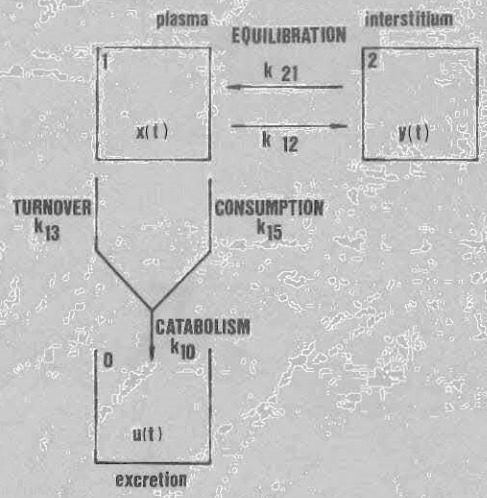
UNIVERSITY OF LEUVEN, BELGIUM
FACULTY OF MEDICINE
DEPARTMENT OF MEDICAL RESEARCH
LABORATORY OF BLOOD COAGULATION

PLASMINOGEN AND PROTHROMBIN METABOLISM IN MAN



Désiré COLLEN
Aangesteld navorser N.F.W.O.

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
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Detection and quantitation of in vivo coagulation and/or fibrinolysis

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NOTICE

The format of this monograph, which is not an official publication, complies with the "aggregaatsthesis" requirements in use at the University of Leuven, Belgium. The contents are based on investigations in which the author has taken the initiative but which have been carried out in collaboration with several other people. Therefore, reference to material contained in this monograph should be made by means of the following papers or abstracts : (published or submitted before 1 June 1, 1974) :

COLLEN, D., ONG, E.B. and JOHNSON, A.J. – Human plasminogen : in vitro and in vivo evidence for the biological integrity of NH_2-terminal glutamic acid plasminogen. (Submitted).

COLLEN, D., TYTGAT, G., CLAEYS, H., VERSTRAETE, M. and WALLEN, P. (1972) – Metabolism of plasminogen in healthy subjects. Effect of tranexamic acid. *J. Clin. Invest., 51,* 1310.

ROUVIER, J., COLLEN, D., SWART, A.C.W. and VERSTRAETE, M. – Prothrombin metabolism in healthy subjects and in two patients with congenital hypoprothrombinemia. *Proc. Boerhaave course on prothrombin, held at Leiden, The Netherlands on May 10-11, 1974.* (In press).

COLLEN, D. and VERMYLEN, J. (1973) – Metabolism of iodine-labeled plasminogen during streptokinase and reptilase therapy in man. *Thromb. Res., 2,* 239.

COLLEN, D., ROUVIER, J. and VERSTRAETE, M. (1972) – Metabolism of iodine labeled plasminogen and prothrombin in cirrhosis of the liver. *Clin. Res., 20,* 483 (Abstract).

COLLEN, D., TRICOT, J.P., SEMERARO, N. and VERMYLEN, J. – Turnover of labeled fibrinogen, plasminogen and prothrombin during physical exercise in man. (Submitted as abstract).

COLLEN, D. and VERSTRAETE, M. (1971) – Metabolism of plasminogen in clinical conditions with activation of the fibrinolytic system. *II Cong. Int. Soc. Thrombosis Haemostasis, Oslo, Norway.* Abstr. vol. p. 116 (Abstract).

COLLEN, D. (1973) – Plasminogen turnover studies in man. *IV Cong. Int. Soc. Thrombosis Haemostasis, Vienna, Austria.* Abstr. vol. p. 118 (Abstract).

COLLEN, D., ROUVIER, J., SWART, A.D.W. and VERSTRAETE, M. – Labeled prothrombin metabolism in man. *Proc. Boerhaave course on prothrombin, held at Leiden, The Netherlands on May 10-11, 1974.* (In press) (Abstract).

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LIST OF ABBREVIATIONS

ACD	acid-citrate-dextrose
ADP	Adenosine diphosphate
APTT	activated partial thromboplastin time
$A^{1\%}_{280\ nm}$	absorbancy at 280 nm of a 10 mg/ml protein solution
cpm	counts per minute
CTA	Committee on Thrombolytic agents
DEAE	Diethylaminoethyl
EACA	epsilon aminocaproic acid
E.F.A.	euglobulin fibrinolytic activity
FDP	fibrin(ogen) degradation products
FPT-test	Fibrin polymerization time test
IU	International Units (streptokinase)
KIU	Kallikrein inhibitor units
MDH	Michigan Department of Health
MW	molecular weight
NIH	National Institutes of Health
p-NPGB	para-nitrophenyl-para-guanidinobenzoate
O.D.	optical density
p	probability
SD	standard deviation
SDS	sodium dodecyl sulphate
t 1/2	half-life
TCA	trichloroacetic acid
t.i.d.	ter in die
tris	trishydroxymethylaminomethane
UK	urokinase
U.S.P.	United States Pharmacopeia

INTRODUCTION

The normal kinetics of in vivo coagulation and fibrinolysis, their interrelation and importance in the formation and removal of fibrin in the body, are as yet poorly understood.

Turnover studies with labeled tracers of components which are specifically consumed during activation of either system, might be well suited to the investigation of the dynamics of in vivo coagulation and fibrinolysis in physiological and pathological conditions in man. Such investigations however require the availability of highly purified, undenatured and functionally intact labeled tracers. Moreover, experimental conditions have to be used in which consumption in the different catabolic pathways can be differentiated from basic protein turnover.

Until recently only fibrinogen, which is a substrate for both thrombin and plasmin, was available for turnover studies. We have investigated its metabolism in normal subjects and in some pathological conditions and found this approach to be very sensitive for the quantitation of fibrinogen consumption, even in the presence of normal plasma fibrinogen levels (1-3).

A more direct evaluation of the kinetics of in vivo coagulation and fibrinolysis might be obtained from turnover studies of prothrombin, the final proenzyme of the coagulation cascade and of plasminogen, the fibrinolytic proenzyme.

The aim of the present study was to prepare and characterize valid labeled metabolic tracers of prothrombin and plasminogen and to evaluate the kinetics of their synthesis and breakdown in healthy subjects and in some experimental and clinical conditions with abnormal turnover of different origin.

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2. TYTGAT, G., COLLEN, D. and VERMYLEN, J. (1972) – Metabolism and distribution of fibrinogen. II. Fibrinogen turnover in polycythaemia, thrombocytosis, haemophilia A, congenital afibrinogenaemia and during streptokinase therapy. *Brit. J. Haemat., 22,* 701.
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Chapter I

PREPARATION AND CHARACTERIZATION OF LABELED PLASMINOGEN AND PROTHROMBIN

Section I :

PREPARATION, CHARACTERIZATION AND TURNOVER CHARACTERISTICS OF PLASMINOGEN PREPARATIONS WITH NH_2-TERMINAL GLUTAMIC ACID AND WITH NH_2-TERMINAL LYSINE*

Studies using labeled plasminogen as a tracer of plasminogen metabolism in humans require highly purified labeled preparations which are physicochemically and metabolically indistinguishable from the endogenous molecules. When we started our studies, two types of human plasminogen with markedly different physicochemical properties, were described in the literature or known to us from personal communications. Robbins *et al.* (1-3) obtained a plasminogen preparation from Cohn fraction III, which consisted of multiple molecular forms with isoelectric points from pH 6.4 to 8.5, all with NH_2-terminal lysine and a molecular weight of approximately 85,000. Wallén and Wiman (4, 5) obtained multiple plasminogen forms with isoelectric points from pH 6.0 to 6.6 and NH_2-terminal glutamic acid, which had a similar electrophoretic mobility on starch-gel electrophoresis to that of plasminogen in plasma. These authors suggested that NH_2-terminal glutamic acid plasminogen corresponded to the circulating molecular forms and that NH_2-terminal lysine forms were "probably derived from the native molecules by partial proteolytic degradation".

Since the preparations of Robbins *et al.* and of Wallén and Wiman were both obtained in low yield by a time-consuming multiple-step procedure, it was not clear if both types of plasminogen represented selected native molecules, or if one type represented partially degraded derivatives of the other which appeared during the

* The information in this section will be reported and discussed in more detail in a forthcoming paper :
D. COLLEN, E.B. ONG and A.J. JOHNSON : Human plasminogen : in vitro and in vivo evidence for the biological integrity of NH_2-terminal glutamic acid plasminogen (Submitted).

purification procedure. Therefore, we studied the turnover characteristics of both types of plasminogen in a small series of healthy subjects. The finding of markedly different catabolic rates placed us in a position of doubt concerning the metabolic characteristics of native human plasminogen.

In 1970, Deutsch and Mertz (6) published their affinity chromatography procedure for the isolation of human plasminogen. This high yield single step purification method enabled us to reinvestigate the problem of the physicochemical characteristics of native plasminogen. This study was carried out in the laboratory of Dr. A.J. Johnson in New York in co-operation with Dr. E.B. Ong. By using some minor modifications (e.g. addition of the plasmin inhibitor Trasylol to prevent proteolytic degradation during purification) of the affinity chromatography method, plasminogen was obtained from fresh frozen plasma with greater than 90 % yield and about 90 % purity (7, 9). The metabolic properties of this plasminogen preparation, before and after further purification by gel filtration and DEAE-Sephadex chromatography, were studied in an effort to resolve the problem of the metabolic properties of native human plasminogen.

MATERIALS AND METHODS

Preparation of plasminogen

The biological homogeneity and metabolic characteristics of six plasminogen preparations, obtained by ion exchange or affinity chromatography using buffers prepared with pyrogen-free water, were studied in normal volunteers. For convenience they are designated as follows. Plasminogen-W was the DE-A plasminogen prepared from Cohn fraction III or from fresh frozen plasma, according to Wallén and Wiman (4, 5). Plasminogen-R was prepared from Cohn fraction III by the method of Robbins *et al.* (8). Plasminogen AC-FFP was prepared by affinity chromatography from fresh frozen plasma as described elsewhere (7, 9). Plasminogen AC-FFP, DE-A was the main protein peak obtained by Sephadex G-150 gel filtration and DEAE-Sephadex chromatography (5) of plasminogen, prepared by affinity chromatography of fresh frozen plasma. Plasminogen AC-CF III, DE-A was the first peak and plasminogen AC-CF III, DE-B the second peak eluted on DEAE-Sephadex chromatography (5) of plasminogen, prepared by affinity chromatography of Cohn fraction III. Plasminogen-A was prepared from fresh frozen plasma by the method of Abiko *et al.* (10), without the use of specific solubilizing agents such as lysine or EACA.

The specific activities of the preparations were in the range of 21-25 CTA units per mg protein. Plasminogen AC-FFP, plasminogen AC-FFP, DE-A, plasminogen-W and plasminogen-A had less than 0.5 % (and usually less than 0.1 %) spontaneous proteolytic activity as compared with the total activatable plasminogen, and plasminogen-R had about 2 % spontaneous activity.

Physicochemical properties of the purified plasminogen preparations

Cellulose acetate gel electrophoresis, performed as described by Claeys *et al.* (11), revealed that plasminogen-W, plasminogen AC-FFP, plasminogen AC-FFP, DE-A and plasminogen AC-CF III, DE-A moved as beta-globulins whereas plasminogen-R and plasminogen AC-CF III, DE-B had a mobility closer to that of the gamma-globulins.

Isoelectric focusing, performed as described elsewhere (7, 9), showed that plasminogen AC-FFP and plasminogen AC-CF III, DE-A contained multiple molecular forms with isoelectric points between pH 6.1 and 7.1, whereas plasminogen AC-CF III, DE-B consisted of multiple forms with isoelectric points between pH 6.5 and 8.5. The isoelectric points of plasminogen-W, as reported by Wallén and Wiman, range from pH 6.0 to 6.6 and of plasminogen-R, as reported by Robbins *et al.*, from pH 6.7 to 8.5.

SDS-polyacrylamide gel electrophoresis (7, 9) showed one main band for all plasminogen preparations with molecular weights of 90,000 ± 3,500 (plasminogen AC-FFP), 90,000 ± 2,800 (plasminogen AC-CF III, DE-A) and 85,900 ± 2,800 (plasminogen AC-CF III, DE-B). The differences in molecular weight between plasminogen AC-FFP, and plasminogen AC-CF III, DE-A on the one hand and plasminogen AC-CF III, DE-B on the other hand, were statistically significant ($p < 0.01$).

NH_2-terminal amino acid analysis, performed as described elsewhere (7, 9), showed only glutamic acid for plasminogen AC-FFP and plasminogen AC-CF III, DE-A and lysine and valine for plasminogen AC-CF III, DE-B. The NH_2-terminal residue of plasminogen-W is glutamic acid (5) and that of plasminogen-R lysine or valine (1).

Labeling and in vitro evaluation of the purified plasminogen preparations

Labeling was performed with $Na^{131}I$ or $Na^{125}I$, as described in chapter II. The average substitution level was 0.25 atoms of iodine per molecule of plasminogen. The free iodide was less than 2 % of the protein bound radioactivity. No change in specific activity or electrophoretic mobility was observed after labeling.

Gel filtration of mixtures of trace amounts of labeled plasminogen and human plasma on Sephadex G-200 equilibrated with 0.1 M EACA - 0.1 M NaCl - 0.05 M tris buffer pH 9.0, showed elution of radioactivity and enzymatic plasminogen activity at the same volume. This finding indicated that the purification and labeling procedures did not result in detectable changes in the shape of the molecules and that all the label was protein bound. However, other studies (5) have indicated that in the absence of EACA, plasminogen with NH_2-terminal lysine is eluted at a slightly smaller volume than NH_2-terminal glutamic acid plasminogen.

Metabolic studies

Turnover studies of intravenously injected plasminogens were carried out as

follows : ^{125}I-plasminogen AC-FFP and ^{131}I-plasminogen-R simultaneously in one subject (BY), ^{125}I-plasminogen AC-FFP and ^{131}I-plasminogen AC-CF III, DE-B in one subject (DF), ^{131}I-plasminogen AC-FFP, DE-A and ^{125}I-plasminogen-W in two subjects (RV and DM), ^{125}I-plasminogen AC-FFP, DE-A and ^{131}I-plasminogen AC-CF III, DE-B in one subject (DP), ^{131}I-plasminogen-W and ^{125}I-plasminogen-R in two subjects (HC and SL), ^{131}I-plasminogen AC-CF III, DE-B in one subject (GS), ^{125}I-plasminogen AC-FFP in one subject (BR) and ^{125}I-plasminogen-A in three subjects (HW, AI and LI). Results of routine hematologic and liver function tests and a complete coagulation work-up were normal in all subjects.

The procedure for the metabolic studies was as described in chapter II. The plasma radioactivity data versus time, expressed as a fraction of the first sample were plotted on a semilogarithmic scale and fitted with a sum of two exponential terms $x(t) = C_1 e^{-a_1 t} + C_2 e^{-a_2 t}$.

RESULTS

Pertinent clinical and tracer data on the various plasminogen preparations in the normal subjects are summarized in table I. Examples of turnover curves are shown in fig. 1 and fig. 2.

The data indicate that plasminogen preparations with NH_2-terminal glutamic acid, lower isoelectric points, beta-globulin mobility on cellulose acetate, faster anodic mobility on polyacrylamide gel electrophoresis and a higher molecular weight (plasminogen-W, plasminogen AC-FFP, plasminogen AC-FFP, DE-A, plasminogen AC-CF III, DE-A and plasminogen-A) have a half-life in the plasma of 1.9 to 2.6 days, whereas the plasminogen preparations with NH_2-terminal lysine (or valine), higher isoelectric points, gamma-globulin mobility on cellulose acetate, slower anodic mobility on polyacrylamide gel electrophoresis and a lower molecular weight (plasminogen-R, plasminogen AC-CF III, DE-B) have a half-life of less than one day.

The plasma radioactivity disappearance curves obtained with plasminogen AC-FFP gradually levelled off, indicating that this preparation was still contaminated with a small amount of protein with a longer half-life (fig. 1 A). The same phenomenon was observed, though to a lesser extent, with plasminogen AC-FFP, DE-A and AC-CF III, DE-A, if, after affinity chromatography, the samples were equilibrated with the starting buffer of the DEAE-Sephadex chromatography by gel filtration on Sephadex G-25. Inclusion of a gel filtration step on Sephadex G-150 was found necessary to remove a small amount of protein with a longer half-life. Labeled preparations, purified in this way, gave straight semilogarithmic decay curves down to less than 1 % of the initial plasma radioactivity (fig. 1 B).

The level of non-TCA precipitable radioactivity reached a maximum after 1 day of less than 2 % of the total plasma radioactivity for plasminogen-W, plasminogen AC-FFP, plasminogen AC-FFP, DE-A and plasminogen AC-CF III, DE-A, compared with 2 to 5 % for plasminogen-R and plasminogen AC-CF III, DE-B. Following this

Table I

Clinical and Metabolic Data on Healthy Subjects Infused with Various Plasminogen Preparations

Preparation	Label	Subject	Sex	Age (yr)	Weight (kg)	Plasma volume (ml/kg)	Plasma radioactivity curve $x(t) = C_1 e^{-a_1 t} + C_2 e^{-a_2 t}$ C_1	a_1	C_2	a_2	t 1/2 for a_1 (days)
AC-FFP	^{125}I	BY	F	44	72	34	0.44	0.30	0.56	1.7	2.3
		BR	F	44	104	37	0.49	0.29	0.51	1.4	2.4
		DF	M	40	88	41	0.50	0.27	0.50	1.4	2.6
AC-FFP,DE-A	^{131}I	RV	M	27	80	37	0.42	0.33	0.58	1.5	2.1
		DM	M	63	81	40	0.46	0.34	0.54	1.5	2.0
	^{125}I	DP	F	54	80	–	0.35	0.34	0.64	1.7	2.0
AC-CF III,DE-B	^{131}I	DP	F	54	80	31	0.54	0.69	0.46	2.0	1.0
		DF	M	40	88	44	0.40	0.87	0.60	2.3	0.8
		GS	F	57	64	39	0.47	0.99	0.53	2.8	0.7
W	^{131}I	HC	M	27	78	39	0.42	0.31	0.58	1.2	2.2
		SL	M	28	58	50	0.50	0.29	0.50	0.9	2.4
	^{125}I	RV	M	27	80	30	0.40	0.36	0.60	1.7	1.9
		DM	M	63	81	–	0.39	0.33	0.61	1.5	2.1
R	^{125}I	HC	M	27	78	41	0.42	0.99	0.58	2.3	0.7
		SL	M	28	58	51	0.46	0.87	0.54	2.0	0.8
	^{131}I	BY	F	44	72	33	0.33	0.87	0.67	3.5	0.8
A	^{125}I	HW	M	49	59	42	0.50	0.29	0.50	1.7	2.4
		AI	M	22	75	–	0.51	0.43	0.49	2.0	1.6
		LI	M	23	69	33	0.45	0.41	0.55	2.7	1.7

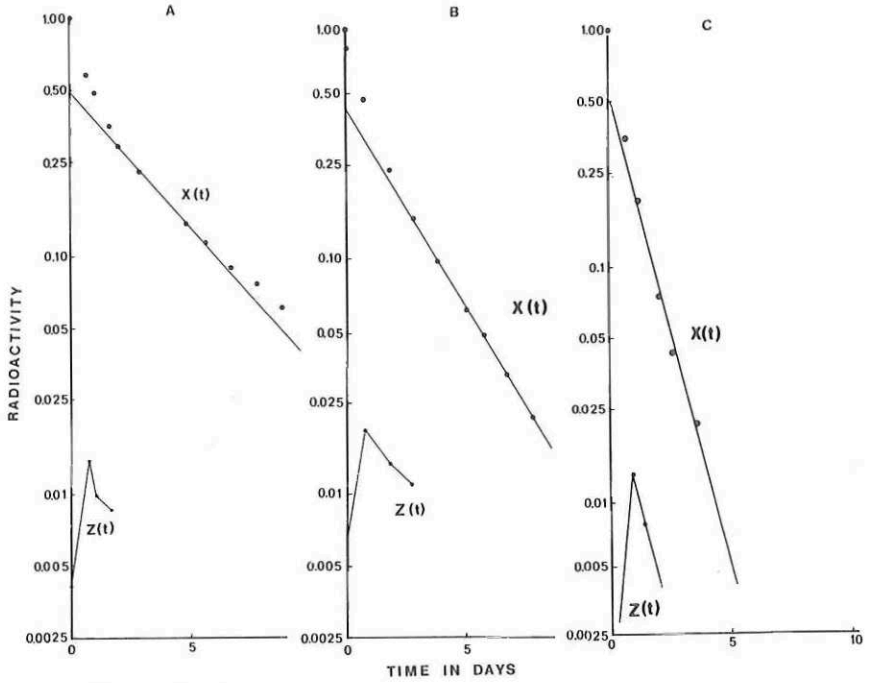

Fig. 1 – *Metabolic data on three plasminogen preparations obtained by affinity chromatography.*
A. ^{125}*I-plasminogen AC-FFP (Subject D.F.)--t 1/2=2.6 days*
B. ^{131}*I-plasminogen AC-FFP, DE-A (Subject D.M.)--t 1/2=2.0 days*
C. ^{131}*I-plasminogen AC-CF III, DE-B (Subject D.F.)-- t 1/2=0.8 days*
x(t) = plasma radioactivity, expressed as a fraction of the first sample
z(t) = non-TCA precipitable radioactivity in plasma.

rise it paralleled the plasma radioactivity.

The amount of radioactivity excreted daily in the urine was virtually a constant fraction of the mean plasma radioactivity during the same interval, except during the first day when it was less. The latter finding indicated that the faster disappearance of the plasma radioactivity during the first days was mainly due to transfer of labeled plasminogen to the extravascular space and not to rapid clearing of denaturated plasminogen. The fractional daily urinary excretion of label was markedly higher for plasminogen-R, which is in agreement with its much shorter plasma half-life. Plasminogen-A, prepared from plasma without using specific solubilizing agents such as lysine or EACA, behaved very similarly to plasminogen AC-FFP, plasminogen AC-FFP, DE-A and plasminogen-W, both in vivo and on polyacrylamide gel electrophoresis.

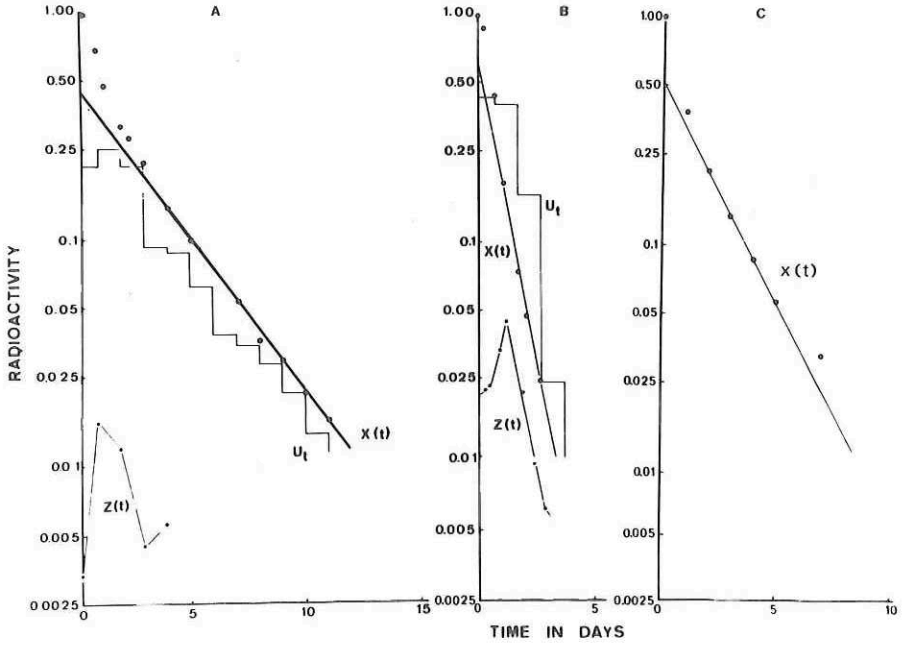

Fig. 2 – *Metabolic data on plasminogen preparations obtained by the method of Wallén and Wiman (4, 5), Robbins et al. (1), and Abiko et al. (10).*
A. ^{131}*I-plasminogen-W (Subject H.C.)--t 1/2 = 2.2 days*
B. ^{125}*I-plasminogen-R (Subect H.C.)--t 1/2=0.7 days*
C. ^{125}*I-plasminogen-A (Subject A.I.)--t 1/2=1.6 days*
x(t) = plasma radioactivity
z(t) = non-TCA precipitable radioactivity in plasma
u_t *= fractional daily urinary excretion of label.*

DISCUSSION

The present study was undertaken to establish the metabolic characteristics of human plasminogen.

The plasma radioactivity half-life of NH_2-terminal lysine plasminogen, prepared according to Robbins *et al.* (1), was less than one day compared with 1.9 to 2.6 days for NH_2-terminal glutamic acid plasminogen, prepared according to Wallén and Wiman (4, 5). In view of the low yield of the purification procedure, it was not clear whether these plasminogens represented selected native forms or partially degraded derivatives generated during the purification procedure. An attempt therefore was made to prepare undegraded "native" plasminogen as quantitatively and rapidly as possible, by using a modification of the one-step affinity

chromatography procedure of Deutsch and Mertz (6). Thus plasminogen was obtained from fresh frozen plasma (plasminogen AC-FFP) with greater than 90 % yield and a specific activity of 22.3 ± 1.3 CTA units per mg protein (7, 9). The plasma radioactivity half-life of this plasminogen preparation, was very similar to that of plasminogen prepared according to Wallén and Wiman (4, 5). However, a gradual levelling-off of the plasma radioactivity decay curve was observed, most probably due to the presence of small amounts of contaminating protein with a longer half-life. These contaminants were apparently removed by gel filtration on Sephadex G-150 and DEAE-Sephadex chromatography (Plasminogen AC-FFP, DE-A).

By way of comparison, the metabolic properties of plasminogen preparations obtained by affinity chromatography from Cohn fraction III were investigated. These studies revealed that the plasma radioactivity half-life of plasminogen with NH_2-terminal glutamic acid and lower isoelectric points (plasminogen AC-FFP ; AC-FFP, DE-A ; AC-CF III, DE-A and W) ranged from 1.9 to 2.6 days, whereas it was usually less than 1 day for plasminogen with NH_2-terminal lysine or valine and higher isoelectric points (plasminogen AC-CF III, DE-B and R). Both types of plasminogen were microheterogeneous (multiple isoelectric points), but they behaved as homogeneous proteins in the turnover experiments. The faster in vivo breakdown of plasminogen with NH_2-terminal lysine was confirmed by a significantly higher fractional daily urinary excretion of label.

Conformational changes in the plasminogen molecule have been observed in the presence of EACA (5, 12). It is not known however whether the original conformation is completely restored after removal of this agent, which is commonly used in most purification procedures. Since the native conformation is probably desirable for normal metabolism and function, a comparative preparation of plasminogen was made without the use of specific solubilizing agents such as lysine or EACA, according to the method of Abiko *et al.* (10). After radio-iodination and injection into normal subjects, the plasma disappearance rate for this preparation (plasminogen A) was essentially the same as for low isoelectric point plasminogen prepared in the presence of EACA. Thus the use of EACA in the purification procedure apparently did not affect the metabolic properties of the final product, suggesting that the conformational changes are completely reversible.

In conclusion, the present study indicates that plasminogen, suitable for metabolic studies, may be obtained from fresh frozen plasma by affinity chromatography in the presence of the inhibitor Trasylol, followed by Sephadex G-150 gel filtration and DEAE-Sephadex chromatography. This material contains several molecular forms with isoelectric points ranging from pH 6.1 to 7.1, all with NH_2-terminal glutamic acid and a molecular weight of 90,000, and has a half-life in plasma of 1.9 to 2.6 days ; it probably represents the native circulating molecules.

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Section II :

PURIFICATION AND CHARACTERIZATION OF LABELED "NATIVE" PLASMINOGEN, CONTAINING ALL MOLECULAR FORMS PRESENT IN NORMAL PLASMA

The studies, reported in the previous section give further support to the concept that native human plasminogen with NH_2-terminal glutamic acid and a molecular weight of approximately 90,000 constitutes the native molecule. The present experiments were designed to evaluate the physicochemical properties of native plasminogen before and after labeling, in order to demonstrate the absence of denaturation. The plasminogen preparations were obtained by the method of Wallén and Wiman, or by affinity chromatography, Sephadex G-150 gel filtration and DEAE-Sephadex chromatography.

Preparation of labeled plasminogen

Before March 1971, human plasminogen was prepared according to the method of Wallén and Wiman (1, 2), starting from fresh frozen plasma. The specific activity, as measured by caseinolytic digestion after urokinase (UK) activation (3), was 23-25 CTA units* per mg protein. The spontaneous caseinolytic activity was less than 0.5 % of the maximum activity after UK activation. The preparations were labeled with $Na^{131}I$ or with $Na^{125}I$, according to the method of McFarlane, as described in chapter II. Initially fraction DE-IIb as specified by Wallén and Wiman was labeled and further purified (2), in order to remove denatured protein which might have been produced during the labeling procedure ; but such a precaution was subsequently found to be unnecessary.

After March 1971, human plasminogen was prepared from fresh frozen plasma by affinity chromatography, Sephadex G-150 gel filtration and DEAE-Sephadex chromatography. The details of the procedure have been described elsewhere (6). The specific activity of the preparations averaged 25 CTA units per mg protein, the spontaneous proteolytic activity was less than 0.1 %. The final recovery was approximately 50 % of the plasma plasminogen, mainly due to losses in the DEAE-Sephadex chromatography. This step was included however as a safety measure to remove small amounts of degraded plasminogen which were sometimes produced in the previous steps. However, this final preparation still contained the 12 main molecular forms normally present in the plasma of healthy individuals (6). The preparations were labeled with $Na^{131}I$ or $Na^{125}I$ with a substitution level of approximately 0.25 atoms of iodine per molecule of plasminogen. The specific activity after labeling was unchanged.

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* In comparison with lot 8 standard plasmin prepared by the Michigan State Department of Public Health, Lansing, Michigan, kindly supplied by Dr. A.J. Johnson, New York University Medical Center, New York, N.Y., U.S.A.

In vitro evaluation of labeled plasminogen

Sephadex G-200 gel filtration of a trace amount of labeled plasminogen in 3 ml of normal human plasma revealed a single radioactivity peak, corresponding to the plasma plasminogen peak (fig. 1).

Immunoelectrophoresis of purified plasminogen revealed a single precipitin line in the beta-globulin region when using a horse antiserum against human serum (fig. 2).

An identical precipitin line was obtained with purified plasminogen and plasma, using a rabbit antiserum raised against purified plasminogen. A single precipitin line was found on immunoelectrophoresis of a mixture of purified plasminogen and a trace amount of labeled plasminogen (fig. 3). Autoradiography revealed a concentration of label in the precipitin line.

Lysine-agarose chromatography, by the method of Brockway and Castellino (7), of a trace amount of labeled plasminogen in 50 ml normal human plasma revealed elution of radioactivity and plasminogen in two main peaks (fig. 4). The relative amounts of radioactivity, protein and plasminogen activity (not shown) present in

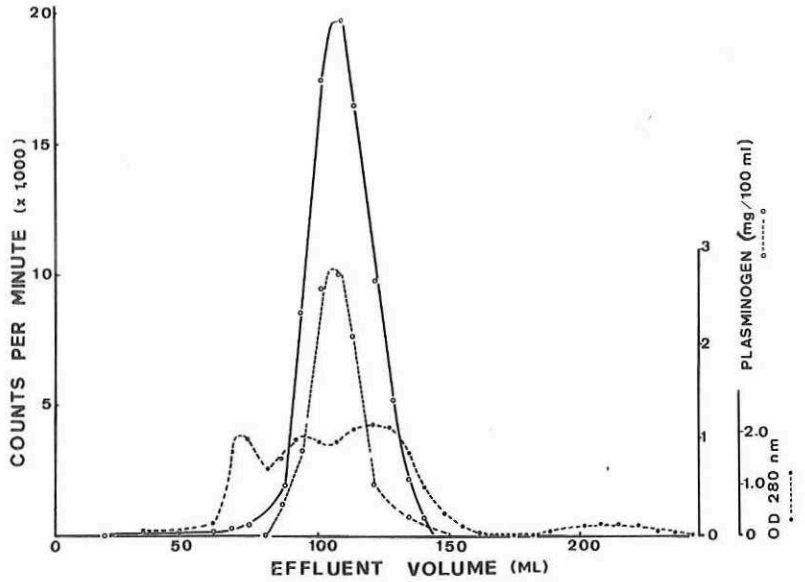

Fig. 1 – *Sephadex G-200 gel filtration of a mixture of a trace amount of labeled plasminogen with 3 ml human plasma. Radioactivity and enzymatic plasminogen activity were eluted at the same volume.*

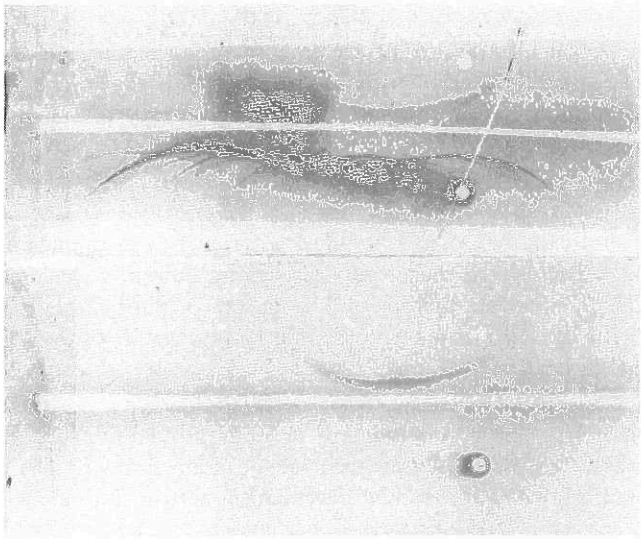

Fig. 2 – *Immunoelectrophoresis in 1 % Agarose gel in Veronal buffer at pH 8.6*
- Top slide : *Upper well, 5 µl plasminogen ; lower well, 5 µl human plasma ; trough, 50 µl commercial horse antiserum against human serum (Hyland).*
- Bottom slide : *Upper well, 5 µl plasminogen ; lower well, 5 µl human plasma ; trough, 50 µl rabbit antiserum raised against purified plasminogen.*

the two peaks, were very similar. In addition some radioactivity was eluted at the beginning of the gradient. This amount was smaller when labeled preparations were used which had been stored for only days or weeks (ref. 6, p. 40) and larger (though still below 10 % of the total) in labeled preparations which had been kept frozen for months (fig. 4).

