Prothrombin as Co-Factor of the Circulating Anticoagulant in Systemic Lupus Erythematosus?

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A. Introduction

During the past 10 years, the haematological symptoms of systemic lupus erythematosus (S. L. E.) have gradually become more clearly defined (5, 13). The haematological morphologist has described the L. E. cell phenomenon. In many cases the immunohaematologist has found, beside biologically false positive syphilis reactions and a positive cephaline-cholesterol flocculation test, auto-immune-antibodies against erythro-, leuko- and thrombocytes (21). The patients may or may not have an haemolytic anaemia, leukopenia or thrombocytopenia.

In some patients with S. L. E., the coagulation physiologist has discovered a *circulating anticoagulant* and a more or less pronounced *hypoprothrombinaemia*, which may occur with it, or alone (1, 3, 4, 6, 10, 12—15, 18, 23, 26). However, the causal relationship between these two coagulation disorders is not yet known.

In this article we wish to call attention to some observations concerning the physiopathology of coagulation, in a man suffering from S. L. E., which may elucidate the interrelationship between hypoprothrombinaemia and circulating anticoagulant.

B. Case History

A Dutchman of unmixed race, born in 1923, now 35 years old, baker by trade, had suffered from L. E. of the skin since his 28th year of life. In May 1957, he was referred to the out-

patient department for general examination on account of a slightly increased ESR, varying thrombocytopenia and a slightly prolonged prothrombin time.

In the family history only pseudo-xanthoma elasticum D a r i e r in the father was reported.

The patient underwent an appendectomy in 1943 and a herniotomy in 1956. No signs of haemorrhagic diathesis were then determined.

In 1952, the patient was treated with gold and bismuth for a histologically verified *lupus erythematosus* of the facial skin. Later, in 1955, he was treated with nivaquine.

In the spring of 1956, a considerable epistaxis occurred for the first time; it was arrested by means of thermocautherization. In August of the same year a haemorrhage was found at the edge of an old tympanic perforation, and, at the same time *subcutaneous haematomas* were found on the legs. The cause proved to be *thrombocytopenia* (18 000 — 20 000 thrombocytes/mm³); the bleeding time was 11 minutes, the coagulation time normal.

Clinical observation and extensive laboratory examination in the autumn of 1956 did not suggest an organic abnormality. The patient had no fever, he did not feel ill. On account of the thrombocytopenia, treatment with Prednisone was begun, producing an increase of the thrombocyte count to more than 100 000/mm³.

In the winter of 1956—57, the patient caught a cold, with cough and a moderately increased ESR; in the chest X-ray an intensified, streaked peribronchial pattern was observed.

On April 16th, 1957, bleeding from the gums developed. The bleeding and coagulation times were normal; the thrombocyte count, 154 000/mm³; but for the first time an *increased prothrombin time* was found (22 sec. compared with a control of 14 sec.). The ESR was 58 and 65 mm during the first and second hours respectively.

On May 7th, 1957, the first examination in our out-patient department took place; the patient had no complaints and no abnormalities were evident on physical examination, except a very moderate L. E. involving the skin above the cheek-bones and to a smaller degree the forearm, and an inconspicuous, non-painful, subcutaneous haematoma on the right thigh. On röntgen examination and ECG, nothing abnormal was detected. Blood pressure was 140/90 mm Hg. There was no rise in temperature.

Laboratory examination (see table 1): moderate *urobilinuria,* good kidney function, no signs of parenchymatous disease of the kidneys. ESR, blood morphology, blood chemistry and paper electrophoresis, in the range of normal. *Cephalin-cholesterol flocculation* test: + + + +. K a h n -, M e i n i c k e - and W a s s e r m a n n -*reactions, strongly positive.* Treponema pallidum immobilisation test, negative. C o o m b s' *test, weakly positive* (1 : 4 +, 1 : 16 ±). *Reaction for complete thrombocyte antibodies, positive.* Complement level of serum, lowered. Serum Iron 102 µg per 100 ml.

An extensive investigation into the causes of the urobilinuria and the slightly *increased indirect reaction* of *bilirubin* with the aid of quantitative liver function tests and determination of the survical time of the erythrocytes, is still lacking. Signs of *hyperplasia of erythropoietic tissue* were found in the *bone marrow,* as early as in 1956.

C. Investigation into the Anomalies of the Haemostasis

a) Methods

The blood to be examined was withdrawn with hollow ground V₂A-steel needles. To obtain *plasma* the blood was decalcified immediately on withdrawal with 1/10 mol. Na-oxalate in a ratio of 9 : 1. *Plasma rich in platelets* was obtained by centrifuging (1500 r.p.m.) for 5—10 minutes. *Plasma poor in platelets* was obtained after centrifuging for 30 minutes at 3000 r.p.m. (distance between the centre of the tubes of blood and the centre of rotation: 15 cm). BaSO₄-

