that the coagulation factors under discussion are plasma glycoproteins that are synthesized in the liver (1, 4). The few reports indicating that these factors are also synthesized outside the liver i.e. in the bone marrow, are obviously mistaken (19, 57). Coumarol congeners thus inhibit the synthesis of certain specific glycoproteins in the liver.

Intake of a sufficiently large dose of Marcoumar blocks the synthesis completely. The factors then disappear from the blood with a velocity that is determined by a constant specific for each clotting factor and by the concentration of this clotting factor. The level of the clotting factors thus will show an exponential decay, as indicated in fig. 1.

The reaction constant of this decay is most easily expressed as its biological half life time (B.H.T.), i.e. the time that is necessary for a given concentration of the clotting factor in plasma to reach half its original value. This B.H.T. is essentially independent of the way in which the synthesis is blocked. Its value is the same in liver destruction due to poison, genetically determined inability to synthesize or blockade by absence of functional vitamin K (18, 24). The

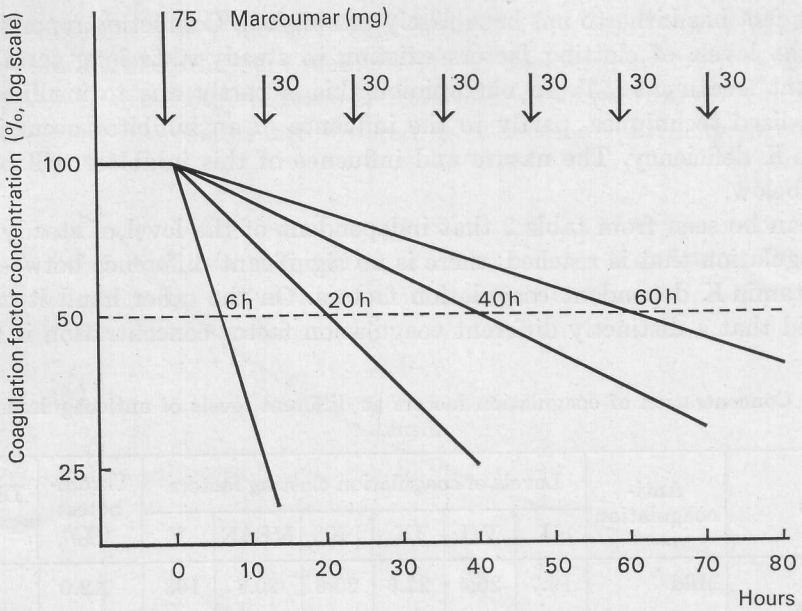


Fig. 1. Schematic view of the disappearance of the vitamin K dependent coagulation factors from the blood after intake of large doses of a coumarin congeners. The factors disappear from the blood with a velocity determined by their half-life time.

biological half life times of different clotting factors are illustrated in table 1. From this table it is directly clear that there is no connection between turnover rate of a coagulation factor and its vitamin K dependancy. This is an important point to keep in mind for the discussion of MARTIUS' theory on the mode of action of vitamin K. With high and maintained doses of Marcoumar the clotting factors II, VII, IX and X will eventually disappear from the blood.

When Marcoumar is administered in daily doses that do not completely block the synthesis a steady state of anticoagulation will be reached in which all four coagulation factors involved are decreased to the same level (25, 27).

Table 1. Biological half-life time of coagulation factors.

| Factor | Name | B.H.T. (h) | Vit. K Dep. |
|---|---|---|---|
| I | fibrinogen | 109 ± 13 | — |
| II | prothrombin | 60 | + |
| V | accellerin | 5–10 | — |
| VII | convertin | 6 | + |
| VIII | A.H.F.-A | 14 | — |
| IX | A.H.F.-B | 20 | + |
| X | Stuart-Prower F. | 40 | + |
| XII | Hageman F. | 60 | — |

This fact has hitherto not been firmly established. Conflicting reports exist as to the levels of clotting factors existing in steady-state long term anticoagulant treatment (53). In our opinion this is partly due to insufficiently standardized techniques, partly to the influence of an inhibitor occurring in vitamin K deficiency. The nature and influence of this inhibitor will be discussed below.

It can be seen from table 2 that independent of the level of steady-state anticoagulation that is reached, there is no significant difference between the four vitamin K dependent coagulation factors. On the other hand it can be observed that a distinctly different coagulation factor concentration is found

Table 2. Concentration of coagulation factors at different levels of anticoagulant treatment.

| Subjects | Anti-coagulation | Levels of coagulation clotting factors | | | | | | Thrombotest (%) | Discrepancy |
|---|---|---|---|---|---|---|---|---|---|
| | | II | VII | IX | X | MEAN | V | | |
| Normals | Mild | 19.7 | 20.4 | 22.6 | 20.6 | 20.8 | 103 | 12.0 | + |
| Coronary infarction | Deep | 12.5 | 13.8 | 12.0 | 12.0 | 12.6 | 97 | 7.3 | + |
| Coronary infarction | Moderately Deep | 15.8 | 14.9 | 16.2 | 14.3 | 15.3 | 105 | 8.2 | + |
| Vit.-K-deficiency | None | 36.9 | 38.7 | 30.8 | 35.5 | 35.5 | 116 | 24.0 | + |
| Liver disease | None | 34.5 | 37.4 | 34.4 | 39.7 | 36.5 | 47 | 37.3 | — |