On polyacrylamide gel electrophoresis at pH 8.3, six to seven main components were observed in the purified plasminogen preparations (fig. 5). All bands contained radioactivity, as shown by autoradiography, and enzymatic activity, as shown by enzymography performed according to the method of Heberlein and Barnhart (8).

Sephadex G-200 gel filtration of mixtures of a trace amount of labeled plasminogen with normal human plasma, which were activated to varying degrees with urokinase, revealed a progressive and parallel disappearance of radioactivity and enzymatic activity eluted in the plasminogen position. The elution profile of radioactivity showed two additional radioactivity peaks, corresponding to the void volume and to a position with an apparent molecular weight of roughly 150,000-200,000.

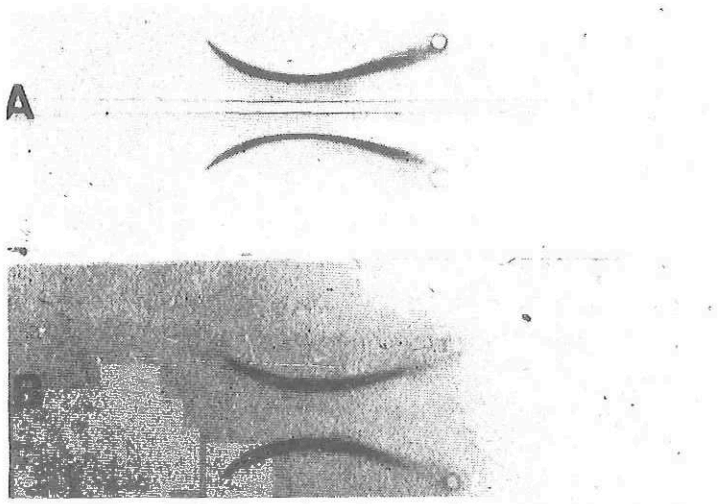

Fig. 3 – *Immunoelectrophoresis of a mixture of labeled and unlabeled plasminogen.*

A. Immunoelectrophoresis in 1 % Agarose in Veronal buffer pH 8.6. Wells : 3 µl plasminogen (8 mg/ml). Trough : 50 µl rabbit antiserum raised against the purified plasminogen.

B. Autoradiography of A.

Conclusion

The purity of the plasminogen preparations used in this study was demonstrated by their high specific activity, low spontaneous proteolytic activity and homogeneity on Sephadex gel filtration and immunoelectrophoresis. The two peaks on lysine-agarose chromatography and the six to seven bands on polyacrylamide gel electrophoresis were also found in fresh plasma (1, 6, 7). Our recent studies (6) have revealed this heterogeneity to be due to the fact that human plasminogen occurs in twelve main molecular forms in the circulation of every individual (6). Indeed, the two peaks, separated by lysine-agarose chromatography, each contain 5-6 main bands on polyacrylamide gel electrophoresis at pH 8.3 with staggered overlapping of the band patterns and an anodal shift of two positions of the pattern of the second peak as compared to that of the first.

No changes in enzymatic or physicochemical properties could be detected after labeling. It therefore appears that the purified labeled plasminogen preparations satisfy the in vitro criteria of purity and physicochemical identity with the native, circulating plasminogen.

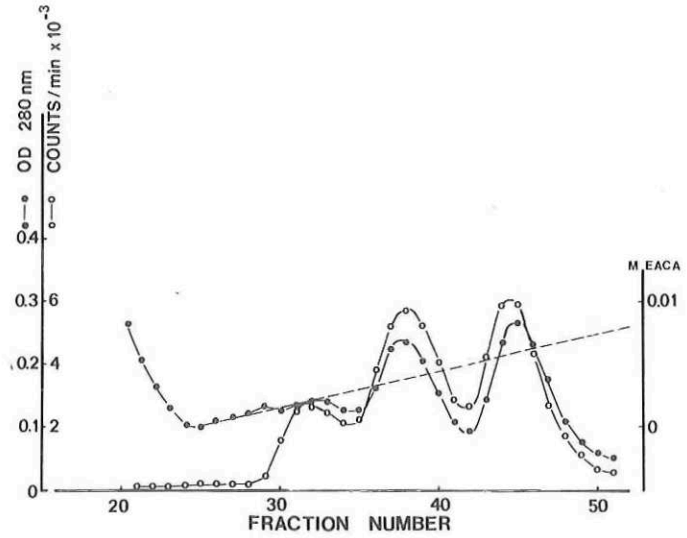

Fig. 4 – *Lysine-agarose chromatography of a trace amount of labeled plasminogen in 50 ml human plasma. Column size 0.9 x 30 cm ; buffer 0.1 M phosphate pH 7.5 containing 5 KIU Trasylol per ml ; flow rate 15 ml/hr ; gradient composed of 150 ml 0.1 M phosphate as starting buffer and 150 ml 0.1 M phosphate - 0.01 M EACA as limiting buffer. The labeled plasminogen preparation used in the chromatography had been kept frozen for six months.*

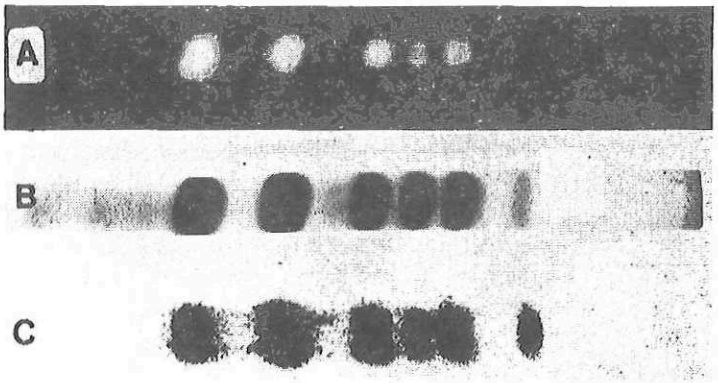

Fig. 5 – *Polyacrylamide gel electrophoresis of 10 µl of a mixture of labeled and unlabeled plasminogen (8 mg/ml).*
A. Enzymography performed according to Heberlein and Barnhart (8).
B. Polyacrylamide gel stained with amido black.
C. Autoradiography of the polyacrylamide gel.

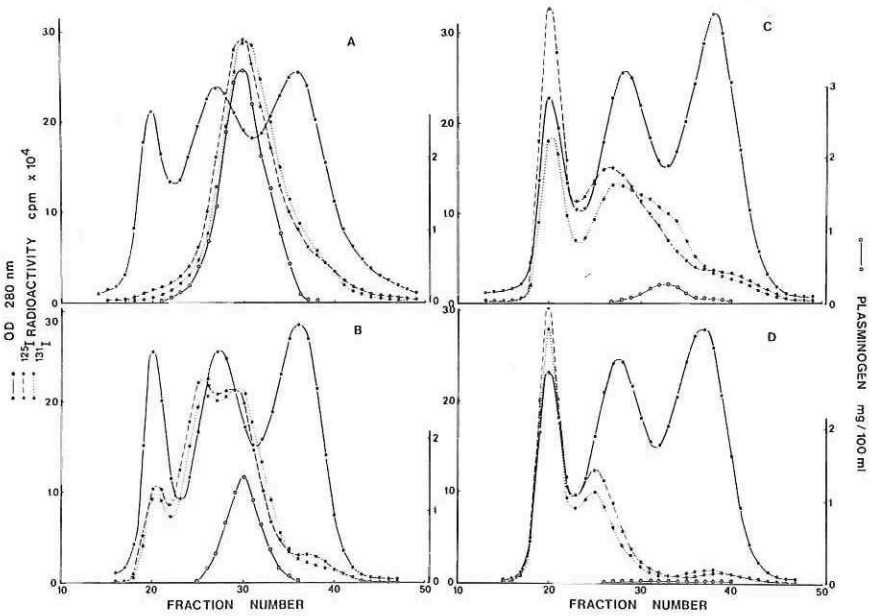

Fig. 6 – *Sephadex G-200 gel filtration of a mixture of a trace amount of ^{125}I-plasminogen A1 (first peak from lysine-agarose) and ^{131}I-plasminogen A2 (second peak from lysine-agarose) with 3 ml human plasma, on a 2.5 x 40 cm column equilibrated with 0.05 M tris - 0.1 M NaCl – 0.315 % Na_3-citrate-0.01 M EACA, pH 9.0. The samples were activated with increasing amounts of urokinase for 30 min at room temperature and the reaction stopped by addition of EACA to a final concentration of 0.1 M.*

A. non activated sample

B. 100 CTA units urokinase per ml plasma

C. 200 CTA units urokinase per ml plasma

D. 500 CTA units urokinase per ml plasma.

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Section III :

PREPARATION AND CHARACTERIZATION OF LABELED PROTHROMBIN

Human prothrombin was purified from fresh or fresh frozen ACD plasma according to Shapiro and Waugh (1), by successive adsorption on DEAE-cellulose and barium citrate, followed by precipitation with ammonium sulphate. This material ("step 3" prothrombin) contained 300-500 % (mean 375 %) of the normal plasma level of prothrombin or 950-1600 (mean 1180) Iowa units of prothrombin per O.D. unit (at 280 nm) and 300-500 % (mean 370 %) factor IX per O.D. unit, indicating an approximately 375 fold purification of both coagulation factors. The mean contaminating factor VII and X activity was 60 % per O.D. unit. Sephadex G-100 gel filtration ("step 4") performed as described by Shapiro and Waugh (1) resulted in a further purification but did not separate factor II and IX activity. After labeling of this material and injection in man the plasma radioactivity disappearance curve gradually levelled off from the sixth day after injection of the tracer* which is indicative of contamination with protein with a longer half-life in plasma. Therefore, prothrombin was further purified by gel filtration on Sephadex G-100 in 0.1 M K_2HPO_4 - 0.1 M KH_2PO_4 buffer pH 6.8 and by hydroxylapatite chromatography according to Swart (2). The prothrombin rich fractions after Sephadex G-100 gel filtration ("step 4" prothrombin) were adsorbed on a 1 x 20 cm hydroxylapatite column, equilibrated with 0.1 M K_2HPO_4 - 0.1 M KH_2PO_4 and eluted with an increasing ionic strength gradient, obtained with 150 ml 0.1 M K_2HPO_4 - 0.1 M KH_2PO_4 as starting buffer and 0.25 M KH_2PO_4 - 0.25 M K_2HPO_4 as limiting buffer. The buffers used in the last two steps were prepared with sterile pyrogen-free water. This purified material ("step 5" prothrombin) contained 1450-1800 (mean 1680) Iowa units prothrombin per O.D. unit or 2000-2450 (mean 2280) Iowa units per mg protein (extinction coefficient $A^{1\,\%}_{280\ nm}$ =13.6 (1)) and was devoid of factor V, VII, IX and X activity. No thrombin activity could be measured. The recovery from plasma, based on prothrombin activity determinations, was approximately 15 %.

SDS-polyacrylamide gel electrophoresis of this material in the presence of dithiothreitol revealed a single band with a molecular weight of 72,500 (fig. 1). Polyacrylamide gel electrophoresis at pH 8.3 revealed one band and also in some cases a trace component with a slower mobility. Immunoelectrophoresis against a rabbit antiserum raised against this material revealed a single precipitin line.

Step 5 prothrombin was labeled with $Na^{131}I$ or $Na^{125}I$ as described in chapter II with an average substitution level of 0.25 atoms of iodine per molecule of prothrombin.

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* In the hands of Dr. S. Shapiro, this preparation results in straight semilogarithmic disappearance curves up to at least 10 days after injection (personal communication).

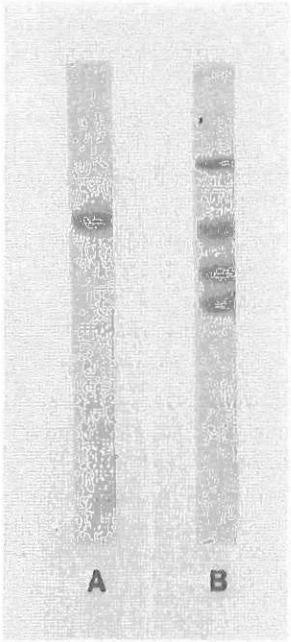

Fig. 1 – *SDS-polyacrylamide gel electrophoresis of purified prothrombin.*
A. Step 5 prothrombin.
B. Reference mixture containing phosphorylase A (MW 94,000) and reduced fibrinogen (alpha chain, MW, 70,900 ; beta chain, MW, 60,400 and gamma chain, MW, 50,700).

In vitro evaluation of labeled prothrombin

Sephadex G-200 gel filtration of mixtures of a trace amount of labeled prothrombin and 3 ml human plasma showed elution of radioactivity and enzymatic prothrombin activity at the same volume (fig. 2), indicating that the purification and labeling procedures did not result in detectable changes in the shape of the molecules and that all of the label was protein bound.

Polyacrylamide gel electrophoresis at pH 8.3 of a mixture of labeled and unlabeled prothrombin showed one main protein band and a trace component with a slower mobility. The main protein band contained 97 % of the total radioactivity applied to the gel.

Immunoelectrophoresis of a mixture of purified prothrombin with a trace

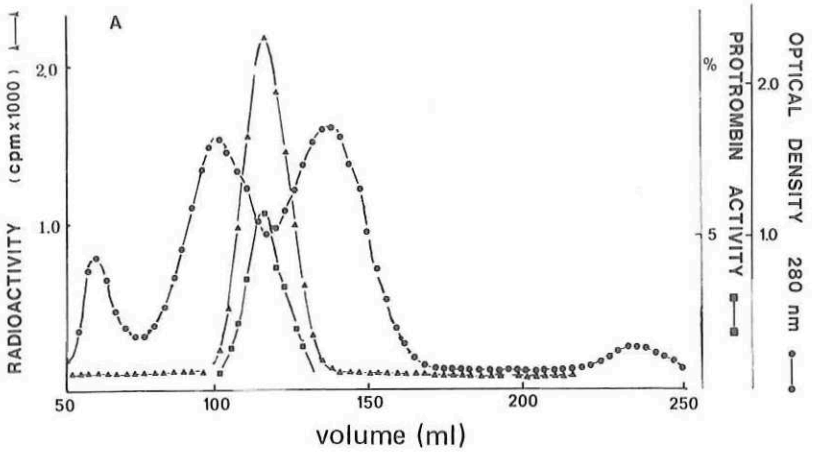

Fig. 2 – *Sephadex G-200 gel filtration of a mixture of a trace amount of labeled prothrombin and 3 ml human plasma. Column size 2.5 x 45 cm, flow rate 15 ml/hr, buffer 0.15 M NaCl - 0.01 M citrate, pH 7.0. Radioactivity and enzymatic prothrombin activity were eluted at the same volume.*

amount of labeled prothrombin as well as a mixture of human plasma and a trace amount of labeled prothrombin, against a rabbit antiserum raised against step 5 prothrombin gave a single precipitin line in which a concentration of isotope was revealed by autoradiography (fig. 3).

Sephadex G-200 gel filtration of mixtures of a trace amount of labeled prothrombin with normal human plasma (defibrinated by heating at 56° C for 3 min), which were activated to varying degrees with tissue thromboplastin and bovine serum, revealed a progressive disappearance of radioactivity in the elution position of prothrombin. In these experiments the activation was interrupted by addition of citrate and the samples were immediately applied to the column. The surface of the radioactivity peak remaining in this position was proportional to the amount of residual prothrombin in the material applied to the column. In the fully activated samples, less than 10 % of the label was eluted in the prothrombin position. The elution profile of radioactivity showed two additional radioactivity peaks, corresponding to the void volume and a position with an apparent molecular weight of 20,000-40,000, presumably representing thrombin-α_2-macroglobulin and prothrombin activation fragments. If the samples were not immediately chromatographed increasing amounts of radioactivity were eluted just before the prothrombin position, presumably as a result of thrombin-antithrombin III complex formation.

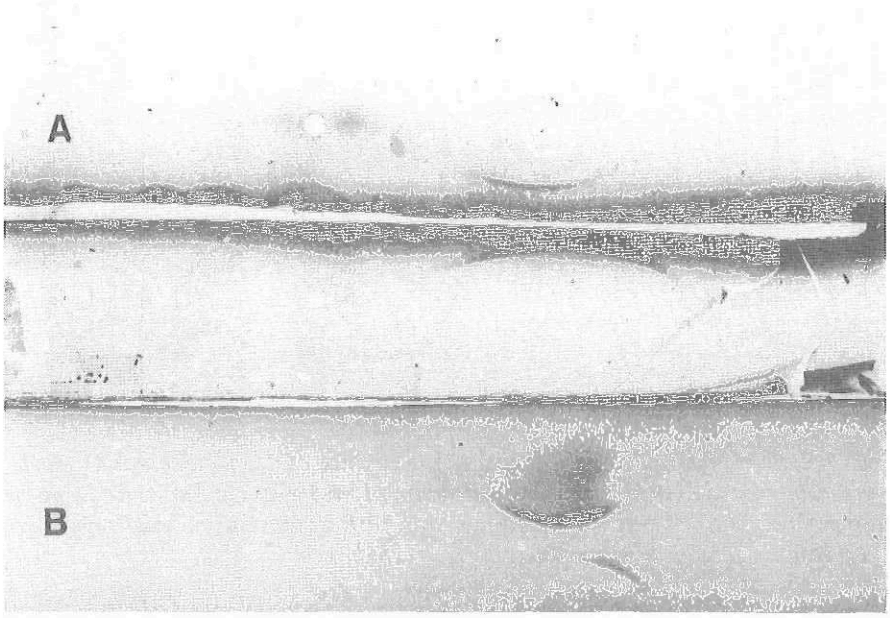

Fig. 3 – *Immunoelectrophoresis and autoradiography.*
A. Immunoelectrophoresis in 1 % Agarose in Veronal buffer pH 8.6.
Lowel well : mixture of 3 µl purified prothrombin (8 mg/ml) and a trace amount of labeled prothrombin.
Upper well : mixture of 3 µl plasma and a trace amount of labeled prothrombin.
Trough : 50 µl rabbit antiserum raised against purified prothrombin.
B. Autoradiography of A.

Conclusion

The purity of the prothrombin preparation used in this study was demonstrated by its high specific activity, absence of significant contaminating thrombin, factor V, VII, IX and X activity, and homogeneity on Sephadex gel filtration, immunoelectrophoresis and polyacrylamide gel electrophoresis. No changes in enzymatic or physicochemical properties could be detected after labeling.

It is therefore concluded that the purified labeled prothrombin preparation satisfies the in vitro criteria of purity and physicochemical identity with the native, circulating prothrombin.

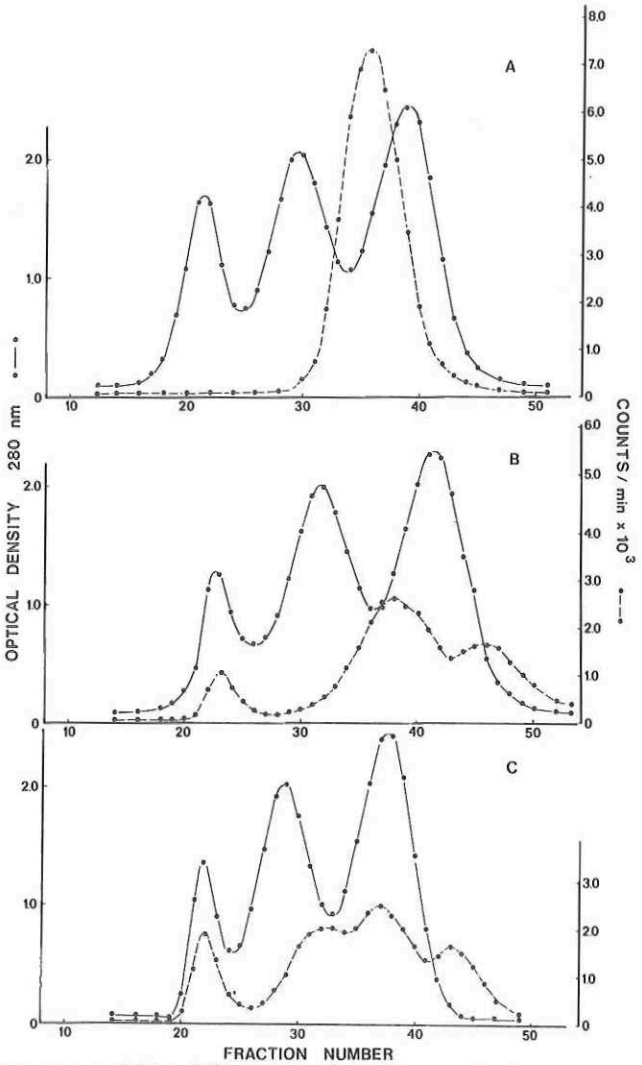

Fig. 4 – *Sephadex G-200 gel filtration of a mixture of a trace amount of labeled prothrombin with 3 ml heat-defibrinated human plasma, on a 2.5 x 40 cm column equilibrated with 0.15 M NaCl - 0.01 M phosphate - 0.01 M citrate pH 7.5. The samples were activated with tissue thromboplastin and bovine serum, the reaction stopped at different time intervals by adding citrate. The residual prothrombin was measured and the sample applied immediately after centrifugation for 1 min.*
A : 32 % prothrombin, no activation ; B : 21 % prothrombin, 1 min activation ; C : 6 % prothrombin, 2 min activation.
Note the progressive appearance of a radioactive peak just before the prothrombin position (presumably thrombin-antithrombin III complex).

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Chapter II

THE NORMAL METABOLISM OF PLASMINOGEN AND PROTHROMBIN

Section I :

PROCEDURE FOR THE METABOLIC STUDIES

The tracer proteins used for the present experiments were prepared from fresh or fresh frozen plasma or from Cohn fraction III, obtained from healthy blood donors without previous history of jaundice or hepatitis. Since the introduction of the immunological tests for hepatitis antigen (Hb-ag), all plasmas were screened before use by counter-electrophoresis and/or by radio-immuno assay (Abbott, Austria®). Approximately 1 mg of the purified materials was again tested with the radio-immuno assay. These materials were generally found to be free of Hb-antigen. On one occasion a dubious result was obtained and this preparation was discarded.

Labeling of the proteins was performed according to the method of McFarlane (1), with some modifications. The protein was dissolved in or diluted with an equal volume of 0.2 M Na_2HPO_4 to a concentration of 2-5 mg per ml. The iodinating solution (consisting of 1.2 ml KI (52.2 mg/liter), 1.2 ml KIO_3 (13.7 mg/liter), 4mCi of $Na^{125}I$ or $Na^{131}I$ in approximately 50 μl bicarbonate buffer and 0.2 ml N HCl, per 20 mg protein) was added dropwise. After stirring for 5 min. at room temperature, unbound iodide was removed by passage through a 1.5 x 3 cm column of Amberlite IRA 401, saturated with chloride. The amount of remaining free iodide, determined as non-TCA precipitable radioactivity, was less than 2 % of that bound to the protein. The solutions were sterilized by filtration, divided into small portions and stored at -20° C until use. The labeling efficiency was usually between 15 and 30 % with an average substitution level of approximately 0.25 (and always less than 1) atoms of iodine per molecule of protein.

Each batch of tracer preparations was subjected to pyrogen testing in rabbits. The pyrogen test was performed according to the pharmacopea regulations (Belgian Pharmacopea, p. 1-40 ; U.S.P. XVII, p. 863). A dose per kg body weight equivalent to approximately 5 times the amount used for the turnover studies in man, was injected into three rabbits. Spot checks for sterility were always satisfactory.

The subjects under investigation were given 500 mg of potassium iodide perorally twice or three times before the infusion of the labeled materials and once daily during the study. Five to 20 μCi of the tracer proteins were injected intravenously. In the first turnover studies with labeled plasminogen, the tracer was diluted in 150 ml of 0.9 % sodium chloride and infused over 30 min. This slow administration was thought to minimize any in vivo activation of the fibrinolytic system in the event of a pyrogenic reaction, although pyrogenicity tests in rabbits had been negative. In all subsequent studies the radioactive plasminogen solution was injected with a syringe over a period of less than 30 sec. In none of the subjects were side effects observed after the infusion or injection.

Ten or 20 ml blood samples were drawn 10 min after the end of the injection or 5 min after the end of the infusion and at different time intervals for up to 12 days. The blood was collected in polystyrene tubes, containing trisodium citrate (final concentration 0.315 %) and in some experiments also aprotinin[1] (final concentration 50 KIU/ml) and tranexamic acid[2] (final concentration 0.05 M) to prevent in vitro activation and digestion of plasminogen. Five ml blood samples were occasionally taken on potassium oxalate (final concentration 0.25 %) for the determination of components of the fibrinolytic system. At least 7 and up to 21 blood samples were taken from each subject. Daily quantitative urine collections were made in some of the subjects throughout the observation period.

Two ml of each of the following were pipetted into duplicate counting tubes : radioactive plasma, plasma supernatant after protein precipitation with an equal volume of 10 % trichloroacetic acid, and urine. After completion of the experiment, the radioactivity was measured in a well-type scintillation counter (Gamma-guard, Autowell Counting System ; or Hewlett Packard, Model 5385) with a sensitivity of between 625,000 and 800,000 cpm/μCi against a background of 30-80 cpm.

The amount of radiation absorbed by the thyroid, and the tracer diluent (plasma and interstitial fluids) was estimated in the following ways.

The thyroidal and total body radioactivity were determined by serial thyroid scanning and total body counting in three subjects after injection of 40 microcurie ^{131}I-fibrinogen.* Under the conditions of regular intake of cold iodine before and during the turnover experiment, no accumulation of tracer could be demonstrated (parallel decay of thyroidal radioactivity, plasma radioactivity and total body radioactivity). Moreover the maximal radioactivity measured in the thyroid region never exceeded the maximum permissible dose of 1.1 microcurie (ICRP, 1959).

The amount of radiation (D) absorbed in a critical organ with mass m after intake of a single dose of I_o microcurie of an isotope of which a fraction f is deposited in the organ is (2) :

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1. Trasylol® Bayer, Leverkusen Germany.
2. Cyklokapron®, Kabi AB, Stockholm, Sweden

* These determinations were performed by Dr. J. Colard, SCK, Mol, Belgium.

$$D_{rem} = \frac{74.f.I_o.T.\ \Sigma\ (RBE)n.\ (1-e^{\frac{-0.693\ t}{T}})}{m}$$

with T, the effective half-life being $T = \frac{T_b \cdot T_r}{T_b + T_r}$

and T_b = biological half-life
T_r = radioactivity half-life

Σ (RBE)n : effective absorbed energy per disintegration

The mass of the distribution fluids of labeled plasminogen and prothrombin is approximately 5 kg and the biological half-life less than 3.5 days.

The total radioactivity ($t \to \infty$), absorbed by these fluids is :

a) for 20 microcurie ^{131}I-labeled tracer (T_r = 8 days; Σ (RBE)n = 0.41 (3))

$$D_{rem} = \frac{74 \times 20 \times 2.4 \times 0.41}{5000} = 0.3$$

b) for 20 microcurie ^{125}I-labeled tracer (T_r = 60 days; Σ (RBE)n = 0.085 (3))

$$D_{rem} = \frac{74 \times 20 \times 3.3 \times 0.085}{5000} = 0.083$$

The amounts of radioactivity injected in the present turnover studies thus gave rise to a total irradiation of less than 0.5 rem.

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Section II :

THE NORMAL METABOLISM OF PLASMINOGEN. EFFECT OF TRANEXAMIC ACID

The present study was undertaken to evaluate the characteristics of the synthesis and catabolism of plasminogen in healthy subjects. It should be stressed once more that the labeled preparations used had NH_2-terminal glutamic acid and a molecular weight of approximately 90,000 and contained all the twelve main molecular forms present in normal plasma. The results of the present study are thus representative of *whole* plasma plasminogen.

The plasminogen preparations used before March 1971 were obtained by the method of Wallén and Wiman and those used after this by a combination of affinity chromatography on lysine-substituted agarose, Sephadex G-150 gel filtration and DEAE-Sephadex chromatography.

Metabolic studies

The control group consisted of 21 normal volunteers comprising the author and colleagues from the blood coagulation laboratory and the university hospital (DC, HC, GG, SL, LV, RV and DJ), students (VB, DK, BE, DR, SJ and RJ) and hospitalized patients without organic disease (EQ, JH, CL, CJ, CG, DC, DM and DJ). Results of routine hematological and liver function tests and a complete coagulation analysis were normal in all subjects.

The procedure for the metabolic studies was as described in the previous section and the analysis of tracer data as described in appendix II. The laboratory procedures are described in appendix I. Between 12 and 21 blood samples were taken from each subject. In some of the subjects the effect of tranexamic acid (EQ, JH and CL) or physical exercise (VB, DK, DR and SJ) on the turnover of labeled plasminogen was studied, starting on the 5th day after injection. In these individuals the normal plasminogen turnover characteristics were calculated on 7 to 10 data points obtained during the control period. In some of the subjects pooled urine samples were collected every 24 hr throughout the observation period.

Plasminogen metabolism was studied in a patient with Behçet's syndrome who had a markedly prolonged euglobulin clot lysis time and reduced euglobulin fibrinolytic activity on bovine fibrin plates.

The effect of a continuous 4 day intravenous infusion of heparin on the plasminogen turnover was studied in one subject. The amount of heparin was adjusted to achieve at least a three-fold increase in clotting time.

The in vivo effect of tranexamic acid, a potent inhibitor of plasminogen activation (1), on the turnover of labeled plasminogen was studied in five normal men. Three of them received 1 g of tranexamic acid t.i.d. for 4 days and two received 2 g t.i.d. for 2.67 days. These doses were considered adequate for inhibition of the fibrinolytic system in vivo (1).

RESULTS

Table I summarizes the clinical and laboratory data for the normal subjects. The mean plasma plasminogen concentration, determined by comparison of the caseinolytic activity of acid treated plasma (2) with that of purified plasminogen, after activation with urokinase, was 20.3 ± 2.6 mg/100 ml. Subsequent recovery studies using affinity chromatography (3, 4) have however revealed that the normal plasma plasminogen level averages 11.5 mg per 100 ml. Possible explanations for this discrepancy are given in the discussion. Since daily fluctuations of the plasma

Table I

Clinical and laboratory data on control subjects

	Sex	Age (y)	Weight (kg)	Plasma plasminogen (mg/100 ml)	Plasma volume (ml/kg)
DC	M	27	83	20.3	50
HC	M	27	78	19.4	39
GG	M	26	80	20.9	31
SL	M	28	58	21.2	50
LV	M	25	75	21.5	43
EQ	M	28	74	20.4	52
JH	M	42	58	20.6	55
CL	M	40	65	18.8	39
CJ	M	51	72	23.6	52
CG	M	44	65	23.3	49
DC	M	52	54	16.8	49
DJ	M	24	79	19.4	37
DM	M	63	81	22.7	40-40
RV	M	27	80	23.4	30-37
DJ	M	48	51	–	–
VB	M	24	71	18.5	38
DK	M	24	87	24.7	39
BE	M	24	62	17.1	35
DR	F	23	50	20.0	42
SJ	F	23	54	18.4	39
RJ	F	21	56	14.2	35
Mean		33	68	20.3	42
SD		12	12	2.6	7

levels of plasminogen were negligible, the subjects were assumed to be in a steady state during the experimental period, implying that the amount of plasminogen synthesized daily equaled the amount catabolized.

