The generation of thrombin in whole plasma

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Biochemical possibilities and physiological realities

by

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INTRODUCTION

It is not difficult, in 1985, to give a scheme of the biochemical reactions that cause blood to clot. There is general consensus on the mechanism that causes plasma coagulation, either when triggered by contact with glass or orther foreign surfaces or when recalcified in the presence of an excess of tissue thromboplastin, that is, via the intrinsic or extrinsic pathway.

Much is known about the individual reactions that constitute these pathways, especially since in the last few years obtaining the individual clotting factors in a pure and functional state is no problem any longer in the specialized laboratory, so that kinetic studies on isolated steps of the coagulation mechanism can reveal the details of the biochemical mechanism involved.

It may seem that the bulk of investigations in the field has been done and that sufficient knowledge has been gathered to explain how thrombin is formed during haemostasis and thrombosis.

It is slightly uncomfortable then to realise that we do not know much about the mechanism of thrombin formation *in vivo,* or even *ex vivo,* i.e. in whole plasma, unless we know what reactions that are biochemically possible govern the kinetics of the process.

The standard schemes of blood coagulation do not answer some simple, every day questions that every hematologist is able to formulate, such as : Why do hemophiliacs bleed whereas the proband of Factor XII deficiency, Mr. Hageman, had no bleeding problems and died of thrombosis ? Why do a large proportion of Factor XI deficient patients show no signs of a haemorrhagic tendency

whereas others do ? Why is it that heparin hardly influences the thromboplastin time and, in the same concentrations has a marked influence both on the thrombin time and the activated partial thromboplastin time ? Why do some kinds of thrombopathy show clotting disorders whereas most do not etc. etc.

Even evident questions like : « If thrombin is necessary to activate factor V (or factor VIII) and if on the other hand factor V_a (factor $VIII_a$) is necessary to form thrombin, then what sets the system going in the first place ? », do not find a ready answer.

In fact the main lines of the coagulation scheme as it is common knowledge at present, explain that thrombin forms in the test tube in the presence of either an excess of thromboplastin or of kaolin and phospholipid. It does not however answer how the mechanism is triggered, regulated and limited *in vivo,* or even in whole plasma under conditions that approach the *in vivo* situation, such as the presence of limited amounts of thromboplastin, blood platelets etc. etc.

In the course of the years many reactions between different clotting factors have been described apart from the main pathways of intrinsic and extrinsic coagulation. Some of them have been found in whole plasma, others in purified preparations. In fact so many of these *cross reactions* have been established that, on the one hand, it seems easy to explain away some of the problems mentioned above by inserting one or more of these reactions where necessary, in the existing reaction schemes. On the other hand, there is hardly any indication as to which of the cross reactions are indeed important *in vivo* and which are not.

In an attempt to approach this problem we will a) give an outline of the classical coagulation mechanism b) make an inventarisation of the most important cross reactions and c) discuss a possible approach to some of the questions posed.

A. THE CLASSICAL COAGULATION MECHANISM

1. Activation by limited proteolysis

The mechanism of activation by limited proteolysis is central in the bloodcoagulation reaction sequence. In fact the principle of a proenzyme enzyme conversion has been discovered in blood coagulation when Pekelharing in 1894 isolated a thromboplastin – and fibrinogen free fraction of plasma that could be activated by tissue

thromboplastin and Ca^{++} so as to render a solution that could clot a preparation of fibrinogen (1, 2).

The studies on digestive proteolytic enzymes ((chymo)trypsin, pepsin etc) and their zymogens have made proenzyme → enzyme conversions of proteolytic enzymes one of the main subjects of classical enzymology (3, 4).

The main chain of tissue thromboplastin induced proteolytic activations is * :

$$VII \rightleftarrows X \rightarrow II$$

For intrinsic coagulation the main chain of activations is (5) :

$$PK \rightleftarrows XII \rightarrow XI \rightarrow X \rightarrow II$$

All the non-activated clotting factors participating in these chains are proenzymes of serine proteases, the activated enzymes consequently are serine proteases.