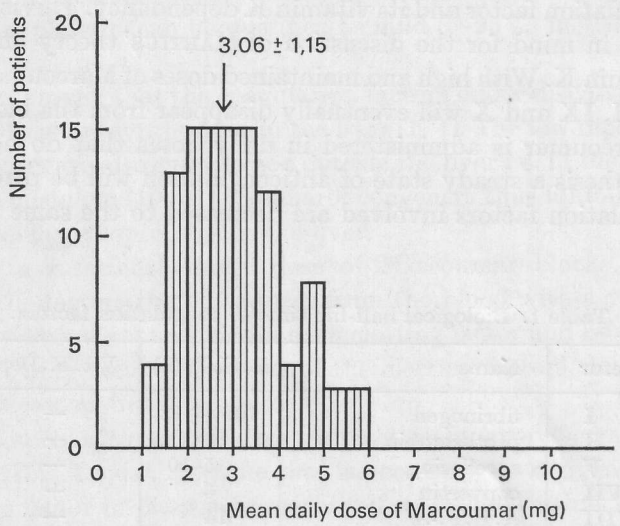


Fig. 2. Frepuention-distribution diagram of the mean daily dose of Marcoumar necessary to obtain a coagulation factor level of 15%. Calculated for 100 patients over a 150 days' period.

when a different assay method (»Thrombotest«) is used. The cause of this discrepancy between different methods will be explained later. From table 2 it can be seen to occur only in cases of relative or absolute vitamin K deficiency and not when the factors are lowered as a cause of parenchymatous liver disease.

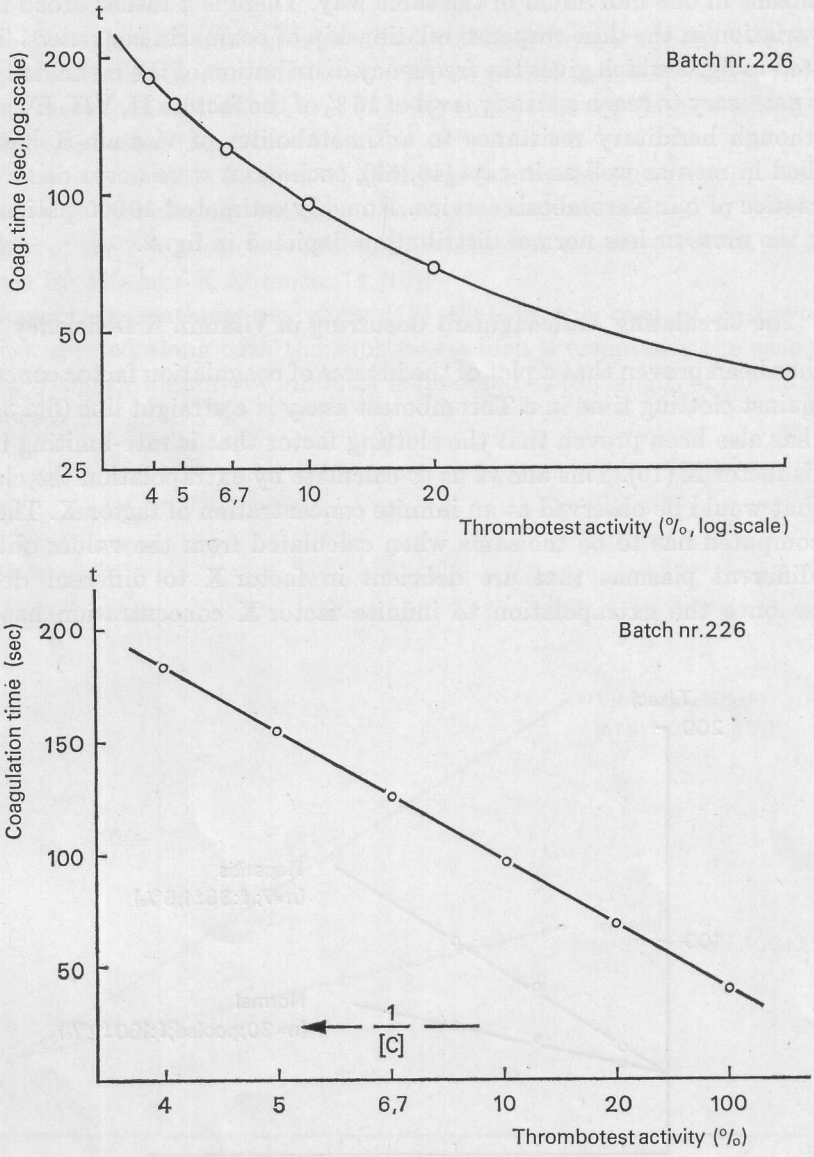


Fig. 3. Two different ways of plotting clotting time as a function of plasma dilution in a Thrombotest experiment. The rectilinearity of the plot of the inverse of the concentration against clotting time is evident.

An unchanging level of a coagulation factor in the blood means that the rate of production equals the rate of decay. When the level of all four coagulation factors comes down to the same percentage of the normal level, this means that the velocity of synthesis is inhibited to the same extent for all four of these factors. The same amount of Marcoumar thus affects the four synthesis mechanisms in one individual in the same way. There is a rather broad individual variation in the dose response relationship of coumarin congeners. This is indicated in fig. 2 which gives the frequency distribution of the mean daily dose that is necessary to reach a steady level of 15 % of the factors II, VII, IX and X.

Although heriditary resistance to antimetabolites of vitamin K has been described in men as well as in rats (46, 59), such cases were never observed in the practice of our Thrombosis service. Roughly estimated 10000 patients fell within the more or less normal distribution depicted in fig. 2.

### The Circulating Anticoagulant Occurring in Vitamin K Deficiency

It has been proven that a plot of the inverse of coagulation factor concentration against clotting time in a Thrombotest assay is a straight line (fig. 3) (15).