The turnover study shown in Fig. 1 is similar to those obtained for all the subjects.

The plasma radioactivity data x(t) plotted against time were approximated by a sum of two exponentials by graphic curve peeling or by computer fitting. The tracer data (x_i) were given a weight 1 and x_i^{-2} in the computer program and the graphically determined values were used as starting values in the program. The parameters of x(t), obtained by graphic analysis are shown in table II. The mean

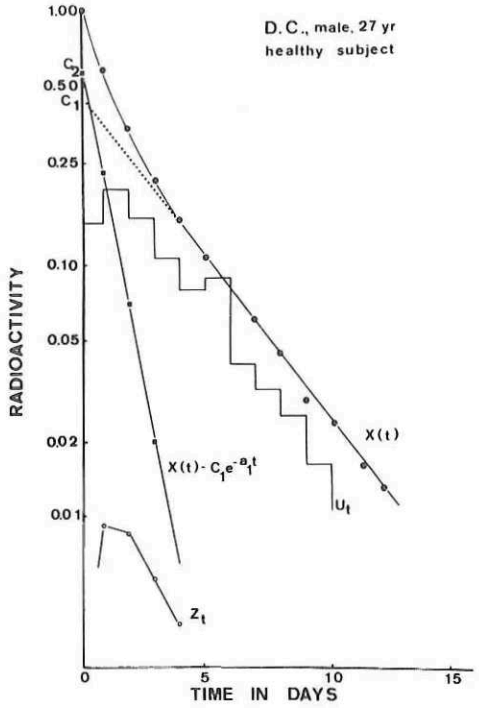

Fig. 1 – *Plasminogen metabolism in a control subject. x(t) = plasma radioactivity ; u_t = fractional daily urinary excretion of label ; z_t = non-TCA precipitable radioactivity in plasma. Graphic curve peeling in a sum of two exponential terms. The straight linear terminal portion of the plasma radioactivity x(t) is extrapolated to the ordinate to obtain the intercept C_1. The slope of this line is $-a_1$. By subtracting the extrapolated line from the original curve $x(t) - C_1 e^{-a_1 t}$ a new line is obtained $C_2 e^{-a_2 t}$ for which the slope $-a_2$ and intercept value C_2 are determined.*

Table II

Plasminogen tracer data of the control subjects

	Preparation	Batch number	$x(t) = C_1 e^{-a_1 t} + C_2 e^{-a_2 t}$				
			C_1	a_1	C_2	a_2	t 1/2 for a_1 (days)
DC	W457A-CF III	11.06.70	0.44	0.29	0.56	1.16	2.40
HC	W457A-CF III	11.06.70	0.42	0.31	0.58	1.16	2.25
GG	W457A-CF III	11.06.70	0.36	0.29	0.64	1.26	2.40
SL	W457A-CF III	11.06.70	0.50	0.29	0.50	0.87	2.42
LV	W457A-CF III	11.06.70	0.34	0.32	0.66	1.39	2.20
EQ	W457A-CF III	16.07.70	0.37	0.26	0.53	1.73	2.65
JH	W457A-CF III	16.07.70	0.58	0.28	0.42	1.73	2.50
CL	W457A-CF III	16.07.70	0.43	0.35	0.56	0.99	2.00
CJ	W8084-FFP	13.12.70	0.53	0.34	0.47	1.39	2.05
CG	W8084-FFP	13.12.70	0.44	0.40	0.56	1.54	1.75
DC	W8084-FFP	13.12.70	0.40	0.40	0.60	1.16	1.75
DJ	W8084-FFP	13.12.70	0.43	0.39	0.57	1.73	1.85
DM	W8084-FFP	13.12.70	0.39	0.33	0.61	1.54	2.10
RV	W8084-FFP	13.12.70	0.37	0.36	0.63	1.73	1.95
Mean			0.43	0.329	0.56	1.31	2.16
SD			0.07	0.046	0.07	0.32	0.29
DM	AC-FFP, DE-A-I	25.03.71	0.46	0.34	0.54	1.50	2.00
RV	AC-FFP, DE-A-I	25.03.71	0.42	0.33	0.58	1.50	2.10
DJ	AC-FFP, DE-A-II	13.03.73	0.58	0.33	0.42	1.06	2.10
VB	AC-FFP, DE-A-II	13.03.73	0.53	0.28	0.47	2.68	2.50
DK	AC-FFP, DE-A-II	13.03.73	0.49	0.24	0.51	1.08	2.90
BE	AC-FFP, DE-A-II	13.03.73	0.41	0.30	0.59	1.73	2.30
DR	AC-FFP, DE-A-II	13.03.73	0.41	0.28	0.59	2.17	2.50
SJ	AC-FFP, DE-A-II	13.03.73	0.41	0.30	0.59	1.51	2.30
RJ	AC-FFP, DE-A-II	13.03.73	0.50	0.28	0.50	1.39	2.50
Mean			0.47	0.30	0.53	1.62	2.36
SD			0.06	0.03	0.06	0.52	0.28
Total mean			0.44	0.32	0.56	1.43	2.24
SD			0.07	0.04	0.07	0.43	0.29

value of the equation describing the evolution of the plasma radioactivity was $x(t) = 0.44e^{-0.32t} + 0.56e^{-1.43t}$. The results obtained by computer analysis were practically identical.

The plasma plasminogen half-life was 2.16 ± 0.29 days for the preparations obtained by the method of Wallén and Wiman and 2.36 ± 0.38 days for the preparations obtained by affinity chromatography. The overall value of the plasma radioactivity half-life was 2.24 ± 0.29 days.

The amount of non-TCA precipitable label never exceeded 2 % of the total plasma radioactivity, which it paralleled after the first day.

The distribution of radioactivity in serial plasma samples was studied by gel filtration on Sephadex G-100 (fig. 2). The radioactivity was eluted in one main peak which corresponded to the enzymatically measured plasminogen peak. A very small peak, probably representing low molecular weight breakdown products and free iodide, was eluted at the total volume of the column.

The amount of radioactivity excreted daily in the urine was approximately a constant fraction of the mean plasma radioactivity, except during the first day when it was less. The latter finding indicated that the initial fall in plasma radioactivity was mainly due to transfer of labeled plasminogen to the extravascular compartment and not to rapid clearing of denatured plasminogen.

The metabolic parameters calculated from the plasma and urine tracer data are summarized in Table III. The fractional catabolic rate constant, as determined from

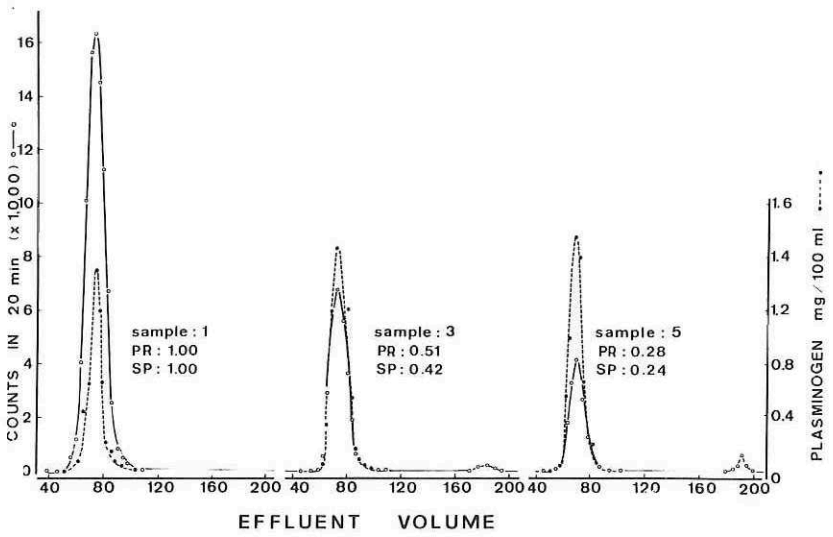

Fig. 2 – *Sephadex G-100 gel filtration of serial plasma samples taken during a turnover study of labeled plasminogen in a healthy subject. The radioactivity and enzymatic activity are eluted at the same position. PR : plasma radioactivity ; SP : surface under the radioactivity peak.*

Table III

Calculated metabolic parameters of the control subjects

	Fractional catabolic rate constant		Absolute catabolic (synthetic) rate (mg/kg/d)	Fractional transcapillary transfer rate constant	Intravascular fraction
	$k_{10,p}$	$k_{10,u}$		k_{12}	IV
DC	0.50	0.56	5.1	0.28	0.58
HC	0.54	0.64	4.1	0.26	0.58
GG	0.57	–	3.7	0.34	0.51
SL	0.47	0.44	4.7	0.15	0.67
LV	0.65	0.41	6.0	0.38	0.49
EQ	0.38	0.36	4.1	0.11	0.65
JH	0.43	–	4.8	0.46	0.65
CL	0.55	0.54	4.0	0.16	0.64
CJ	0.53	0.55	6.6	0.31	0.65
CG	0.68	0.64	7.8	0.36	0.59
DC	0.66	–	5.1	0.20	0.61
DM	0.63	–	5.6	0.43	0.52
RV	0.72	–	6.3	0.50	0.50
DJ	0.70	–	4.5	0.46	0.56
Mean	0.57	0.52	5.2	0.31	0.59
SD	0.11	0.10	1.2	0.13	0.06
DM	0.58		5.3	0.38	0.58
RV	0.60		5.2	0.41	0.55
DJ	0.46		–	0.17	0.71
VB	0.48		3.4	0.22	0.58
DK	0.40		3.8	0.27	0.60
BE	0.59		3.5	0.56	0.51
DR	0.58		4.9	0.82	0.49
SJ	0.57		4.1	0.44	0.53
RJ	0.47		2.4	0.37	0.60
Mean	0.53		4.1	0.48	0.57
SD	0.07		1.0	0.25	0.06
Total mean	0.55		4.8	0.38	0.58
SD	0.10		1.2	0.20	0.06

the plasma radioactivity disappearance ($k_{10,p}$), was 0.55 ± 0.10 ; and as determined from the urinary excretion of radioactivity ($k_{10,u}$), it was 0.52 ± 0.10. The absolute catabolic (synthetic) rate, obtained by multiplying the intravascular plasminogen pool by the fractional catabolic rate constant ($k_{10,p}$), was 4.8 ± 1.2 mg/kg per day. The intravascular fraction (IV) was 0.58 ± 0.06 and the fractional transcapillary efflux rate constant (k_{12}) 0.38 ± 0.20 of the plasma pool per day.

In a patient with Behçet's syndrome (fig. 3) the plasma plasminogen concentration (20.0 mg/100 ml) and the serum antiplasmin level were normal. However, the euglobulin clot lysis time was markedly prolonged (usually more than 24 hr), and the euglobulin fibrinolytic activity on unheated bovine fibrin plates was always much lower than in the controls tested at the same time. The plasma radioactivity disappearance rate of both ^{125}I-plasminogen (t 1/2 = 2.05 days) and ^{131}I-fibrinogen (t 1/2 = 4.25 days) were in the normal range.

The metabolism of labeled plasminogen and prothrombin were studied simultaneously in one subject during and after continuous infusion of heparin (30,000 units per day). There was no change in the plasma plasminogen half-life (t 1/2 –

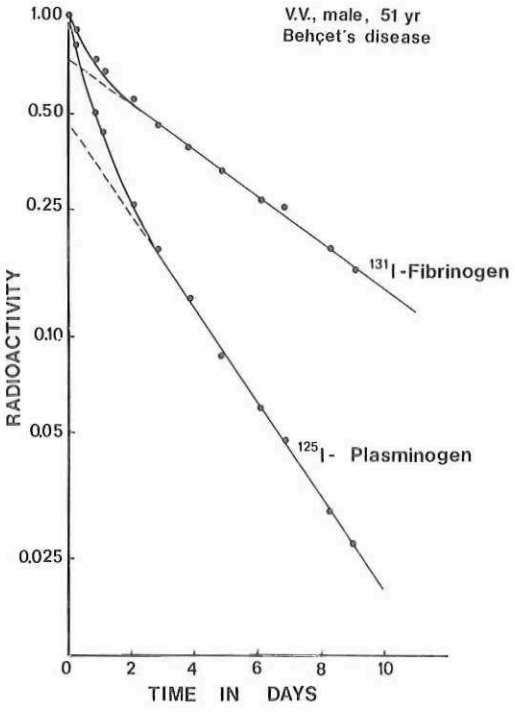

Fig. 3 – *Metabolism of ^{125}I-plasminogen and ^{131}I-fibrinogen in a patient with Behçet's syndrome, associated with a decreased euglobulin fibrinolytic activity. The figure shows the decay of both tracers in the plasma.*

1.95 days) after discontinuance of the anticoagulant (fig. 4).

The effect of in vivo inhibition of the fibrinolytic system by tranexamic acid on the turnover of labeled plasminogen was studied in five normal subjects. Three received 1 g perorally t.i.d. ; two showed no statistically significant change in the slope of the regression line from the plasma radioactivity data obtained during both experimental periods, whereas the third subject showed a small but significant shortening of the plasminogen half-life (from 2.5 to 2.1 days) during in vivo inhibition of the fibrinolytic system. Two other subjects who received 2 g tranexamic acid t.i.d. showed a markedly increased plasminogen turnover (fig 5 shows this increase in one of them).

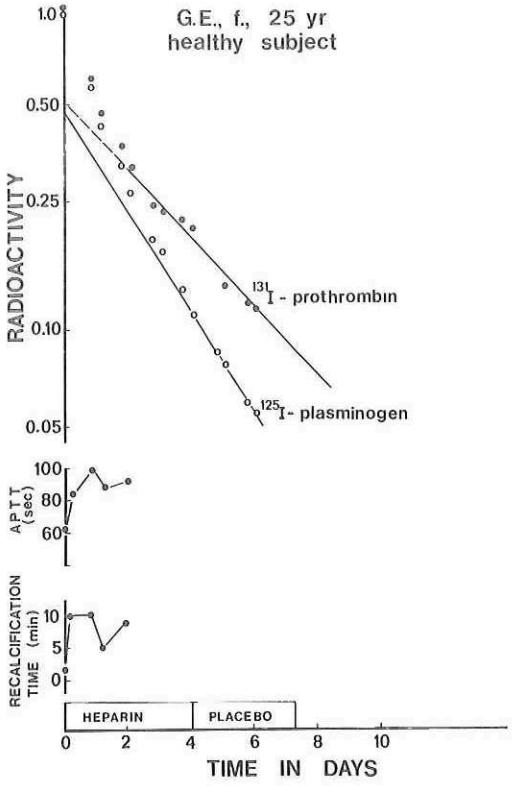

Fig. 4 – *Turnover of ^{125}I-plasminogen and ^{131}I-prothrombin during and after a continuous heparin infusion over 4 days in a normal subject.*
Heparin : continuous intravenous infusion of 30,000 units heparin in 1000 ml saline per day.
Placebo : infusion of 1000 ml saline per day.

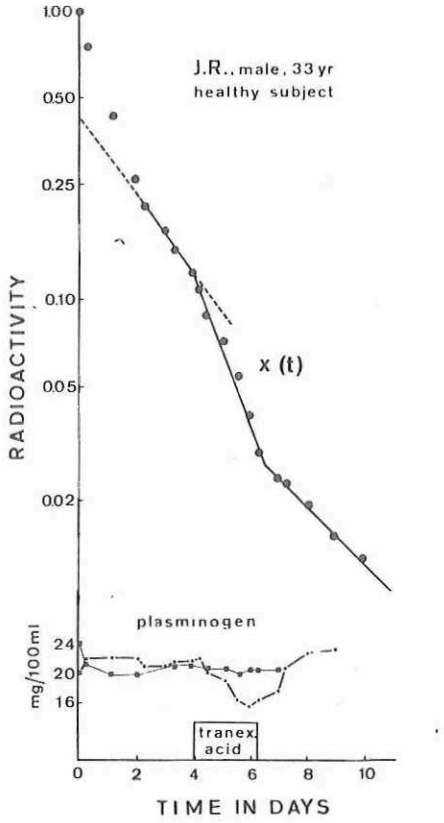

Fig. 5 – *Plasminogen metabolism in a healthy subject before and during in vivo inhibition of the fibrinolytic system with 2 g tranexamic acid t.i.d.*
x(t) = plasma radioactivity
● – ● = *enzymatically determined plasminogen*
■ – ■ = *immunoreactive plasminogen.*

The plasma radioactivity half-life in these two men decreased from 1.8 to 1.2 days and from 2.1 to 1.2 days, respectively. One subject from whom complete urine collections were obtained, showed an increase in the fractional catabolic rate constant as determined from the urinary excretion of label ($k_{10,u}$) during tranexamic acid administration, indicating that the increased plasma radioactivity disappearance was due to accelerated plasminogen catabolism. After the drug was discontinued, the plasminogen half-life in both subjects returned to normal. During fibrinolytic inhibition, the enzymatically measured plasminogen level decreased progressively but the concentration of immunoreactive plasminogen in plasma remained unchanged.

DISCUSSION

The metabolism of plasminogen was studied in 21 healthy subjects to determine its normal turnover characteristics. The biological integrity of the labeled plasminogen was shown by the low initial urinary excretion of label. After the equilibration period, the plasma radioactivity dropped steadily to less than 1 % of the initial value, indicating the absence of detectable amounts of contaminating protein with a significantly longer half-life.

The mean plasma plasminogen concentration, determined by comparison of the caseinolytic activity of acid treated plasma (2) with that of purified plasminogen, after activation with urokinase was 20.3 ± 2.6 mg/100 ml. The experimental conditions of our assay are described in detail elsewhere (5)*. This value agrees very well with the data of Rabiner *et al.* (9), who obtained 20.6 ± 0.36 mg/100 ml using a radioimmunoassay. However, affinity chromatography with greater than 90 % yield (based on activity recovery using the standard caseinolytic assay) and about 90 % purification yields only 12.8 ± 1.7 mg/100 ml plasma (4, 7). As determined by this method the plasma plasminogen level would be approximately 11.5 mg per 100 ml plasma, implying that our data on the plasma plasminogen level are over-estimated by almost 100 %.

The plasma radioactivity data were fitted with a sum of two exponential terms. The metabolic parameters calculated from these function parameters, using a two-compartment mammillary model, were : fractional catabolic rate constant 0.55 ± 0.10 of the plasma pool per day ; plasma radioactivity half-life 2.24 ± 0.29 days ; fractional transcapillary efflux rate 0.38 ± 0.20 of the plasma pool per day ; intravascular fraction 0.58 ± 0.06 and absolute catabolic (synthetic) rate 4.8 ± 1.2 mg/kg per day.

In view of the controversy over a continuous physiological balance between fibrin formation and fibrinolysis (11), and our finding of a rapid plasminogen turnover in normal subjects, we tried to evaluate the extent to which plasminogen was consumed in the fibrinolytic pathway by studying a patient with Behçet's syndrome who had low circulating plasminogen activator activity (decreased euglobulin fibrinolytic activity and prolonged euglobulin clot lysis time). Because both his plasminogen and fibrinogen metabolism were normal the primary pathway

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* However, using the standard CTA caseinolytic assay (6), the specific activity of highly purified plasminogen was 25 CTA units per mg and that of plasma 2.5 - 3.0 CTA units per ml, corresponding to 10 - 12.5 mg per 100 ml (3, 4, 7).
Upon recalibration of our plasminogen assay system against the original CTA standard plasmin (MDH lot #8) the caseinolytic activity of acidified and urokinase-activated plasma was calculated to be 3.5 ± 0.6 CTA units per ml and that of purified plasminogen 21 CTA units per mg. It is therefore possible that part of the discrepancy between the specific activity of plasminogen determined in our system and in the CTA caseinolytic assay is due to incomplete activation of the plasminogen in the former. Indeed, purified NH_2-terminal glutamic acid plasminogen is more slowly activated than the plasminogen in acidified plasma (7, 8). However the origin of the difference in caseinolytic activity of acidified and urokinase activated plasma (2.75 versus 3.5 CTA units per ml) remains unexplained.

of plasminogen catabolism appears to be metabolic rather than fibrinolytic.

In an attempt to confirm this hypothesis, we gave 1 g of tranexamic acid t.i.d. perorally to three normal subjects, a dose known to inhibit the fibrinolytic system in vivo (1). Two of them showed no change in plasminogen turnover, but the plasma radioactivity half-life was slightly but significantly shortened in the third. Two normal subjects were therefore given a 2 g dose of the fibrinolytic inhibitor t.i.d. Both showed a marked increase in plasminogen catabolism, which subsided when the drug was discontinued. These findings suggested that tranexamic acid has a direct but reversible effect on plasminogen metabolism. Conformational changes in the plasminogen molecule in the presence of antifibrinolytic amino acids such as epsilonamino caproic acid (2) or tranexamic acid (12) may possibly be responsible for the enhanced catabolism and therefore tranexamic acid cannot be used to test the relative importance of the activation of plasminogen on its turnover rate. Rabiner *et al.* (9), using an immunological method, reported a depressed plasma plasminogen level during administration of EACA in five hemophiliacs, a finding which we could not confirm in the present short-term experiments with tranexamic acid.

In order to rule out the remote possibility of significant plasminogen consumption secondary to in vivo coagulation, we evaluated the effect of heparin anticoagulation on plasminogen turnover in one normal subject. The plasma radioactivity disappearance rate remained unchanged.

The foregoing results led to the conclusion that under normal conditions the bulk of plasminogen is catabolized neither by primary fibrinolytic activation nor by consumption secondary to intravascular coagulation.

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Section III :

THE METABOLISM OF PROTHROMBIN IN HEALTHY SUBJECTS AND IN TWO PATIENTS WITH CONGENITAL HYPOPROTHROMBINEMIA

The metabolism of prothrombin has recently been investigated under normal (1-3), hypocoagulable (2) and hypercoagulable (4) conditions in humans. However, in these studies it was not demonstrated that the purified labeled prothrombins used were metabolized in the same way as untreated native prothrombin in fresh plasma.

In the present study, we report data on the kinetics of prothrombin synthesis and catabolism in normal subjects, using a highly purified labeled prothrombin preparation devoid of factors VII, IX and X activity.

The biological integrity of the labeled prothrombin was investigated by comparing its turnover rate with the disappearance rate of prothrombin activity after transfusion of fresh plasma into two patients with congenital hypoprothrombinemia.

METHODS

The control group consisted of 16 subjects, comprising the author and colleagues from the coagulation laboratory and the university hospital (JV, JR, NB, DH, MV, RV, DC, GB, SJ, NS, and RV) and patients without organic disease (JL, AV, JC, GM and PS). Results of routine hematological and liver function tests and complete coagulation analysis were normal in all individuals.

The procedure for the metabolic studies was as described in chapter II and the analysis of the tracer data as described in appendix II. Between 9 and 20 blood samples were taken from each subject. In some of them (DC, GB, SJ, NS and RV) the influence of physical exercise on the turnover of labeled prothrombin was studied, starting on the sixth day after the injection of the tracer. In these individuals, the normal prothrombin turnover characteristics were calculated on 8 to 11 data points, obtained during the control period. Pooled urine samples were collected every 24 hr throughout the observation period from some of the subjects.

The metabolism of prothrombin was studied in two patients (LR and JTR) with congenital hypoprothrombinemia.* Their coagulation defect has been described previously (5). Both subjects had a prothrombin level of 4 to 6 per cent of normal as estimated by the two stage prothrombin assay and by Laurell immunoelectrophoresis. The metabolism of untreated prothrombin was studied in both patients by enzymatic and immunological prothrombin assay after transfusion of 500 ml fresh plasma. In one of the patients, the turnover of labeled prothrombin was studied at the same time.

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* These patients were under the care of Dr. Haanen and his staff at the University of Nijmegen, The Netherlands, where these turnover studies were performed.

The effect of a continuous 4 day intravenous infusion of heparin, in a dose sufficient to achieve at least a three-fold increase in the clotting time, on the prothrombin turnover was studied in one subject.

RESULTS

Table I summarizes the clinical and laboratory data on the normal subjects. The mean plasma prothrombin concentration was 301 ± 37 Iowa units per ml or 13.2 ± 1.3 mg per 100 ml. A conversion factor of 2,280 Iowa units per mg of prothrombin, as derived from our purified preparations was used to convert enzymatic prothrombin activity to protein concentration. The plasma prothrombin concentration measured by the immunoelectrophoretic technique of Laurell, using a monospecific rabbit antiserum, raised against purified prothrombin was 13.3 ±

Table I

Clinical and laboratory data on the control subjects

	Age (y)	Weight (kg)	Plasma prothrombin			Plasma volume ml/kg
			Enzymatic assay		Immuno assay	
			Iowa U/ml	mg/100 ml	mg/100 ml	
JV	32	68	335	14.7		43
JR	32	86	315	13.8		36
NB	26	74	310	13.6	15.1	38
DH	25	71	310	13.6	15.3	37
MV	45	92	315	13.9	12.9	41
RV	28	67	305	13.3	12.9	35
JL	48	70	300	13.1	11.6	35
AV	59	74	310	13.6	11.6	45
JC	57	49	315	13.9	12.9	38
GM	23	81	310	13.6	13.3	47
PS	35	51	385	16.9	13.9	51
DC	30	85	271	11.9	–	31
GB	26	88	236	10.4	–	39
SJ	29	65	261	11.5	–	40
NS	25	66	230	10.0	–	39
RV	30	75	303	13.3		33
Mean	34	73	301	13.2	13.3	39
SD	11	12	37	1.6	1.3	5

1.3 mg/100 ml. Daily fluctuations of the prothrombin levels in plasma were negligible. It is therefore reasonable to assume that the subjects were in steady state with respect to prothrombin metabolism, implying that the amount of prothrombin synthesized daily equaled the amount catabolized. Results of a representative study are shown in fig. 1.

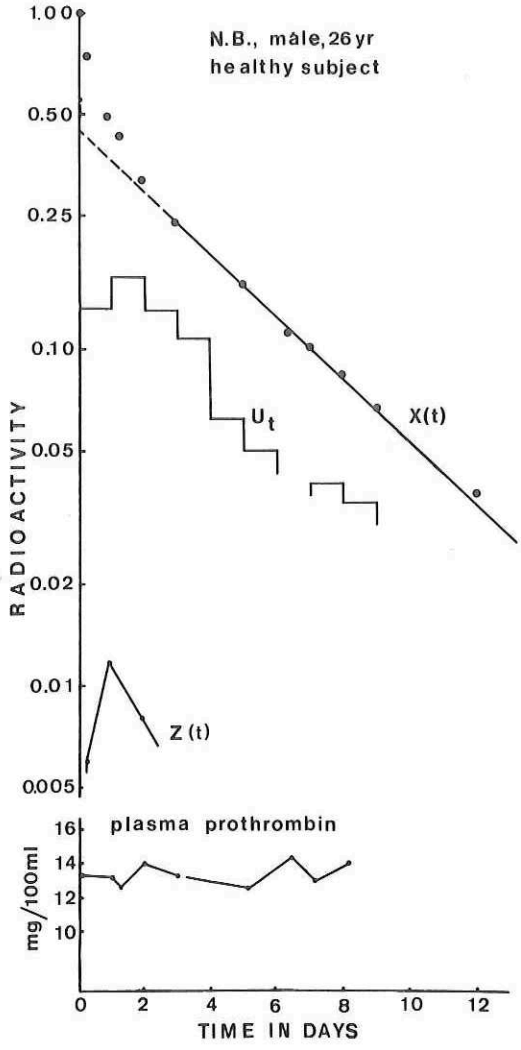

Fig. 1 – *Prothrombin metabolism in a control subject.*
x(t) : plasma radioactivity ;
u_t : fractional daily urinary excretion of label ;
z(t) : non-TCA precipitable radioactivity in plasma.

Similar results were obtained in all subjects. The plasma radioactivity x(t) versus time could readily be approximated by a sum of two exponential terms, using data up to approximately 10 days after injection of the tracer. The tracer data obtained by graphical curve peeling on semilogarithmic paper are summarized in table II. The mean value of the equation, describing the evolution of plasma radioactivity was $x(t) = 0.49e^{-0.23t} + 0.51e^{-1.42t}$. The plasma radioactivity half-life after equilibration was 3.04 ± 0.28 days.

The amount of non-TCA precipitable radioactivity in plasma never exceeded 2 % of the total plasma radioactivity, which it paralleled after the first day.

The distribution of radioactivity in serial plasma samples was investigated by gel filtration on Sephadex G-100. Throughout the study the radioactivity was eluted in one main peak corresponding to the enzymatically measured prothrombin. A small peak, (less than 5 % of the total) probably representing small circulating breakdown products and free iodide was eluted at the total volume of the column.

Except for the first day, the daily urinary excretion of label was approximately a

Table II

Prothrombin tracer data on the control subjects

	$x(t) = C_1 e^{-a_1 t} + C_2 e^{-a_2 t}$				
	C_1	a_1	C_2	a_2	t 1/2 for a_1 (days)
JV	0.50	0.23	0.50	1.40	3.00
JR	0.54	0.26	0.46	1.40	2.65
NB	0.45	0.21	0.55	1.25	3.25
DH	0.41	0.21	0.59	1.40	3.30
MV	0.52	0.20	0.48	1.15	3.50
RV	0.45	0.21	0.55	1.15	3.35
JL	0.43	0.24	0.57	0.92	2.85
AV	0.50	0.24	0.50	1.73	2.85
JC	0.52	0.23	0.48	1.15	3.00
GM	0.49	0.23	0.51	1.38	3.00
PS	0.50	0.19	0.50	1.26	3.60
DC	0.56	0.26	0.44	1.31	2.71
GB	0.55	0.25	0.45	0.97	2.79
SJ	0.49	0.23	0.51	2.24	3.00
NS	0.47	0.24	0.53	2.04	2.88
RV	0.47	0.24	0.53	1.98	2.95
Mean	0.49	0.23	0.51	1.42	3.04
SD	0.04	0.02	0.04	0.38	0.28

constant fraction of the corresponding mean plasma radioactivity. During the first day a smaller radioactivity fraction was excreted, probably due to equilibration of released isotope with the body iodide pool. The absence of a high excretion of label during the first day indicated that the initial fall in plasma radioactivity was mainly due to transfer of labeled prothrombin to the extravascular compartment and not to rapid clearing of denatured protein.

The metabolic parameters, calculated from the plasma and urine tracer data are summarized in table III.

The results were as follows. The fractional catabolic rate constant, as determined from the plasma disappearance rate of radioactivity ($k_{10,p}$) was 0.40 ± 0.04 of the plasma pool per day and as determined from the urinary excretion of radioactivity

Table III

Calculated metabolic parameters on the control subjects

	Fractional catabolic rate constant		Absolute catabolic (synthetic) rate (mg/kg/day)	Fractional transcapillary transfer rate constant	Intravascular fraction
	$k_{10,p}$	$k_{10,u}$		k_{12}	IV
JV	0.40	–	2.50	0.42	0.58
JR	0.42	–	2.06	0.37	0.63
NB	0.39	0.38	2.02	0.40	0.54
DH	0.42	0.37	2.12	0.49	0.50
MV	0.33	0.30	1.87	0.33	0.60
RV	0.38	–	1.75	0.35	0.55
JL	0.42	–	1.92	0.21	0.58
AV	0.42	–	2.61	0.56	0.57
JC	0.37	–	1.98	0.30	0.62
GM	0.40	–	2.56	0.42	0.58
PS	0.33	–	2.90	0.40	0.58
DC	0.40	–	2.40	0.32	0.65
GB	0.38	–	2.72	0.20	0.67
SJ	0.42	–	2.48	0.83	0.54
NS	0.45	–	2.07	0.74	0.53
RV	0.45	–	2.33	0.71	0.53
Mean	0.40		2.27	0.44	0.58
SD	0.04		0.34	0.18	0.05

($k_{10,u}$) approximately 0.35 of the plasma pool per day. The absolute catabolic (synthetic) rate, obtained by multiplying the intravascular prothrombin pool with the fractional catabolic rate constant ($k_{10,p}$) was 2.27 ± 0.34 mg/kg per day. The intravascular fraction (IV) was 0.58 ± 0.05 and the fractional transcapillary efflux rate constant (k_{12}) 0.44 ± 0.18 of the plasma pool per day.

The transfusion of 500 ml fresh plasma into the patients with congenital hypoprothrombinemia resulted in an increase in the prothrombin levels from 5.7 to 15.5 % in patient LR and from 5.8 to 18.5 % in patient JTR as measured by the two stage prothrombin assay, and from 6.7 to 9.9 % in patient LR and from 4.3 to 12.7 % in patient JTR as measured by the immunological assay of Laurell. However the precipitin rockets in the patients were broader than in normal plasma, resulting in a less precise quantitation of the immunoreactive protein from the height of the rockets (fig. 2).