Tab.   1

	7/5/57	29/10/57	12/2/58	23/4/58	normal
Urinanalysis					
Sediment	no pathol.		no pathol.		
Protein and glucose	negativ		negativ		
Urobilinogen	++		+++		
Specific weight			1.025		
Phenolred excretion	normal				
Blood analysis[*])					
ESR (Westergren)					
mm per 1/2 h.	2/5		3/31		
Haemoglobin g%/o	14.7	15.9	15.9	16.2	
Erythrocyte count					
mill./cmm	4.5	5.12	4.64	4.99	
Reticulocyte count %/oo			6	6	
Leucocyte count	9 000	7 800	7 000	8 000	
Eosinoph. gran. count		50	110	70	
Neutr. gran. count	6 100	4 900	4 100	6 100	
Monocyte count	600	300	1 200	600	
Lymphocyte count	2 300	2 600	1 700	1 300	
Platelet count	135 000	75 000	125 000	137 500	200—300 000
Osmot. resist. erythr.			0.48—0.36		0.48—0.38%/o
Urea mg%/o	18		29		NaCl
Bilirubin mg%/o			0.98	0.89	
Iron gamma%/o	102				
Total protein g%/o	8,4		8.4	8.4	
Electrophoretic pattern					
(paper-el.)					
albumin %/o	68.5	73.3	69.2	66.5	69.8±1.5
alpha 1 globulin %/o	3.0	3.7	3.2	5.8	3.3 ± 0.3
alpha 2 globulin %/o	3.8	4.3	5.5	4.4	6.3 ± 0.6
beta globulin %/o	6.2	5.3	7.5	8.6	7.9 ± 0.8
gamma globulin %/o	18.4	13.4	14.6	14.7	12.9 ± 1.3
Coombs - test dir.	1/16 ±	1/4 +; 1/16±			
Free-platelet-antibodies					
(Dausset)	+	slightly +			
L.E.-cel-phaenomenon					
(Zimmer et al.)	negativ	negativ		negativ	
Wasserman-,					
Meinicke-,					
Kahn reaction	++++	++++			
Cephalin-cholestrol-					
flocculation	++++				
(Hanger)					

plasma or serum, is plasma or serum which has been shaken for 3 minutes with BaSO₄, 50 mg per ml, at room temperature for complete removal (adsorption) of prothrombin, factors VII, IX, X and *Stuart-Prower*-factor. The *tissue thromboplastin* was prepared according to O w r e n ' s original method (19), and stored in portions of 2 ml in the deep freeze, at a temperature of — 25° C. The CaCl₂ solution was 1/40 mol. The dilution fluid was a *buffer substance* (M i c h a e l i s) with a pH of 7.42. All coagulation tests were performed at a *constant temperature* of 37° C (water bath). The test tubes were of ordinary Thüringer glass, with a round bottom, a diameter of 16 mm and a length of 96 mm. Determination of the *prothrombin* time was according to the method of Q u i c k (22). The *prothrombin* (factor II) was determined with a one-stage and a two-stage method (16), in dilutions of 1/40 — 1/400 in order to exclude a disturbing action of the anticoagulant. Factors V and VII-complex were determined according to a one stage method (16). In performing the *thromboplastin-generation test,* we followed the directions given by B i g g s (2) and H i c k s (9); the *thrombin-generation test* is modified according to v a n d e r P o l (20). In the *prothrombin consumption test* 0.1 ml serum was incubated with equal parts of thromboplastin and CaCl₂, and after 60 seconds 0.1 ml bovine BaSO₄-plasma, diluted 1 : 5 with buffer (instead of fibrinogen) was added to the incubated mixture. The methods of determining *antithrombins* were as described earlier (17). The *thrombocytes* were counted according to v a n H e r w e r d e n ' s method (8) (normal values 200 000 — 300 000 per cu. mm); the activity of *thrombocyte factors* 3 and 4 was tested in the thromboplastin-generation test with or without the addition of 2 µg heparin per ml CaCl₂; the results were compared with those obtained with normal thrombocytes under the same experimental conditions. In *thromboelastography* H a r t e r t ' s (7) directions were accurately followed. The *anticoagulant* in the serum protein was localized by means of continuous paper electrophoresis, in which 12 fractions were obtained, and the anticoagulative characteristics of the different tractions were tested by determination of the coagulation time in the following system.

> 0.1 ml platelet-poor normal plasma
> 0.1 ml thromboplastin diluted 1/100 with buffer
> 0.1 ml protein traction to be tested
> 0.1 ml CaCl₂.

b) Results

In 1955, the patient showed a *thrombocytopenia;* in 1957, in addition to the thrombocytopenia be showed a moderately *prolonged prothrombin time* and a *slightly prolonged coagulation time.* The *clot formation* was haemophilia-like, as is clearly shown by the thromboelastogram (fig. 1).
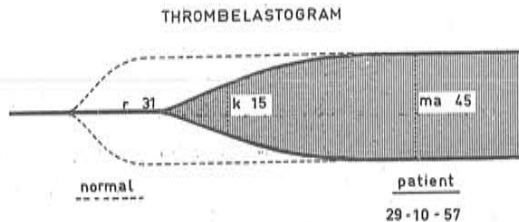
THROMBELASTOGRAM

F i g . 1 : Thromboelastogram.

Retraction was *delayed* by the decreased coagulability and, in accordance with the reduced thrombocyte count, *slightly diminished* (for example, in the presence of a normal haematocrit value and a thrombocyte count of 135 000 per ml at 37° C, 39.3% of serum was expressed within 24 hours; after mixing with 50% normal blood the amount of serum expressed under the same circumstances was 45%).

T a b . 2 : Investigation into the Haemostasis

Bleeding time (D u k e) : 2—4 min. R u m p e l - L e e d e : negative
Coagulation time: 8—15 min. (n. 3—8) Thrombocytes: 75—135 000
Thrombelastogram: r = 26—31 min.; k = 14—16 min.; ma = 45 mm
Recalcification time (H o w e l l) : 220—240 sec. (n. 90—110)

Prothrombin time (Q u i c k) : 23—25 sec. (n. 12.5 sec.)
    factor I   = fibrinogen = antithrombin I (C l a u s s) : 100%
    factor II   = *prothrombin:* one stage *16—25%,* two stage: *idem*
    factor V   = proaccelerin (K o l l e r) : 50%
    factor VII/X = proconvertin / S t u a r t - factor (O w r e n) : 60%
    antithrombin II and V = a. immédiate: normal

Thromboplastin generation (B i g g s) : normal
    (factors VIII, IX, PTA and H a g e m a n - factor normal)

Antithrombin III = a. progressive: plasma: normal, serum: increased (fig. 2)

Clot retraction: delayed (haemophilia-like)
            only slightly diminished,
            no fibrinolysis.

