E. KOWALSKI, Z. LATAŁŁO, S. NIEWIAROWSKI

THE FUNCTION OF FACTOR VII IN BLOOD COAGULATION AND HEMOSTASIS

Laboratory of Clinical Biochemistry, Institute of Hematology, Warsaw, Poland

INTRODUCTION

Progress in modern coagulation research is intimately connected with examination of the coagulation system of patients with hemorrhagic diathesis. The abnormal findings in the blood plasma of these patients allow conclusions to be drawn as to the existence of various blood clotting components and their interactions, and the building up of the complicated coagulation scheme. From this point of view we examined a patient with Factor VII deficiency to clear up the function of this Factor in physiological blood clotting.

CASE REPORT

H. D., a 34 year old male had bleedings from early childhood. In 4th year of life he bled several weeks after tooth extraction. In 1955 he suffered severe bleedings from the alimentary tract. In 1956 and 1957 he had macroscopical hematuria. During his whole life he has suffered from gum bleedings and prolonged oozing after skin cuts. No other important anamnestic data could be obtained. The physical examination of the patient revealed nothing important besides clubbing of the fingers. The liver and spleen were not palpable. Radiological and routine laboratory examinations including liver function tests showed no abnormality. Only the albumin globulin ratio was decreased. (Albumin 39%, alfa$_1$ 7,7%, alfa$_2$ 9,2%, beta 16,2%, fibrinogen 10,6%, gamma globulin 16,9%) *. Total protein 7,2%.

FAMILY HISTORY

There is no family history of abnormal bleeding except that of his sister who suffered bleeding from the alimentary tract. Unfortunately we had no opportunity to examine exactly the blood clotting system of the patient's family.

Examination of blood clotting system and hemostasis.

Capillary fragility (Rumpel-Leede) — negative.

* Tiselius apparatus.

Platelets (Fonio) — 233.000.
Bleeding time (Duke) — 1 min 20 sec. — 6 min (normal 2—4 min).
Clot resistance test (*Copley* 9) — positive.
Clot retraction — normal.
Clotting time (Lee — White) normal.
Plasma recalcination time: 82 sec. — 134 sec. (normal 60—180 sec.).
Thrombin time of oxalated plasma — 5,5 sec. (normal 6,5 sec.).
One stage prothrombin time using rabbit brain thromboplastin (*Quick* 30) * 48—113 sec. (normal 13—16 sec.).

Factor V (modification of Wolf's method 39) 20—28 sec. (normal range 20—28 sec.).
Factor VII in plasma (*Koller* et al. 18) — 55—110 sec. (normal range 25—35 sec.) The Factor VII level is approximately 8% normal.
Factor VII in serum — 51 sec. (normal 20 sec.).
Two stage prothrombin (*Leonow* 22) — 20 units.
Two stage prothrombin modificated by adding purified Factor VII** to the incubation mixture — 324 units (normal 220—350 units).
Prothrombin consumption test (*Quick* 30) — Serum prothrombin time, tested with brain tromboplastin, calcium chloride and bovine deprothrombinized plasma, was significantly prolonged.

1 hour serum	—	126 sec.	(normal	20—30 sec.)
3 ,, ,,	—	115 sec.	,,	30—40 sec.)
24 ,, ,,	—	172 sec.	,,	above 50 sec.)

Fibrinogen level (*Quick* 30) 2,6—5,64 mg/ml — slightly elevated fibrinolysis in whole plasma (*Kopeć* and *Niewiarowski* 19) — sometimes enhanced.
Fibrinolysis in plasma euglobulins (*Kowarzyk* and *Buluk* 21) — normal.
Antiplasmin (*Niewiarowska* 26) — normal.
Plasminogen (*Kowalski et al* 20) — normal.
Correction of the one-stage prothrombin time of patient's plasma:

Plasma	Prothrombin time:
Patient's plasma	69 sec.
Normal plasma	15 sec.
Patient's plasma + Normal plasma 1:1	15,5 sec.
Patient's plasma + Normal plasma 1: 5	19,2 sec.
Patient's plasma + Normal plasma 1 : 10	64 sec.