The disappearance rate of the transfused prothrombin from the plasma was studied by measuring the time evolution of the difference between the prothrombin baseline value and the prothrombin levels after transfusion of fresh plasma. Differences of more than 20 % above the baseline value were only obtained during the first 4 days after the transfusion. The mean values of these differences obtained in the two patients, expressed as a fraction of the differences in the first sample, are shown in fig. 3. These data show that the mean disappearance rate of infused unlabeled prothrombin in the patients is indistinguishable from the mean disappearance rate of labeled prothrombin in the 16 control subjects.

The disappearance rate of enzymatic prothrombin activity and radioactivity from the plasma after simultaneous infusion of 500 ml plasma and injection of

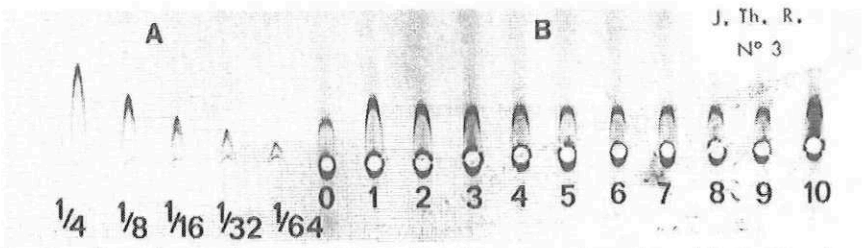

Fig. 2 – *Quantitative immunoelectrophoretic assay of prothrombin in the plasma of a patient with congenital hypoprothrombinemia before and after transfusion of 500 ml fresh plasma.*

A. dilutions of pooled normal plasma

B. undiluted plasma of the patient

○ : before transfusion

1 : 30 min after the end of the transfusion

2-10 : at daily intervals after the transfusion.

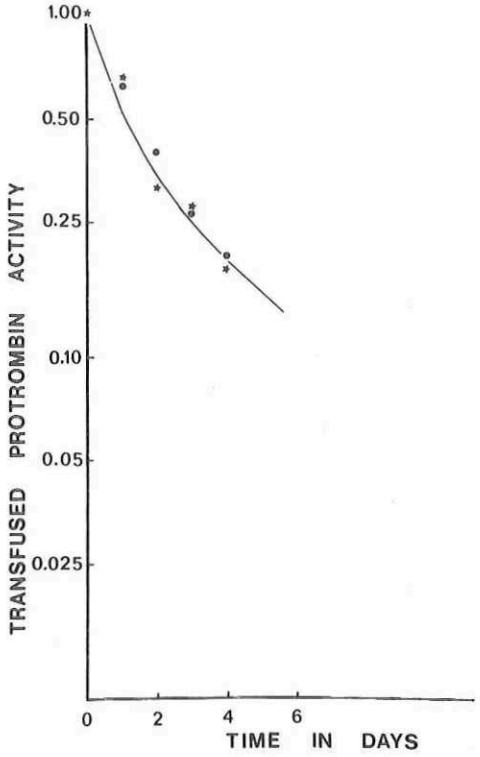

Fig. 3 – *Mean disappearance rate of infused prothrombin from the plasma in two patients with congenital hypoprothrombinemia*
★ *enzymatic assay, mean of the two patients*
● *immunological assay, mean of the two patients*
Solid line : mean disappearance rate of radioactivity in the control group.

labeled prothrombin in patient LR is shown in fig. 4. Within the errors of the biological assay, no difference in the plasma disappearance rate of unlabeled and labeled prothrombin was observed. Moreover, after equilibration, the plasma half-life of labeled prothrombin was 3.1 days, which is the mean value obtained in the control group.

The metabolism of labeled prothrombin and plasminogen was studied simultaneously during and after continuous infusion of heparin in one normal subject (section II, fig. 4). There was no change in the plasma prothrombin half-life (t 1/2 = 3.0 days) after discontinuance of the anticoagulant.

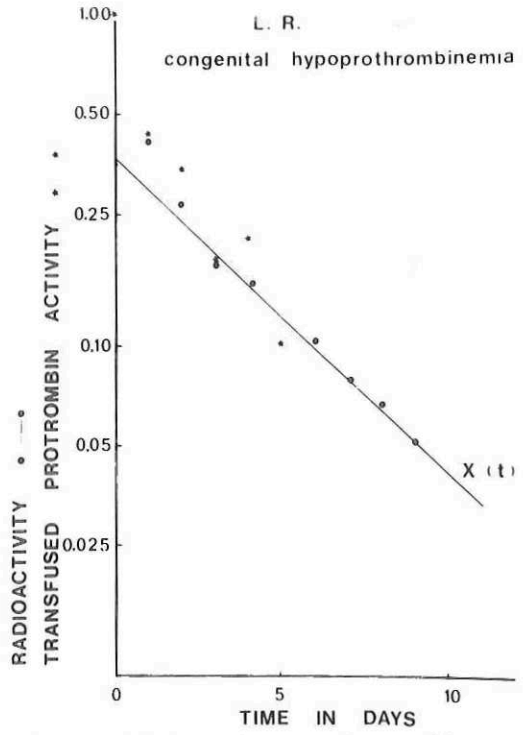

Fig. 4 – *Prothrombin metabolism in a patient with congenital hypoprothrombinemia, after transfusion of 500 ml fresh plasma and injection of labeled prothrombin.*

- ● *plasma radioactivity*
- ★ *enzymatic prothrombin activity : difference between measured level and baseline value.*

DISCUSSION

The metabolism of labeled prothrombin was studied in 16 control subjects to determine its normal turnover characteristics. The biological integrity of the labeled prothrombin was demonstrated by the low initial urinary excretion of radioactivity, and by the presence of a relatively high intravascular fraction after equilibration with the extravascular fluids. After equilibration, the plasma radioactivity dropped steadily over a period of at least 10 days, indicating the absence of detectable amounts of contaminating proteins with a longer half-life. The separation of prothrombin from the other factors of the prothrombin complex by hydroxylapa-

tite chromatography did not cause degeneration of the in vitro or in vivo characteristics of the prothrombin molecule, but instead resulted in a further purification.

The mean plasma prothrombin concentration determined by comparison of the enzymatic activity of plasma in the two stage prothrombin assay with that of purified prothrombin was 13.2 ± 1.6 mg/100 ml. Using quantitative immunoelectrophoresis a nearly identical value was obtained.

The plasma radioactivity data were fitted with a sum of two exponential terms. The metabolic parameters, calculated from these function parameters using a two-compartment mammillary model were : fractional catabolic rate constant 0.40 ± 0.04 of the plasma pool per day ; plasma radioactivity half-life 3.04 ± 0.28 days ; fractional transcapillary fraction 0.58 ± 0.05 and absolute catabolic (synthetic) rate 2.27 ± 0.34 mg/kg per day.

The mean plasma radioactivity half-life of 3.04 ± 0.28 days in our series is in line with the data of Shapiro and Martinez (2), obtained with step 4 prothrombin, larger than the value reported by Takeda (3) but shorter than the results obtained by Benamon-Djiane *et al.* (1).

Direct proof that the turnover of labeled proteins reflects the metabolism of their endogenous native counterparts is difficult to obtain. However, the disappearance rate of enzymatic and immunoreactive prothrombin activity after transfusion of 500 ml fresh plasma into our two hypoprothrombinemic patients was indistinguishable from the disappearance rate of radioactivity from the plasma of the normal subjects. This finding strongly suggests identical biological behavior of the purified labeled substrate and the untreated prothrombin in plasma and is further substantiated by the similar disappearance rates of prothrombin activity and radioactivity after simultaneous transfusion of fresh plasma and injection of labeled prothrombin into one hypoprothrombinemic patient.

Studies on the prothrombin metabolism in four severe hemophiliacs (2) indicated that, under normal conditions, only a small fraction of the prothrombin turnover at the most can be due to consumption by continuous in vivo coagulation. These results are further substantiated by our finding that heparin infusion had no influence on the turnover of prothrombin in a normal subject.

The normal disappearance rate of plasma radioactivity in a patient with hypoprothrombinemia clearly demonstrates that prothrombin metabolism in man follows first order kinetics over a large range of plasma prothrombin concentrations and is in line with our previous observations on the metabolism of labeled fibrinogen (6).

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Chapter III

PLASMINOGEN METABOLISM DURING STREPTOKINASE AND REPTILASE THERAPY IN MAN

Streptokinase infusion in man results in activation of the fibrinolytic system, leading to depletion of fibrinogen and plasminogen, decrease in antiplasmin activity and appearance of fibrin(ogen) degradation products in the plasma. It is well established that streptokinase infusion can induce thrombolysis.

Reptilase infusion results in conversion and depletion of fibrinogen, a less marked decrease in plasminogen and antiplasmin levels, and appearance of fibrin(ogen) degradation products in the plasma. On the basis of these observations, secondary activation of the fibrinolytic system has been postulated and considered as potentially thrombolytic (1).

Although there is obvious consumption of plasminogen during both therapeutic regimens, the dynamics of its turnover have not been investigated quantitatively. Labeled plasminogen could be a useful tool for the study of the fate of this molecule during these therapeutic approaches because its degradation rate can be studied independently of synthesis and because the formation and disappearance rates of radiolabeled activation and/or breakdown products can be measured quantitatively.

MATERIALS AND METHODS

The metabolism of plasminogen during *streptokinase* (Kabikinase®) therapy was studied in six patients with thromboembolic disease. The therapy consisted of an initial dose of 250,000 to 750,000 IU, as individually specified (table I) infused over 30 min, and a maintenance dose of 100,000 IU per hr for 72 hr. The labeled plasminogen was injected 10 min prior to the start of the thrombolytic treatment in four patients, during the maintenance therapy in one patient (FG), and 2.8 days before the start of the streptokinase infusion in one patient (DV). In the last patient a simultaneous turnover study of ^{125}I-plasminogen and ^{131}I-fibrinogen was performed.

Table I

Clinical and laboratory data on the patients

	Sex	Age (yr)	Weight (kg)	Plasma plasminogen (mg/100 ml)	Initial dose
A. *Streptokinase* :					
MD	F	45	44	21.5	500,000 IU
LM	F	71	65	17.5	250,000 IU
FV	M	72	68	–	750,000 IU
FM	M	64	45	27.0	250,000 IU
FG	M	68	72	17.7	250,000 IU
DV	M	69	73	–	250,000 IU
B. *Reptilase* :					
MH	M	45	93	21.3	
RV	F	47	50	9.3	
LM	F	71	65	20.4	
EV	M	49	78	22.9	
SG	M	72	61	19.9	

The metabolism of plasminogen during *reptilase* (Defibrase®, lot 460) therapy was studied in five patients with thromboembolic disease. This therapy consisted of intravenous infusions of 2 ml reptilase over 1 hr at intervals as individually specified. The labeled plasminogen was injected 10 min prior to the first reptilase infusion in three subjects (MH, RV and SG), 1.7 days before the start of the therapy in one patient (EV) and 1 day after the first infusion in one patient (LM). To investigate whether reptilase had a direct effect on the turnover of plasminogen, labeled plasminogen was preincubated for 1 hour at 37° C with 2 μl reptilase per ml of plasminogen solution. This reptilase concentration is at least three times higher than the maximal possible concentration in vivo. After incubation this material was injected intravenously into one patient.

In the first experiments, blood samples were taken on the anticoagulants and fibrinolytic inhibitors described in chapter 2. In later experiments 0.1 ml of a 0.01 M solution of para-nitrophenyl-para-guanidinobenzoate (in 20 % dimethyl formamide – 80 % acetonitrile) (plasmin and activator inhibitor) was added per 10 ml blood for the streptokinase patients and 0.1 ml of an anti-reptilase antiserum (kindly supplied by Pentapharm) per 10 ml blood for the reptilase patients.

The clinical and laboratory data on the patients are summarized in table I.

The procedure for the analysis of tracer data were as described in chapter II. Laboratory tests were performed as described in appendix I. In the reptilase experiments, the plasma radioactivity data could be fitted satisfactorily with a

function $x(t) = Ce^{-at}$; the fractional catabolic rate constant (k_{10}) was calculated from the area under the plasma radioactivity disappearance curve (A) by the formula

$$k_{10} = \frac{1}{A} = \frac{a}{C} \qquad (2).$$

RESULTS

Plasminogen catabolism during streptokinase therapy

Labeled plasminogen was injected into four patients (LM, FM, MD, and FV) 10 min prior to the start of the streptokinase infusion. In two of the patients (MD and FV) who received a loading dose of 500,000 and 750,000 units respectively, depletion of fibrinogen and plasminogen to below 5 % of the pre-infusion value was already observed by the end of the initial 30 min infusion of streptokinase. In the two patients (LM and FM) who received a loading dose of 250,000 units, a depletion of fibrinogen and plasminogen was obtained after 4 hrs and 6 hrs respectively.

In all patients the change in plasma radioactivity with time could be described by a sum of two exponential terms $x(t) = C_1 e^{-a_1 t} + C_2 e^{-a_2 t}$. The parameters of x(t) are summarized in table II. A marked acceleration of the disappearance rate of plasma radioactivity was observed (t 1/2 : 0.50 to 0.75 days). The data from the patient M.D. are shown in fig. 1. The increased rate of plasminogen catabolism was matched by an increased rate of excretion of label into the urine. The level of radioactive breakdown products not precipitated by trichloroacetic acid never exceeded 5 % of the total plasma radioactivity.

Gel filtration of serial plasma samples on Sephadex G-200 showed that during streptokinase infusion the plasma radioactivity was no longer confined to the plasminogen peak. In patient LM in whom depletion of plasminogen was not obtained until 4 hr after the start of the streptokinase infusion we noted (fig. 2) : a) a parallel decrease of radioactivity and of plasminogen activity in the fractions eluted at the plasminogen position, and b) an equivalent increase in radioactivity at two positions, one corresponding to the void volume and the second just before the globulin position. No measurable plasminogen-streptokinase activator was found in the latter of these two peaks. Similar patterns of radioactivity were also observed in plasma samples from the two patients MD and FV. In patient FG, in whom labeled plasminogen was injected during the maintenance therapy when the plasma plasminogen was undetectable and the fibrinogen level was 32 mg/100 ml, an increased plasma radioactivity disappearance rate (t 1/2 : 0.75 days) was observed. There was already a marked change in the elution pattern of radioactivity in the sample taken 10 min after the injection (fig. 3).

Table II

Plasminogen tracer data on the streptokinase patients

	Plasma volume (ml/kg)		$x(t) = C_1 e^{-a_1 t} + C_2 e^{-a_2 t}$				
			C_1	a_1	C_2	a_2	t 1/2 for a_1 (days)
MD	38		0.47	1.26	0.53	7.0	0.55
LM	45		0.36	1.07	0.64	3.5	0.65
FV	45		0.80	1.07	0.20	7.0	0.65
FM	54		0.31	1.38	0.69	1.0	0.50
Mean			0.49	1.20	0.52	4.6	0.59
SD			0.22	0.15	0.22	2.9	0.08
FG	40		0.44	0.92	0.56	2.0	0.75
DV	40	before SK	0.49	0.39	0.51	1.2	1.80
		during SK	0.65	0.99	0.35	1.7	0.70

In addition to the two radioactivity peaks corresponding to peaks -1 and -2 in fig. 2, some radioactivity was still eluted in the plasminogen position. This peak however had virtually disappeared one hour after injection of the labeled plasminogen.

In patient D.V., the plasma radioactivity half-life of fibrinogen (t 1/2 : 4.5 days) and plasminogen (t 1/2 : 1.8 days) were both within the normal range (fig. 4). Infusion of streptokinase resulted in an abrupt increase of both disappearance rates, very similar to that occurring in patients in whom streptokinase therapy was started immediately after injection of the tracer proteins.

Plasminogen catabolism during reptilase therapy

Infusion of reptilase resulted in a gradual decrease of fibrinogen until, after 12 to 24 hours, it became undetectable by either of the methods (cfr. appendix I) used. The plasma plasminogen decreased on average over this period from approximately 20 to between 7.5 and 11 mg per 100 ml in patients MH, LM, GS and VE with normal liver function, and from 9.3 to 3 mg per 100 ml in patient RV with disturbed liver function. A marked increase in the amounts of incoagulable fibrin(ogen) degradation products was found in all five patients. However, there was no significantly increased fibrinolytic activity in the euglobulin fraction using bovine fibrin plates.

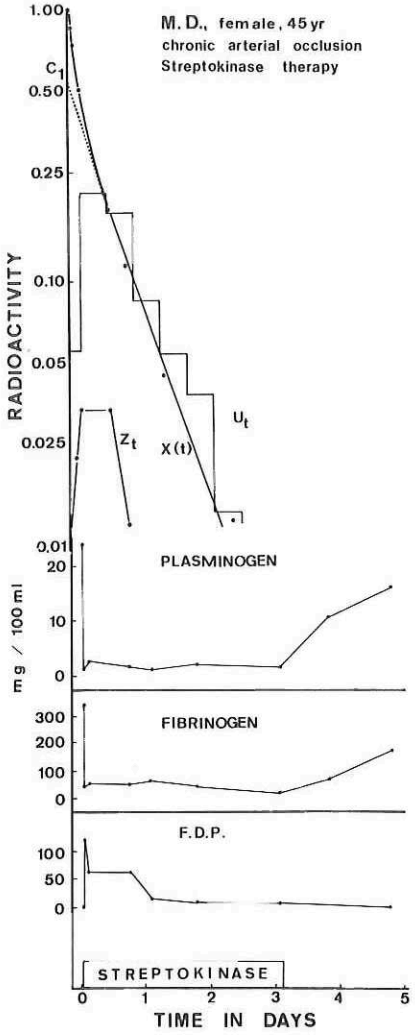

Fig. 1 – *Metabolism of labeled plasminogen during streptokinase therapy.*
x(t) : plasma radioactivity
z_t : non-TCA precipitable radioactivity in plasma
u_t : fractional urinary excretion of label per 12 hr
F.D.P. : fibrinogen degradation products in serum.

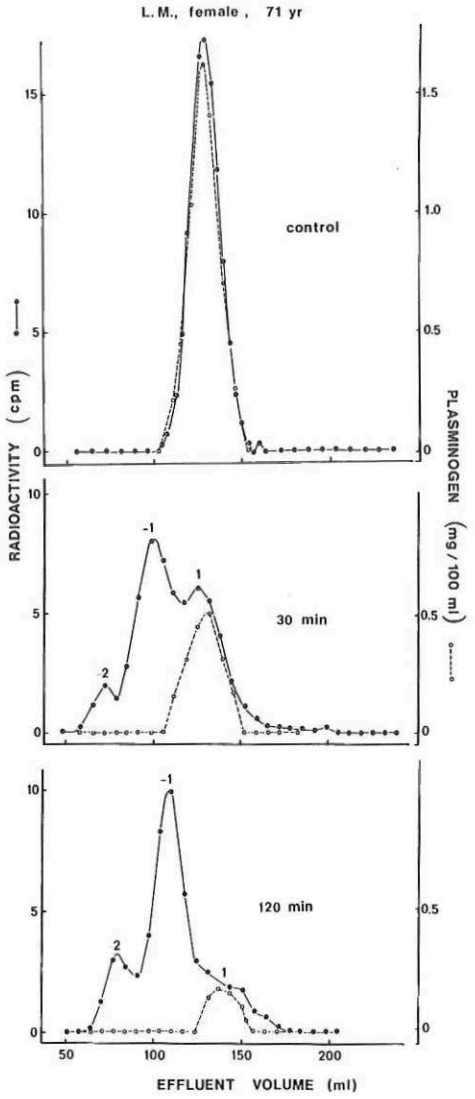

Fig. 2 – *Sephadex G-200 gel filtration of serial plasma samples taken before (control) and 30 min and 2 hr after initiation of streptokinase therapy.*

– *peak 1 : elution position of plasminogen*

– *peak-1 : apparent molecular weight roughly 150,000-200,000*

– *peak-2 : at position of void volume.*

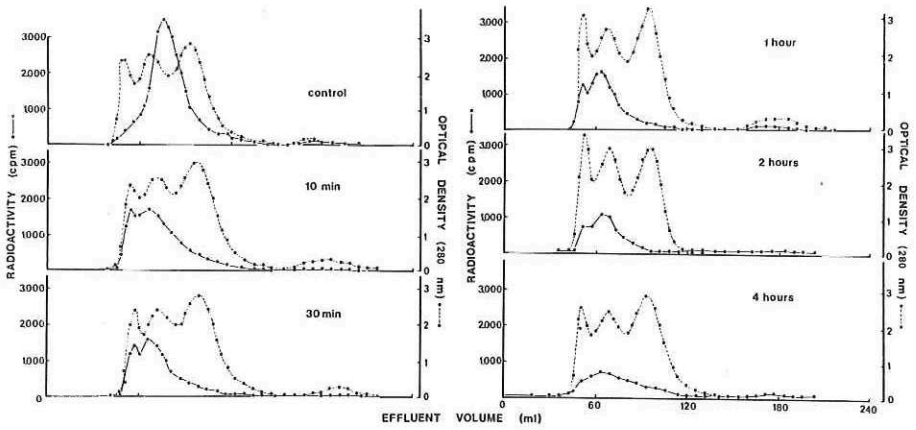

Fig. 3 – *Sephadex G-200 gel filtration of serial plasma samples taken after injection of labeled plasminogen during the maintenance dose of streptokinase. Control : mixture of 5 µl labeled plasminogen and 3 ml plasma.*

In all patients a marked acceleration of the plasma radioactivity disappearance rate was noted, as shown for LM and RV in fig. 5.

In two patients (MH and RV) to whom repeated reptilase infusions were administered at 24 hr intervals, the disappearance rate of plasma radioactivity could be described by a single exponential term, with a half-life of 0.45 to 0.80 days over the entire period of data collection (table III). In the three other patients in whom

Table III

Plasminogen tracer data on the reptilase patients

	Plasma volume (ml/kg)	$x(t) = C_e^{-at}$		
		C	a	t 1/2 (days)
MH	44	1.00	1.54	0.45
RV	54	1.00	0.87	0.80
LM	37	1.00	1.54	0.45
EV	50	0.31	0.92	0.75
SG	39	1.00	1.73	0.40
Mean	45		1.32	0.57
SD	7		0.40	0.19

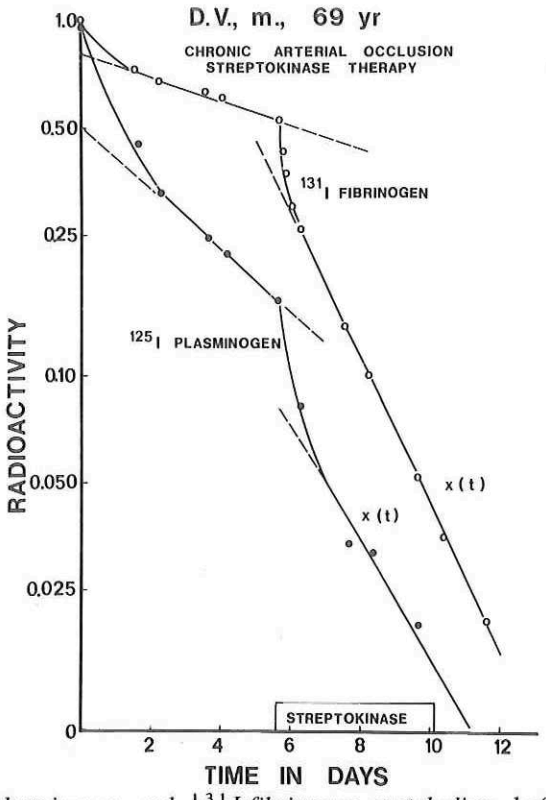

Fig. 4 – ^{125}*I-plasminogen and* ^{131}*I-fibrinogen metabolism before and during streptokinase therapy in a patient with thromboembolic disease.*
x(t) : plasma radioactivity.

the enzyme infusion was only given once after the injection of labeled plasminogen, the plasma radioactivity disappearance rate was similarly enhanced during the first day (Table III). Thereafter however a gradually normalizing plasminogen turnover was noted, concomitant with a gradual rise in plasma fibrinogen and plasminogen levels. In the patient in whom labeled plasminogen was injected 24 hr after the first reptilase infusion (LM), at a time when marked hypofibrinogenemia was already present, the initial enhanced disappearance rate of plasma radioactivity due to a second infusion of the enzyme was identical to that seen in the other patients.

The fractional catabolic rate of plasminogen in the five patients during the first day after the enzyme infusion was 0.87 to 1.75 times the plasma pool per day.

In one patient (RV) labeled plasminogen preincubated with reptilase was injected after completion of the first turnover study. The plasma disappearance rate of this preincubated labeled plasminogen was within the normal range (t 1/2 : 1.65 days).

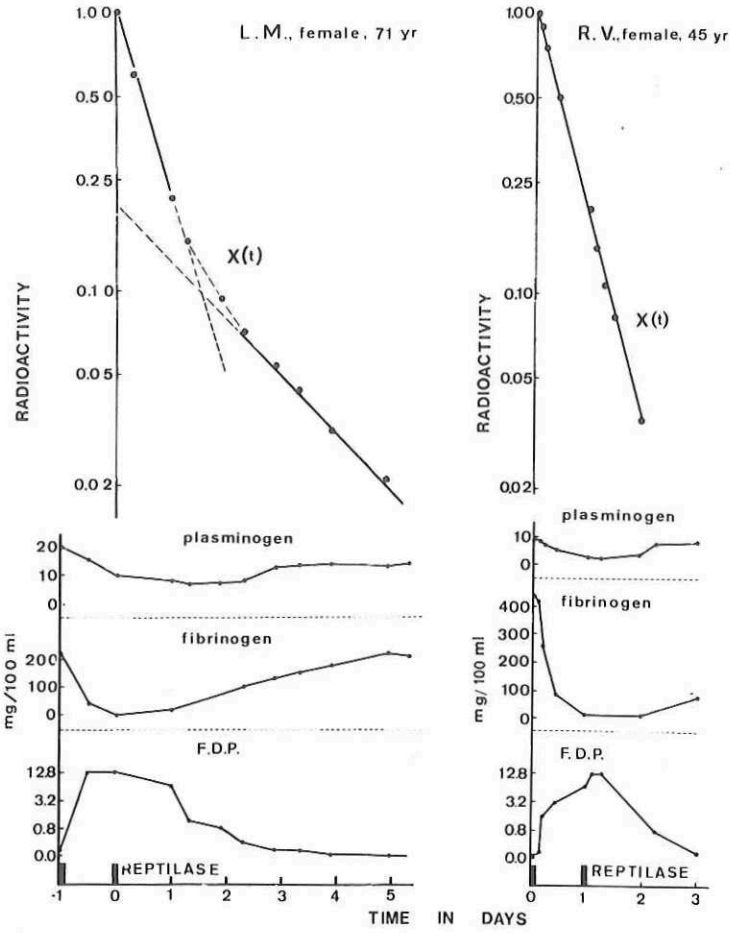

Fig. 5 – *Catabolism of plasminogen during reptilase therapy.*
x(t) : plasma radioactivity
F.D.P. : fibrin(ogen) degradation products in serum.

Sephadex G-200 gel filtration of serial plasma samples obtained during reptilase infusion showed the progressive appearance of small amounts (less than 10 per cent) of radioactivity eluted before the plasminogen peak and corresponding to the elution positions of the additional radioactivity peaks observed during streptokinase therapy. This change in elution pattern was studied in detail in patient AJ (see chapter VI). Referring back to the gel filtration patterns of patient LM and RV, small amounts of radioactivity eluted with a smaller volume were also found to be present (fig. 6). This phenomenon had been overlooked previously (3).

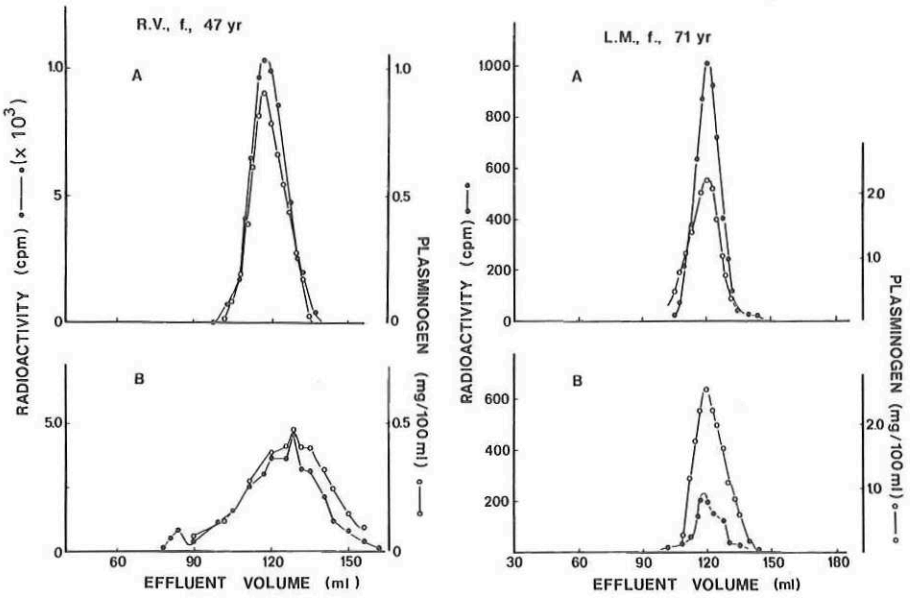

Fig. 6 – *Sephadex G-200 gel filtration of serial plasma samples taken 10 min. and respectively 4 and 24 hrs after injection of labeled plasminogen in patients during reptilase therapy.*

DISCUSSION

The metabolism of labeled plasminogen was studied in patients during streptokinase and reptilase therapy in order to study the kinetics of plasminogen catabolism under these conditions and to investigate the formation and disappearance rate of radiolabeled activation and/or breakdown products of plasminogen.

Activation of the fibrinolytic system by streptokinase resulted in a marked increase in the plasma radioactivity disappearance rate, the half-life being decreased to 0.50-0.75 days. The streptokinase infusion drastically changed the molecular distribution of radioactivity as shown by Sephadex G-200 gel filtration of serial plasma samples. Except in patients LM and FM, in whom a somewhat slower plasminogen depletion was obtained, virtually no radioactivity was found at the plasminogen elution position after 1 hr. Consistently, additional radioactivity peaks were observed at the void volume and at an elution position just before the gamma-globulin peak. These peaks, accounting for most of the plasma radioactivity, presumably represent circulating radioactive plasmin-α_2-macroglobulin and plasmin-α_1-antiplasmin complexes, although no further identification has as yet been carried out.

In the patient LM the plasma plasminogen level decreased more slowly and reached a value of less than 5 % of the pre-infusion level only after 4 hours. This course of events was mirrored by a correspondingly slow decrease in plasminogen-bound radioactivity in the gel filtration samples, indicating that the labeled plasminogen preparation has retained its functional integrity in vivo.

In the reptilase-treated patients a marked increase in the plasma radioactivity disappearance rate was noted. Gel filtration revealed that the evolution of plasma radioactivity remained essentially indicative of the turnover of plasminogen. However, gel filtration of serial plasma samples on Sephadex G-200 (3 patients, see also chapter VI) revealed the presence of small amounts of radioactivity eluted in the positions of the additional peaks observed during streptokinase therapy. These findings, although still preliminary, give further support to the hypothesis that reptilase infusion is accompanied by a secondary fibrinolytic response.

Surprisingly the tracer data could be adequately approximated by a single exponential term over the entire period of data collection in the patients in whom the enzyme infusions were repeated at 24 hr intervals. Such behavior can only be explained by a non-linear stimulation of the consumption of plasminogen by the discontinuous reptilase infusions. However, the present data allow the calculation of a mean fractional catabolic rate of plasminogen during the reptilase infusion. If we assume that the breakdown of plasminogen occurs in the intravascular compartment, the fractional catabolic rate is represented by the inverse of the area under the plasma radioactivity disappearance curve (2). Thus fractional catabolic rates of 0.87 and 1.54 times the plasma pool per day were calculated for the two patients. Disregarding the physiological turnover of 0.55 of the plasma pool per day, an amount of plasminogen equivalent to 0.32 and 0.99 times that present in this pool was catabolized per day as a result of the therapy.

In patient MH with a normal pre-infusion level of plasminogen (20 mg/100 ml plasma), the concentration decreased to 10 mg per 100 ml plasma the day after the first infusion. During the period of apparently steady turnover the amount of plasminogen consumed as a result of the reptilase infusion was thus 10 mg per 100 ml plasma per day. The rate of synthesis during that period was 15 mg per 100 ml plasma per day. Compared to the normal rate of synthesis under physiological conditions of approximately 10 mg per 100 ml plasma per day, the rate of synthesis was thus increased by 50 %.

In the patient with abnormal liver function and a pre-infusion level of 9.3 mg of plasminogen per 100 ml plasma, an increase in the fractional catabolic rate of plasminogen of 0.32 times the plasma pool per day was observed. At the same time the plasma plasminogen was decreased to a stable level of 2.5 mg per 100 ml. Consequently, the amount of plasminogen consumed as a result of the reptilase therapy was approximately 0.8 mg per 100 ml plasma per day. The synthesis rate during this period was 2.2 mg per 100 ml per day. Assuming that under normal conditions this patient would have had a normal fractional catabolic rate (as demonstrated during the turnover experiment with reptilase incubated plasminogen), her plasma plasminogen level would have been maintained at a level of

9.5 mg per 100 ml by a synthesis rate of approximately 5 mg per 100 ml per day. These data suggest that during reptilase therapy the rate of synthesis was decreased in this patient.