2. Heterogeneous biocatalysis

The proteolytic activations shown above can be obtained when the molecules are solved in the aqueous phase. They are accelerated up to 100.000 fold however by the presence of a phospholipid interface and specific protein cofactors. To illustrate this mechanism we will take the activation of prothrombin as an example. Factor X_a is capable to generate thrombin for prothrombin in free solution, but only in a very ineffective mechanism (6, 7, 8) Hanahan & Pappahadjopoulos (9) were the first to observe that an active prothrombinase exist only in preparations that contain the three components FX_a, FV and phospholipid. (The question of factor V activation will be discussed later). Hemker et al (10) showed that the generation of prothrombinase activity can be described as the reversible formation of a complex of FX_a, FV and phospholipid. In a series of very elegant experiments Rosing et all (11) later showed that phospholipid deminish the K_m of the prothrombin conversion (a typical change would be from 3000 nM to 30 nM) whereas Factor V_a increases the turnover number (k_{cat}) about 1000 fold.

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* Arrows indicate activation steps, double arrows mean mutual activation. Brackets indicate multienzyme complexes.
PK = prekallikrein, TF = Tissue Factor, PL = phospholipids.
Roman numerals indicate the factors.

Further investigations (11-18) showed that the change in K_m is caused by the fact that the lipid bound enzyme has a higher affinity for the substrate then the free enzyme has. The change in k_{cat} is probably brought about by an alignment of the active-site of Factor X_a to the vulnerable sites of Factor II caused by their mutual interactions with Factor V_a.

In 1967 Hemker and Kahn (19) found that the Factor X activating enzyme is a complex of the Factors $VIII_a$ and IX_a and phospholipid, completely comparable to the prothrombinase complex. Later van Dieijen et al (20) showed that in this complex the kinetic effects of phospholipids (on K_m) and Factor $VIII_a$ (on k_{cat}) were similar to those of PL and Factor V_a in the prothrombinase complex.

Apart from their kinetic effect on k_{cat}, the factors V_a and $VIII_a$ also serve to better bind their resp. enzymes (FX_a and FIX_a) to phospholipid (21, 22).

The available data, primarily those coming from the laboratory of Nemerson (23) indicate clearly that Factor VII and tissue thromboplastin form a complex that is again comparable to prothrombinase. In this case the protein cofactor and the phospholipid are intimately bound but the mechanistic-role of the protein factor, like Factor V_a, seems to be enhancing the efficiency of the enzyme whereas the lipid serves to booster the affinity for the substrate. The most obvious difference with the other complexes resides in fact that tissue thromboplastin does not arise from an on the spot combination of the protein cofactor and the lipid but is a tight complex, shed as such by wounded cells. We will not enlarge upon the surface reactions and cofactors of contact activation here for reasons that will be discussed later.

We can summarize the reactions of the classical coagulation pathways as follows :

Extrinsic pathway.

$(VII, TT, PL) \rightleftarrows (X, V_a, PL) \rightarrow II$

Intrinsic pathway.

$PK \rightleftarrows XII \rightarrow XI \rightarrow (IX, VIII_a, PL) \rightarrow (X, V_a, PL) \rightarrow II$

An overview of the coagulation reactions is not complete if no account is given of the way in which thrombin formation is limited. Apart from the trivial possibility of substrate exhaustion, more often then not silently assumed to be the clotting delimiter in the

older literature, there are two main mechanisms to be considered : scavenging of coagulation proteases by antithrombin in III, α_2 macroglobulin and other antiproteases (see 24 for a review) and breakdown of the protein cofactors (FV_a and $FVIII_a$) by activated protein C (together with protein S) (25, 26). Protein C and protein S are vitamin K dependent proteins circulating in the plasma (27, 28). Protein C is activated by thrombin adsorbed onto thrombomodulin (29). Because thrombomodulin occurs at the surface of intact endothelium this mechanism may help to limit thrombin formation to wounded areas.

Among the anti-proteases AT III is extremely important because of the fact that its activity can be enhanced by heparin (30-32) which makes it the lever on which this important family of antithrombotic drugs acts.