It has also been proven that the clotting factor that is rate-limiting in this assay is factor X (15). This allows us to calculate by extrapolation the clotting time that would be observed at an infinite concentration of factor X. The time thus computed has to be the same when calculated from the values obtained with different plasmas that are deficient in factor X to different degrees, because once the extrapolation to infinite factor X concentration has been

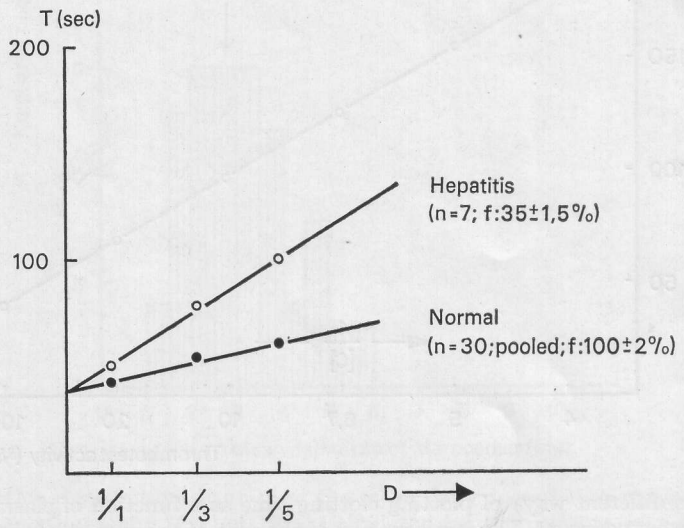


Fig. 4. The clotting-time — dilution graph in chronic hepatitis (from ref. 14).

carried out, the values from which the extrapolation has been effected are no longer of importance.

Fig. 4 shows a representative experiment in which a pooled plasma of patients with parenchymatous liver disease is demonstrated to have the same minimal clotting time as normal plasma has, thus behaving as one would expect.

When we do the same experiment with plasma from a patient with absolute or relative vitamin K deficiency however, we find that extrapolation to infinite clotting factor concentration gives clotting times that are distinctly higher than was to be expected (fig. 5).

We have been able to show that this is due to the fact that in vitamin K deficiency a circulating anticoagulant occurs that acts as a competitive inhibitor of the coagulation reaction. This inhibitor we called **PIVKA**: **P**rotein **I**nduced by **V**itamin **K** **A**bsence (14, 16).

Elementary mathematics show (13) that in the case of a competitive inhibitor diluted along with the substrate–which is essentially the case we are dealing with–the amount of inhibitor is rendered as the length of the horizontal line dotted in fig. 5.

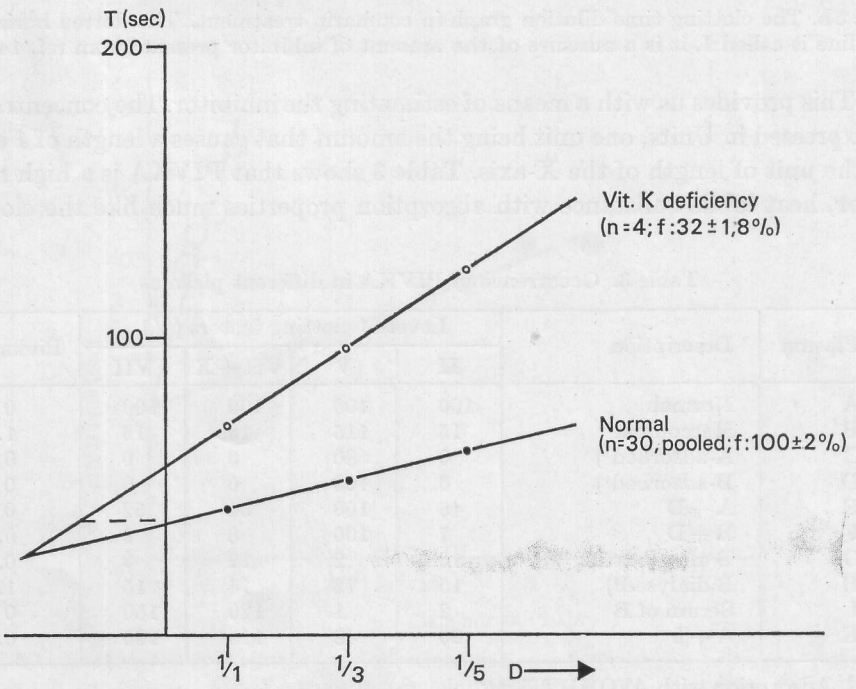


Fig. 5a. The clotting time — dilution graph vitamin K deficiency.