In the three other patients in whom the reptilase infusions were not repeated at 24 hr intervals the initial increase of the plasma radioactivity disappearance rate and the decrease of the plasma plasminogen level was very similar to that in patient MH.

In the patient who was already defibrinogenated by a previous infusion of reptilase, a similarly enhanced plasma radioactivity disappearance rate was observed during the first 24 hr after a further reptilase infusion. This was followed by a 24 hr period during which the radioactivity disappeared at a slower rate, then followed by a normal turnover rate of the remaining radioactive plasminogen. These results confirm the finding that during reptilase therapy the plasma radioactivity mainly represents unaltered circulating plasminogen. From extrapolation of the linear part of this curve one can estimate that at least 50 % of the total plasminogen pool is consumed within 48 hr by a single reptilase infusion in an already defibrinogenated patient.

The present results indicate that labeled plasminogen is a useful tool for the quantitative study of the in vivo consumption of plasminogen and of the formation and disappearance rate of labeled activation and/or breakdown products of plasminogen in patients in whom fibrinolysis and/or fibrin formation have been induced therapeutically.

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Chapter IV

METABOLISM OF PLASMINOGEN AND PROTHROMBIN IN CIRRHOSIS OF THE LIVER

The levels of plasminogen and prothrombin in plasma are frequently subnormal in patients with cirrhosis of the liver. This may be the result of diminished synthesis, or excessive consumption, or both.

Increased plasminogen consumption may be caused by in vivo activation of the fibrinolytic system or may be a consequence of intravascular coagulation which has been shown to occur in this disease (1). Increased fibrinolytic activity in cirrhotic plasma has been found by several authors (2-4), but not by others (5). However, liver cirrhosis patients are prone to the development of increased fibrinolytic activity after stimulation by nicotinic acid injection (6-8). This has been ascribed to excess availability and delayed clearance of plasminogen activator (6) and may be potentiated by decreased plasma antiplasmin levels (7).

Plasminogen consumption may be related to intravascular coagulation through activation of the fibrinolytic system (secondary fibrinolysis) (9), or through adsorption of plasminogen onto fibrin evolving in vivo (10) with subsequent enhanced clearing. Secondary fibrinolysis has been accepted on the basis of circulating fibrin degradation products in defibrination syndromes, although no unequivocal evidence for its existence has been obtained. Adsorption of plasminogen onto fibrin has been found by some authors (10), but not by others (11).

Decreased plasma levels of coagulation factors and particularly of the vitamin K dependent factors, II, VII, IX and X have frequently been reported in cirrhosis of the liver. The pertinent literature data have recently been reviewed by Walls and Losowski (12). In general a decrease in these factors has been ascribed to an impaired hepatic synthesis. However, intravascular coagulation, which has been shown to occur in liver cirrhosis (1), might contribute to the hemorrhagic diathesis by consumption of clotting factors, aggravating the decreased levels of these components.

The actual levels of plasminogen and prothrombin in plasma are the resultant of an equilibrium between synthesis and catabolism and therefore do not reflect the dynamics of their turnover. The study of the metabolism of labeled plasminogen

and prothrombin could be useful in the study of the kinetics of synthesis and breakdown and in evaluation of the absolute amounts of protein catabolized in the different metabolic pathways.

The aim of the present study was to quantitate the kinetics of the synthesis and breakdown of plasminogen and prothrombin in cirrhosis of the liver.

MATERIALS AND METHODS

The metabolism of labeled plasminogen was studied in 4 patients and that of labeled prothrombin in 6 patients with cirrhosis of the liver. The diagnosis of liver cirrhosis was documented by physical examination and laboratory findings and was proven histologically in the ones whose coagulation patterns permitted peritoneoscopy and biopsy (table I). A clinical diagnosis was accepted on the basis of long standing jaundice, signs of portal hypertension and biochemical evidence of liver cell failure. None of these patients had noticeable bleeding during the experimental period as determined by clinical observations and hematocrit determinations. During the study the patients were kept on salt-poor diet, in some of them supplemented with a chronic treatment with diuretics. A practically constant body weight was maintained for all patients throughout the study.

The procedure for the metabolic studies and the analysis of tracer data was as described in chapter II.

Anticoagulation was performed by continuous intravenous infusion of heparin (15-20 IU/kg per hr) for 2 to 3 days – a dose that resulted in at least a twofold prolongation of the activated partial thromboplastin time.

RESULTS

Plasminogen metabolism

Plasminogen metabolism in cirrhosis of the liver

Table II summarizes the plasminogen laboratory and tracer data for the 4 patients with cirrhosis of the liver. The mean plasma plasminogen concentration in the cirrhotic patients was 10.8 ± 5.1 mg/100 ml or 5.3 ± 1.3 mg/kg. Daily fluctuations of the plasminogen levels in plasma were negligible in these patients, implying that the amount of plasminogen catabolized daily equaled the amount synthesized.

The plasma radioactivity x(t) versus time could readily be approximated by a sum of two exponential terms. The mean value of the equation, describing the evolution of plasma radioactivity was $x(t) = 0.61e^{-0.48t} + 0.39e^{-2.0t}$ ($t\ 1/2$: 1.48 ± 0.24 days).

The distribution of radioactivity in serial plasma samples was investigated by gel filtration on Sephadex G-100. Throughout the study, the radioactivity was eluted

Table I

Clinical data and laboratory results in liver cirrhosis patients

	Age (yr)	Weight (kg)	Albumin (g/100 ml)	Gamma globulin (g/100 ml)	Bilirubin (mg/100 ml)	Alkaline phosphatases (I.U.)	G.O. transaminase	G.P. transaminase	Platelet count ($\times 10^{-3}/mm^3$)	Plasma fibrinogen (mg/100 ml)	Thrombin time (sec)	Hematocrit (per cent)	Hepatomegaly (cm)	Esophageal varices	Ascites	Previous hepatic coma	Liver histology	Fibrinogen degradation products (μg/ml)
AN	46	51	2.25	2.43	2.7	233	60	19	127	160	26	35	12	–	+	–	+	5
VP	34	70	4.48	1.77	1.3		67	35	129	253	17	43	9	–	–	+	+	
FV	55	53	2.55	3.12	1.8	117	37	16	40	200	22	33					+	
FD	67	59	3.05	2.84	4.9	200	37	25	98	140	23	42			+			
GD	54	68	2.06	2.61	4.4	155	42	29	46	80	31	36	3	+	+	+	+	
MK	67	72	3.68	2.16	1.2	112	70	46	42	160	21	40	2	–	+	–		4
VD	58	74	2.57	1.70	0.9	194	42	38	280			41	13	–	–	–	+	5
RH	44	63	2.47	3.44	8.3	333	73	20	232	154	25				+			
Mean	53	64	2.89	2.51	3.19	192	54	29	124	164	24	39	8					
SD	11	9	0.82	0.62	2.55	76	16	10	90	53	4	4	5					

Table II

Laboratory and plasminogen tracer data

	Plasma volume (ml/kg)	Plasma plasminogen (mg/100 ml)	$x(t) = C_1 e^{-a_1 t} + C_2 e^{-a_2 t}$					
			C_1	a_1	C_2	a_2	t 1/2 for a_1 (days)	t 1/2* for a_1 (days)
AN	46	8.3	0.60	0.55	0.40	2.0	1.25	2.10
VP	56	17.4	0.58	0.39	0.42	1.3	1.80	
FV	42	11.7	0.64	0.48	0.36	2.3	1.45	1.90
FD	47	5.6	0.62	0.49	0.38	2.3	1.40	
Mean	48	10.8	0.61	0.48	0.39	2.0	1.48	2.0
SD	6	5.1	0.02	0.07	0.02	0.5	0.24	
Controls								
Mean	42	20.3	0.44	0.32	0.56	1.4	2.24	
SD	7	2.6	0.07	0.04	0.07	0.4	0.29	

* During heparin infusion.

in one main peak, corresponding to the enzymatically measured plasminogen peak. A small peak, probably representing small circulating breakdown products and free iodide was eluted at the total volume of the column.

The metabolic parameters, calculated from plasma and urine data are summarized in table III.

The results were as follows : fractional catabolic rate constant as determined from the plasma disappearance of radioactivity ($k_{10,p}$) 0.67 ± 0.09 of the plasma pool per day, absolute catabolic rate 3.3 ± 1.4 mg/kg per day, intravascular plasminogen fraction 0.71 and fractional transcapillary efflux rate constant 0.38 ± 0.12 of the plasma pool per day.

Plasminogen metabolism in cirrhosis of the liver compared to the control group

In the cirrhotic patients the intravascular fraction was significantly increased ($p < 0.01$) ; the plasma plasminogen concentration was significantly decreased ($p < 0.01$). A significant decrease was noted in the plasminogen half-life ($p < 0.01$) and an increase in the fractional catabolic rate ($p < 0.05$). The transcapillary efflux rate did not differ in the two series. A significant positive correlation was found in the cirrhotic group between the plasma plasminogen level and the synthetic rate ($p < 0.01$) but not between the plasma plasminogen level and the fractional catabolic rate.

Table III

Metabolic parameters of plasminogen

	Fractional catabolic rate constant			fract. transcap. transfer rate	intra-vascular fraction	synthetic rate (mg/kg/day)
	$k_{10,p}$	$k_{10,u}$	$k_{10,p}$*	k_{12}	IV	
AN	0.77	0.71	0.44	0.35	0.71	2.9
VP	0.55	0.41		0.22	0.72	5.2
FV	0.67	0.55	0.49	0.46	0.71	3.3
FD	0.70			0.48	0.70	1.8
Mean	0.67	0.56		0.38	0.71	3.3
SD	0.09			0.12		1.4
Controls						
Mean	0.55	0.52		0.38	0.58	4.8
SD	0.10	0.10		0.20	0.06	1.2

* During heparin infusion.

Influence of in vivo inhibition of coagulation on the plasminogen turnover in cirrhosis

Anticoagulation was performed over 3 days by monitored infusion of heparin in 2 subjects (AN and VF) in whom the plasminogen half-life was markedly shortened. The evolution of the plasma radioactivity and pertinent coagulation parameters are illustrated for 1 patient in fig. 1. In contrast to our findings in a normal subject, heparin anticoagulation induced a prolongation of the plasminogen half-life from 1.25 to 2.10 and from 1.45 to 1.90 days.

Prothrombin metabolism

Prothrombin metabolism in cirrhosis of the liver

Table IV summarizes the prothrombin laboratory and tracer data for the 6 patients with cirrhosis of the liver. The mean plasma prothrombin concentration was 5.7 ± 1.9 mg/100 ml. Daily fluctuations of the plasma prothrombin levels were also negligible. The immunologically determined plasma prothrombin level was similar to that enzymatically determined. This observation excludes the possibility that in liver cirrhosis the decreased plasma prothrombin level could be due to synthesis of an abnormal prothrombin molecule (e.g. deficient vit. K dependent "finalization" of the molecule).

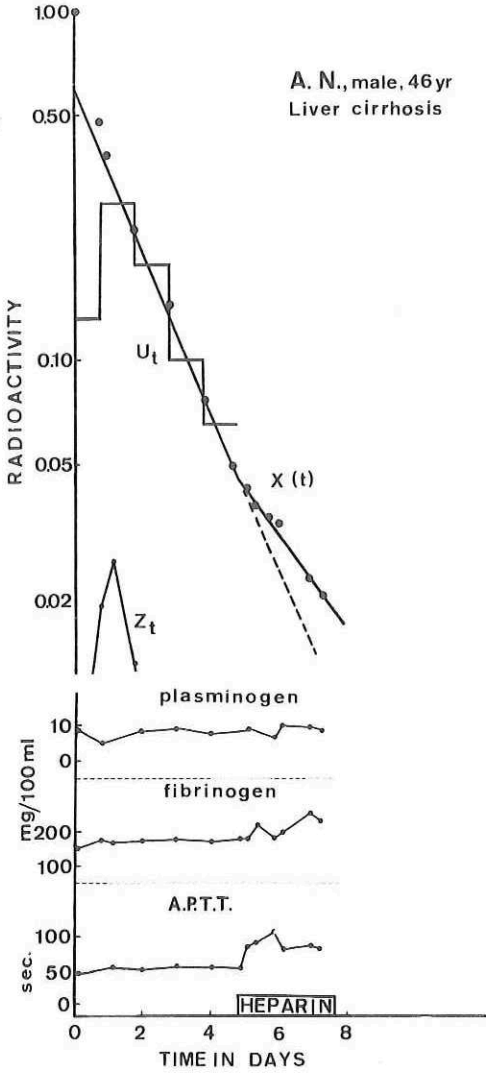

Fig. 1 – *Plasminogen metabolism before and during anticoagulation in a patient with liver cirrhosis.*
x(t) : plasma radioactivity
u_t : fractional daily urinary excretion of label
z_t : non TCA-precipitable radioactivity in plasma
A.P.T.T. : activated partial thromboplastin time.

Table IV

Laboratory and prothrombin tracer data

	Plasma volume (ml/kg)	Plasma prothrombin (mg/100 ml)		$x(t) = C_1 e^{-a_1 t} + C_2 e^{-a_2 t}$					
		Enzymatic assay	Immuno assay	C_1	a_1	C_2	a_2	t 1/2 a_1	t 1/2*
AN	39	4.4		0.54	0.308	0.46	1.7	2.25	2.70
VP	56	7.0		0.62	0.315	0.38	1.7	2.20	
CD	44	3.1	3.7	0.60	0.252	0.40	1.7	2.75	
MK	49	6.1	4.6	0.62	0.257	0.38	1.7	2.70	
VD	40	8.4	8.0	0.58	0.288	0.50	1.4	2.40	3.70
RH	43	5.2		0.68	0.295	0.32	1.4	2.35	2.80
Mean	45	5.7		0.59	0.286	0.41	1.6	2.44	
SD	6	1.9		0.06	0.024	0.06	0.2	0.23	
Controls									
Mean	39	13.3		0.49	0.23	0.51	1.4	3.04	
SD	5	1.3		0.04	0.02	0.04	0.4	0.28	

* During anticoagulation.

The plasma radioactivity x(t) versus time could readily be approximated by a sum of two exponential terms. The mean value of the equation, describing the evolution of plasma radioactivity, was $x(t) = 0.59e^{-0.286t} + 0.41e^{-1.6t}$ (t 1/2 : 2.44 ± 0.23 days). As was observed in the normals, the amount of non-TCA precipitable radioactivity never exceeded 2 % of the total plasma radioactivity, the daily urinary excretion of label was approximately a constant fraction of the corresponding mean plasma radioactivity and the radioactivity of serial plasma samples was eluted in one main radioactivity peak on Sephadex G-100 gel filtration.

The metabolic parameters, calculated from the plasma tracer data are summarized in table V. The results were as follows : fractional catabolic rate constant ($k_{10,p}$) 0.434 ± 0.049 of the plasma pool per day, absolute catabolic rate 1.12 ± 0.49 mg/kg per day, intravascular fraction 0.67 ± 0.05 and fractional transcapillary efflux rate constant (k_{12}) 0.37 ± 0.07.

Prothrombin metabolism in cirrhosis of the liver compared to the control group

In the cirrhotic patients the plasma prothrombin concentration was significantly decreased ($p < 0.01$). A significant decrease was noted for the prothrombin half-life

Table V

Metabolic parameters of prothrombin

	Fractional catabolic rate constant			Fract. transcap. transfer rate constant	Intra-vascular fraction	Synthesis rate (mg/kg/day)
	$k_{10,p}$	$k_{10,u}$	$k^{*}_{10,p}$	k_{12}	IV	
AN	0.494	0.434	0.409	0.46	0.63	0.85
VP	0.457	0.396		0.38	0.69	1.79
CD	0.380	-		0.41	0.66	0.52
MK	0.398	-		0.35	0.70	1.07
VD	0.483	-		0.36	0.60	1.62
RH	0.395	0.480		0.25	0.75	0.86
Mean	0.434	0.437		0.37	0.67	1.12
SD	0.049			0.07	0.05	0.49
Controls						
Mean	0.40	0.349		0.44	0.58	2.27
SD	0.04			0.18	0.05	0.34

* During anticoagulation.

($p < 0.01$). The increase of the fractional catabolic rate was only of borderline significance ($p \doteq 0.10$). The absolute amount of prothrombin synthesized daily was lower in the cirrhotic patients ($p < 0.01$). A significant positive correlation was found in the cirrhotic group, between the plasma prothrombin level and the synthetic rate ($r = 0.75$, $p < 0.05$) but not between the plasma prothrombin level and the fractional catabolic rate ($r = 0.15$, $p > 0.1$).

Influence of in vivo inhibition of coagulation on the prothrombin turnover in cirrhosis

Anticoagulation over 3 days was performed by monitored infusion of heparin in 3 subjects. The evolution of the plasma radioactivity and pertinent coagulation parameters are illustrated for one patient in fig. 2. In contrast to our findings in a normal subject, heparin anticoagulation induced a prolongation of the prothrombin half-life from 2.25 to 2.70, from 2.35 to 2.80 and from 2.40 to 3.70 days. In none of the patients however, was the prolongation of the plasma radioactivity half-life accompanied by a significant rise in the plasma prothrombin level.

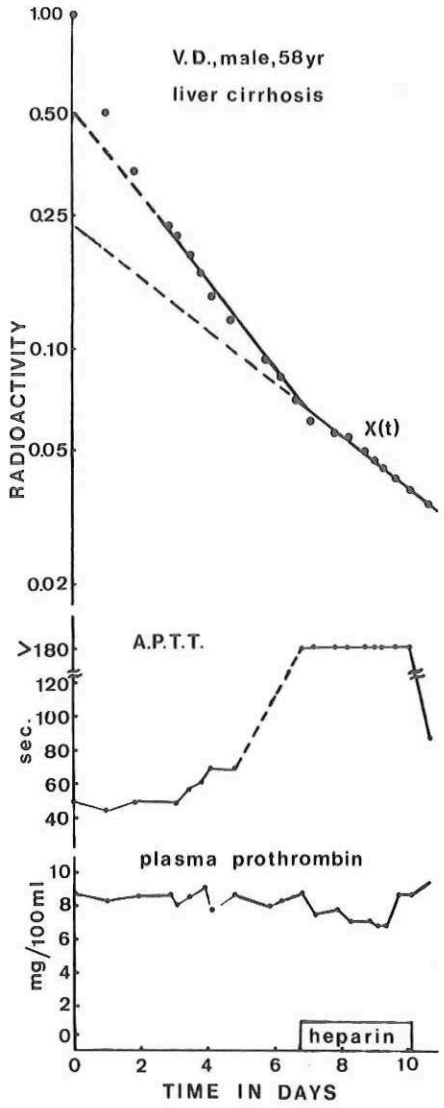

Fig. 2 – *Prothrombin metabolism before and during anticoagulation in a patient with liver cirrhosis.*
x(t) : plasma radioactivity
A.P.T.T. : activated partial thromboplastin time.

DISCUSSION

The plasminogen half-life of 1.48 ± 0.24 days in a group of 4 cirrhotic patients is significantly shorter than the value of 2.24 ± 0.29 days obtained in a group of 21 healthy subjects ($p < 0.01$) whereas the fractional catabolic rate of 0.67 ± 0.09 is significantly higher than the control value of 0.55 ± 0.10 ($p < 0.05$). The absolute amount of plasminogen synthesized daily of 3.3 ± 1.4 mg/kg is however significantly lower than the control value of 4.8 ± 1.2 mg/kg ($p < 0.05$).

The mean prothrombin half-life of 2.44 ± 0.23 days in the 6 cirrhotic patients is significantly shorter than the half-life of 3.04 ± 0.28 days in the control group ($p < 0.01$), whereas the mean fractional catabolic rate of 0.434 ± 0.049 of the plasma pool per day in cirrhosis is significantly higher than the control value of 0.40 ± 0.04 ($p \triangleq 0.10$). The mean quantity of prothrombin catabolized daily of 1.12 ± 0.49 mg/kg in cirrhotics is significantly lower than the control value of 2.27 ± 0.34 mg/kg ($p < 0.05$).

It is very unlikely that the accelerated fractional breakdown of both plasminogen and prothrombin results from a generalized enhanced protein catabolism or excessive protein loss in cirrhosis of the liver as discussed elsewhere (1). The increased plasminogen and prothrombin turnover supports the concept of increased catabolism by an additional metabolic pathway, different from the physiological turnover, which is operative in cirrhosis of the liver.

The normalizing effect of heparin infusion on the turnover of labeled plasminogen in two of the patients and on the turnover of labeled prothrombin in three of the patients supports the concept that their increased turnover is at least partially and probably mainly related to intravascular coagulation.

Several factors have been invoked in the pathogenesis of the in vivo activation of the coagulation system in cirrhosis of the liver : sluggish circulation and local clotting in the expanded collateral circulation and the congested spleen, defective clearing from the circulation of activated clotting factors, and in vivo hemolysis with liberation of thromboplastin-like substances.

Two hypotheses can be put forward to relate the accelerated plasminogen catabolism to in vivo coagulation : secondary activation of the fibrinolytic system (9) or adsorption of plasminogen onto fibrin evolving in vivo (10). The absence of overt signs of fibrinolytic activity (no increased euglobulin fibrinolytic activity, no increase in circulating fibrin split products) does not necessarily invalidate the hypothesis of secondary activation of the fibrinolytic system. Indeed the dynamic study of the plasminogen turnover could be much more sensitive in detecting accelerated consumption than the static picture of circulating levels of plasminogen, plasminogen activator or fibrin(ogen) degradation products. If and to what extent adsorption of plasminogen onto fibrin occurs in vivo cannot as yet be evaluated experimentally. Moreover dissent has been expressed concerning the phenomenon of adsorption of plasminogen onto fibrin in vitro (11).

The finding of a decreased absolute amount of plasminogen and prothrombin catabolized per day in cirrhosis of the liver as compared to the control groups does

not militate against the hypothesis of consumption of a part of both proteins by in vivo coagulation. Indeed, consumption of coagulation factors by basic protein turnover and by chronic, low grade intravascular coagulation are apparently reactions of first order kinetics which proceed at a constant fractional rate (3). The absolute amounts of protein which are consumed in each pathway therefore depend on the actual plasma levels of these factors, which are the resultant of equilibria between synthesis and catabolism.

From the data obtained before and during heparin infusion in our two patients with labeled plasminogen and in our three patients with labeled prothrombin, it is possible to calculate the amounts of plasminogen and prothrombin catabolized in each pathway as discussed in appendix II.

In patient AN the slope of the radioactivity disappearance curve for plasminogen before anticoagulation was 0.55, corresponding to a fractional catabolic rate of 0.77 of the plasma pool per day. During anticoagulation the slope was 0.33, corresponding to a fractional catabolic rate of 0.44. The difference between the two fractional catabolic rates is 0.33 and represents the fraction of the plasma plasminogen pool which is consumed per day, most likely by intravascular coagulation. The corresponding amount of consumed plasminogen is 1.25 mg/kg per day. The lack of a significant increase in the plasminogen level during the short-term anticoagulation period, which one would expect from its retarded catabolism, suggests that the synthesis was not operating at the maximal level during the anticoagulation period. Indeed, the absence of plasminogen increase during anticoagulation could not be explained, in our experience, by an effect of heparin on the plasminogen assay or on the circulating plasminogen level. It is also possible however that the prolongation of the plasma radioactivity half-life is overestimated, due to the presence of a small amount of contaminating protein with a longer half-life, of which the contribution to the total plasma radioactivity would be more significant during the anticoagulation period. This artifact can however not be very significant, because with the same labeled preparation the turnover curves in DC (chapter II), GK (appendix III) and MD (chapter III) were obtained. In none of these turnover curves a significant levelling of the plasma radioactivity disappearance curves was observed.

It has recently been demonstrated in cell-free systems that heparin is a potent inhibitor of protein synthesis (13, 14). Whether the absence of plasminogen increase during anticoagulation in vivo is due to the same phenomenon remains to be established.

In patient FV the slope of the radioactivity disappearance curve for plasminogen before anticoagulation (a_1) was 0.48, corresponding to a fractional catabolic rate of 0.67 of the plasma pool per day. During anticoagulation, the slope was 0.36 corresponding to a fractional catabolic rate of 0.49. The difference between the two fractional catabolic rates of 0.18 of the plasma pool per day corresponds to an amount of 0.88 mg of plasminogen per kg body weight, consumed per day.

If we accept that the difference in fractional catabolic rate of 0.12 of the plasma pool per day between the cirrhotic patients and the control group is due to

consumption of plasminogen, the average amount of plasminogen consumed per day in addition to physiological breakdown is 0.12 x 5.3 = 0.64 mg/kg per day.

On the other hand, the difference in synthetic rate between the cirrhotic and control subjects is 1.5 mg/kg per day which is considerably higher than the difference in catabolic rate.

In patient AN the slope of the radioactivity disappearance curve for prothrombin before anticoagulation was 0.308, corresponding to a fractional catabolic rate of 0.494. During anticoagulation the slope was 0.257, corresponding to a fractional catabolic rate of 0.409. The difference between the two fractional catabolic rates is 0.085, and represents the fraction of the plasma prothrombin pool which is consumed per day by intravascular coagulation. The corresponding amount of consumed prothrombin is 0.15 mg/kg per day. Because this is only 9 % of the amount of prothrombin present in the plasma, it is logical that the plasma prothrombin level did not rise significantly during the short term anticoagulation.

Similarly, the amount of prothrombin catabolized per day in connection with intravascular coagulation in patient VD is 0.26 mg/kg per day, this is only 8 % of the amount of prothrombin present in the plasma.

In general, if we accept that the difference in fractional catabolic rate of 0.030 of the plasma pool per day between the cirrhotic and the control subjects is completely due to consumption by intravascular coagulation, the average amount of prothrombin consumed per day in this pathway is 0.030 x 2.59 = 0.08 mg/kg per day. On the other hand, the decrease in synthesis rate between the cirrhotics and the control group is 1.15 mg/kg per day.

In conclusion, our results on the metabolism of plasminogen and prothrombin in patients with cirrhosis of the liver, indicate that two mechanisms are responsible for the abnormal plasminogen and prothrombin turover : (1) increased fractional catabolic rate, possibly mainly in connection with continuous low grade intravascular coagulation and (2) impaired synthesis, most probably by the diseased liver. The actual level of circulating plasminogen and prothrombin is however determined by the residual synthetic capacity.

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Chapter V

METABOLISM OF FIBRINOGEN, PLASMINOGEN AND PROTHROMBIN DURING STRENUOUS EXERCISE IN MAN

Physical exercise induces changes in the results of laboratory tests of hemostasis, which include shortening of the clotting time (or such tests as the recalcification or partial thromboplastin time), an increase in factor VIII, increased platelet adhesiveness and ADP-induced aggregation, and increased euglobulin fibrinolytic activity (for review see ref. 1). Such changes in the coagulation or fibrinolysis tests can also be encountered in thrombotic or fibrinolytic conditions respectively. However, the extent to which, if indeed at all, these abnormalities reflect an actual systemic prothrombin and/or plasminogen activation and fibrinogen to fibrin conversion is unknown.

In order to quantitate the fibrinogen consumption in association with physical exercise and the relative role therein of in vivo activation of the coagulation and fibrinolytic system, we studied the metabolism of labeled fibrinogen, plasminogen and prothrombin before, during, and after exertion in young healthy volunteers. In order to amplify possibly marginal effects we decided to subject the individuals to repeated exhausting physical exercise.

MATERIALS AND METHODS

Twelve healthy volunteers, laboratory personnel and students, participated in the study. The metabolism of ^{131}I-labeled fibrinogen was studied in all subjects, combined with ^{125}I-plasminogen in four and with ^{125}I-prothrombin in five of them.

The procedure for the injection of tracer proteins and sampling of blood (at least twice daily) was as described in chapter II. In total 17 to 21 blood samples of 5 to 15 ml were obtained over a period of 9 or 10 days. The normal turnover of the labeled proteins was followed for 4-5 days. During this period the subjects performed their normal duties but were asked to refrain from taking part in sports.

The physical exercise was started on the morning of the 5th (or 6th) day after

injection of the labeled materials and repeated after 10 min of rest during which, in some of the subjects blood samples were taken to determine the influence of exercise on the coagulation and fibrinolytic profile. The blood samples for estimation of the plasma radioactivity during the period of physical exercise were taken just before the exertion in order to exclude or at least minimize the influence of hemoconcentration on the plasma radioactivity.

The physical exertion was obtained on a bicycle ergometer with a work load of 180 Watts per second (1200 kgm per min), until exhaustion. Most of the individuals were exhausted within 5 min. Two of the subjects who performed for over 15 min at this load were subsequently exhausted with 250 or 300 Watts per second. The exertion procedure was repeated on the evening of the same day and the morning and evening of the following day. Thus each individual was subjected in total to eight sessions of exhaustive exercise.

Auxilliary laboratory tests were performed as described in appendix I.

Analysis of tracer data

The observed changes in the plasma radioactivity disappearance rates were subjected to statistical analysis as follows.

The plasma radioactivity data, obtained before the exercise period (6-11 measurements), were fitted by a sum of two exponential terms $x(t) = C_1 e^{-a_1 t} + C_2 e^{-a_2 t}$. The limiting slope ($a_1$) of the radioactivity disappearance curve was calculated by subtracting the contribution of the second term of the equation describing the evolution of the plasma radioactivity from the experimental data, and by linear regression analysis of the logarithm of this corrected plasma radioactivity versus time. The slope of the plasma radioactivity disappearance curve during the two days of physical exercise (5 data points) was calculated by linear regression analysis of the logarithm of the plasma radioactivity versus time.

The fractional catabolic rate constant during exercise (k^*_{10}) was calculated from equation (20), derived in appendix II.

RESULTS

The clinical and laboratory data on the 12 healthy volunteers are summarized in table I.

Influence of physical exercise on laboratory tests for the coagulation and fibrinolytic system

Physical exercise induced a shortening of the partial thromboplastin time and an increase in the factor VIII activity in plasma, obtained within 5 min after the end of the exertion (fig. 1).

Table I

Clinical and laboratory data

	Age (yr)	Sex	Weight (kg)	Plasma fibrinogen (mg/100 ml)	Plasma plasminogen (mg/100 ml)	Plasma prothrombin (mg/100 ml)
JT	25	M	72	244	–	–
JV	34	M	72	296	–	–
AC	24	M	70	167	–	–
DK	24	M	87	179	21.5	–
VB	24	M	71	130	16.1	–
SJ	23	F	54	208	16.0	–
DR	23	F	50	191	17.4	–
DC	30	M	85	208	–	11.9
NS	29	M	65	170	–	10.0
GB	26	M	88	187	–	10.4
SJ	25	M	66	261	–	11.5
RV	30	M	75	186	–	13.3
Mean	26		71	202	17.8	11.4
SD	4		12	45	2.6	1.3

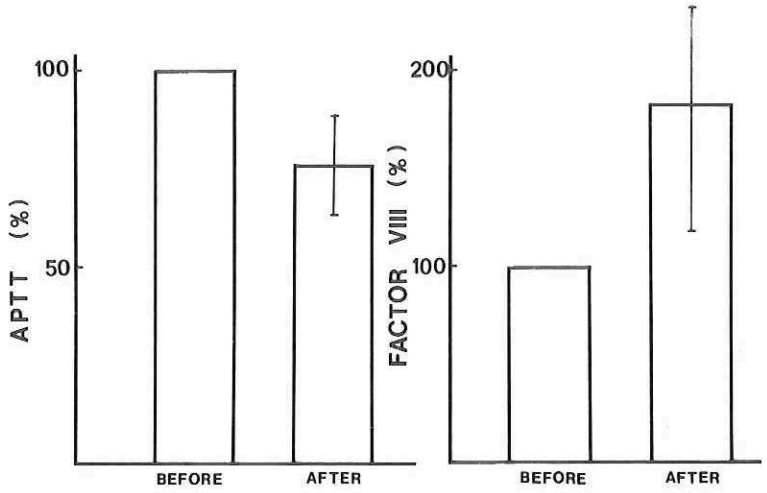

Fig. 1 – *Influence of physical exercise on the activated partial thromboplastin time and the factor VIII activity in plasma. The values are means of 4 determinations on plasma obtained just prior to and within 5 min. after the end of the exertion. The values before exercise are set at 100% in each individual to allow a direct comparison. The vertical lines represent ± s (standard deviation) of the mean.*

Immunological determination of factor-VIII showed a parallel increase in factor-VIII-related antigen following exercise (fig. 2).

The fibrinolytic activity in plasma, estimated as the euglobulin clot lysis time and the euglobulin fibrinolytic activity on bovine fibrin plates, increased markedly following physical exercise (fig. 3).