Prothrombin consumption: delayed, serumprothr. after 3 h.: < 1%
Platelet factor 3 and 4: normal
Circulating anticoagulant: +++.

The serum antithrombin-III was markedly increased (fig. 2).

Analysis of the causes of the prolonged prothrombin time revealed primarily a *marked decrease of prothrombin* (see table 2); however, since a drop in the prothrombin level to 25—16% could not cause more than 3—5 seconds' increase of the prothrombin time and only a few minutes' increase of the thromboelastographic reaction time, and both were more prolonged in the patient, further clotting disorders were sought. In mixing tests it was found that the coagulation time of the patient's plasma could not be normalized by adding normal plasma. This suggested the presence of a *circulating anticoagulant.* When the patient's plasma was mixed with increasing amounts of normal plasma, containing
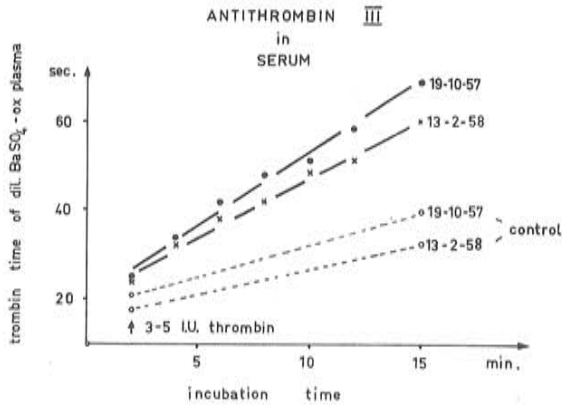
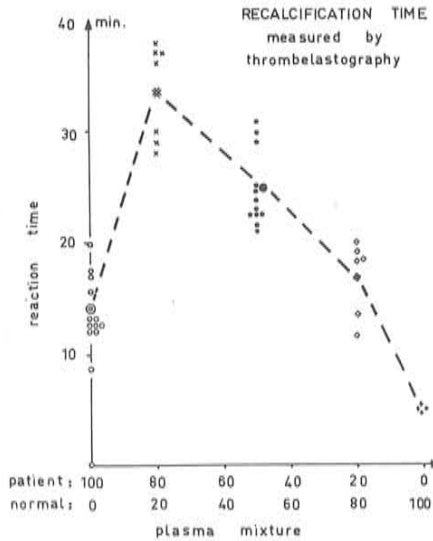Fig. 2: Activity of antithrombin III in patient's serum, as compared with that of normal serum.

Fig. 3: Thromboelastographic reaction times in the patient's and in normal platelet-poor plasma and in various mixtures of these plasma's.

thrombocytes, the prolongation of the recalcification time remained approximately the same up to a ratio of one part patient's plasma to five parts normal plasma. When it was mixed with thrombocyte-poor plasma, the coagulation time became even longer (fig. 3).

A similar observation was made in determining the prothrombin times of plasma mixtures; a relative prolongation was seen when undiluted thrombo-

plastin was used, and an absolute prolongation when the thromboplastin was diluted 1 : 10 with buffer substance (fig. 4).
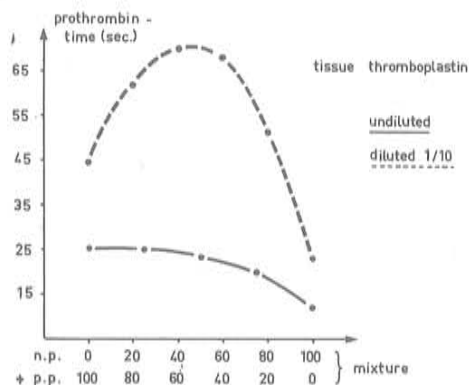

F i g . 4 : Q u i c k's one-stage prothrombin time in patient's plasma (p. p.), normal plasma (n. p.) and various mixtures of p. p. and n. p. The full line shows the values obtained with undiluted thromboplastin, and the dotted line the values with diluted thromboplastin (1 part thromboplastin and 9 parts buffer).

The formation of thromboplastin in H i c k s ' test and the formation of thrombin in the thrombin-generation test were very clearly inhibited when these were applied to mixtures of patient's and normal plasma (fig. 5 and 6). However, in B i g g s ' thromboplastin-generation test the inhibition in mixing experiments did not appear.
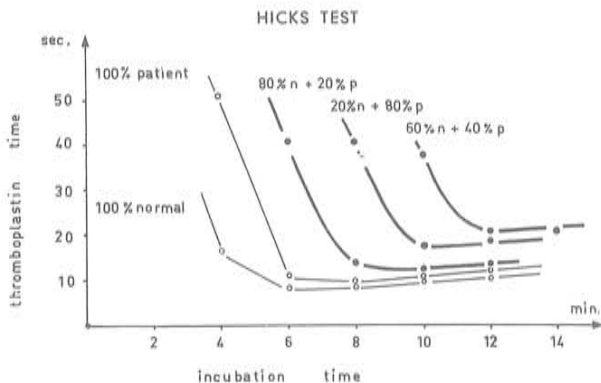

F i g . 5 : Thromboplastin formation in the screening test according to H i c k s . Thromboplastin for- mation was unmistakably delayed and diminished when the test was applied to mixtures of n. p. and p. p.; this was most marked in the ratio 60% n. p. and 40% p. p.