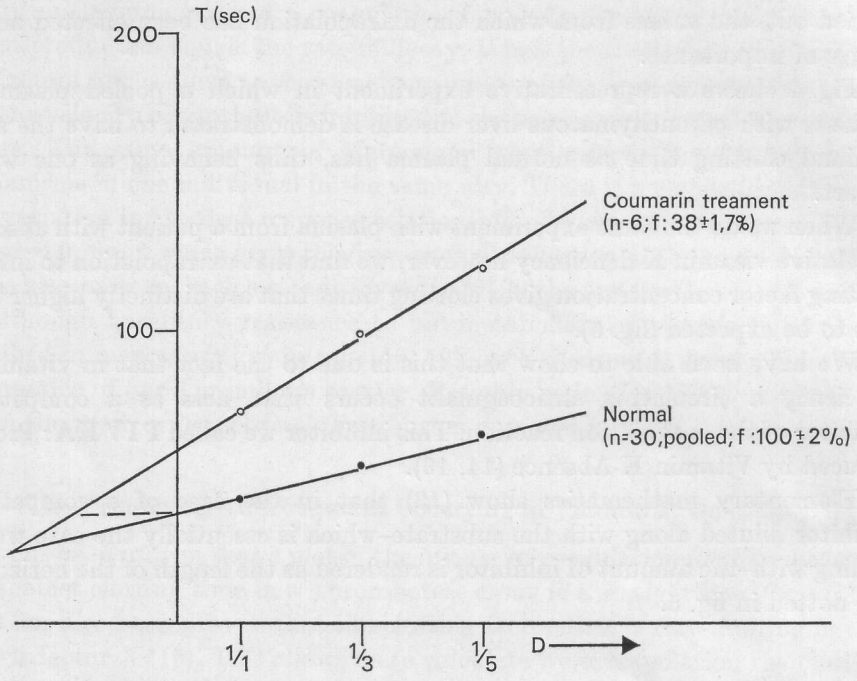


Fig. 5b. The clotting time dilution graph in coumarin treatment. The dotted horizontal line is called I, it is a measure of the amount of inhibitor present (from ref. 14).

This provides us with a means of estimating the inhibitor. The concentration is expressed in Units, one unit being the amount that causes a length of 1 equal to the unit of length of the X-axis. Table 3 shows that PIVKA is a high molecular, heat labile substance with absorption properties much like the clotting

Table 3. Occurrence of PIVKA in different plasmas

| Plasma | Description | Level of clotting factors (%) | | | | Pivka (U) |
|---|---|---|---|---|---|---|
| | | II | V | VII + X | VII | |
| A | Normal | 100 | 100 | 100 | 100 | 0 |
| B | Marcoumar | 15 | 115 | 18 | 16 | 1.5 |
| C | A-adsorbed[1]) | 0 | 80 | 0 | 0 | 0 |
| D | B-adsorbed[1]) | 0 | 100 | 0 | 0 | 0 |
| E | A + D | 45 | 100 | 54 | 52 | 0 |
| F | B + D | 7 | 100 | 8 | 9 | 0.8 |
| G | B after heating[2]) | 10 | 2 | 12 | 9 | 0.1 |
| H | B dialysed[3]) | 15 | 72 | 14 | 15 | 1.1 |
| I | Serum of B | 2 | 1 | 120 | 150 | 0 |
| K | A + I | 60 | 48 | 115 | 120 | 0.1 |

1 Adsorption with $Al(OH)_3$ 2.5 mg/ml.
2 4 hours at 45°C.
3 24 hours at 4°C.

factors II, VII, IX, and X. We have not yet been able to get this inhibitor in a purified form. Preliminary experiments indicate that separation on TEAE columns is possible, but at an extremely low yield.

We were able to localise the site of action of PIVKA at the level of factor X (table 4, fig. 6, 7, 8, 9). The data of table 4 are already very suggestive of this.

Table 4. Specific clotting factor estimations in severely vit. K-deficient patients

| Reagent | Buffer | Pat. R | | Pat. B | |
|---|---|---|---|---|---|
| | | 1/1 | 1/10 | 1/1 | 1/10 |
| Factor II cong. | 46 | 42 | 45 | 37 | 40 |
| Factor II art. | 89 | 46 | 65 | 38 | 56 |
| Fact. V art. | 195 | 15 | 19 | 16 | 19 |
| Factor VII/X art. | 149 | *200* | 110 | *158* | 123 |
| Factor VII cong. | 106 | 96 | 104 | 73 | 87 |
| Factor X acquired def. | 46 | *95* | *47* | *84* | *51* |
| Factor X cong. | 50 | *76* | *55* | *71* | *55* |

Patient R: Attempted suicide by prolonged intake of Sintrom
Slope t–D curve: 1%. Inhibitor: 1.8 U.

Patient B: Bile obstruction by carcinoma of the stomach
Slope t–D curve: 2%. Inhibitor: 2.0 U.

The figures give the means of ten clotting time estimations expressed in seconds.

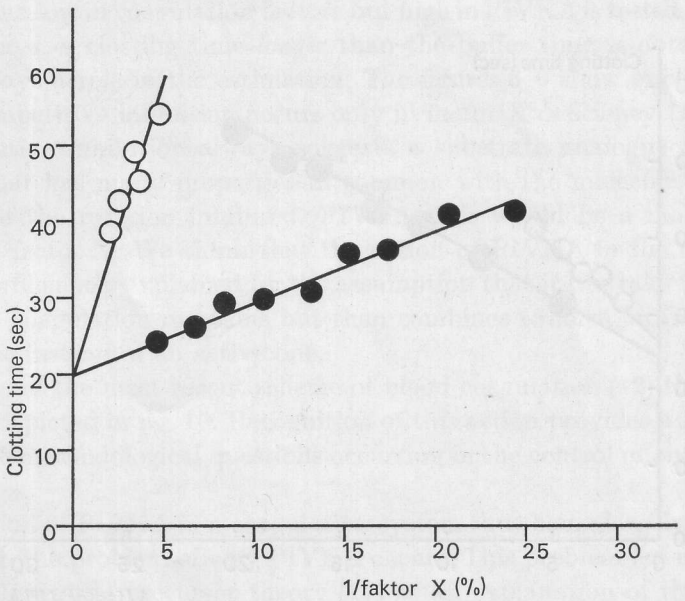


Fig. 6. The clotting time — clotting factor concentration relationship in a Factor X assay.
upper curve: in presence of PIVKA.
lower curve: in absence of PIVKA.
The inverse of the clotting factor concentration is plotted along the abcissa (from ref. 11).