B. THE CROSS REACTIONS

1. Activations of clotting factors by thrombin

It has been shown that both Factor VIII and Factor V have to be activated before they can play their role as a protein cofactor, and that thrombin is the enzyme that brings about these activations (33-36). Thrombin-activated Factor V ($F \cdot V_a$) in a purified state, when kept under the right conditions is relatively stable. The activation state of factor VIII is always a transitory phenomenon, ending in inactivation. The activation of Factor V has been described in terms of protein chemistry. The one chain molecule of Factor V is cleaved in three places by thrombin and two of the four resulting fragments recombine under the influence of Ca^{++} to form Factor V_a. The mode of interaction between Factor VIII and thrombin is still essentially unknown. It has been described that in a human system Factor X_a can activate Factor VIII (37).

At this moment it may considered to be established beyond any doubt that activation of the Factors V and VIII is obligatory for them to take part in the coagulation mechanism. This does not mean however that we know whether these reactions are physiologically important. To be more exact : it is essential to know whether these activations play a rate limiting role under physiological conditions and what the physiological activator is. From the work of Hurlet et al (38) it may be concluded that the activation of V and VIII does not occur *in vivo* in the time course of normal haemostasis but

this again does not allow conclusions as to its factual importance. Such conclusions might be drawn if an aberrant prothrombin were known that yield a thrombin incapable of activating Factor V, *prothrombin Metz* (39, 40) might be a candidate.

2. Activation of platelets by thrombin

Thrombin is the most potent physiological platelet activator (41), a concentration of 0.1 to 1.0 nM will suffice to trigger a half maximal release reaction. All other activators of human platelets need concentrations that are one or more orders of magnitude greater in order to cause the same response.

Among the proteins released by platelets are Factor V, antithrombin III and heparin neutralizing proteins (platelet Factor 4). The amount of Factor V sequestered in the platelets is roughly equal to the amount present in the plasma therefore a complete release will double the Factors V content of platelet rich plasma (42). The thrombin that causes the release reaction will also activate the released Factor V. It has been shown that this activation rather then the release reaction itself is the rate limiting factor for the generation of Factor V activity from triggered platelets (43). The concentration of Factor V in platelet poor plasma is about 25 nM whereas that of its partner, Factor X is around 200 nM. This may lead one to think that the contribution of platelet Factor V may be important in vivo. The aggregation of platelets at sites where the haemostatic mechanism is active will cause a further increase in the ratio of platelet Factor V to plasma factor V. Still patients with a storage pool deficiency that are unable to release Factor V from their platelets do not have an important haemorrhagic diathesis (44). It seems that only patients lacking Factor V in both platelets *and* plasma do show a haemorrhagic syndrome (45). This may be explained by the generally recognized fact that the normal level of any clotting factor represents a large functional excess. As a rule the level of any clotting factor must drop significantly below 10 % before a decrease of the clotting function becomes apparent.

A second procoagulant function of platelets induced by thrombin together with collagen is the platelet « flip-flop » reaction discovered by Bevers et al. (46). This reaction consists of a transbilayer movement of the procoagulant, negatively charged phospholipids (primarily phosphatidyl serine) that as a rule in the resting

cell are to be found almost exclusively at the inside face of the membrane. In the presence of collagen and thrombin platelets produce these procoagulant phospholipids at the outside of the cell without the cell being disrupted. The precise molecular mechanism of this reaction is not yet clear. Anyhow, platelets thus activated, offer large amounts of binding sites for the Factors IX_a, $VIII_a$, X_a and V_a at their outer surface so that prothrombinase and the Factor X activating enzyme can readily form at their surface there. One patient has been described in which this mechanism is defective, she suffers from a mild haemorrhagic diathesis (47).

It has been reported that collagen activated platelets can start coagulation via a Factor XI dependent mechanism and that ADP activation of platelets triggers coagulation via Factor XII (48). These findings remain to be confirmed. The recent observation that platelets release a potent inhibitor of Factor XI_a, so that Factor IX activation by FXI_a hardly proceeds in the presence of activated platelets (49) makes one doubt the importance of contact activation for in vivo thrombin generation.