Finally, both with the use of tissue thromboplastin and with intrinsic thromboplastin, there was marked inhibition of the coagulation process if
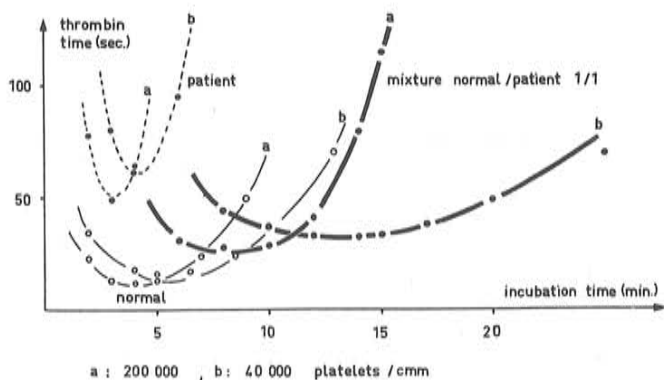
a : 200 000 , b : 40 000   platelets / cmm

F i g . 6 : Thrombin-generation test in normal plasma (————————), patient's plasma (— — — —) and mixed plasma (———————), using various quantities of thrombocytes. Thrombin formation was found to be markedly delayed, particularly at lower thrombocyte counts, in mixtures of n. p. and p. p.

normal plasma was mixed with BaSO₄-plasma of the patient; this was especially clear when diluted tissue thromboplastin was used. If, on the other hand, patient's plasma was mixed with patient's BaSO₄-plasma the inhibition decreased as the amount of patient's plasma was diminished; this decrease was even more distinct when the patient's plasma was mixed with normal BaSO₄-plasma (fig. 7 and 8).

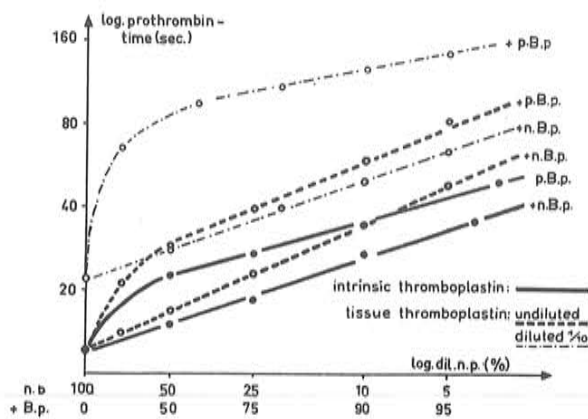F i g . 7 : Q u i c k's one-stage prothrombin times and B i g g s' thromboplastin times in n. p. and its dilutions in BaSO₄-plasma (B. p.), both from a normal subject (n. B. p.) and from the patient (p. B. p.). The interrupted lines represent the prothrombin times as determined with undiluted thromboplastin (————————) on the one hand, and with thromboplastin diluted with buffer 1 : 10 (—·—·—·—), the full bold lines represent thromboplastin times according to B i g g s (= clotting times of the substrate under the influence of normal intrinsic thromboplastin).
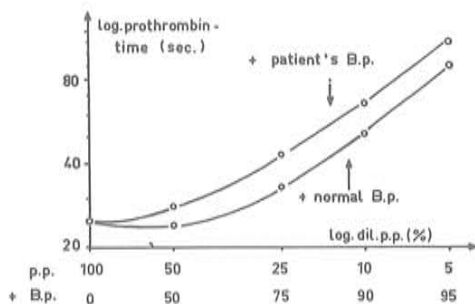
F i g . 8 : Q u i c k ' s one-stage prothrombin times in p. p. and its dilutions in B. p. from a normal subject and in patient's B. p. A striking feature is that the prothrombin time of the 50% dilution in normal B. p. (1 part p. p. and 1 part normal B. p.) is slightly shorter even than the patient's own prothrombin time.

Analogous mixing tests were done with hypoprothrombinaemic plasma and patient's BaSO₄-plasma, in which there was hardly any inhibition (fig. 9).

In order to investigate the influence of the anticoagulant on the amount of prothrombin consumed during and after clotting, BaSO₄-serum from the patient was added to normal blood (fig. 10).
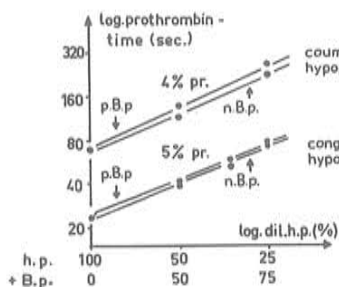

Fig. 9

Fig. 10

F i g . 9 : Test arrangement analogous to that in Fig. 7, but replacing n. p. by hypoprothrombinaemic plasma (h. p.) obtained from a subject with congenital hypoprothrombinaemia on the one hand (5% prothrombin; prothrombin time 23 sec.), and from a patient treated with marcoumar on the other hand (4% prothrombin; prothrombin time 78 sec.). When the h. p. is diluted in the patient's BaSO₄ plasma, the prolongation of the prothrombin time is hardly more marked than in the case of dilution in normal BaSO₄ plasma.
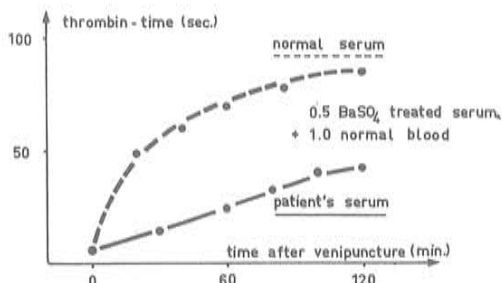
F i g . 1 0 : Prothrombin consumption. Since the patient's blood contained only a small quantity of prothrombin, the influence of the anticoagulant on the prothrombin consumption was determined as follows : 0.5 ml. patient's BaSO₄-serum was added to 1 ml. freshly obtained normal blood of the same blood group. Prothrombin consumption in this mixture was clearly retarded and slightly decreased (full line). The dotted line shows the prothrombin consumption under the influence of normal BaSO₄ serum.