The plasma levels of fibrinogen, plasminogen and prothrombin, and the amounts of fibrinogen degradation products in the serum were not influenced by physical exercise.

Influence of physical exercise on the metabolism of labeled fibrinogen, plasminogen and prothrombin

The tracer data are summarized in table II. Representative turnover curves are shown in fig. 4. During physical exercise a significant shortening of the plasma radioactivity half-life of fibrinogen (from 3.84 to 3.30 days, $p < 0.01$) and of plasminogen (from 2.56 to 2.30 days, $p < 0.01$) but not of prothrombin (from 2.87 to 2.96 days, $p > 0.2$) was observed.

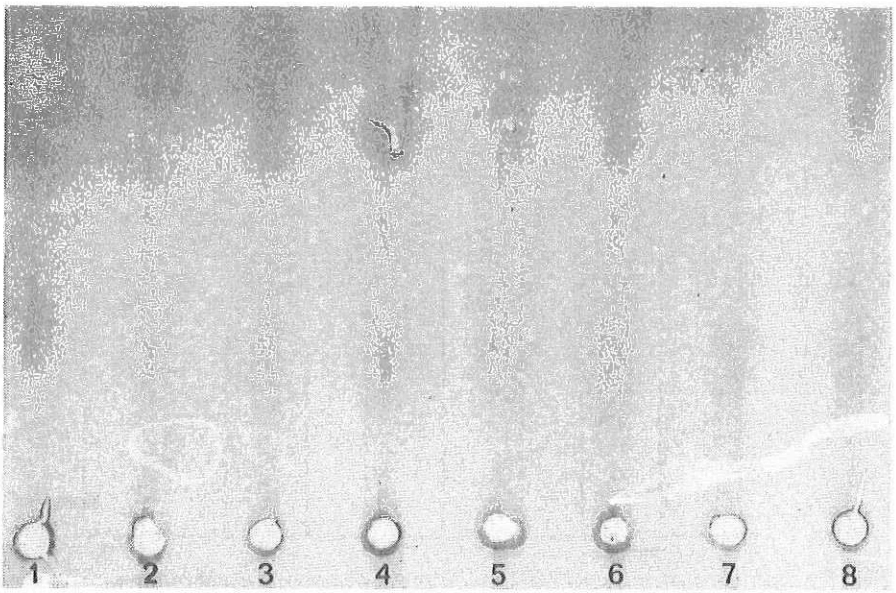

Fig. 2 – *Influence of intermittent physical exercise on the level of factor VIII-related antigen in the plasma of one healthy individual, determined by the method of Laurell. Odd numbers : just prior to exercise. Even numbers : within 5 min after the exercise.*

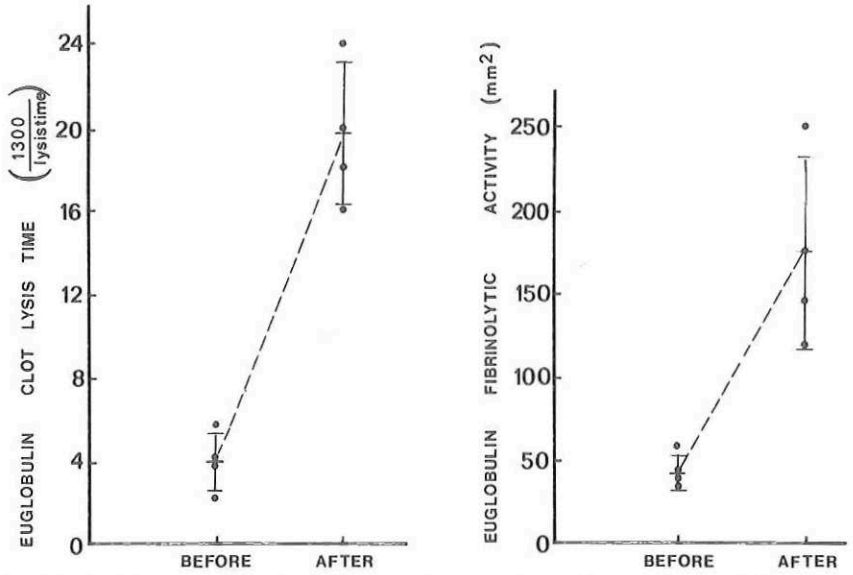

Fig. 3 -- *Influence of physical exercise on the fibrinolytic activity in the euglobulin-fraction of plasma as determined by the euglobulin clot lysis time and the fibrinolytic activity on bovine fibrin film. Each dot represents the mean of 4 determinations on the euglobulin fraction of plasma, obtained just prior to and within 5 min after the end of the exertion in the same subject. The vertical line represents ± s (standard deviation) of the mean.*

The main metabolic parameters calculated before and during physical exercise are summarized in table III. The fractional catabolic rate constants and absolute catabolic rates of fibrinogen and plasminogen but not of prothrombin, are significantly higher during the exercise period than before ($p < 0.01$ and $p < 0.050$ respectively).

DISCUSSION

The present study was undertaken to determine the significance of the alleged hypercoagulable and fibrinolytic state induced in plasma by physical exercise, at the level of plasminogen and/or prothrombin activation and of fibrinogen consumption.

Following exertion, the well known increase in euglobulin fibrinolytic activity (2, 3), factor VIII level (4) and factor VIII-related antigen (1, 5), and shortening of the activated partial thromboplastin time (3) were observed.

Table II

Tracer data

	Plasma volume ml/kg	$x(t) = C_1 e^{-a_1 t} + C_2 e^{-a_2 t}$						
		C_1	a_1	C_2	a_2	t 1/2	a_1^*	t 1/2*
A. *Fibrinogen*								
JT	39	0.79	0.165	0.21	1.10	4.20	0.187	3.70
JV	34	0.85	0.197	0.15	1.76	3.50	0.217	3.20
AC	41	0.82	0.177	0.17	0.94	3.90	0.239	2.90
DK	43	0.80	0.156	0.20	1.30	4.40	0.164	4.25
VB	36	0.70	0.171	0.30	1.73	4.05	0.198	3.50
SJ	42	0.72	0.188	0.28	1.61	3.70	0.255	2.70
DR	41	0.74	0.180	0.26	2.00	3.85	0.207	3.35
DC	33	0.74	0.198	0.26	0.92	3.50	0.207	3.35
NS	37	0.63	0.169	0.37	1.58	4.10	0.217	3.20
GB	39	0.78	0.217	0.22	0.86	3.20	0.213	3.25
SJ	40	0.75	0.195	0.25	1.58	3.55	0.213	3.25
RV	33	0.63	0.170	0.37	1.69	4.10	0.235	2.95
Mean	38	0.75	0.182	0.25	1.42	3.84	0.213	3.30
SD	4	0.06	0.017	0.07	0.38	0.35	0.024	0.40
B. *Plasminogen*								
DK	39	0.49	0.237	0.51	1.08	2.95	0.257	2.70
VB	38	0.53	0.279	0.47	2.68	2.50	0.330	2.10
SJ	39	0.41	0.303	0.59	1.51	2.30	0.338	2.05
DR	42	0.41	0.277	0.59	2.17	2.50	0.296	2.35
Mean	40	0.46	0.274	0.54	1.86	2.56	0.305	2.30
SD	2	0.06	0.027	0.06	0.71	0.28	0.037	0.30
C. *Prothrombin*								
DC	31	0.56	0.256	0.44	1.31	2.70	0.201	3.45
NS	40	0.47	0.241	0.53	2.04	2.90	0.239	2.90
GB	39	0.55	0.248	0.45	0.97	2.80	0.243	2.85
SJ	39	0.49	0.231	0.51	2.24	3.00	0.227	3.05
RV	32	0.47	0.235	0.53	1.98	2.95	0.272	2.55
Mean	36	0.51	0.242	0.49	1.71	2.87	0.236	2.96
SD	4	0.04	0.010	0.04	0.54	0.12	0.026	0.33

* During the two day period of physical exercise.

Table III

Metabolic parameters

	Fractional catabolic rate constant		Fractional transcapillary transfer rate	Intra-vascular fraction	Absolute catabolic (synthetic) rate (mg/kg/day)	
	k_{10}	k^*_{10}	k_{12}	IV	before	during
A. *Fibrinogen*						
JT	0.201	0.229	0.161	0.822	19.1	21.8
JV	0.227	0.251	0.204	0.867	22.8	25.3
AC	0.204	0.281	0.101	0.862	14.0	19.2
DK	0.189	0.199	0.195	0.824	14.5	15.3
VB	0.234	0.273	0.404	0.730	11.0	12.8
SJ	0.254	0.345	0.336	0.753	21.8	30.1
DR	0.236	0.272	0.417	0.763	18.5	21.3
DC	0.249	0.261	0.137	0.796	17.1	17.9
NS	0.252	0.330	0.439	0.670	15.9	20.8
GB	0.230	0.255	0.099	0.836	16.8	18.6
SJ	0.250	0.274	0.292	0.781	26.1	28.6
RV	0.255	0.361	0.478	0.667	15.7	22.2
Mean	0.231	0.278	0.272	0.781	17.8	21.2
SD	0.022	0.047	0.139	0.067	4.2	5.0
B. *Plasminogen*						
DK	0.394	0.436	0.273	0.602	3.30	3.66
VB	0.482	0.580	0.926	0.579	2.95	3.55
SJ	0.573	0.663	0.442	0.528	3.58	4.14
DR	0.571	0.618	0.823	0.485	4.17	4.52
Mean	0.505	0.574	0.616	0.548	3.50	3.97
SD	0.085	0.098	0.309	0.052	0.52	0.45
C. *Prothrombin*						
DC	0.396	0.302	0.323	0.646	1.46	1.11
NS	0.452	0.448	0.742	0.533	1.81	1.79
GB	0.373	0.364	0.200	0.665	1.51	1.48
SJ	0.426	0.418	0.830	0.543	1.91	1.87
RV	0.441	0.522	0.719	0.533	1.88	2.62
Mean	0.418	0.411	0.563	0.584	1.71	1.69
SD	0.033	0.083	0.282	0.066	0.21	0.42

* During the two day period of physical exercise.

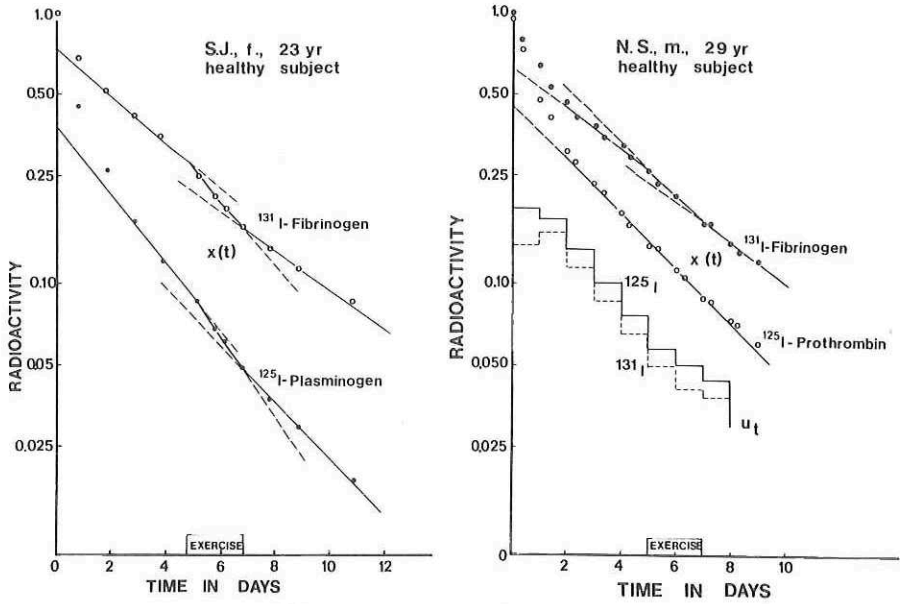

Fig. 4 - *Metabolism of ^{131}I-fibrinogen and ^{125}I-plasminogen (left side) and of ^{131}I-fibrinogen and ^{125}I-prothrombin (right side) before and during physical exercise in a healthy subject.*
x(t): plasma radioactivity
u_t : fractional daily urinary excretion of label.

The shortening of the activated partial thromboplastin time has been ascribed to an increased factor XII level (3). In 6 tested subjects however, we did not find an increase in the factor XII content of plasma. Prentice *et al.* (1) have ascribed the shortening of the Stypven-time after exercise to a release into the plasma of microparticulate material with thromboplastic activity. However, since in the activated partial thromboplastin time an excess of such material is added to the plasma, this explanation does not seem adequate to explain the shortened partial thromboplastin time. The origin of the alleged hypercoagulable state following physical exercise thus remains obscure.

The euglobulin fibrinolytic activity was tested in 4 subjects before and during each standard exercise period. A marked and reproducible increase was observed in all cases. In agreement with the data of Prentice *et al.* (1), however, this was not accompanied by an increase of fibrinogen degradation products in serum, nor of a change in the plasminogen or antiplasmin level in plasma.

During strenuous physical exercise, the plasma radioactivity disappearance rate of fibrinogen and plasminogen but not of prothrombin increased significantly. Theoretically there are at least four possible explanations for this phenomenon.

The first possibility is that physical exercise in addition to a loss of plasma fluid (hemoconcentration) also results in a loss of protein (shift to the extravascular space). There are however two arguments against this hypothesis : a) the plasma protein levels are completely normalized within 30 min after physical exercise (6), long before the time at which blood samples were taken in our experiments and b) we did not observe an increased disappearance rate of prothrombin, a protein which has a larger distribution space than fibrinogen.

The second possibility is that during physical exercise, there is a generalized increased protein catabolism. To the best of our knowledge (and after discussion with Prof. J. Poortmans, Laboratoire de l'effort, University of Brussels) there is no evidence in favor of this point of view. Furthermore the steady decay of prothrombin argues against this hypothesis.

It is theoretically possible that the increased plasma radioactivity disappearance would be caused by proteinuria which we did not estimate in our subjects. Furthermore, during exercise plasmic fibrinogen degradation products could be demonstrated immunologically in post-exercise urine (7), but only in trace amounts. However the amount of fibrinogen consumed during exercise (on the average 240 mg per day) is at least an order of magnitude greater than the amount of fibrinogen degradation products in the urine (7).

Finally it is possible that the increased fibrinogen and plasminogen metabolism during physical exercise is due to consumption. In view of the known exercise-induced changes in the coagulation-fibrinolytic profile this hypothesis probably explains most, if not all of the increased turnover.

The fractional catabolic rate of fibrinogen in the 12 healthy subjects increased from 0.231 to 0.278 of the plasma pool per day. This accelerated catabolism (0.047 of the plasma pool per day) corresponded to a consumption of 3.4 mg fibrinogen per kg per day as a consequence of 4 periods of exhausting exercise. Per exertion this represents approximately 0.85 mg per kg or 1.1 % of the plasma fibrinogen. The average total exercise-induced fibrinogen consumption was approximately 240 mg per day.

The fractional catabolic rate of plasminogen increased from 0.505 to 0.574 of the plasma pool per day, corresponding to an exercise-induced consumption of 0.07 of the plasma plasminogen pool or 0.47 mg per kg per day. Per exertion this represents approximately 0.12 mg per kg or 1.7 % of the plasma pool per day. The average total exercise-induced plasminogen consumption was approximately 33 mg per day.

On the basis of a molecular weight of 330,000 for fibrinogen and 90,000 for plasminogen, about 2 molecules of fibrinogen were consumed per molecule of plasminogen, (on the basis of a normal plasma plasminogen level of 20 mg/100 ml), as a result of exercise.

Physical exercise had no influence on the metabolism of labeled prothrombin,

suggesting that the fibrinogen consumption is the result not of intravascular coagulation but of fibrinogenolysis.

Recently Harpel and Mosesson (8) found that the plasmin-α_2-macroglobulin complex retains a small fraction (less than 0.1 %) of the fibrinogenolytic activity of the unbound enzyme and that this residual activity is protected from the action of other plasma inhibitors. It may therefore be possible that the fibrinogen consumption which we have observed in association with physical exercise is caused by an increase in the circulating plasmin-α_2-macroglobulin complex, as a result of increased activation of plasminogen.

The plasmin generated in the blood following physical exercise binds partly to α_2-macroglobulin and partly to the α_1-globulin inhibitor. The relative amounts bound to each of the inhibitors are not very well known but our gel filtration experiments on samples obtained from patients during streptokinase therapy suggest that approximately 1/3 of the plasmin generated in vivo binds to α_2-macroglobulin and 2/3 to the α_1-globulin. Thus, upon physical exhaustion approximately 0.5 % of the circulating plasminogen, after activation to plasmin, would bind to α_2 macroglobulin. Although this complex has less than 0.1 % of the fibrinogenolytic activity of the uncomplexed plasmin, it nevertheless seems responsible for the observed fibrinogen consumption. Probably its half-life in plasma of several hours and its protection from the action of other circulating inhibitors are responsible for this phenomenon.

In view of the fact that exercise activates both the coagulation and the fibrinolytic systems, as determined by in vitro assay techniques, it was until now unclear whether physical exertion would enhance or diminish a thrombotic tendency. Our data indicate that, during exercise, activation of the fibrinolytic system (plasminogen consumption) largely exceeds activation of the coagulation system (prothrombin consumption) in vivo, and that therefore exercise may have a favourable effect in preventing thrombosis. The fibrinolytic potential generated by physical exercise might exceed the amount of fibrinogenolytic activation, observed in our experiments, as 1) the released plasminogen activator may have a strong affinity for fibrin and 2) the plasmin-antiplasmin complex could dissociate in the presence of fibrin.

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Chapter VI

METABOLISM OF THE TWO MAJOR FORMS OF PLASMINOGEN, SEPARATED BY LYSINE-AGAROSE CHROMATOGRAPHY

Section I :

TURNOVER OF THE TWO MAJOR FORMS OF PLASMINOGEN IN HEALTHY SUBJECTS

The turnover studies with labeled plasminogen, reported in the previous chapters were carried out with plasminogen preparations, containing the multiple molecular forms present in single donor plasma. The derived kinetic parameters are thus representative of the whole plasma plasminogen.

Using lysine-agarose chromatography with EACA gradient elution (1) plasminogen is separated in two fractions, which each display six bands on polyacrylamide gel electrophoresis at pH 8.3, with staggered overlapping (2). Thus human plasminogen occurs in twelve main molecular forms in the circulation.

Our studies (2) have indicated that the plasminogen components, separated by lysine-agarose chromatography, have small but significant differences in amino acid composition. Because their molecular weights and NH_2-terminal and COOH-terminal amino acids are identical, the observed differences are probably due to differences in primary structure.

The heterogeneity, displayed upon polyacrylamide gel electrophoresis at pH 8.3 seems to be due to a variation in sialic acid content (2).

The aim of the present study was to evaluate the turnover kinetics of the two major forms of human plasminogen, isolated by lysine-agarose chromatography, in healthy subjects.

MATERIALS AND METHODS

Preparation and characterization of the labeled plasminogen

Plasminogen was purified from fresh frozen plasma by affinity chromatography and

Sephadex G-25 gel filtration. From the resulting material, the two plasminogen forms, termed plasminogen A1 (first peak) and plasminogen A2 (second peak) were separated by lysine-agarose chromatography, using EACA gradient elution. The details of the procedure are described elsewhere (2). After concentration by ultrafiltration the two fractions were further purified by Sephadex G-150 gel filtration and DEAE-Sephadex chromatography as described in chapter I and labeled with $Na^{125}I$ or $Na^{131}I$ as described in chapter II.

The in vitro evaluation of the labeled materials has been reported elsewhere (2).

Metabolic studies

The control group consisted of 8 normal volunteers. The procedure for the metabolic studies was as described in chapter II and the analysis of tracer data as described in appendix II.

RESULTS

Table I summarizes the clinical and laboratory data for the normal subjects. The turnover study shown in fig. 1 is similar to those obtained for all the subjects. For both plasminogen forms, the plasma radioactivity data x(t) plotted against time

Table I

Clinical and laboratory data on the healthy subjects

	Age (yr)	Weight (kg)	Plasma volume (ml/kg)	
			^{125}I-Plg A1	^{131}I-Plg A2
HC	30	78	46	34
IC	43	72	45	35
BN	75	72	52	31
UV	30	84	–	–
PG	25	65	42	42
KV	33	82	37	37
PV	27	58	40	38
GV	77	65	–	
Mean	43	72	43	36
SD	21	9	5	4

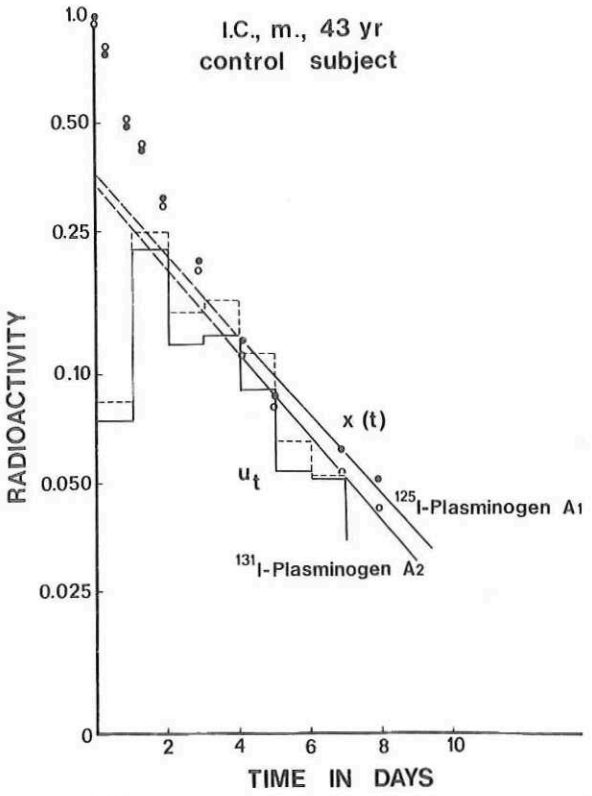

Fig. 1 — *Turnover of ^{125}I-plasminogen A1 and ^{131}I-plasminogen A2 in a healthy subject.*
x(t) : plasma radioactivity.
u_t : fractional daily urinary excretion.

were approximated by a sum of two exponential terms. The parameters of the equation describing the decay of the plasma radioactivity for both forms, are summarized in table II. The two plasminogen fractions behaved very similarly to one another and to the total plasminogen in the turnover experiments. The plasma radioactivity half-life was 2.34 ± 0.23 days for plasminogen A1 and 2.25 ± 0.17 days for plasminogen A2.

The daily amount of radioactivity excreted in the urine was very similar for both fractions. The fractional catabolic rate constant determined from the plasma radioactivity disappearance expressed as a fraction of the plasma pool per day ($k_{10,p}$) was 0.55 ± 0.07 for plasminogen A1 and 0.60 ± 0.06 for plasminogen A2.

Table II

Tracer data of the two plasminogen forms, isolated by lysine-agarose chromatography

	FIRST PEAK Plasminogen A1					SECOND PEAK Plasminogen A2				
	$x(t) = C_1\ e^{-a_1 t} + C_2\ e^{-a_2 t}$					$x(t) = C_1\ e^{-a_1 t} + C_2\ e^{-a_2 t}$				
	C_1	a_1	C_2	a_2	t 1/2 for a_1 (days)	C_1	a_1	C_2	a_2	t 1/2 for a_1 (days)
HC	0.34	0.315	0.66	1.16	2.20	0.35	0.330	0.67	1.26	2.10
IC	0.35	0.252	0.62	1.16	2.75	0.34	0.277	0.66	1.16	2.50
BN	0.34	0.301	0.66	1.26	2.30	0.33	0.308	0.67	1.39	2.25
UV	0.45	0.322	0.55	1.54	2.15	0.40	0.330	0.60	1.26	2.10
PG	0.44	0.289	0.56	1.54	2.40	0.37	0.289	0.63	1.39	2.40
KV	0.49	0.272	0.51	1.54	2.55	0.45	0.289	0.55	1.39	2.40
PV	0.50	0.295	0.50	1.73	2.35	0.47	0.315	0.53	1.73	2.20
GV	0.36	0.338	0.64	1.39	2.05	0.31	0.338	0.69	1.26	2.05
Mean	0.41	0.298	0.59	1.42	2.34	0.38	0.310	0.63	1.36	2.25
SD	0.07	0.028	0.07	0.21	0.23	0.06	0.023	0.06	0.17	0.17

CONCLUSION

The metabolism of the two major plasminogen forms, separated by lysine-agarose chromatography was studied in 8 healthy subjects to determine if the presumed differences in primary structure were accompanied by differences in turnover characteristics. The two plasminogen fractions behaved very similarly to one another and to the total plasminogen in the turnover experiments.

Siefring and Castellino (3) have very recently performed turnover studies with in vivo labeled ^{14}C-plasminogen in rats and rabbits. These authors also found only minor differences between the two forms but surprisingly obtained very short plasma radioactivity half-lifes, ranging from 9 to 14 hr.

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2. COLLEN, D. (1973) – *The microheterogeneity of human plasminogen.* Doctorate thesis. Faculty of Sciences, University of Leuven, Belgium.
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Section II :

TURNOVER OF THE TWO MAJOR FORMS OF PLASMINOGEN DURING STREPTOKINASE AND REPTILASE THERAPY

In vitro experiments have revealed that, upon activation with small amounts of streptokinase or urokinase (molar ratios 1/2500 to 1/500), the two major forms of plasminogen are converted to plasmin at different rates (1).

In an effort to determine whether these in vitro findings are biologically significant, the metabolism of both forms was studied during streptokinase therapy in two patients and during reptilase therapy in one patient.

MATERIALS AND METHODS

The metabolism of ^{125}I-plasminogen A1 and ^{131}I-plasminogen A2 was studied in two patients with chronic thromboembolic disease. The therapy consisted of an initial dose of 500,000 IU over 10 hr in the first patient (AJ) and 500,000 IU over 48 hr in the second patient (LV). These low priming doses were selected to obtain a progressive activation of the fibrinolytic system for investigative purposes. At the end of this experimental period, the patients were further treated with streptokinase according to the classical scheme (maintenance dose of 100,000 IU per hr for 72 hr).

The labeled plasminogens were injected 10 min prior to the start of the streptokinase infusion in the first patient (AJ) and 1 day before the therapy in the second one (LV).

The metabolism of both plasminogen fractions during reptilase therapy was studied in patient AJ on a separate occasion (before the turnover study during streptokinase). The tracers were injected two days after the first infusion of 2 ml reptilase, at a time when the plasma fibrinogen level was 120 mg/100 ml and the plasminogen 9.3 mg/100 ml. The enzyme infusion was repeated once after the injection of tracer.

Blood samples were taken after different time intervals on trisodium citrate and p-NPGB in the streptokinase patients and on trisodium citrate, p-NPGB and anti-reptilase antiserum in the reptilase experiment in the amounts described in chapter III.

The clinical and laboratory data on the patients are summarized in table I.

RESULTS

Turnover of the two major forms of plasminogen during streptokinase therapy

In patient AJ, infusion of 500,000 IU streptokinase over 10 hr resulted in a progressive decrease of the plasma fibrinogen from 250 to 100 mg/100 ml and of

Table I

Clinical and laboratory data on the patients

	Sex	Age (yr)	Weight (kg)	Plasma plasminogen (mg/100 ml)	Initial dose of streptokinase
A. *Streptokinase*					
AJ	M	62	55	8.0	500,000 IU over 10 hr
LV	M	51	73	15.2	500,000 IU over 48 hr
B. *Reptilase*					
AJ	M	62	55	9.3	

the plasma plasminogen from 8.0 to approximately 5 mg/100 ml (fig. 1). The plasma radioactivity disappearance rate of both plasminogens was markedly and similarly increased to a half-life of 0.5 days.

Gel filtration of serial plasma samples on Sephadex G-200 (fig. 2) showed that during streptokinase infusion the plasma radioactivity was partially and progressively converted to elution positions corresponding to the two additional radioactivity peaks which probably represent I-plasmin-α_2-macroglobulin and I-plasmin-α_1-antiplasmin complexes. Fig. 2 clearly illustrates that plasminogen A1, which is more easily converted to plasmin in vitro, is also more easily converted to the smaller elution volumes on Sephadex G-200.

In patient LV, infusion of 500,000 IU streptokinase over 48 hr resulted in a progressive decrease of fibrinogen (after a transient increase to 550 mg/100 ml) from 350 to 200 mg/100 ml and a progressive decrease of the plasma plasminogen from 15 to 6 mg/100 ml (fig. 3). The plasma radioactivity disappearance rate of both plasminogens, injected 1 day before the start of the streptokinase infusions, was also markedly and similarly increased to a half-life of approximately 0.75 days.

Gel filtration of serial plasma samples on Sephadex G-200 (fig. 4), revealed only small amounts of radioactivity in the additional elution positions of the plasmin-inhibitor complexes. In this patient the different rate of conversion of the two plasminogen forms to earlier elution positions was not observed.

Turnover of the two major forms of plasminogen during reptilase infusion in a patient with thromboembolic disease

In patient AJ ^{125}I-plasminogen A1 and ^{131}I-plasminogen A2 was injected two days after a prior reptilase infusion, at a time when the plasma fibrinogen was 120

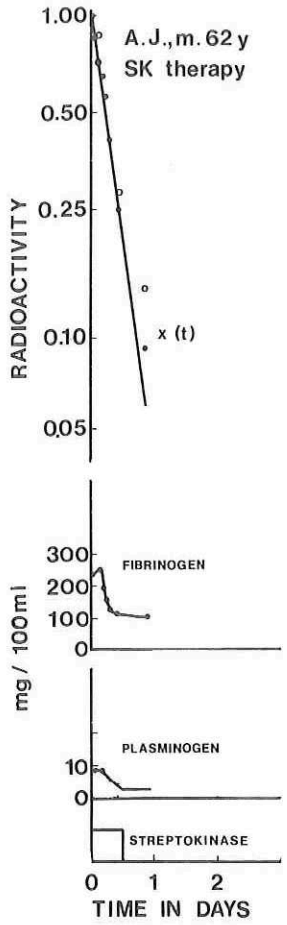

Fig. 1 – *Metabolism of ^{125}I-plasminogen A1 (○) and ^{131}I-plasminogen A2 (●) during infusion of 500,000 IU streptokinase over 10 hr in a patient with thromboembolic disease.*
x(t) : plasma radioactivity.

mg/100 ml and the plasma plasminogen 10 mg/100 ml. The enzyme infusion was repeated once after injection of the tracers and resulted in a progressive decrease of the plasma fibrinogen to an undetectable level and of plasminogen to 5 mg/100 ml plasma (fig. 5).

The disappearance rate of the plasma radioactivity was initially (during the first day) markedly and similarly enhanced for both plasminogens. Thereafter a

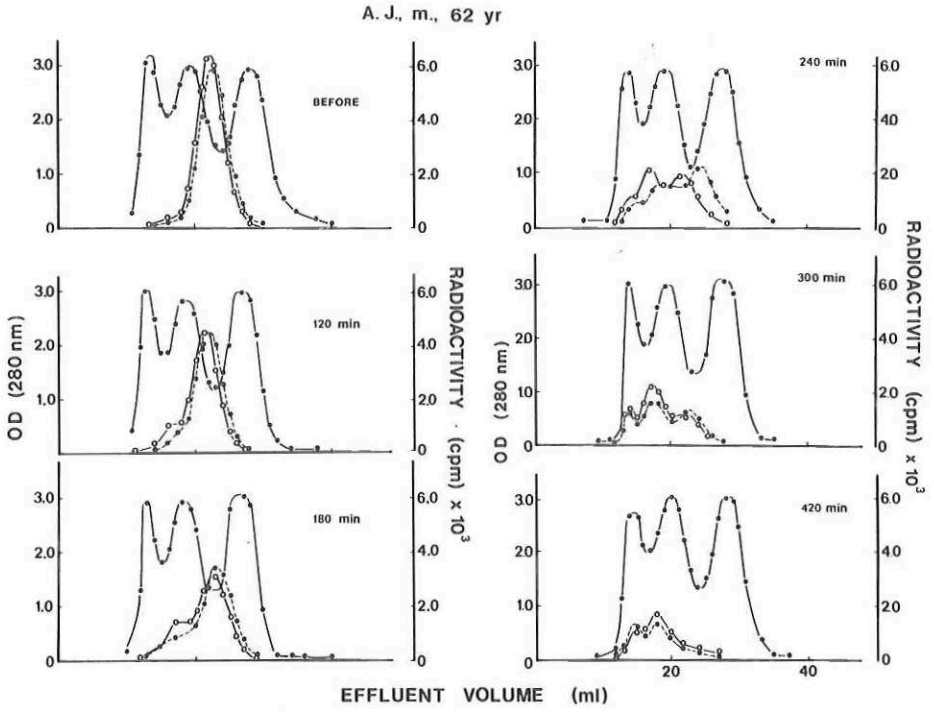

Fig. 2 – *Sephadex G-200 gel filtration of serial plasma samples taken before (control) and at different time intervals after initiation of a streptokinase infusion of 500,000 IU over 10 hr in a patient with chronic thromboembolic disease. Column size 2.5 x 40 cm ; buffer system 0.05 M tris - 0.1 M NaCl - 0.1 M EACA pH 9.0 ; flow rate 20 ml/hr ; room temperature ; fraction volume 3.5 ml.*

○—○ : ^{125} *I-plasminogen A1*
●----● : ^{131} *I-plasminogen A2*
●—● : *OD at 280 nm*

gradually normalizing plasminogen turnover was noted, concomitant with a gradual rise of the plasma fibrinogen and plasminogen levels. The results of the plasma radioactivity disappearance were thus very similar to those obtained with whole plasminogen (chapter III).