3. The Josso loop

In the classical view contact factors and haemophilic factors form the intrinsic pathway and the importance of the role of the contact factors is derived from the recognized importance of the anti-haemophilic factors. The activating action of Factor VII on Factor IX invalidates this argument. The first indications that the action of the antihaemophilic factors (FVIII and FIX) is not confined to the coagulation pathway started by the contact factors were obtained by Biggs and Nossell (50), Josso (51) was the first to postulate that Factor VII can activate Factor IX so that the antihaemophilic factors play a role in thromboplastin triggered coagulation. This means that Factor X can be activated either directly by Factor VII and tissue thromboplastin or indirectly by Factor IX_a (together with Factor $VIII_a$) that, in its turn has been activated by Factor VII. It is easy to see that the function of this pathway will anyhow be dependent upon the amount of thromboplastin available. The contribution of the direct, one-step action of Factor VII_a on Factor X_a formation will be constant in time and roughly proportional to the concentration of thromboplastin. The contribution via the pathway VII → IX → X will be small in the beginning of the

reaction but will increase proportionally with time as the Factor X activating enzyme – i.e. Factor IX_a-builds up. Therefore the reinforcement loop constituted by the antihaemophilic factors – which we propose to call the Josso loop after its discoverer – will gain in importance when clotting is started by smaller amounts of thromboplastin.

The early observations on the interconnections between the extrinsic and the intrinsic pathway did not get the attention they deserved until Østerud and Rappaport drew attention to the fact that the Factor VII thromboplastin complex is capable of activating Factor IX in a partially purified system (52). Later Zur and Nemerson (53) Jesty and Silverberg (54) and Marlar and Griffin (55) established this pathway without any reasonable doubt.

The physiological importance of the Josso loop is difficult to ascertain because of the thromboplastin dependent and hence time dependent effect discussed above. It is tempting to use the Josso-loop mechanism as a tentative explanation for the clinical observation that haemophiliacs tend to bleed in thromboplastin poor organs such as joints, but this can hardly be accepted as a proof of its importance. Jesty and Silverberg (54) calculate that the activation of Factor X by Factor VII_a is 6 to 7 times faster than the activation of Factor IX. Zur and Nemerson (53) find a ratio of 10 of the theoretical maximal velocities but argue that the actual ratio will be completely dependent upon the thromboplastin concentration. Van de Besselaar et al. (56) conclude from observations in deficient human plasma's that the Josso loop may be of no importance in human plasma. Clearly the issue is not settled as yet.

Kalousek et al. (57) have reported that Factor X is able to activate Factor IX. This would constitute a mutual activation interaction that could enhance Factor X activation even without activation of Factor IX by Factor VII. Their experiments have been carried out in purified systems that did not contain protein cofactors (F · V and F ·VIII). Any indication as to the physiological significance of this interaction is lacking at this moment.

4. Activation of Factor VII

The current view on the starting mechanism of coagulation is based on the observation that the *pro*enzyme Factor VII has a non-neglectable enzymatic activity (58, 59). Once it adsorbes onto tissue

thromboplastin, the activity of Factor VII is enhanced so as to become sufficiently important to start the clotting process. It has been observed however that there exists a more active form of Factor VII, the two chain Factor VII_a. This form can be generated from the one chain form in a number of different ways. Altman and Hemker (60) showed, as early as 1968, that the contact activation mechanism can enhance Factor VII activity *in vitro*. The cold activation of Factor VII, involving kallikrein and different other proteins has been well established. It has also been described that Factor VII can be activated by Factor IX_a and by Factor X_a (61, 62). A very interesting suggestion is made by Silverberg and Jesty (63), when they claim that a complex of Factor VII, tissue thromboplastin and Factor X_a in the proteolytically active species.