Properties of the anticoagulant: Protamine sulphate did not neutralize the anticoagulant action. After heating to 56° C for 30 min., after dialysis or after

treatment with BaSO₄, the anticoagulant capacity was practically unchanged in plasma as well as in serum. The anticoagulant properties of the serum were localized in the gamma fraction (fig. 11).
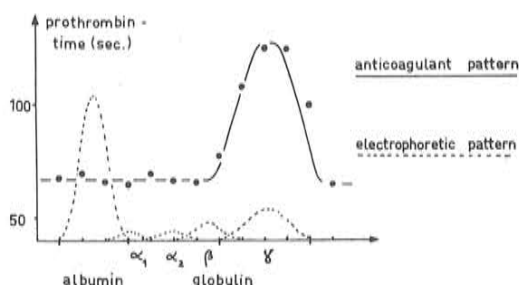

Fig. 11: Localization of the anticoagulant in the serum protein. The anticoagulant was found in the γ-globulins after electrophoretic separation of serum proteins.
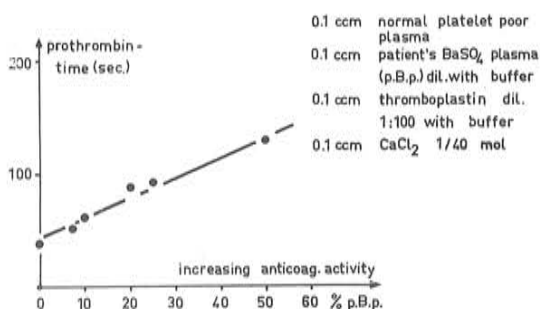
Fig. 12: Measuring the activity of the anticoagulant, we determined the influence of the patient's B. p. on the Quick one-stage prothrombin time of normal plasma, using thromboplastin diluted with buffer (1 : 100). In our case, 20% patient's B. p. caused a 100% increase in prothrombin time (from 42 to 84 sec.).

Quantitative determination of the anticoagulant was done by measuring the effect of varying amounts of patient's BaSO₄-plasma, (plasma diluted with buffer 1 : 2, 1 : 5, 1 : 10, 1 : 20 etc.) on the prothrombin time of normal plasma using thromboplastin diluted to 1 : 100. The prothrombin time of normal plasma to which only buffer had been added was 42 seconds at this dilution of thromboplastin. A curve of the prothrombin time as influenced by the different amounts of the anticoagulant was made, and from this the concentration was calculated at which the prothrombin time was doubled; in our patient this was at a concentration of over 20% (dilution 1 : 5) (fig. 12). The lower this percentage, the more active is the anticoagulant.

D. Discussion

The dermatological diagnosis of L. E. of the skin, has been established since the patient's 28th year. He is now 35 years old. L. E. of the internal organs, however, is not clinically evident. The patient feels perfectly healthy, and the principal functions of the internal organs seem to be normal. Only the varying increase of the ESR, the moderate increase of indirectly reacting bilirubin, the increased urinary urobilin excretion, the weak positive antiglobulin reaction, the *strong positive cephalin-cholesterol flocculation test and the strong positive syphilis reactions* were evidence of S. L. E. The L. E. cell phenomenon was repeatedly negative. We believe we have found another known symptom of S. L. E. in the pathological blood coagulation. S w i f t (26) described a similar case with butterfly-shaped L. E. of the facial skin, of more than 10 years standing, in which besides positive syphilis reactions, a positive cephalin-cholesterol flocculation test and a positive L. E. cell phenomenon, a circulating anticoagulant and thrombocytopenia were found.

Analysis of the coagulation (Table 2) has revealed a *marked deficiency of prothrombin.* The value has decreased from 25% to 16% of normal within one year. Such a deficiency explains the decreased formation of thrombin and the markedly increased activity of antithrombin III in the serum, but does not sufficiently account for the pathology of the thromboelastogram, and certainly not for the degree of increase in the prothrombin time (21). Thus, a further aberration of coagulation must exist, besides the prothrombin deficiency.

According to the standard tests our patient's blood also contained a *circulating anticoagulant:* the patient's plasma caused inhibition of the coagulation time when added to normal plasma. However it was most peculiar that in some cases the coagulation time of the mixture should become even longer than that of the patient's own plasma. This is surprising because the prolonged coagulation time of plasma with a circulating anticoagulant, usually recedes in linear proportion with the increase of dilution in normal plasma. In this respect the action of antithrombin II (heparin), antithrombin V (17) and anti-thromboplastinogen (27) should be born in mind. Therefore, it must be assumed that the *circulating anticoagulant in the patient can be further activated by a factor in normal plasma.* This increased inhibition of coagulation after addition of normal plasma to patient's plasma is not only expressed in thromboelasto-graphy by H a r t e r t' s method (fig. 3), in the thrombin-generation test (fig. 6) and in determination of the prothrombin time by Q u i c k' s method (fig. 4), but also in H i c k s' thromboplastin-generation test (fig. 5).

The most obvious explanation for this phenomenon is found in the assumption that the activity of the circulating anticoagulant is dependent on the

presence of a *factor* of normal plasma, *which is present in patient's plasma in a decreased amount.* Such a factor with activating properties is also called a co-factor; in this case it would be the co-factor of the anticoagulant.

An analogous situation is found with heparin, which can only exert its anti-coagulant action in co-operation with a plasma co-factor (activator).

The decreased prothrombin level in the patient's blood suggests the *hypo-thesis that prothrombin itself may be the postulated co-factor of the anti-coagulant.* According to this hypothesis, the inactive anticoagulant would acquire its anticoagulant properties in the presence of prothrombin, and the activity of the anticoagulant would thus increase with the amount of pro-thrombin available. In the case of our patient, we establish the level of inactive anticoagulant arbitrarily at 100% and that of the co-factor at 16%, according to the prothrombin level found at the time of these experiments. For the sake of simplicity, we assume further that in normal plasma 0% inactive anticoagulant accompanies 100% prothrombin, in other words that there is no anticoagulant activity similar to that of the patient in normal plasma, although it is indeed possible if not probable that normal plasma contains also a certain amount of such anticoagulant activity. According to this reasoning, a mixture of one part patient's plasma and one part normal plasma, results in a ratio of 50% inactive anticoagulant, 58% co-factor (prothrombin). In this proportion, the anticoagulant can of course, be more active than in our patient, where the ratio is 100 : 16%. However, if the patient's plasma is mixed with normal plasma from which the co-factor (prothrombin) has been removed by adsorption to $BaSO_4$, the mixture contains 50% inactive anticoagulant, but only 8% co-factor; the anticoagulative action in this ratio would be weaker than in our patient.