Sephadex G-200 gel filtration of serial plasma samples, obtained after the reptilase infusion showed the progressive appearance of small amounts of radioactivity eluted before the plasminogen peak and corresponding to the elution positions of the additional radioactivity peaks observed during streptokinase infusion (cfr. chapter III). The behavior of the two tracers was however very similar.

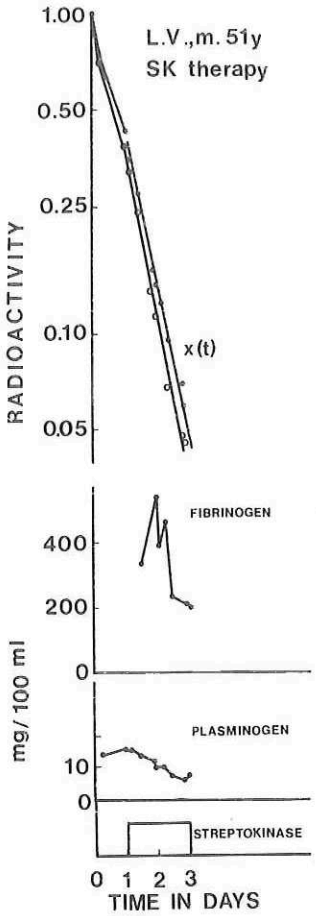

Fig. 3 – *Metabolism of ^{125}I-plasminogen A1 (○) and ^{131}I-plasminogen A2 (●) during infusion of 500,000 IU streptokinase over 48 hr in a patient with thromboembolic disease.*
x(t) : plasma radioactivity.

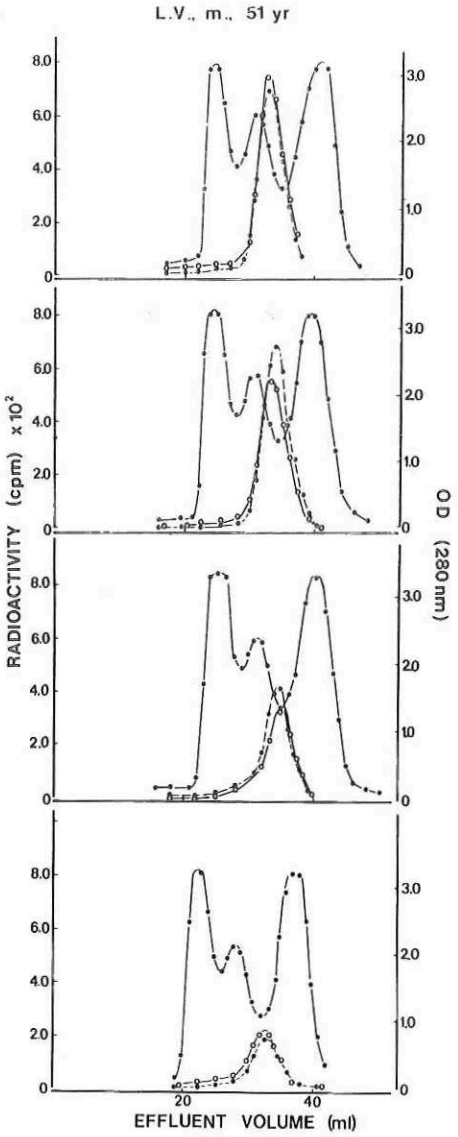

Fig. 4 – *Sephadex G-200 gel filtration of serial plasma samples taken before (control) and at different time intervals after initiation of a streptokinase infusion of 500,000 IU over 48 hr. Conditions of chromatography as described in fig. 2.*

○—○ : ^{125}*I-plasminogen A1*

●- - -● : ^{131}*I-plasminogen A2*

●—● : *OD at 280 nm*

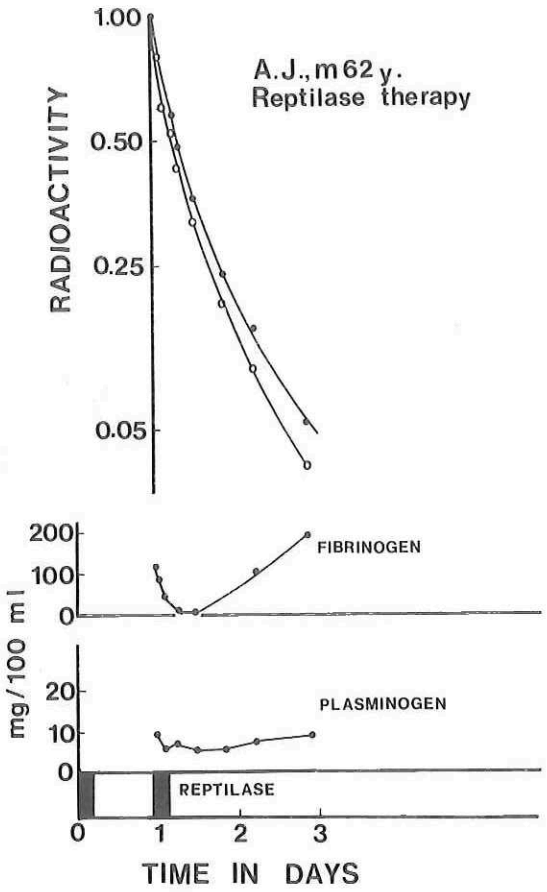

Fig. 5 – *Turnover of ^{125}I-plasminogen A1 (○) and ^{131}I-plasminogen A2 (●) following a reptilase infusion.*
x(t) : plasma radioactivity

CONCLUSION

The metabolism of the two major forms of plasminogen, isolated by affinity chromatography was studied in preliminary experiments in an effort to find out if the different activation properties of the two forms, observed in vitro, had a biological role.

Activation of the fibrinolytic system with low priming doses of streptokinase, resulted in a marked but similar increase in the plasma radioactivity disappearance rate of both plasminogens, the half-life being decreased to 0.50 to 0.70 days. The

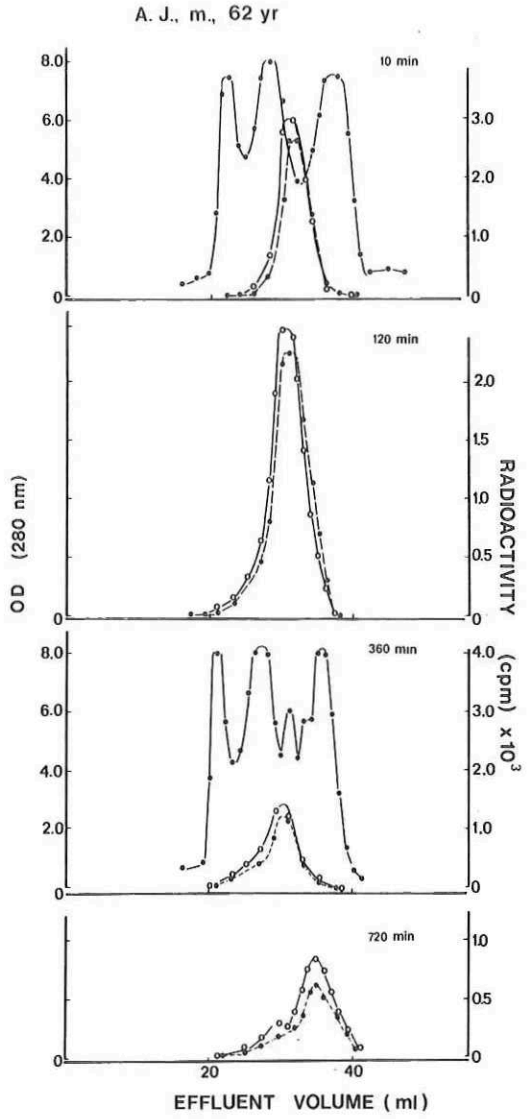

Fig. 6 – *Sephadex G-200 gel filtration of serial plasma samples taken before and at different time intervals after infusion of reptilase. Conditions of chromatography as described in the legend of fig. 2.*

○—○ : ^{125}*I-plasminogen A1*

●- - -● : ^{131}*I-plasminogen A2*

●—● : *OD at 280 nm*

streptokinase infusion changed the molecular distribution of radioactivity, as discussed in chapter III. In this respect the two plasminogens also behaved very similarly although in one patient a somewhat faster conversion of the radioactivity to the additional elution positions was observed.

Reptilase infusion also resulted in significant and similar consumption of both types of plasminogen. Gel filtration revealed that the bulk of the plasma radioactivity remained confined to the plasminogen position, but also clearly revealed the presence of small amounts of radioactivity, eluted in the positions of the additional radioactivity peaks observed during streptokinase therapy.

In conclusion, it appears that the behavior of both types of plasminogen is very similar if not identical during streptokinase and reptilase therapy, which are model conditions for the study of the consumption of plasminogen following primary fibrinolytic activation and in association with in vivo coagulation.

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Section III :

TURNOVER OF THE TWO MAJOR FORMS OF PLASMINOGEN DURING STRENUOUS PHYSICAL EXERCISE

During strenuous physical exercise the fractional catabolic rate of plasminogen in 4 healthy subjects increased from 0.505 to 0.575 of the plasma pool per day, corresponding to a plasminogen consumption of 33 mg per day. These data were obtained with labeled plasminogen preparations containing the multiple molecular forms of plasminogen, and in particular the two major forms isolated by lysine-agarose chromatography. Because differences in activation kinetics of the two forms were observed in vitro, it remained possible that exertion induced a preferential consumption of one form (presumably the first fraction which is more easily activated).

To test this hypothesis, simultaneous turnover studies of the two plasminogen forms were performed in 4 healthy volunteers before and during physical exercise.

MATERIALS AND METHODS

The metabolism of ^{125}I-plasminogen A1 and ^{131}I-plasminogen A2 was studied in four healthy male volunteers.

The procedure for the injection of tracer proteins and the sampling of blood (twice daily) was as described in chapter II. In total 15 to 18 blood samples were obtained over a period of 7 to 9 days. The normal turnover of the labeled proteins was followed for 4-5 days. The physical exercise was performed as described in chapter V. The tracer data were analyzed as described in appendix II.

RESULTS

The tracer data on both plasminogens in the healthy subjects are summarized in table I.

A representative turnover curve is shown in fig. 1. During the physical exercise the plasma radioactivity half-life of plasminogen A1 shortened from 2.36 ± 0.17 to 2.20 ± 0.23 days and that of plasminogen A2 from 2.28 ± 0.15 to 2.10 ± 0.23 days. The fractional catabolic rate increased from 0.518 ± 0.043 to 0.568 ± 0.07 of the plasma plasminogen pool per day for plasminogen A1 and from 0.559 ± 0.035 to 0.626 ± 0.06 of the plasma plasminogen pool per day for plasminogen A2.

Table I

Influence of physical exercise on the turnover of the two plasminogen forms, isolated by lysine-agarose chromatography

	$x(t) = C_1\ e^{-a_1 t} + C_2\ e^{-a_2 t}$							Fractional catabolic rate constant	
	C_1	a_1	C_2	a_2	t 1/2 for a_1 (days)	a_1^*	t* 1/2 for a_1^* (days)	k_{10}	k_{10}^*
Plasminogen A1									
UV	0.45	0.322	0.55	1.54	2.15	0.365	1.90	0.570	0.670
PG	0.44	0.289	0.56	1.54	2.40	0.289	2.40	0.530	0.530
KV	0.49	0.272	0.51	1.54	2.55	0.295	2.35	0.469	0.517
PV	0.50	0.295	0.50	1.73	2.35	0.322	2.15	0.504	0.559
Mean	0.47	0.295	0.53	1.59	2.36	0.318	2.20	0.518	0.568
SD	0.03	0.021	0.03	0.10	0.17	0.035	0.23	0.043	0.070
Plasminogen A2									
UV	0.40	0.330	0.60	1.26	2.10	0.375	1.85	0.592	0.714
PG	0.37	0.289	0.63	1.39	2.40	0.289	2.40	0.577	0.577
KV	0.45	0.289	0.55	1.39	2.40	0.330	2.10	0.512	0.608
PV	0.47	0.315	0.53	1.73	2.20	0.338	2.05	0.556	0.606
Mean	0.42	0.306	0.58	1.44	2.28	0.333	2.10	0.559	0.626
SD	0.05	0.020	0.05	0.20	0.15	0.035	0.23	0.035	0.060

* During the two day period of physical exercise.

CONCLUSION

During strenuous physical exercise, the catabolism of the two major forms of plasminogen, isolated by lysine-agarose chromatography was slightly though significantly increased. These turnover studies thus extend the observation in four healthy subjects, reported in chapter V. However, no significant difference was found between the two plasminogen forms. It thus seems that the differences in activation rate, observed in vitro are not biologically significant, at least not in this condition of repeated transient in vivo activation of the fibrinolytic system. Thus the exact biological role of this heterogeneity of plasminogen remains to be established.

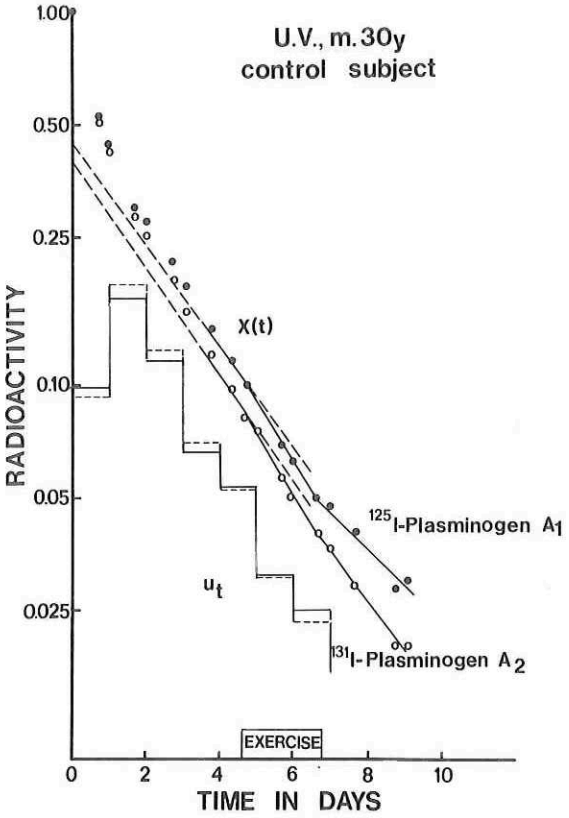

Fig. 1 – *Metabolism of ^{125}I-plasminogen A1 and ^{131}I-plasminogen A2 in a healthy subject before and during strenuous physical exercise.*
x(t) : plasma radioactivity
u_t : fractional daily urinary excretion of label.

Chapter VII

SUMMARY AND CONCLUSIONS

The normal kinetics of in vivo coagulation and fibrinolysis and their interrelation and relative role in the formation and removal of fibrin in the body are as yet poorly understood.

Turnover studies with labeled tracers which are specifically consumed during activation of either system might be well suited to investigate the kinetics of in vivo coagulation and fibrinolysis. Such a dynamic approach should be more specific and sensitive than the static picture obtained by the determination of the plasma levels of coagulation and fibrinolysis reactants or even reaction products such as fibrin(ogen) degradation products.

Indeed, the actual levels of these components will only be abnormal if the rates of their formation and removal are grossly unbalanced. Previous turnover studies with radiolabeled fibrinogen from our laboratory and others have shown that such kinetic studies may be valuable for the detection and quantitation of fibrinogen consumption in states of altered coagulability.

The aim of the present study was to obtain information of the kinetics of in vivo coagulation and fibrinolysis as derived from turnover studies of prothrombin, the final proenzyme of the coagulation cascade, and of plasminogen, the fibrinolytic proenzyme. Therefore it was necessary to prepare and characterize valid labeled metabolic tracers of prothrombin and plasminogen and to evaluate the kinetics of their synthesis and breakdown in healthy subjects and in some experimental and clinical conditions with abnormal turnover.

Chapter I deals with the preparation and characterization of labeled plasminogen and prothrombin.

In view of the controversy over the physicochemical properties of human plasminogen and of our finding of a difference in in vivo behaviour between plasminogen prepared according to Robbins *et al.* and according to Wallén and Wiman, it was necessary to study the biological homogeneity and metabolic characteristics of several plasminogen preparations. The preparations with NH_2-terminal glutamic acid, lower isoelectric points and a molecular weight of

approximately 90,000, had a half-life in vivo of approximately 2.0 to 2.5 days, compared to less than 1.0 day for preparations with NH_2-terminal lysine, higher isoelectric points and a molecular weight of approximately 84,000, irrespective of the method of purification. These metabolic data thus support the view that plasminogen isoenzymes with NH_2-terminal glutamic acid represent the native, circulating molecular forms.

The method of choice for the preparation of high quality plasminogen, suitable for metabolic studies consisted of the following steps : a) affinity chromatography of fresh frozen plasma on lysine-substituted agarose in the presence of the plasmin inhibitor Trasylol (more than 90 % recovery of plasminogen with approximately 90 % purity), b) Sephadex G-150 gel filtration (removes contaminants with a longer in vivo half-life) and c) DEAE Sephadex chromatography (removes small amounts of degraded plasminogen). Such preparations after labeling with radioiodine have a high specific activity and low spontaneous proteolytic activity and are indistinguishable from infractionated plasma plasminogen on Sephadex gel filtration and immunoelectrophoresis. They contain the twelve main molecular forms present in the circulation of every individual.

Using a combination of the methods of Shapiro and Waugh and of Swart, human prothrombin was highly purified from fresh or fresh frozen plasma. This material was devoid of thrombin, factor V, VII, IX and X activity. After labeling, no changes in enzymatic or physicochemical properties could be detected, and the material behaved very similarly to unfractionated plasma prothrombin on Sephadex gel filtration and immunoelectrophoresis.

It was therefore concluded that labeled plasminogen and prothrombin preparations could be obtained which both satisfied the in vitro criteria of purity and physicochemical identity with their native, circulating counterparts.

Chapter II summarizes our findings on the normal metabolism of plasminogen and prothrombin.

The biological integrity of the tracer proteins and the presence of, at the most, negligible amounts of contaminating proteins was demonstrated in vivo by low trichloroacetic acid soluble plasma radioactivities, by a relatively high intravascular fraction after equilibration, by the absence of a rapid initial urinary excretion of label and by a steady exponential decay of the plasma radioactivity after equilibration.

The plasma radioactivity disappearance rate x(t) for both proteins could be adequately described by a sum of two exponential terms $x(t) = C_1 e^{-a_1 t} + C_2 e^{-a_2 t}$ by graphical curve peeling on semilogarithmic paper or by non-linear exponential curve fitting with a Gauss-Newton procedure. Therefore the metabolism of plasminogen and prothrombin was represented by a two compartment mammillary model in which it is assumed that the compartments 1 and 2 represent the plasma and extravascular space respectively, that synthesis and catabolism occur in close association with the plasma, in which rapid mixing occurs, and that the exchange and breakdown rates are proportional to the amounts present. From this model and the values of the exponents and coefficients of the equation describing the plasma

radioactivity disappearance rate, it is then possible to estimate physiologically meaningful metabolic parameters such as the fractional catabolic rate constant, the transcapillary efflux and reflux rate constants, and the relative sizes of the intravascular and extravascular pools.

Plasminogen turnover studies in 21 healthy subjects performed with preparations containing all the molecular forms present in plasma, gave the following results : plasma plasminogen concentration 20.3 ± 2.6 mg/100 ml ; equation describing the evolution of the plasma radioactivity $x(t) = 0.44e^{-0.32t} + 0.56e^{-1.43t}$; plasma radioactivity half-life 2.24 ± 0.29 days ; fractional catabolic rate constant as determined from the plasma radioactivity disappearance rate ($k_{10,p}$) 0.55 ± 0.10 of the plasma pool per day and 0.52 ± 0.10 as determined from the urinary excretion of radioactivity ($k_{10,u}$) ; absolute catabolic (synthetic) rate 4.8 ± 1.2 mg/kg per day ; intravascular fraction 0.58 ± 0.06 ; and fractional transcapillary efflux rate constant (k_{12}) 0.38 ± 0.20 of the plasma pool per day.

The plasminogen (and fibrinogen) turnover rate was normal in a patient with Behçet's syndrome and low circulating plasminogen activator activity, and in a healthy subject during anticoagulation with heparin. These findings support the concept that under normal conditions the primary pathway of plasminogen catabolism is not via fibrinolytic activation or consumption secondary to intravascular coagulation.

Prothrombin turnover studies in 16 healthy subjects gave the following results : plasma prothrombin concentration 13.2 ± 1.6 mg/100 ml ; equation describing the evolution of the plasma radioactivity $x(t) = 0.49e^{-0.23t} + 0.51e^{-1.42t}$; plasma radioactivity half-life 3.04 ± 0.28 days ; fractional catabolic rate constant determined from the plasma radioactivity disappearance rate ($k_{10,p}$) 0.40 ± 0.04 of the plasma pool per day ; absolute catabolic (synthetic) rate 2.27 ± 0.34 mg/kg per day ; intravascular fraction 0.58 ± 0.05 ; and fractional transcapillary efflux rate constant (k_{12}) 0.44 ± 0.18 of the plasma pool per day.

The plasma disappearance rate of prothrombin activity in two patients with congenital hypoprothrombinemia after transfusion of 500 ml fresh plasma was very similar to the plasma disappearance rate of radioactivity in the controls. The identical behavior of labeled prothrombin and the unfractionated prothrombin in fresh plasma was further demonstrated by a similar disappearance rate of both radioactivity and enzymatic activity after simultaneous infusion of plasma and labeled prothrombin into one of the hypoprothrombinemic patients.

The prothrombin turnover remained unchanged in one healthy subject during and after a continuous heparin infusion. This observation extends the findings of Shapiro and Martinez on the metabolism of prothrombin in four hemophiliacs and supports the concept that the main pathway of prothrombin turnover is not via thrombin formation.

Chapter III deals with the metabolism of plasminogen during streptokinase therapy in six patients, and during reptilase therapy in five patients, with thromboembolic disease.

A markedly enhanced plasma radioactivity disappearance rate (t 1/2 : 0.50 to 0.75

days) was observed after injection of radiolabeled plasminogen before or during streptokinase therapy. Sephadex G-200 gel filtration of serial plasma samples revealed a still faster disappearance rate of plasminogen-bound radioactivity and the appearance of radioactive complexes, eluted at the void volume (presumably plasmin-α_2-macroglobulin complex) and with an apparent molecular weight of roughly 150,000 (presumably plasmin-α_1-antiplasmin). Thus, the evolution of the plasma radioactivity during streptokinase therapy mainly reflected the distribution and catabolism of these complexes.

A markedly enhanced plasminogen turnover which could be described by a single exponential term (t 1/2 : 0.45 - 0.80 days) was also observed during reptilase therapy. Upon gel filtration on Sephadex G-200 of plasma samples collected on anti-reptilase antiserum, it was observed that besides a main radioactivity peak corresponding to the plasma plasminogen position, small amounts of radioactivity were eluted in the position of the additional radioactive peaks observed during streptokinase therapy. The decay of the plasma radioactivity during reptilase therapy remained essentially representative of the metabolism and distribution of plasminogen. A two to three-fold increase in the mean fractional catabolic rate was observed in the patients, corresponding to a daily reptilase-induced consumption of up to 10 mg of plasminogen per 100 ml plasma.

In chapter IV, we attempted to use these labeled proteins to elucidate the kinetics of in vivo synthesis and breakdown of plasminogen and prothrombin in cirrhosis of the liver. The metabolism of labeled plasminogen was studied in four patients with severe liver cirrhosis, and that of labeled prothrombin in six. Compared to the normals, the cirrhotic patients showed an increased fractional catabolic rate and a decreased synthetic rate, resulting in subnormal levels of plasminogen or prothrombin. In the cirrhotics, the plasma concentration correlated to the synthetic rate, but not to the fractional catabolic rate, for both proteins.

Heparin infusion prolonged the half-life of plasminogen in plasma in two cirrhotics from 1.25 to 2.10 and from 1.45 to 1.90 days and the half-life of prothrombin in three cirrhotics from 2.25 to 2.70, from 2.35 to 2.80 and from 2.40 to 3.70 days.

These results, although obtained on a small series of patients, indicate that the abnormal turnover of plasminogen and prothrombin in cirrhosis of the liver depends on two mechanisms : increased breakdown which is at least partially reversible by heparin administration, and impaired synthesis. The decreased plasma levels however are mainly caused by the decreased synthetic ability.

The effect of physical exercise on the metabolism of labeled fibrinogen, plasminogen and prothrombin is studied in chapter V. Exhausting physical exercise, repeated four times per day for two days was performed in twelve healthy subjects. The exertion resulted in the well-known changes in the coagulation (shortening of the partial thromboplastin time and increase of factor VIII) and fibrinolytic (increased euglobulin fibrinolytic activity) tests but did not induce changes in the concentration of fibrinogen, plasminogen or prothrombin in the plasma.

During physical exercise in the 12 subjects, an increased catabolism of fibrinogen

(12 subjects) and plasminogen (4 subjects) but not of prothrombin (5 subjects) was observed. The fractional catabolic rate of fibrinogen increased from 0.231 to 0.278 of the plasma pool per day, resulting in a fibrinogen consumption of 240 mg per day or 3.4 mg/kg per day. The fractional catabolic rate of plasminogen increased from 0.505 to 0.574 of the plasma pool per day, resulting in a plasminogen consumption of 33 mg per day or 0.47 mg/kg per day. On the basis of a molecular weight of 330,000 for fibrinogen and 90,000 for plasminogen, about 2 molecules of fibrinogen were consumed per molecule of plasminogen. The physical exercise had no influence on the metabolism of labeled prothrombin suggesting firstly that the observed increases in the disappearance rates of the fibrinogen- and plasminogen-bound radioactivity in plasma are not due to redistribution during exertion and secondly that the fibrinogen consumption is the result not of intravascular coagulation but fibrino(geno)lysis.

Chapter VI reports on turnover studies performed with the two major forms of plasminogen, isolated by lysine-agarose chromatography with EACA gradient elution. We have previously suggested that these two forms are genetically determined isozymes, probably caused by gene duplication. In addition we have shown that these two forms have different activation kinetics in vitro.

In a group of 8 healthy subjects, the turnover characteristics of the two plasminogens were very similar to each other. In 2 patients during streptokinase and 1 patient during reptilase therapy, no clear-cut differences were observed in the kinetics of the in vivo consumption of the two plasminogens. In 4 healthy subjects a small but significant increase in the turnover of the two plasminogens was observed. Thus the possibly different biological role of the two plasminogens remains to be established.

In conclusion, the present study has demonstrated that turnover studies with labeled plasminogen and prothrombin may be useful tools for very sensitive detection and quantitation of in vivo activation of the coagulation and/or fibrinolytic system. The method is complicated in the sense that highly purified and biologically intact labeled preparations are required and that several blood samples during a sufficiently long observation period are necessary. However, the sensitivity of the method and the fact that the synthesis and breakdown kinetics can be studied separately largely compensate for its complexity. It thus seems to us that for the study of some problems in the field of hemostasis and thrombosis the present approach might yield important information which cannot be obtained from static measurements on blood samples.

Appendix I

AUXILIARY LABORATORY TECHNIQUES

A. Biologic coagulation and fibrinolytic and liver function assays

Fibrinogen in plasma was determined with the FPT-test described by Vermylen *et al.* (1) or by the method of Blombäck and Blombäck (2). Fibrin(ogen) degradation products in serum, prepared by collecting blood on aprotinin (250 KIU/ml) and thrombin (20 NIH U/ml), were determined by the method of Merskey *et al.* (3).

Plasminogen was determined by caseinolysis, usually performed as described by Claeys *et al.* (4) and in some instances by the standard CTA caseinolytic assay (5). Plasminogen in plasma was determined after neutralization of the inhibitors by acidification (6) and converted to mg/100 ml by comparison with purified plasminogen. The antiplasmin activity of plasma was determined as described by Amery *et al.* (7). The euglobulin fibrinolytic activity was measured on bovine fibrin plates according to Astrup and Mullertz (8) or by the euglobulin clot lysis time (9). The euglobulin clot lysis time (T) was converted to fibrinolytic activity (V) by the equation

$$V = \frac{1300}{T} .$$

Prothrombin was assayed by a one stage (10) and two stage (11) method, factor IX according to Soulier and Larrieu (12), factor VIII according to Vermylen and Verstraete (13) and factor XII as described by Haanen *et al.* (14). The activated partial thromboplastin time was measured according to Proctor and Rapaport (15).

The following additional routine coagulation and fibrinolytic tests were performed as referred to elsewhere (16) : clotting time, one stage prothrombin time, Owren time, factor VII-X, factor V, thrombin time, platelet count. The following liver function tests were performed according to standard laboratory procedures : serum protein assay and electrophoresis, bilirubin, alkaline phosphatases, serum glutamic-oxaloacetic and glutamic-pyruvic transaminases.

Protein was determined by the method of Lowry (17) or by absorbancy measurements at 280 nm accepting $A^{1\%}_{280\,nm}$ to be 17.0 for plasminogen (18) and 13.6 for prothrombin (19).

B. Electrophoretic and immunoelectrophoretic techniques

Plasminogen, prothrombin and factor VIII were determined by Laurell's immunologic method (20), using monospecific rabbit antisera raised against purified plasminogen or prothrombin or a commercial anti-factor VIII antiserum (Nordic).

Polyacrylamide gel electrophoresis at pH 8.3 and at pH 4.3 was performed by the method of Davis, as described in detail elsewhere (21). Enzymography of the plasminogen-gels was performed according to Heberlein and Barnhart (22) as modified by Wallén and Wiman (23). SDS-polyacrylamide gel electrophoresis was described by the method of Weber and Osborn (24) as modified by McDonagh *et al.* (25).

Immunolectrophoresis was performed according to Scheidegger (26).

C. Analytical chromatographic techniques

Gel filtration on Sephadex G-200 of mixtures of trace amounts of labeled plasminogen or prothrombin and human plasma, or of serial plasma samples obtained during turnover studies were performed at room temperature on 2.5 x 45 cm columns at a flow rate of approximately 15 ml per hr. Three ml fractions of serial plasma samples were used for the chromatography ; the eluate, collected in 3 ml fractions was analyzed for radioactivity and for enzymatic activity. In some experiments the columns were calibrated with proteins of known molecular weight. In other experiments the relative elution position of radioactivity was referred to the elution position of the three main peaks of the plasma proteins.

In the gel filtration experiments with labeled plasminogen, the columns were equilibrated and developed with 0.1 M NaCl - 0.05 M tris buffer pH 9.0 with or without the addition of 0.1 M EACA to prevent plasminogen activation on the column. In the prothrombin experiments a 0.1 M NaCl - 0.05 M phosphate - 0.01 M citrate buffer, pH 7.5 was used.

Lysine-agarose chromatography of 3 ml plasma samples obtained during turnover studies with labeled plasminogen or on a mixture of 50 ml plasma with a trace amount of labeled plasminogen were performed at room temperature on 0.9 x 30 cm columns, equilibrated with 0.1 M phosphate buffer pH 7.5 (containing 5 KIU Trasylol per ml) at a flow rate of 15 ml per hr. After removal of non-adsorbed protein with the equilibration buffer, the column was eluted with a linear gradient consisting of 150 ml 0.1 M phosphate pH 7.5 as starting buffer and 150 ml 0.1 M phosphate - 0.01 M EACA pH 7.5 as limiting buffer. Fractions of 4-5 ml were collected and analyzed for radioactivity and protein content.

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Appendix II

MATHEMATICAL MODEL FOR THE METABOLISM OF PLASMINOGEN AND PROTHROMBIN IN PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS IN MAN

In physiological conditions, protein molecules are continuously synthesized, are released into the blood stream in which they are rapidly mixed and pass through capillary walls into the extravascular fluids or are catabolized in the breakdown sites. Extravascular protein molecules traverse the interstitial fluids, enter lymph or blood capillaries and return to the circulation after different time intervals.

The analysis of the turnover kinetics of labeled plasma proteins aims at the estimation of the amounts of protein synthesized and destroyed (in one or more catabolic pathways) per day, of the pool size of the proteins in the plasma and the interstitial fluids, and of the exchange rates between the pools.