* Brain thromboplastin — outdated antirabbic vaccin (mfd by Warszawska Wytwórnia Surowic i Szczepionek, Warsaw) was used as a source of thromboplastin. This preparation does not contain inhibitor as tested at various dilutions with normal plasma.

** Factor VII was purified according to Wagner et al. (38). This preparation contained only negligible amounts of prothrombin.

Patient's plasma +
Normal serum 1:1 23 sec.
Patient's plasma +.
Plasma of patient receiving dicoumarol 1:1 62,5 sec.

Results of thromboplastin generation test performed by the method of Duckert et al. (11) are shown in table I. This test performed with all isolated components of the first coagulation stage of the patient's plasma is normal, as well as that with mixed single components. The clotting of normal and patient's plasma with its own thromboplastin is normal.

Table I

Thromboplastin generation in Factor VII deficient plasma from patient H. D.

Date	Thromboplastic generation mixture of			Source of substrate plasma	Incubation time of T.G. mixt. min.				Prothrombin time of substrate plasma with brain thrombopl.	
	Serum	$BaSO_4$ plasma	Platelets		2	4	6	8	N	HD
					Clotting time of substrate plasma (sec)					
19.8	N	N	N	N	49	24	15	18,5	16	78
	N	N	N	HD	33	16,2	13,5	14,5		
	HD	HD	N	N	43	19	14	15		
	HD	HD	N	HD	19	11	11,5	15		
23.8	N	N	N	HD	34	15	13	14	16	107
	N	N	N	N	33	13	15	—		
	HD	HD	N	HD	23	18	11	13		
	HD	HD	N	N	38	17	13	13		
	HD	HD	HD	N	25	10,5	13	13		
	N	N	HD	N	12	12	10	13		
30.8	N	N	N	N	35	16	15	17	16	87
	HD	HD	HD	HD	20,5	14	11	12		
	HD	HD	NN	N	39	18	15	16		
	HD	HD	N	HD	21	13,5	11	12,5		

N = normal HD = patient

It can be seen from table II that Vitamin K has no influence on the clotting anomaly. Blood transfusion partially corrects it. Interactions of patient's serum and serum fractions containing Factor VII with brain thromboplastin: see table III. Old patient's serum was incubated for 10 minutes with brain thromboplastin and calcium ions. At various time intervals bovine, Seitz filtered plasma (devoid of Factor VII) was added. It can be seen that the clotting time in the mixture is significantly longer than the clotting time in the control system containing normal serum. During the incubation of thromboplastin and serum the clotting time was shortened. For further investigation of this phenomena patient's serum fractions containing Factor VII were obtained by adsorbing old serum on

$BaSO_4$. The precipitate of $BaSO_4$ was eluted with sodium citrate and the eluate dialysed against tap water for 24 hours. By this procedures the minimal amounts of Factor VII contained in patient's plasma were concentrated. In experimental conditions analogous to those with serum, similar results were obtained.

Table II

Influence of blood transfusion and Vitamin K on the one-stage prothrombin time and factor VII of the patient

	One stage prothrombin time (sec.)	Factor VII (sec.)
Before transfusion	110	105
15 min. after transfusion of blood (250 ml)	44	80
Before Kavitan (Menadione)	83	56,4
2 h. after Kavitan (50 mg)	68	53,8
Before K_1	85	90
2 h. after K_1 (50 mg)	113	108

Table III

Interaction of Factor VII with brain thromboplastin

Incubation mixture	Incubation times of mixture (min.)													
	0		1		2		4		6		8		10	
	Clotting times of substrate plasma with incubation mixture (sec)													
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
I. Thromboplastin + Ca^{++} + serum of H. D.	60	—	34	—	25	100	31	—	—	—	33	—	31	—
Control	21	60	13	—	14	55	12	63	13	75	—	—	14	—
II. Thromboplastin + Ca^{++} + S. F. c. F. VII of H. D.	51	105	31	—	21	90	19,2	90	16,8	90	15,5	—	16,8	100
Control	20	50	11	—	10	45	12	50	12	50	9	—	—	60