This reasoning is the basis of our consideration about the results of our mixing tests. In the interpretation of the results, it should be taken into account that every decrease of prothrombin in itself increases the prothrombin (or coagulation) time.

Fig. 7 shows that when normal plasma (0% inactive anticoagulant and 100% co-factor) was diluted with patient's $BaSO_4$-plasma (100% inactive anti-coagulant and 0% co-factor), a highly progressive inhibition of the coagulation process (prolongation of the prothrombin time) appeared. The inhibition is much more pronounced when the determinations are made with diluted thrombo-plastin. The maximum inhibition is reached at the mixing ratio of about 1 : 1 (50% inactive anticoagulant and 58% co-factor).

Fig. 8 shows the effect of dilution of patient's plasma (100% inactive anti-coagulant and 16% co-factor) with patients $BaSO_4$-plasma (100% inactive anticoagulant and 0% co-factor) in curve A, and that of patient's plasma with

normal BaSO4-plasma (0% inactive anticoagulant and 0% co-factor) in curve B. Curve A demonstrates that the coagulability remained initially unchanged with a progressive dilution of the co-factor from 16% to 8%. This constancy of the prothrombin time is, on the one hand the result of the reduced action of the anticoagulant due to diminution of the co-factor (prothrombin) which leads to shortening of the prothrombin time, and, on the other, a consequence of the decrease in prothrombin, which prolongs the prothrombin time. If the inactive anticoagulant is diluted together with the co-factor, as in dilution of patient's plasma in normal BaSO4-plasma (curve B) the prothrombin time in the 1 : 2 dilution may even be reduced by 1—2 sec.; the anticoagulative action is then not only diminished by the decrease of the co-factor, but also by the decrease of the inactive anticoagulant, which more than compensates the prolongation of the prothrombin time through a lowered prothrombin level.

Fig. 9 demonstrates the results in a experiment analogous to the preceding one. However, instead of normal plasma, hypoprothrombinaemic plasma was used, which had been obtained from a patient with congenital hypoprothrombin-aemia*) (prothrombin time 23 sec., prothrombin level 5%) and from a patient with distinct hypoprothrombinaemia caused by coumarin (prothrombin time 78 sec., prothrombin level 4%).

In both cases inhibition of the coagulation mechanism was strikingly less distinct or even hardly present, which proves that the anticoagulant was much less active than when normal plasma (prothrombin level 100%) was used in the same test. This is a strong evidence that prothrombin is identical to the co-factor of the anticoagulant.

Fig. 4 shows the results achieved in the thromboplastin-generation test according to H i c k s ; in this test there is co-factor (prothrombin) in the system, as the plasma is not treated with BaSO4. When the test was only applied to patient's plasma, the formation of thromboplastin was hardly impaired (small amount of anticoagulant, which moreover, was diluted). In mixing experiments, a considerable inhibition was observed with a maximum after the addition of 60% normal plasma (60% co-factor). In B i g g s ' test, the inhibition was practically nil also in mixing experiments since the incubation mixture contained hardly any prothrombin (co-factor); in B i g g s ' test the plasma is treated with BaSO4, and the serum prothrombin level was less than 0.2%. In the incubation mixture the inactive anticoagulant is diluted to such a degree that after the addition of normal substrate (100% co-factor), the coagulation time of the substrate is hardly increased.

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*) We are very grateful to our collegue P. G. Hoorweg, specialist in internal medicine in Amsterdam, for supplying plasma from a patient with congenital hypoprothrombinaemia (21).

After determining the correlation between anticoagulative action and the prothrombin level, we studied again the *data from the literature on L. E. patients with a circulating anticoagulant and hypoprothrombinaemia.*

First, it may be worth mentioning that H i c k s himself, in the publication of his screening test, mentions a case of L. E. with a circulating anticoagulant and hypoprothrombinaemia (17% prothrombin), in which an undisturbed formation of thromboplastin was observed in the original thromboplastin-generation test according to B i g g s , as well as in his screening test. H i c k s did not carry out mixing tests (9).

B o n n i n and co-workers (3) R a m o t and S i n g e r (23) and other authors (14), also found normal thromboplastin formation with the aid of B i g g s ' thromboplastin-generation test. However, they did not conclude from this that no anticoagulative action was possible in a system containing minimal amounts of prothrombin, but thought the anticoagulant did not retard the formation of thromboplastin.

In the two cases recently published by L a u r e l l and N i l s s o n (12), the thromboplastin-generation test according to B i g g s revealed clearly pathological results also with normal substrate. In these cases, however, in which besides almost normal plasma prothrombin levels there was a marked anticoagulative activity, the prothrombin consumption possibly was diminished, similar to the cases 1 and 2 published by F r i c k (6). The high level of residual serum prothrombin would then be responsible for the inhibition of thromboplastin formation in the thromboplastin-generation test according to B i g g s .

It appeared that several other observations mentioned in the literature were in agreement with our postulate of the identity of the co-factor with prothrombin. This is clear for example from the results reported in 1955 by F r i c k (6). In his case 1, in which a definite hypoprothrombinaemia exists besides the circulating anticoagulant, the recalcification time of normal plasma is increased to nearly that of patient's plasma by the addition of only 5% patient's plasma. Such a prolongation can hardly be attributed to the anticoagulative activity of patient's plasma alone, since normally an anticoagulant loses much of its activity in the dilution of 1/20. Considered the much higher co-factor activity of the mixture ($>$ 95% instead of $<$ 20% as in the patient) the anticoagulative activity may, however, be much higher than 1/20 of that of the patient alone, what would explain the prolongation described by F r i c k . The two other cases of F r i c k and those of S w i f t (26) and of B o n n i n and co-workers (3), all with marked anticoagulative activity, showed only a slight prothrombin (co-factor) deficiency; an intensified inhibition in mixing tests was less manifest.