If anywhere, then it is at the level of the activation of Factor VII that every conceivable reciprocal interaction of clotting factors has been described whereas any indication of their physiological importance is lacking. It is evident that all biochemical observations do not necessarily represent reactions that play a role in (patho)physiology. This being said, it must also be mentioned that often conclusions are drawn too quickly from clinical observations. Tradition has it that the scarce observations of a Factor VII deficiency or of any other rare clotting factor deficiency provoke speculations as to the physiological importance of a deficiency of that specific factor. Now some observe a low Factor VII level ($< 5\,\%$) without clinical symptoms whereas others find these patients severly handicapped. The same holds for Factor XI deficiencies and others. In trying to interprete these data one should be aware of the following :

a) any really important bleeding syndrome will lead to death either before or shortly after birth. Only the relatively mild syndromes survive. We remind of the analogy in thrombophilia : AT III and protein C deficiencies are only known in the heterozygeous states probably because complete deficiencies are lethal for the foetus ;

b) any deficiency that does not lead to a clinically important syndrome will more often then not go unnoticed. It must be kept in mind that the physiological levels of clotting factors as a rule represent a large excess of that factor so that a decrease to as low as $< 10\,\%$ of the normal level will not cause any overt disease. The number of deficiencies that are recognized not to cause problems

will therefore depend on chance findings and hence be under-estimated. This is illustrated by the fact that these disorders tend to cluster around laboratories that specialize in research on blood coagulation that are backed up by competent clinicians. We thus see that neither the really important deficiencies nor those without any clinical consequences will be recognized in routine medical practice. Therefore it is very hard – if not impossible – to draw conclusions on the mechanism of the blood coagulation process from the correlation between observed clinical symptoms and the accompanying clotting factor deficiencies.

C. INHIBITORY REACTIONS

One may think of several crosslinks between the reactions that inhibit the clotting process and those that enhance thrombin formation.

The *thrombin feedback reactions* play an important role here. As we have discussed before, thrombin will enhance its own formation by activating the factors V and VIII as well as platelets. Any inhibition of thrombin formation and any reaction that inactivates thrombin therefore will interfere with this positive feedback. At the moment it is completely unknown in howfar antithrombin III inhibits thrombin formation because it prevents the activation of Factor V or Factor VIII etc. Because heparin acts via antithrombin III, this means that the pathways of heparin action in plasma are to a large extend unknown at this moment. Also diminution of the available amount of thrombin will cause a decreased rate of activation of protein C and therefore prolong the mean lifetime of the prothrombin – and Factor X activating complexes. Again no quantitative data are available that allow an estimate of the importance of this conceivable pathway.

In the second place one has to reckon with *competition for clotting proteases* between procoagulant complexes and protease scavengers. Here more data are availalbe. It has been observed for instance (64, 65) that antithrombin III will attack Factor X_a less readily in the presence of phospholipids then in free solution and even less if both phospholipids and Factor $V_{(a)}$ are present. To what extend this phenomenon, observed in (semi)purified systems is operative *in vivo* is anybodies guess. The fact that low molecular weight heparins are efficient antithrombotics and have an enhanced anti Fac-

tor X_a activity suggests that anti-Factor X_a scavenging by AT III-heparin complexes might be of (patho)physiological importance. It has also been shown however that there is no direct relation between anti-Factor X action and antithrombotic properties (66, 67).

What holds for Factor X_a and antithrombin III, in principle holds for all surface bound proteases and all antiproteases. We do

c) Because in the specialized laboratory all the clotting factors are now available in a purified form it is possible to bypass and shortcut pathways by directly adding the purified activated intermediates. Also kinetic studies in whole plasma are now possible because the concentration of one specific factor can be modified between zero and very high concentrations by adsorption of that factor to a specific antibody or by adding the purified factor to the plasma.

d) Clotting factors that have properties different from the normal ones occur in rare congenital disorders or can be produced by chemical modification. If for example the prothrombin, in normal plasma is replaced by aminidated prothrombin, thrombin will still be readily formed by prothrombinase, but this aminidated thrombin will be unable to activate Factors V and VIII. In such a plasma the importance of feedback activation of these factors may be studied.

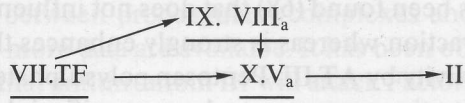
If thus appears that there are several possible ways to integrate the data from modern biochemical studies with the study of the clotting of plasma *ex vivo*.

SUMMARY

We give a review of modern concepts of the mechanism of blood coagulation. Two main principles govern this process :
a) activation of proenzymes by limited proteolysis
b) heterogeneous biocatalysis.