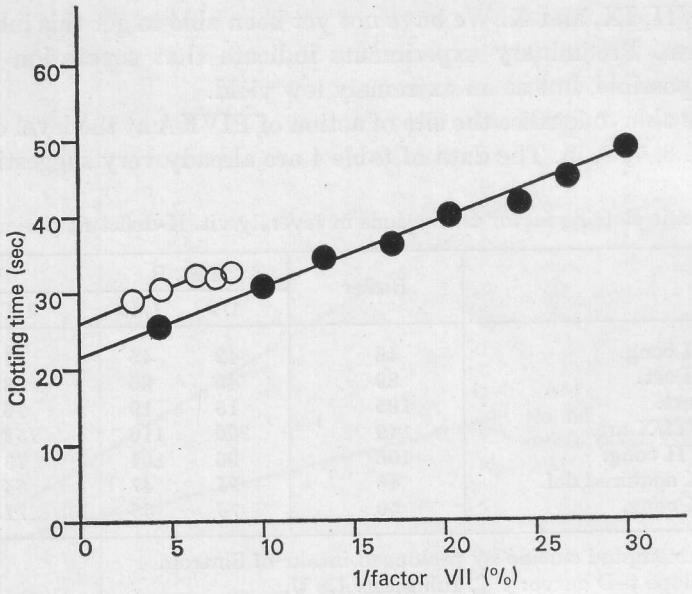


Fig. 7. The clotting time—clotting factor concentration relationship in a Factor VII assay.
upper curve: in presence of PIVKA.
lower curve: in absence of PIVKA.
The inverse of the clotting factor concentration is plotted along the abcissa (from ref. 11).

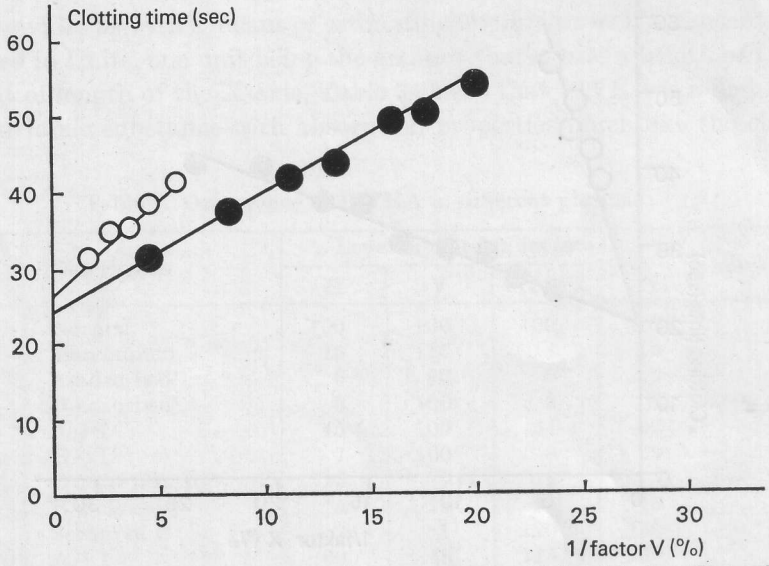


Fig. 8. The clotting time — clotting factor concentration relationship in a Facton V assay.
upper curve: in presence of PIVKA.
lower curve: in absence of PIVKA.
The inverse of the clotting factor concentration is plotted along the abcissa (from ref. 11),

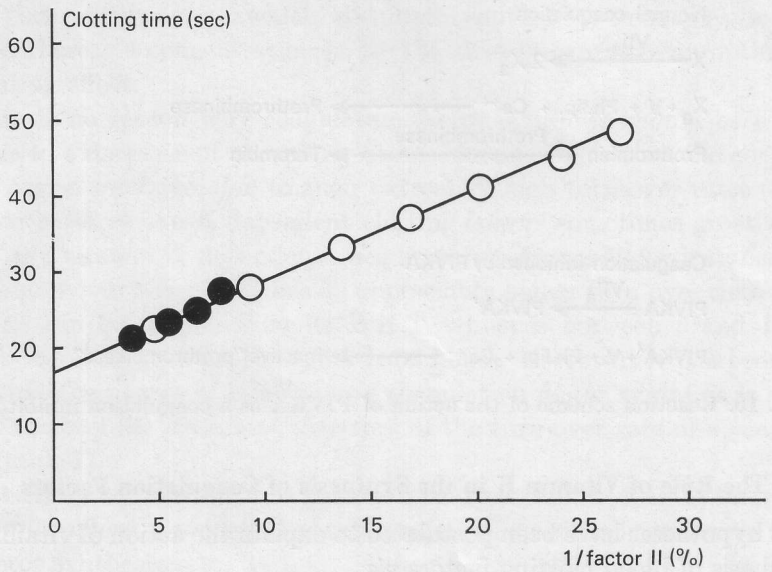


Fig. 9. The clotting time — clotting factor concentration relationspip in a Factor II assay
white dots: in absence of PIVKA.
black dots: in presence of PIVKA.
(from ref. 11).

When plasma low in coagulation factors but high in PIVKA is tested in different specific assays, a clotting time *longer* than the buffer time is obtained when factor X plays a rôle in the estimation. The figures 6–9 show explicitely that marked competitive inhibition occurs only in factor X deficiency (11).

Competitive inhibition always suggests a substrate analogue and thus a molecule that has many properties in common with the molecule that is the substrate of the reaction inhibited. PIVKA thus would be a molecule very much alike factor X. We think that the action of PIVKA in the coagulation system is sufficiently explained by the assumption that it can take the place of factor X in coagulation reactions but then combines to form an inactive prothrombinase instead of an active one.

On basis of the most recent scheme of blood coagulation (12) the action of PIVKA is depicted in fig. 10. Recognition of this action provides a solution for many of the methodological questions occurring in the control of anticoagulant therapy.