In the literature, besides cases with marked anticoagulative activity and varying prothrombin deficiency, cases also occur without anticoagulant, but

with marked hypoprothrombinaemia. The cases 2 and 3 of F r i c k (6), the two patients of S w i f t (26) and the patients of C o n l e y and H a r t m a n (4), B a r k h a n (1) and L a u r e l l and N i l s s o n (12) show hardly any decrease of prothrombin, whereas L e y and co-workers (15), in their patients, describe a nearly complete prothrombin deficiency without a definite anticoagulant activity. Case one of F r i c k (6), our case and those of B o n n i n and co-workers (3) and H i c k s (9) reveal different amounts of circulating anticoagulant with more or less pronounced hypoprothrombinaemia.

In order to comprehend this rather striking variability of the clotting disorder, other considerations are necessary, first of all concerning the *site of action of the anticoagulant* in the coagulation mechanism.

In the intravascular coagulation process the anticoagulant mainly causes inhibition of the rate of formation of thromboplastin and only as a secondary result, a decrease of the amount of thromboplastin formed. This can be concluded from fig. 5. The course of the prothrombin consumption in fig. 10 supports this view.

In the thrombin-generation test (fig. 6) the pathology of thrombin formation in mixed plasma with decreased number of thrombocytes, increases out of proportion; curve B in fig. 6 strongly resembles that of severe thrombocytopenia. One is therefore inclined to regard the anticoagulant as an antagonist to *thrombocyte factor 3*. Fig. 7 demonstrates that the anticoagulant also impedes the activity of the *intrinsic thromboplastin* formed as shown previously by R a m o t and S i n g e r (23) and L a u r e l l and N i l s s o n (12).

Another point of attack of the anticoagulant is the *tissue thromboplastin*. In coagulation tests with diluted tissue thromboplastin this action was very clearly seen (fig. 4 and 7).

Concerning the chemical properties of the inactive anticoagulant and of the inhibited substance, we only know that the former is found in the gamma globulins and possesses some properties of them, while the latter, very probably occurring in thrombocytes, intrinsic thromboplastin and tissue thromboplastin, seems to be a substance from the large group of *phospholipids*.

In order to comprehend the variability of the clotting disorder, it is further necessary to put forward different possibilities in the *pathogenesis of the hypoprothrombinaemia*.

A selective disorder of the production of prothrombin in the liver cell is most improbable, as all known acquired disturbances of the liver cell metabolism, do not cause prothrombin deficiency alone, but a more or less proportional decrease also of factors VII, IX and *Stuart-Prower*-factor. An intensified *peripheral breakdown* of prothrombin is much more probable. This assumption,

however, leads to similar difficulties. Experience has taught that an intensified peripheral breakdown of prothrombin is usually accompanied by an intensified breakdown of other coagulation factors. This applies, e.g. to increased fibrinolysis and fibrinogenolysis, which may be accompanied by prothrombinolysis, as well as to the prothrombin deficiency resulting from pathologically increased intravascular coagulation, in which a marked decrease of fibrinogen is constantly observed. Moreover, patients with such syndromes are usually seriously ill. Since neither one nor the other symptom was present in our patient, and his hypoprothrombinaemia is moreover selective, another process, one specifically attacking the prothrombin, must be responsible. The assumption that, in vivo *prothrombin is consumed in the* (immunological?) *reaction between inactive anticoagulant* (γ-globulin; antibody?) and *plasma phospholipid* (antigen?) *independent on the coagulation mechanism* may be the key to the solution of the problem of the acquired selective prothrombin deficiency in our patient as well as in other L. E. patients. We assume, furthermore, that the amount of circulating inactive anticoagulant as well as the amount of circulating phospholipid (most probably deriving from thrombocytes, and possibly from erythrocytes and endothelial cells), varies considerably in any individual case.

The reaction in vivo can be represented schematically as follows:
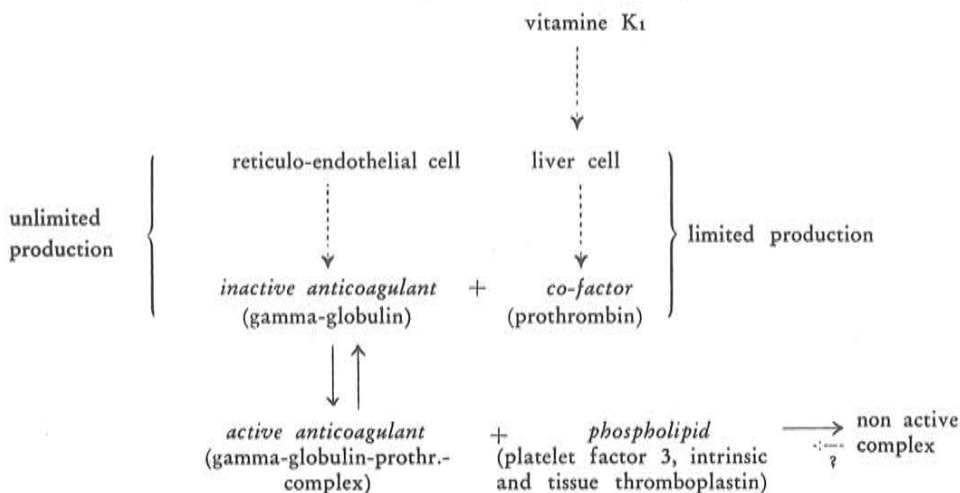
In the diagram it is assumed, that there is, on the one hand, a well equilibrated reaction between circulating inactive anticoagulant, prothrombin and the active anticoagulant (gamma globulin-prothrombin-complex) but that, on the other hand, the reaction between the active anticoagulant and phospholipid is