A-Bovine Seitz filtered plasma (devoid of factor VII) was used as substrate

B-Bovine $BaSO_4$ adsorbed plasma (devoid of prothrombin and Factor VII) was used as substrate

S. F. c. F. VII-serum fraction containing Factor VII.

DISCUSSION

The examination of the blood coagulation system of this patient allows the conclusion that the basic coagulation defect consists of Factor VII deficiency. This conclusion can be drawn from the prolonged one-stage prothrombin time with normal prothrombin tested by the modified two-stage method, and with normal Factor V level; it can be proved by estimation of Factor VII level according to the method of *Koller* et al. (18) and by correction tests.

There are many cases previously described in the literature as Factor VII deficiency. (1, 2, 4—8, 10, 12—14, 23—25, 27, 31, 33—35, 37, 40). However these cases are not homogenous. In the last years it has been possible to separate from them a new clinical entity, the Stuart Factor deficiency (3, 15, 32) as well as Prower Factor deficiency (36). They differ from typical Factor VII deficiency by abnormal thromboplastin generation, which may be corrected by normal and hemophilia B serum. In view of these findings our case belongs rather to the typical Factor VII deficiency group. (1, 2, 13, 16, 17).

It is of interest to note that the majority of cases published previously in the literature as Factor VII deficiency showed some disturbances in blood clotting time and thromboplastin generation, in other cases the thromboplastin generation was not performed. The diagnosis of these conditions should be reestablished.

It is of interest to note the other abnormalities of coagulation and protein system in patient's blood. The fibrinolysis is enhanced and the albumin/globulin ratio is decreased. In this connection the clubbing of the fingers may be of interest. For further considerations in this context these findings are not important, therefore they will not be discussed.

Our interest in the coagulation system of the patient's blood was connected with an almost complete lack of Factor VII. This gave us the possibility of studying the function of Factor VII in normal blood coagulation. The finding of normal result of thromboplastin generation test using all components of the patient's blood as well as single components mixed with adequate normal components of the first coagulation stage allowed the conclusion that Factor VII is not needed for normal thromboplastin generation. Patient's plasma clots in normal time with its own thromboplastin. That means that for intravascular clotting Factor VII is not needed. This statement is in agreement with the view of *Ackroyd*, (1) and *Jürgens* (16) who came to the same conclusions by similar reasoning.

The real role of Factor VII in blood clotting becomes evident from the experiment with brain thromboplastin. From this experiment it can be seen that Factor VII is necessary for adequate thrombin generation with the aid of tissue thromboplastin. This means that it is necessary for "tissue clotting", and bleeding in patients with Factor VII deficiency may by caused by inadequate "tissue hemostasis". The differentiation of "tissue and intravascular hemostasis" may be helpful in better understanding of hemorrhagic and thrombotic phenomena. These contradictory phenomena sometimes occur together in the same patient e. g. during anticoagulant therapy and are difficult to interprete. More exact knowledge of the different blood clotting mechanismus is needed for a more adequate approach and treatment of these cases.

Seegers in his original approach to coagulation research came to similar conclusions. Autoprothrombin I which is derived from prothrombin

may be identical with Factor VII. *Seegers* and his collaborators (28, 29) found, that autoprothrombin I accelerates only lung thromboplastin clotting but no other thrombin generation may be catalysed by autoprothrombin I. However in the present state of knowledge it is not possible to clear up the problem of how far autoprothrombin I is identical with Factor VII or with Stuart Factor.

Note added in proof. Prothrombin time of patient plasma measured with Russer viper venom (supplied kindly by Borrough and Wellcome, London) was normal.

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Otrzymano dnia 17.II.1958 r.