The action of PIVKA in a coagulation system thus being largely solved, we are left with the problem of why PIVKA occurs. This problem led us to postulate the polypeptide precursor theory (17) as an explanation of the action of vitamin K in the synthesis of coagulation factors.

This theory however is better discussed against the background of other theories on vitamin K action.

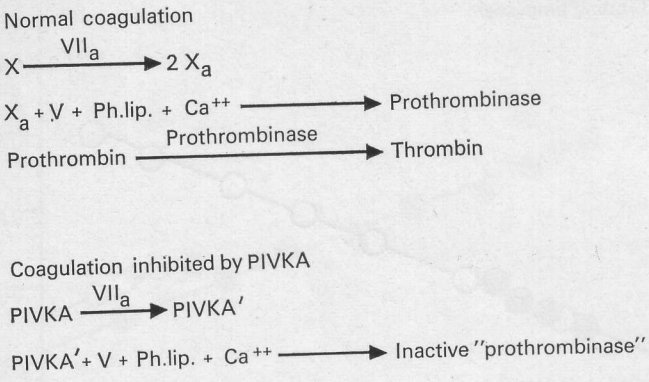


Fig. 10. Reaction scheme of the action of PIVKA as a coagulation inhibitor,

### The Rôle of Vitamin K in the Synthesis of Coagulation Factors

Four hypotheses have been postulated to explain the action of vitamin K in the synthesis of blood clotting factors *viz.*

a) Vitamin K forms part of the coagulation factor molecule (9).

b) Vitamin K is an essential part of the respiratory chain phosphorylation. Coagulation factor synthesis is extremely sensitive to a slight decrease in oxidative phosphorylation (30 up to and including 38).

c) Vitamin K acts at the level of genetic control. It causes the synthesis of m-RNA, carrying the information necessary for the synthesis of factors II, VII, IX, and X, by derepressing the operator that controls the structural genes specific for these factors (42).

d) Vitamin K acts by converting polypeptide precursors into functional clotting factors (2, 17).

As vitamin K has been shown not to be a constituent of preparations containing a high specific activity of the factors II, VII, IX and X, the first hypothesis can be directly disregarded (50, 51).

The second hypotheses, first proposed by Martius looks attractive, because it directly assigns a vital rôle to vitamin K in a very important biochemical process. On first view the theory seems to be supported by the fact that dicumarol acts as an uncoupler (33) of oxidative phosphorylation. A closer look at the problem, however, shows various arguments which makes this theory very improbable indeed.

a) Vitamin K has almost certainly been proven not to be a constituent of the respiratory chain, nor to play a rôle in the mechanism of oxidative phosphorylation in mammals, as has been extensively discussed by Pennock (46).

b) Dicumarol has been shown to uncouple all three phosphorylating sites in the respiratory chain at the same time and to about the same extent, even those sites in which it is impossible to claim a rôle for vitamin K (6, 8).

c) The concentration at which dicumarol optimally uncouples is around $10^{-3}$ M, whereas a concentration of $10^{-5}$ M already can have an optimal anti-coagulating effect.

d) There is no reason why coagulation factor synthesis should be especially sensitive to a decrease of phosphorylation. Martius states that the coagulation factors are so sensitive, due to their extremely high turn-over rates (31). The turn-over rates of non-K dependent clotting factors sometimes greatly exceed that of the vitamin K dependent ones however. Factor V for instance, which is not sensitive to vitamin K, has an appreciably higher turn-over than factor II has. This can be judged from its B.H.T. which is between 5 and 10 hours, whereas the B.H.T. of prothrombin is 60 hours. Moreover, it has been shown above that the degree of inhibition of coagulation factor synthesis is independent of the half life time, and therefore of the turn-over rate of a coagulation factor (table 1).

e) We have never been able to demonstrate that dinitrophenols, known to be potent uncouplers of oxidative phosphorylation, specifically influence coagulation factor synthesis.

f) Patients with dicumarol poisoning never show the signs and symptoms specific of poisoning with uncoupling agents.

The evidence conflicting with an action of vitamin K via the respiratory chain phosphorylation thus seems overwhelming.

As has been shown in the review of PENNOCK, it must be considered likely that vitamin K exerts its action *after* protein synthesis, at least after protein synthesis *sensu strictu* i.e. after polypeptide synthesis (2, 20, 46). This makes hypothesis c) unlikely and is in very good agreement with hypothesis d).

We first postulated the fourth hypothesis as a result of the above cited experiments on PIVKA (17).

The recognition of the inhibiting protein PIVKA under conditions of absence of vitamin K or administration of coumarol congeners, required a postulate to explain why inhibition of the synthesis of one group of proteins should go hand in hand with the appearance of another one. A possible explanation would be that the vitamin K dependent proteins are synthesized in a multistep procedure. This would mean that one or more steps are necessary before the product of ribosomal polypeptide synthesis is converted into a coagulation factor. Inhibition of a later step would then lead to the building up of an intermediate product and this intermediate product would have all the properties that PIVKA showed, i.e. it would be a protein that was very much like the vitamin K dependent clotting factors, and be induced by the absence of functional vitamin K. The polypeptide-precursor theory thus states that in the normal genetically-determined way the parenchymatous liver cells synthesize polypeptides which are direct precursors of the K dependent coagulation factors. The synthesis of these polypeptides is thought to be independent of

vitamin K. In a subsequent step these polypeptides then are converted into the functional coagulation factors. This second step is vitamin K dependent (fig. 11).