strongly shifted towards the reaction product (which possibly will be eliminated from circulation). Thus, with an increasing amount of circulating phospholipid (through accelerated cell-destruction?) increasing amounts of active anticoagulant are consumed; the balance between the active anticoagulant, inactive anticoagulant and prothrombin is shifted in favour of the active anticoagulant; prothrombin is consumed. As the production of prothrombin in the liver cell is most probably limited, the prothrombin level of the blood, in consequence, will decrease. The amount of inactive anticoagulant (gamma-globulin), however, depends wholly on the (unlimited) production intensity of the reticulo-endothelial system. In presence of sufficient inactive anticoagulant, the decrease of prothrombin then is a measure of the amount of circulating phospholipids.

On the basis of this line of thought, the great individual variability in amount of circulating anticoagulant and prothrombin observed in the literature can be explained. When there is much inactive anticoagulant and little circulating phospholipid, consequently little consumption of prothrombin, a marked anticoagulative activity is found in the presence of a fairly normal prothrombin level, and, reversely, when there is little production of inactive anticoagulant and much circulating phospholipid, practically no anticoagulant and a more or less marked loss of prothrombin may be found due to the constant (complete) consumption of the active anticoagulant.

Finally, some *nosological* remarks: the coagulation disorder in patients with systemic L. E. is probably one of the symptoms of an *immune reaction,* in which two factors, important for the normal coagulation process, viz. phospholipid and prothrombin, are concerned. The immunological defence mechanism may be aimed *against products of an increased breakdown of thrombocytes, erythrocytes and vascular endothelial cells, and especially against phospholipids.*

A comparative study of patients in whom hypocoagulability due to a circulating anticoagulant (antithromboplastin) develops in reaction to encephalomalacia (?), cardiac infarction and pulmonary embolism (11) would be interesting. If the circulating anticoagulant in these cases too would only be active in combination with prothrombin, then coumarin therapy, causing lowering of the prothrombin level, would reduce the activity of the circulating anticoagulant; in other words, it would have a paradoxical effect in so far, as the intravascular coagulability would not diminish proportionally to the decrease in prothrombin.

The improvement in the coagulation disturbance after administration of large doses of vitamin K_1, as described by L e y and co-workers, remains obscure (15). Our patient is not yet treated with vitamin K_1.

We hope our paper may be a contribution to a better insight into the coagulation disorders in L. E. and may also lay a basis for further research in this field.

Summary

A 35-year old man with lupus erythematosus of the skin is demonstrated, who also shows signs of systemic L. E.: sometimes slightly increased ESR, varying thrombocytopenia, slightly prolonged coagulation and prothrombin time, positive cephalin-cholesterol flocculation test, biologically false positive syphilis reactions and signs of a slightly increased haemolysis. Electrophoretic examination of the serum proteins presents practically normal values, certainly no hypergammaglobulinaemia. Analytical examination of coagulation reveals hypoprothrombinaemia and an anticoagulant most probably directed against thrombocyte factor 3, intrinsic thromboplastin and tissue thromboplastin. The anticoagulant seems to be only active in the presence of prothrombin. Prothrombin therefore, is considered a co-factor of the anticoagulant. The inactive anticoagulant is localized in the gamma globulins. The possible cause of the hypoprothrombinaemia (immune reaction?) and the data in the literature are discussed.

Résumé

Description d'un cas de lupus erythémateux de la peau présentant simultanément des symptômes d'un lupus erythémateux disséminé (sédimentation des globules rouges légèrement accélérée, thrombopénie variable, temps de prothrombine légèrement allongé, test de flocculation à la céphaline-cholesterine pathologique, test sérologique de la syphilis positif et symptômes d'une légère hémolyse). L'électrophorèse des protéines sériques donne un résultat à peu près normal, en particulier pas d'hypergammaglobulinémie. L'examen de la coagulation sanguine révèle une hypoprothrombinémie et un anticoagulant probablement dirigé contre le facteur 3 plaquettaire, la thromboplastine plasmatique et la thromboplastine tissulaire. Cet anticoagulant ne paraît être actif qu'en présence de prothrombine qui est considérée comme son cofacteur. L'anticoagulant inactif est localisé dans les globulines gamma. Les causes possibles de l'hypoprothrombinémie (réaction immunologique?) sont discutées.

Zusammenfassung

Es wird ein 35jähriger Mann mit Lupus erythematodes der Haut beschrieben, der auch Zeichen eines System-L. E. aufweist: zeitweise leicht gesteigerte Blutsenkung, wechselnde Thrombopenie, etwas verlängerte Gerinnungs- und Prothrombinzeit, positive Kephalin-Cholesterol Flockungsreaktion, falsch posi-

tive Syphilisreaktionen und Zeichen leicht gesteigerter Hämolyse. Elektrophoretische Untersuchung der Serumeiweißkörper ergibt praktisch normale Werte, insbesondere keine Hypergammaglobulinämie. Die Untersuchung der Blutgerinnung ergibt neben einer Hypoprothrombinämie ein Antikoagulans, das höchstwahrscheinlich gegen Thrombozytenfaktor 3, Blut- und Gewebethrombokinase gerichtet ist. Es ist anscheinend nur in Gegenwart von Prothrombin aktiv. Es wird daher angenommen, daß Prothrombin ein Cofaktor des Antikoagulans ist. Das inaktive Antikoagulans findet sich in der Gammaglobulinfraktion. Die mögliche Ursache der Hypoprothrombinämie (Immunreaktion?) wird diskutiert.

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