The former can be considered common knowledge, the second indicates the fact that three clotting enzymes (Factor VII_a, IX_a and X_a) cannot act efficiently unless they are adsorbed on a suitable phospholipid substrate next to a protein cofactor (Tissue Factor (TF), Factors V_a and $VIII_a$). On good grounds the importance of the contact activation system for physiological blood coagulation can be doubted. The antihemophilic factors form a reinforcement loop for the activation of Factor X (Josso loop).

The basis scheme of blood coagulation therefore is :



(The arrows indicate activation, the underlining indicate the phospholipid surface).

This scheme is only the backbone of the reaction sequence, many other reactions have been described. Thrombin e.g. activates the protein cofactors V and VIII. Thrombin also activates blood platelets and induces

them to shed Factor V and to generate patches or procoagulant phospholipids at their surfaces. Also limiting processes are important, e.g. : the thrombin formed is captured by antiproteases present in the plasma and Factors V_a and $VIII_a$ are inactivated by activated protein C.

Protein C is a vitamin K-dependent plasma protein that can be activated by thrombin adsorbed to a surface protein of endothelial cells (thrombomodulin).

Apart from the main lines sketched here, many other interactions between clotting factors have been described. At the moment it is unknown wether they have physiological significance or are only possible under non-physiological test conditions.

It even is an open question at the moment what reactions govern the velocity of thrombin formation in whole plasma.

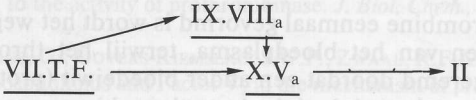
RÉSUMÉ

Nous présontons ici un survol des réactions biochimiques qui amènent à la formation de la thrombine. Deux principes essentiels peuvent être retenus :

a) l'activation de proenzymes par protéolyse limitée et

b) la biocatalyse hétérogène.

Le premier principe est un mécanisme universellement admis en biochimie. Le deuxième indique que trois enzymes de la coagulation (Facteurs VII_a, IX_a et X_a) ne peuvent atteindre leur efficacité maximale que lorsqu'ils sont adsorbés sur une surface de phospholipides et en présence d'un cofacteur (TF. : facteur tissulaire, facteur V_a et facteur $VIII_a$). Si le rôle de l'activation par le contact semble aléatoire, la « boucle Josso » formée pour l'activation du Facteur X, par les facteurs antihémophiliques (Facteur VIII et IX) apparaît importante. Le schéma global de la thrombino-formation peut donc être représenté comme suit :



(Les flèches indiquent l'activation, les traits sous les symboles indiquent la surface phospholipidique).

A côté de ces réactions principales de nombreuses réactions annexes ont été décrites. Ainsi la thrombine rend actifs les cofacteurs V et VIII. De même la thrombine induit une réaction plaquettaire qui conduit a la libération du facteur V contenus dans leurs granules, produisant ainsi des phospholipides procoagulants à leur surface.

La thrombine en milieu plasmatique est inactivée par des protéines inhibitrices, comme l'antithrombine III et l'α_2 macroglobuline.

La thrombine adsorbée à une protéine de la surface de l'endothélium (la thrombomoduline) est capable d'activer une autre protéine plasmatique : la protéine C. Une fois activée, cette protéine détruit les cofacteurs V_a et $VIII_a$ freinant ainsi la thrombinoformation.

Le mécanisme de la thrombinoformation est donc connu dans ces principes. Cependant une question principale reste à être résolue : quelles sont les interactions qui déterminent la vitesse de la thrombinoformation dans le plasma ?

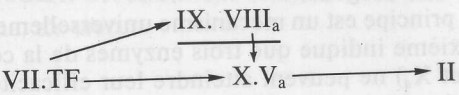
SAMENVATTING

Er wordt een overzicht gegeven van het biochemisch mechanisme van de bloedstolling. Twee principes beheersen dit proces :
a) activering van proenzymen door beperkte proteolyse en
b) heterogene biokatalyse.

Het eerste wordt bekend verondersteld. Het tweede geeft aan dat stollingsenzymen (de factoren VII_a, IX_a, X_a) slechts efficiënt kunnen werken als zij geadsorbeerd zijn aan een phospholipide oppervlak tesamen met een niet enzymatisch hulpeiwit (TF (= weefselfactor), factor V_a en factor $VIII_a$).