It is in accordance with this theory that long term treatment with Marcoumar tends to cause a shift in the immunoelectrophoretic pattern of the plasma. As can be seen from figure 12, the disappearance of precipitation lines at certain points of the immunoelectropherogram goes hand in hand with the

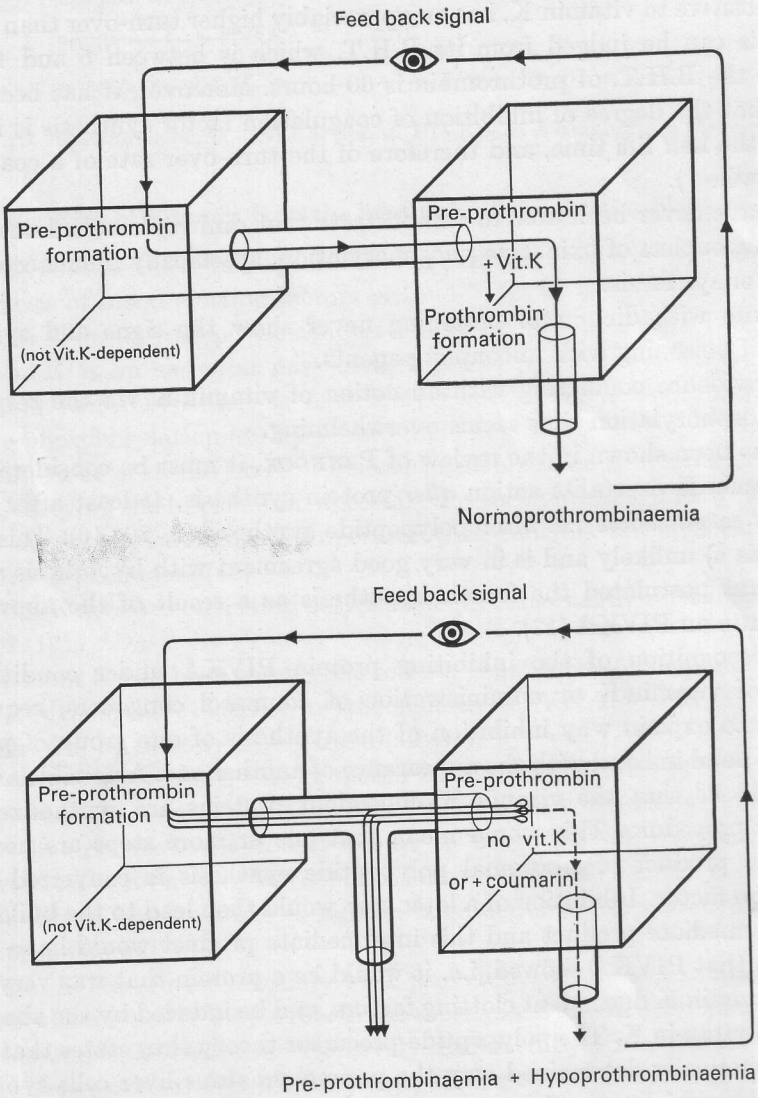


Fig. 11. Schematic view of the postulated mode of generation of PIVKA. In this graph PIVKA is denoted as „preprothrombin" (from ref. 14).

appearance of others. It is not claimed that the precipitation lines shown are caused by the coagulation factors. Other proteins may as well be dependent on vitamin K.

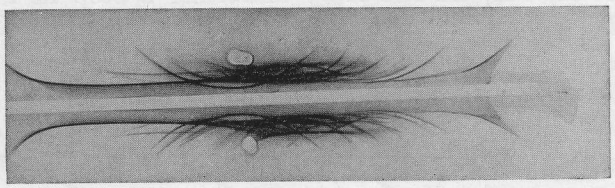

Fig. 12. Immunoelectrophoresis of plasma after prolonged Marcoumar intake. The pathological plasma (top) is compared to a normal plasma. Amido black, protein staining.

On the nature of the second step we can but speculate, but as the factors under discussion have been shown to be glycoproteins (29, 49, 54), and as the conversion protein → glycoprotein is known to occur after protein synthesis (7, 41) it might be possible that this step, or part of it, is under influence of vitamin K.

I will conclude this review with some brief remarks on the effect of vitamin K on the blood coagulation factor synthesis that has been inhibited by Marcoumar (55).

It appears that any dose of vitamin $K_1$ that causes an observable rise of the level of coagulation factors does so by a »switch on« phenomenon. About two hours after oral or intravenous administration of 1 or more mg of vitamin $K_1$ the level of all four clotting factors begins to rise. The velocity of increase is equal for all four clotting factors concerned from the 2nd. to the 8th. hour after administration. After the 8th. hour each of the coagulation factors resumes its own velocity of synthesis (fig. 13).

Only the duration of the effect is influenced by the dose of vitamin $K_1$ given (provided the dose exceeds 1 mg), the increase of the factors is not further accelerated by intake of larger doses of vitamin $K_1$. This is consistent with the view that vitamin $K_1$ and Marcoumar compete for the same biologically active site. The concentration at that site reached by 1 mg of vitamin $K_1$ or more, obviously represents an excess of vitamin $K_1$ and thus completely negates the effect of Marcoumar present.

The cause of the biphasic reappearance is not well understood at the moment. It may be related to the presence of polypeptide precursors of the clotting factors in the liver cell, or to release phenomena from the cell.

The coagulation factor concentration after continued maximal stimulation of the synthesis by vitamin K never exceeds the normal level, neither in healthy

normal volunteers not receiving Marcoumar, nor in volunteers or patients under steady state anticoagulant treatment.

In **conclusion,** it can thus be said that coumarol congeners most probably depress the synthesis of certain plasma proteins among which the coagulation factors II, VII, IX and X by inhibiting a step in their synthesis that occurs after polypeptide synthesis.

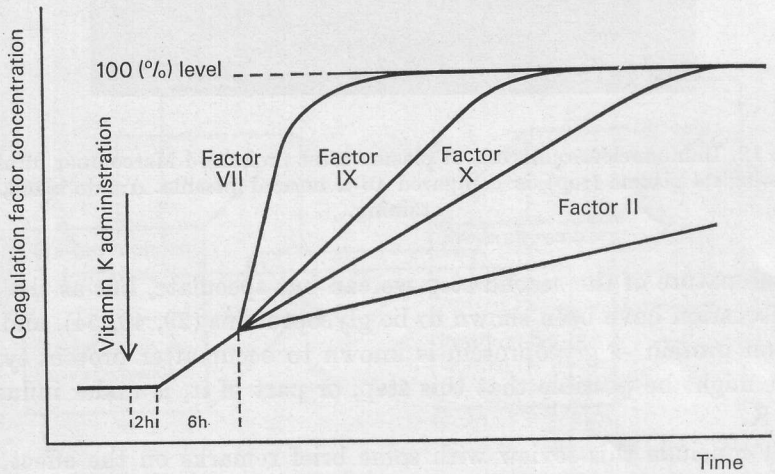


Fig. 13. Schematic view of the reappearance of the vitamin K dependent coagulation factors after vitamin K administration.

## Zusammenfassung: Störungen des Gerinnungsmechanismus durch Vitamin-K-Antagonisten

Verabfolgt man eine ausreichend hohe Dosis eines Vitamin-K-Antagonisten, so verschwinden die Gerinnungsfaktoren II, VII, IX und X aus dem Blut, und zwar mit der gleichen Geschwindigkeitsrate, mit der sie normalerweise eliminiert werden. Dieser Prozeß ist eine Reaktion erster Ordnung. Er läßt sich durch eine Konstante, die biologische Halbwertszeit (BHZ), kennzeichnen. Durch die Dauereinnahme von Cumarinderivaten können die Blutkonzentrationen dieser vier Gerinnungsfaktoren gesenkt werden. Die hierzu erforderlichen Tagesdosen schwanken unter den einzelnen Individuen beträchtlich, während Vitamin K kaum individuelle Wirkungsunterschiede zeigt, sobald sich die Gerinnungshemmung erst eingependelt hat.

Neben der Senkung der Blutspiegel der genannten Gerinnungsfaktoren führt die Langzeitbehandlung mit Cumarinderivaten zu einem anderen Phänomen, das bislang unerkannt geblieben war. Es scheint, daß unter dem Einfluß von Vitamin-K-Antagonisten ein neues Protein gebildet wird, das den Gerinnungsfaktoren II, VII, IX und X hinsichtlich seiner physikochemischen

Eigenschaften sehr ähnelt. Wir haben dieses Protein »protein induced by vitamin K absence« (PIVKA) genannt.

Auf dieses Protein wurden wir durch seine hemmende Wirkung bei Gesamtgerinnungsbestimmungen aufmerksam, wie etwa bei der »Thrombotest«-Bestimmung.

Der Angriffsort der kompetitiven Hemmung ist der Faktor X. Vermutlich ist PIVKA ein dem Faktor X analoges Substrat, das die Umwandlung von Faktor X in Faktor $X_a$ durch Faktor VIIa hemmt und somit ein inaktives, Prothrombin-umwandelndes Enzym darstellt.

Die Gerinnungsfaktoren II, VII, IX und X und PIVKA scheinen gleicher Herkunft zu sein. Wir stellten daher die folgende Hypothese auf: Die Vitamin-K-abhängigen Gerinnungsfaktoren werden in einem Zwei-Phasen-Prozeß synthetisiert. Die erste Phase ist die Eiweißsynthese. Das Produkt der ersten Phase wird in einem Vitamin-K-abhängigen Prozeß in einen biologisch wirksamen Gerinnungsfaktor umgewandelt. Wird die zweite Phase gehemmt, so tritt dieses Intermediärprodukt, das normalerweise im kreisenden Blut nicht gefunden wird, in die Blutbahn über. PIVKA würde also das Produkt der ersten Stufe sein. Beobachtungen an Patienten mit absolutem Vitamin-K-Mangel und mit chronischer Hepatitis unterstützen diese Hypothese. Wir können uns der Theorie nicht anschließen, daß Cumarinderivate an sich die Eiweißsynthese beeinflussen. Vergleichende Untersuchungen von Dicumarol und Dinitrophenol am Menschen und an der Ratte unterstützen unsere Ansicht. PIVKA hat sich bisher noch nicht in reiner Form darstellen lassen. Das erzielte Produkt enthält noch Spuren der Gerinnungsfaktoren II, VII, IX und X. Dicumarol ruft Veränderungen im immunoelektrophoretischen Verhalten der Plasmaproteine hervor.

Bei einer Langzeitbehandlung mit Antikoagulantien bewirkt Vitamin K, daß die Gerinnungsfaktoren erneut im Blut erscheinen. Etwa 2–6 Std. nach Verabfolgung von Vitamin K erscheinen alle 4 Gerinnungsfaktoren gleichzeitig wieder. Ihre Synthese vollzieht sich unter normaler Geschwindigkeit, bis die physiologischen Blutspiegel erreicht sind. Wir haben niemals ein »Überschießen« über normale Konzentrationen hinaus beobachtet. Das von anderen Autoren beschriebene Überschießen läßt sich wahrscheinlich dadurch erklären, daß diese den physiologischen Anstieg der Gerinnungsfaktoren VII und IX mit zunehmendem Alter außer Acht gelassen hatten.

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