Er zijn gronden om aan het belang van de z.g. contactactivering te twijfelen, maar de antihemofilie factoren (IX en VIII) vormen een versterkingslus voor de activering van factor X.

Het grondschema van de bloedstolling wordt zodoende :



(De pijlen duiden op activaties, de strepen onder de symbolen stellen het phospholipide oppervlak voor).

Naast dit basisschema zijn er talrijke andere reacties beschreven. Zo brengt het thrombine in bloedplaatjes een reactie teweeg die zorgt voor het verschijnen van stollingsactieve phospholipide oppervlakken en geactiveerde factor V.

Als het thrombine eenmaal gevormd is wordt het weggevangen door de antiproteasen van het bloedplasma, terwijl het thrombinevormend enzym wordt geremd doordat een ander bloedeiwit (protein C) wanneer het eenmaal geactiveerd is (nl. door thrombine gecomplexeerd met thrombomoduline, een eiwit dat aan de oppervlakten van endotheel cellen voorkomt) de hulpfactoren V_a en $VIII_a$ vernietigt.

Ofschoon in grote lijnen kwalitatief bekend is welke reacties de hoofdlijnen van het stollingsproces vormen en welke andere reacties biochemisch mogelijk zijn blijft het een open vraag welke processen in vivo snelheidsbepalend zijn.

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BESPREKING

MERLEVEDE. – Hetgeen mij opvalt is dat in uw schema de factoren II, VII, IX en X centraal staan. Dit zijn ook de factoren die onder invloed van vitamine K van precursoren omgezet worden in stollingsfactoren. Wat is er eerst gekomen in de conceptuele voorstelling van dit schema : de belangrijke rol van vitamine K of de experimentele vaststelling dat deze factoren centraal staan ?

HEMKER. – De vitamine K-gevoelige stollingsfactoren zijn ook de zymogenen van de proteolytische enzymen van de bloedstolling, als men tenminste het contact activeringssysteem, op basis van de argumenten die ik in mijn voordracht noemde, buiten beschouwing laat.

De experimenten van de laatste paar jaar laten duidelijk zien dat deze proteolytische enzymen de ruggegraat van het bloedstollingsmechanisme vormen. Op deze manier begrijpen wij waarom vitamine K zo'n belangrijke rol speelt. Wij zijn onze studies begonnen om het wezen van de orale antistollingsbehandeling en dus van de werking van vitamine K, beter te begrijpen en dat is uitgemond in onze conceptuele voorstelling van de rol van de proteolytisch enzymen.

VERSTRAETE. – Orale anticoagulantia remmen de synthese van vitamine K afhankelijke stollingseiwitten. De therapeutische dosering van orale anticoagulantia is dusdanig dat de streefwaarden van de betrokken stollingseiwitten tussen 10 tot 15 % van hun uitgangsconcentratie in het bloed bedragen.

In uw kinetische studies, uiteraard in vitro en met gezuiverde stollingsfactoren, hebt U aangetoond dat slechts bij vele lagere concentraties de kinetica van de bloedstolling geremd is. Als men de rol van thrombine op bloedplaatjes buiten beschouwing laat, wordt het dan niet moeilijk de antistollingstherapie rationeel te verklaren ?

HEMKER. – Inderdaad is het zo dat ook bij adequate orale antistolling de plasma concentraties van de stollingsfactoren nog beduidend hoger zijn dan de concentraties die, in een geïsoleerde stap, een half maximale redactiesnelheid geven.

Omdat het geïntegreerde proces van de bloedstolling een sterk niet-lineair en tegengekoppeld systeem is, mag men daaruit echter niet concluderen dat het hele systeem ook wel weinig geremd zal zijn. In feite is het reconstrueren van de kinetica van het gehele systeem op basis van wat wij van de kinetica van de onderdelen weten de opgave die ik me voor de komende tien jaar gesteld heb. Daarom kan ik op uw vraag nog geen exact antwoord geven. Het lijkt echter wel zeker dat de tegenkoppeling via thrombine naar de thrombocyt die u noemde, hierbij een belangrijke rol speelt.