The breakdown of plasma proteins apparently is a first order reaction and synthesis and breakdown seem to occur in or in close relation to the plasma compartment (1). The equilibrium between the protein concentration in plasma and in the interstitial fluids is dynamic. At least two forces (filtration by differences in hydrostatic pressure and diffusion) are responsible for their transfer and act at least in three distinct places (the arterial and the venous ends of the blood capillaries and the ends of the lymph capillaries). In spite of this complex origin, the process of transfer may still be described as a sum of first order processes (2),

The corresponding mathematical model consists of a system of n linear first order differential equations with constant coefficients, n representing the number of compartments. Berman and Schoenfeld (3) have demonstrated that the n compartment mammillary model for the metabolism of plasma proteins can be completely solved if the initial conditions and the constants (C_k) and coefficients (a_k) describing the plasma radioactivity $x(t) = \Sigma C_k e^{-a_k t}$ are known.

The results of a turnover study consist of a series of measurements of plasma radioactivity (x_i) at different time intervals (t_i) after administration of the isotope. For calculation of the metabolic parameters a sum of exponentials has to be fitted to these data in which not only the coefficients (C_k) and exponents (a_k), but also the number of terms n have to be estimated.

We have demonstrated (4,5) that, in view of the limited number (less than 20) and

accuracy (around 5 %) of the plasma radioactivity determinations which can be obtained during turnover experiments with labeled fibrinogen and plasminogen, the approximation of the decay of the plasma radioactivity by a sum of two exponential terms constitutes the optimal approximation of the turnover phenomenon.

Mathematical model for the metabolism of plasminogen and prothrombin in physiological conditions

The plasma radioactivity data (x_i) versus time (t) were fitted with a sum of two exponential terms $x(t) = C_1 e^{-a_1 t} + C_2 e^{-a_2 t}$, by graphical analysis on semilogarithmic paper (curve peeling) and by non-linear exponential curve fitting by a damped Gauss-Newton procedure (6). The accuracy of the estimates of the exponents and coefficients by the two methods were nearly identical

Thus the metabolism and distribution of labeled plasminogen and prothrombin were represented by a two compartment mammillary model (fig. 1), (7,8) although

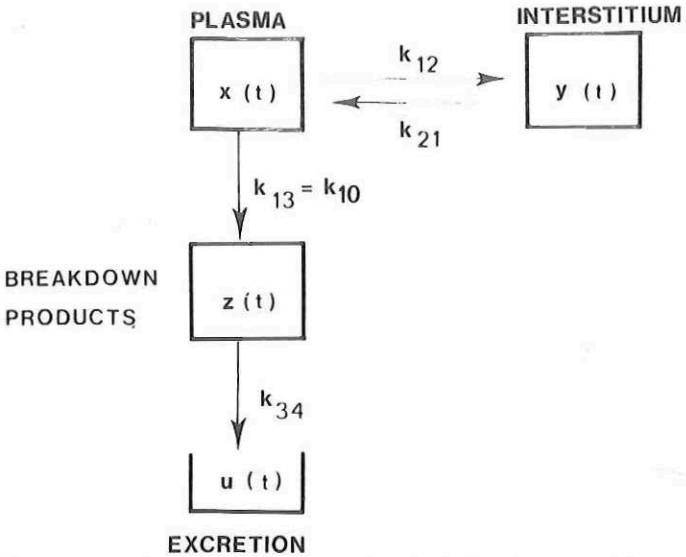

Fig. 1 – *Two compartment mathematical model for the metabolism of labeled plasminogen and prothrombin in physiological conditions. When the radioactive material is injected into plasma, rapid mixing occurs. The total plasma radioactivity at time t is represented by x(t). Radioactivity passes into the interstitium at a rate $k_{12}x(t)$ per day and is catabolized at a rate of $k_{10}x(t)$ per day. Interstitial radioactivity returns to the circulation at a rate $k_{21}y(t)$ per day, y(t) representing the total radioactivity at time t in the interstitium. Radioactive, non TCA-precipitable degradation products in the body represented by z(t), are excreted almost entirely in the urine at a rate of $k_{34}z(t)$, in which the accumulated radioactivity is represented by u(t).*

it is realized that such description constitutes a gross oversimplification of the physiological distribution and breakdown kinetics.

From the exponents and coefficients of the equation describing the decay of the plasma radioactivity it is then possible to estimate physiologically meaningful metabolic parameters such as the fractional catabolic rate constant (k_{10}), the transcapillary efflux (k_{12}) and reflux (k_{21}) rate constant and the relative sizes of the intravascular (IV) and extravascular (EV) pools.

If the plasma radioactivity x(t) is represented by

$$x(t) = C_1 e^{-a_1 t} + C_2 e^{-a_2 t} \text{ with } x(o) = C_1 + C_2 = 1 \tag{1}$$

then the Laplace transform

$$X(s) = \int_o^\infty x(t)\, e^{-st}\, dt \quad \text{of } x(t) \text{ is}$$

$$X(s) = \frac{C_1}{s + a_1} + \frac{C_2}{s + a_2} = \frac{s + C_1 a_2 + C_2 a_1}{s^2 + (a_1 + a_2)s + a_1 a_2} \tag{2}$$

The two compartment mammillary model, (fig. 1) can be described by

$$\frac{dx(t)}{dt} = -(k_{12} + k_{10})\, x(t) + k_{21} y(t) \tag{3}$$

$$\frac{dy(t)}{dt} = k_{12} x(t) - k_{21} y(t)$$

which for x(o) = 1 and y(o) = 0 after Laplace transformation gives

$$\begin{aligned} sX(s) - 1 &= -(k_{12} + k_{10})X(s) + k_{21} Y(s) \\ sY(s) &= k_{12} X(s) - k_{21} Y(s) \end{aligned} \tag{4}$$

Elimination of Y(s) and collection of terms in (4) gives

$$X(s) = \frac{s + k_{21}}{s^2 + (k_{12} + k_{21} + k_{10})s + k_{10} k_{21}} \tag{5}$$

From equations (2) and (5) one can deduce :

for s = 0 that $k_{10} = \dfrac{a_1 a_2}{a_1 C_2 + a_2 C_1}$ (6)

– by comparing the numerators of equations (2) and (5), that

$$k_{21} = C_1 a_2 + C_2 a_1 \tag{7}$$

– by comparing the denominators of equations (2) and (5), that

$$k_{12} + k_{21} + k_{10} = a_1 + a_2 \text{ and } k_{10} k_{21} = a_1 a_2 \text{ , such that} \tag{8}$$

$$k_{12} = \frac{C_1 C_2 (a_2 - a_1)^2}{C_1 a_2 + C_2 a_1} \tag{9}$$

The radioactivity distribution ratio $\frac{EV}{IV}$ is :

$$\frac{EV}{IV} = \lim_{t \to \infty} \frac{y(t)}{x(t)} \tag{10}$$

For $t \to \infty$ equation (1) becomes :

$$x(t) = C_1 e^{-a_1 t} \text{ and } y(t) = D_1 e^{-a_1 t} \tag{11}$$

Thus $$\frac{EV}{IV} = \frac{D_1}{C_1} \tag{12}$$

Since $$\frac{dy(t)}{dt} = -k_{21} y(t) + k_{12} x(t) \tag{13}$$

For $t \to \infty$ this becomes

$$-a_1 D_1 = -k_{21} D_1 + k_{12} C_1 \text{ or } \frac{D_1}{C_1} = \frac{k_{12}}{k_{21} - a_1} \tag{14}$$

From (13) and (15) it appears that

$$\frac{EV}{IV} = \frac{D_1}{C_1} = \frac{k_{12}}{k_{21} - a_1} \tag{15}$$

Substitution of (7) and (9) in (15) gives :

$$\frac{EV}{IV} = \frac{C_1 C_2 (a_2 - a_1)^2}{(C_1 a_2 + C_2 a_1)(C_1 a_2 + C_2 a_1 - a_1)} = \frac{C_1 C_2 (a_2 - a_1)^2}{(C_1 a_2 + C_2 a_1)(C_1 a_2 - C_1 a_1)}$$

$$\frac{EV}{IV} = \frac{C_2(a_2 - a_1)}{C_1 a_2 + C_2 a_1} \tag{16}$$

$$\text{and } IV = \frac{1}{1 + \frac{EV}{IV}} \tag{17}$$

Closely agreeing estimates of the fractional catabolic rate constant (k_{10}), as determined by the disappearance rate of plasma radioactivity ($k_{10,p}$) and by the ratio of the daily urinary radioactivity to the corresponding mean plasma radioactivity ($k_{10,u}$) corrected for the delay in iodile excretion (1, 9) (chapter II, section II), suggest that the kidneys are virtually the sole excretory site, and that no significant accumulation of isotope occurs at the breakdown sites. The presence in the body of radioactive non-TCA precipitable breakdown products, consisting mainly of radioactive iodide and iodotyrosine (10) and distributed in about 8 times the plasma volume (11), calls for the introduction of an additional pool into the metabolic model. The total fractional catabolic rate constant (k_{10}) is related to basic protein turnover, occuring with a rate constant k_{13} and, at least theoretically, to consumption by in vivo activation of the coagulation or fibrinolytic system. Our findings (chapter II, section II and III) suggest that the bulk of plasminogen and prothrombin is not catabolized by either of these systems in healthy subjects. Fig. 1 is the simplest flow diagram, which takes these findings into account. The description requires four differential equations

(1) $\frac{dx}{dt} = -kx(t) + k_{21}y(t)$

with $x(o) = 1$ and $x(\infty) = 0$

(2) $\frac{dy}{dt} = k_{12}x(t) - k_{21}y(t)$ $y(o) = 0,$ $y(\infty) = 0$

(3) $\frac{dz}{dt} = k_{10}x(t) - k_{34}z(t)$ $z(o) = 0,$ $z(\infty) = 0$

(4) $\frac{du}{dt} = k_{34}z(t)$ $u(o) = 0,$ $u(\infty) = 1$

with $k = k_{12} + k_{10}$

The unvarying plasma plasminogen and prothrombin concentration under physiological conditions implies that the system is in steady state, i.e., that the fractional transfer rates are constant.

Mathematical model for the metabolism of plasminogen and prothrombin in pathological conditions

As is shown in chapters III and V and in appendix III, several clinical or experimental conditions may be associated with an increased plasminogen or prothrombin turnover, which cannot be ascribed to an accelerated protein metabolism but would be related to consumption following chronic low grade in vivo activation of the coagulation or fibrinolytic system. At first glance, this consumption apparently follows first order kinetics implying that it occurs at a constant fractional rate, and that the absolute amounts of protein which are consumed in each pathway depend on their actual plasma levels, which are the resultants of equilibrium between synthesis and catabolism. The simplest corresponding flow diagram is represented in fig. 2, in which the observed fractional catabolic rate constant (k_{10}) is due to catabolism by basic protein turnover (k_{13}) and by in vivo consumption (k_{15}).

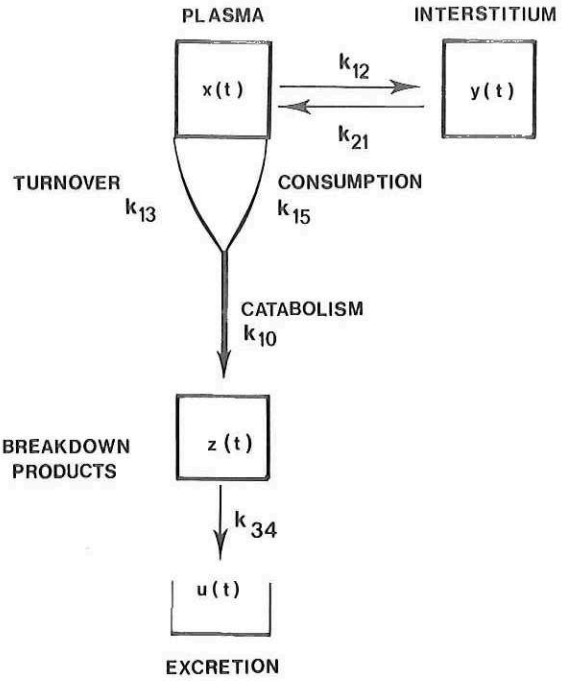

Fig. 2 – *Compartmental model for the metabolism of labeled plasminogen and prothrombin in pathological conditions.*
Breakdown of plasminogen or prothrombin occurs by two metabolic pathways : (1) "basic" protein turnover at a rate $k_{13}x(t)$ per day, and (2) consumption of plasminogen or prothrombin at a rate $k_{15}x(t)$ per day. For further details, see legend fig. 1.

If one assumes firstly that the difference in fractional catabolic rate observed in clinical conditions (as e.g. liver cirrhosis) and in normal subjects is entirely due to in vivo consumption (which may not always be the case), and secondly that the half-life of radioactivity bound to activated molecules is short (as has been shown in chapter III), this consumption may be quantitated as the difference of the two catabolic rates.

Alternatively the extent of in vivo consumption may be estimated in individual cases from the influence of pharmacological inhibition of in vivo coagulation (heparin) on the plasma radioactivity disappearance rate.

If the values of the transfer constants of the system are changed at time T, the following modifications apply :

– the plasma radioactivity is represented by :

$$x'(t) = C_1^* e^{-a_1^* t} + C_2^* e^{-a_2^* t} \text{ with } x(T) = C_1^* + C_2^* \# 1 \qquad (1')$$

or after Laplace transformation by

$$X'(s) = \frac{sx(T) + C_1^* a_2^* + C_2^* a_1^*}{s^2 + (a_1^* + a_2^*)s + a_1^* a_2^*} \qquad (2')$$

– the two compartment mammillary model is described by :

$$\frac{dx'(t)}{dt} = -(k_{12}^* + k_{10}^*)x'(t) + k_{21}^* y'(t) \qquad (3')$$

$$\frac{dy'(t)}{dt} = k_{12}^* x'(t) - k_{21}^* y'(t)$$

which for $x'(o) = x(T)$ and $y'(o) = y(T)$ after Laplace transformation gives :

$$sX'(s) - x(T) = -(k_{12}^* + k_{10}^*)X'(s) + k_{21}^* Y'(s) \qquad (4')$$

$$sY'(s) - y(T) = k_{12}^* X'(s) - k_{21}^* Y'(s)$$

or

$$X'(s) = \frac{sx(T) + k_{21}^* (x(T) + y(T))}{s^2 + (k_{12}^* + k_{21}^* + k_{10}^*)s + k_{10}^* k_{21}^*} \qquad (5')$$

From equations (2') and (5') one can deduce by comparing the denominators that

$$k^*_{12} + k^*_{21} + k^*_{10} = a^*_1 + a^*_2 \tag{18}$$

and

$$k^*_{10} k^*_{21} = a^*_1 a^*_2 \tag{19}$$

Elimination of a^*_2 gives $k^*_{10} = \dfrac{(a^*_1 - k^*_{12} - k^*_{21})\, a^*_1}{a^*_1 - k_{21}}$ (20)

Because infusion of heparin has no effect on the turnover of labeled plasminogen and prothrombin in a normal subject (chapter II), it is reasonable to assume that in vivo inhibition of coagulation with this drug does not affect the reflux (k_{21}) and efflux (k_{12}) rate constants, so that $k_{12} = k^*_{12}$ and $k_{21} = k^*_{21}$. Therefore a change in the evolution of the plasma radioactivity during heparin infusion in patients reflects a modification in the fractional catabolic rate constant, due to inhibition of the in vivo consumption, most probably by interference with in vivo coagulation. Consequently, the fractional catabolic rate constant during heparin infusion can be calculated (20) in which a^*_1 represents the slope of the plasma radioactivity disappearance curve during anticoagulation and k^*_{12} and k^*_{21} the transfer rate constants calculated from the plasma radioactivity disappearance rate before anticoagulation. The fraction of the plasma plasminogen or prothrombin pool consumed per day (k_{15}), secondary to intravascular coagulation is then represented by the difference in fractional catabolic rates before $k_{10} = k_{13} + k_{15}$ during ($k^*_{10} = k_{13}$) anticoagulation.

The influence of physical exercise on the turnover of fibrinogen, plasminogen and prothrombin was calculated from equation (20) using the same assumptions.

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Appendix III

LABELED PLASMINOGEN AND PROTHROMBIN AS TRACERS OF ABNORMAL METABOLISM. OBSERVATIONS IN SOME CLINICAL CONDITIONS

Section I :

METABOLISM OF PLASMINOGEN IN SELECTED CLINICAL CONDITIONS : TWO CASE REPORTS

The usefulness of labeled plasminogen as a tracer of the kinetics of plasminogen synthesis and breakdown in vivo was illustrated in two patients with a history of defibrination.

Patients

The metabolism of plasminogen was studied in a 77 year old patient (KG) with *metastatic carcinoma of the prostate.* Prior to the turnover study he had presented transient episodes of defibrination concomitant with plasma fibrinolytic activity on bovine fibrin film. During the plasminogen turnover study, his platelet count, fibrinogen level, thrombin time and fibrin(ogen) degradation products in serum were stable at nearly normal values, while his plasma plasminogen concentration was increased (33.4 mg/100 ml).

A 23 year old patient (AV) with *missed abortion* for 8 weeks and marked hypofibrinogenemia was treated by continuous infusion of 30,000 IU heparin per day. Two days after admission labour started spontaneously and delivery occurred. Labeled plasminogen was injected immediately after a priming dose of 5,000 IU heparin. The turnover of plasminogen was studied before and after delivery.

Pertinent clinical and laboratory data on the patients are summarized in table I.

RESULTS

The catabolism of plasminogen was markedly accelerated in the patient with prostatic carcinoma (fig. 1). The plasma radioactivity disappearance curve could be

Table I

Clinical and laboratory data

	Age (yr)	Weight (kg)	Plasma Plasminogen (mg/100 ml)	Plasma Volume (ml/kg)	Diagnosis
KG	77	67	33.4	45	Prostatic carcinoma
AV	23	47	8.8	55	Missed abortion
Controls					
Mean	33	68	20.3	42	
SD	12	12	2.6	7	

described by $x(t) = 0.7e^{-0.69t} + 0.3e^{-2.75t}$ (t 1/2 = 1.0 day).

Sephadex G-200 gel filtration of serial plasma samples showed elution of radioactivity in one main peak at the same volume as plasminogen activity and in one small peak at the total volume of the column.

During peroral administration of tranexamic acid 2 g t.i.d., a further shortening of the plasminogen half-life to 0.5 days occurred. This was associated with an almost complete disappearance of the enzymatically measured plasminogen activity and with a decrease of the immunoreactive plasminogen to approximately 50 % of its initial value at the end of the drug administration period. Subsequent intermittent intravenous injection of 5,000 IU heparin every 4 hours resulted in a prolongation of the plasma radioactivity half-life to 1.8 days. A significant rise in the fibrinogen level was also observed.

Four weeks after completion of the first turnover study, during which the patient was treated with oestrogen, ^{125}I-plasminogen and ^{131}I-fibrinogen were injected simultaneously. The plasminogen half-life (t 1/2 : 1.3 days) and the fibrinogen half-life (t 1/2 : 1.55 days, controls 4.14 ± 0.51 days) were both markedly shortened. During this observation period the plasma fibrinogen (300 mg/100 ml) and plasminogen levels (22.5 mg/100 ml) were within the normal range, despite these markedly shortened half-lifes.

The half-life of plasminogen in plasma was considerably shortened (t 1/2 : 0.95 days) during adequate anticoagulation of the patient with missed abortion (fig. 2) as shown by the marked prolongation of the activated partial thromboplastin time (1).

The plasma radioactivity disappearance curve was described by $x(t) = 0.72e^{-0.73t} + 0.28e^{-3.45t}$. During anticoagulation, there was only a slow increase in the plasma fibrinogen and a slow decrease in the fibrin(ogen) degradation

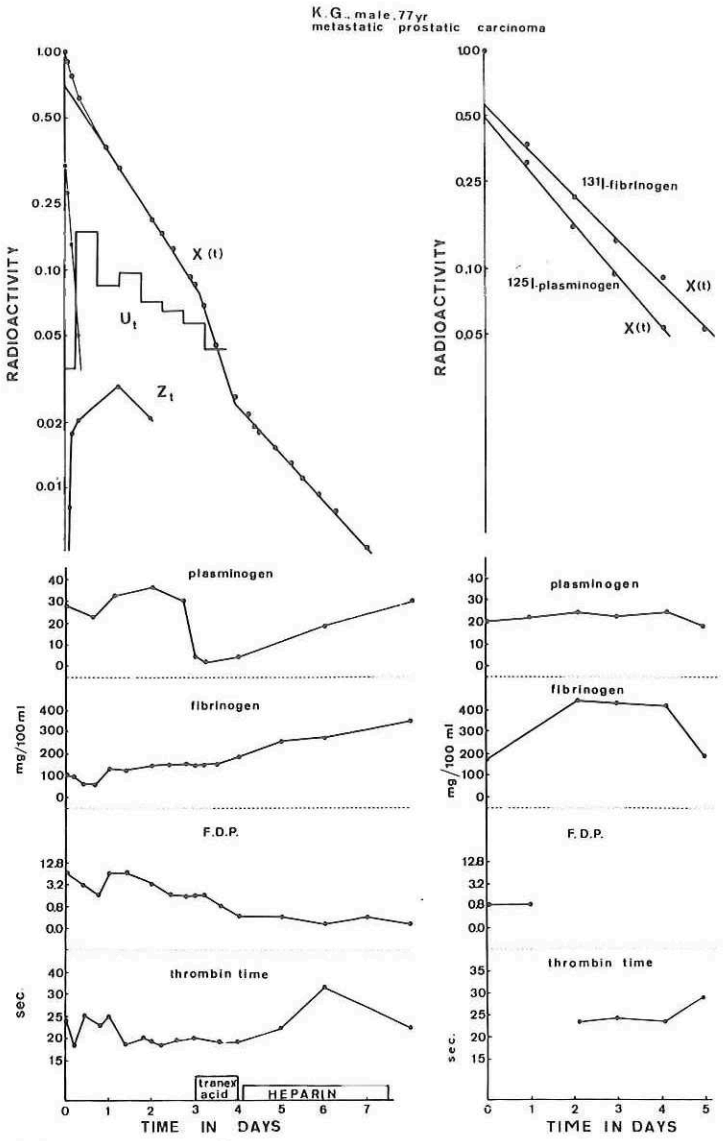

Fig. 1 – *Plasminogen metabolism in a patient with metastatic carcinoma of the prostate. Left side : metabolism of* ^{125}*-I-plasminogen. Right side : metabolism of* ^{125}*-I-plasminogen and* ^{131}*-I-fibrinogen. x(t), plasma radioactivity ;* z_t*, non-TCA precipitable radioactivity in plasma ;* u_t*, fractional urinary excretion of label per 12 hours ; plasminogen, enzymatically determined plasma plasminogen level ; F.D.P., fibrin(ogen) degradation products in serum ; tranexamic acid, in vivo inhibition of the fibrinolytic system by tranexamic acid 2 g t.i.d. ; heparin, anticoagulation by intravenous injection of 5,000 IU heparin per 4 hours.*

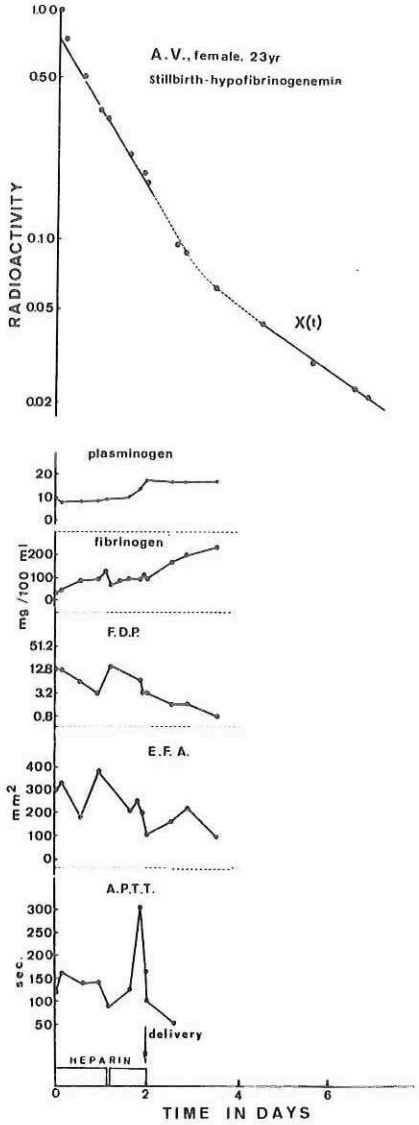

Fig. 2 – *Plasminogen metabolism in a patient with dead fetus syndrome. x(t), plasma radioactivity ; F.D.P., fibrin(ogen) degradation products in serum ; E.F.A., euglobulin fibrinolytic activity on unheated bovine fibrin film ; A.P.T.T., activated partial thromboplastin time ; heparin, continuous intravenous infusion of 30,000 IU heparin per day.*

products in serum. The plasma plasminogen level was below normal and the euglobulin fibrinolytic activity moderately elevated. Inadvertent interruption of the heparin infusion for two hours resulted in a decrease in the activated partial thromboplastin time to 80 seconds, concomitant with a temporary decrease in plasma fibrinogen and increase in fibrin(ogen) degradation products in serum suggesting an exacerbation of intravascular coagulation. The heparin infusion was stopped at the end of the second day because labour started spontaneously. The patient delivered two macerated fetuses, together with about 50 to 100 g clotted blood. There was practically no blood loss post partum. After delivery the plasminogen half-life returned to normal spontaneously (t 1/2 = 2.2 days). The plasminogen and antiplasmin levels were decreased before delivery but also subsequently returned to normal. The euglobulin fibrinolytic activity on unheated bovine fibrin film was moderately increased before delivery but normal thereafter. Lysis was never observed when plasma was applied to fibrin films.

The tracer data and calculated metabolic parameters in the two patients are summarized in tables II and III.

Table II

Plasminogen tracer data

Fitted function $x(t) = C_1\ e^{-a_1 t} + C_2\ e^{-a_2 t}$						
	C_1	a_1	C_2	a_2	t 1/2 for a_1	t 1/2* for a_1
KG	0.70	0.69	0.30	2.75	1.00	1.80
AV	0.72	0.73	0.28	3.45	0.95	2.20
Controls						
Mean	0.44	0.32	0.56	1.43	2.24	
SD	0.07	0.04	0.07	0.43	0.29	

* During anticoagulation in KG and after delivery in AV.

DISCUSSION

The metabolism of labeled plasminogen was studied in two selected clinical conditions in order to demonstrate its usefulness as a tracer of the dynamics of in vivo synthesis and breakdown of plasminogen.

The breakdown of plasminogen was accelerated (t 1/2 : 1.0 day) in the patient with prostatic carcinoma, although his plasma plasminogen remained constantly above the normal level, indicating a poor correlation between the level of

Table III

Calculated metabolic parameters

	Fractional catabolic rate constant			Absolute catabolic rate (mg/kg/day)	Fractional transcapillary transfer rate constant	Intravascular fraction
	$k_{10,p}$	$k_{10,u}$	$k^{*}_{10,p}$		k_{12}	IV
KG	0.89	0.74**	0.48	13.4	0.42	0.78
AV	0.94	–	0.40	4.5	0.55	0.78
Controls						
Mean	0.55			4.8	0.38	0.58
SD	0.10			1.2	0.20	0.06

* During anticoagulation in KG and after delivery in AV.

** Underestimation of the real value due to incomplete urine collections.

plasminogen and its turnover rate. To find out if the accelerated plasminogen disappearance was due to primary activation of the fibrinolytic system, it was decided to study whether in vivo inhibition of fibrinolysis resulted in a prolongation of the plasma radioactivity half-life. Tranexamic acid was therefore administered in a peroral dose of 2 g t.i.d. Unexpectedly*, a shortening of the half-life to 0.5 days occurred. During subsequent anticoagulation, the plasminogen half-life became lengthened to within the normal range. This finding strongly suggested that the acceleration of the breakdown was secondary to in vivo coagulation. The slope of the radioactivity disappearance curve before anticoagulation was 0.69, corresponding to a fractional catabolic rate constant of 0.89. During anticoagulation the slope was 0.39, corresponding to a fractional catabolic rate of 0.48. The difference between the two fractional catabolic rates is 0.41 and this represents the fraction of the plasma plasminogen pool which is consumed per day in connection with intravascular coagulation. The corresponding amount of plasminogen consumed is 6.1 mg/kg per day.

A plasminogen turnover experiment, performed four weeks later during which the patient was treated with oestrogens, revealed a similarly enhanced catabolism.

* This observation led to further studies on the effect of tranexamic acid in a dosage of 2 g t.i.d. on the turnover of labeled plasminogen in normal subjects (see chapter II), which indicated that this drug accelerated the plasminogen turnover, presumably by a direct effect on the plasminogen molecule. It thus became apparent that evaluation of the influence of tranexamic acid on the turnover of labeled plasminogen does not allow detection of plasminogen catabolism via in vivo fibrinolysis.

This invalidates the hypothesis that the normalization of the half-life during anticoagulation in the first study was the result of a spontaneous regression of in vivo coagulation. Whether the accelerated plasminogen catabolism was due to secondary activation of the fibrinolytic system (2) or to adsorption of plasminogen onto fibrin evolving in vivo (3) could not be established with the present method.

In the patient with missed abortion, the plasminogen turnover was markedly increased despite heparin anticoagulation (t 1/2 : 0.95 days) but spontaneously returned to normal after delivery (t 1/2 : 2.2 days). The slope of the radioactivity disappearance curve before delivery was 0.73, corresponding to a fractional catabolic rate of 0.94. After delivery, the slope was 0.32, corresponding to a fractional catabolic rate of 0.40. The difference of 0.54 corresponds to a consumption of 2.6 mg/kg per day, notwithstanding anticoagulation.

Three distinct mechanisms have been proposed for the hypofibrinogenemia in the dead fetus syndrome : generalized intravascular coagulation (5), primary activation of the fibrinolytic system (6), and local intra-uterine coagulation (7). Usually low grade intravascular coagulation would occur, potentially associated with a moderate decrease in plasminogen, which has been ascribed to a secondary activation of the fibrinolytic system (5). The moderate effect of heparin on the fibrinogen level in our patient suggested that intravascular coagulation was occuring. Additional evidence for this was derived from the effect of inadvertent interruption of the heparin infusion on the plasma fibrinogen level and the amount of fibrin(ogen) degradation products in serum. However, if intravascular coagulation alone had been operative, one would have expected a more pronounced correcting effect on the coagulation parameters during therapeutic anticoagulation, and consequently on the plasminogen level and its turnover. This indicated that other mechanisms than generalized intravascular coagulation were still active. One possibility is that the adequate systemic heparinization could not prevent local intra-uterine coagulation with subsequent plasminogen absorption and/or secondary fibrinolytic activation. Another is that fetal material released into the maternal circulation, besides inducing hypofibrinogenemia by intravascular coagulation also activated the fibrinolytic system directly, only the coagulant action being inhibited by heparin.

These two cases demonstrate that abnormal plasminogen metabolism can be detected and quantitated by studying the turnover of labeled plasminogen and that chronic low grade plasminogen consumption apparently follows first order kinetics. Furthermore it appears that the actual level of the plasma plasminogen is a poor indication of its turnover. Indeed, in patient KG an increased catabolism was counterbalanced by a still more increased synthesis, resulting in an elevated plasma plasminogen level. In patient AV the synthetic rate was in the normal range but, due to a more than twofold increase in catabolism, the plasma level was only about 50 % of normal.

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Section II :

METABOLISM OF PROTHROMBIN IN SELECTED CLINICAL CONDITIONS : THREE CASE REPORTS

The metabolism of prothrombin was studied in three selected clinical conditions predisposing to intravascular coagulation in order to demonstrate its usefulness as a tracer of in vivo prothrombin consumption.

Patients

The metabolism of prothrombin was studied in the 77 yr old male (KG) with *metastatic carcinoma of the prostate* (cfr. section I).

A prothrombin turnover study was performed in a 65 yr old female (LC) with *primary polycythemia.* She was admitted with high hematocrit values (63-70 %) and thrombocythemia (500,000-700,000 platelets/mm^3), two conditions known to predispose to intravascular coagulation.

Labeled prothrombin was injected into a 70 yr old male (GV) with known *cirrhosis of the liver,* who was admitted because of increasing jaundice and flapping tremor. During the turnover study a diagnostic ascites puncture revealed malignant cells. The clinical and biochemical findings progressively deteriorated, the patient developed hepatic pre-coma at the end of the study and died shortly thereafter. Obduction revealed a *gastric adenocarcinoma* and a *hepatoma.* There was no intraperitoneal or cerebral bleeding.

The clinical and laboratory data of the patients are summarized in table I.

Table I

Clinical and laboratory data

	Age (yr)	Weight (kg)	Plasma prothrombin (mg/100 ml)	Plasma volume (ml/kg)	Diagnosis
KG	77	67	13.3	34	Prostatic carcinoma
LC	65	50	12.1	40	Polycythemia vera
GV	78	59	5.6	62	Cirrhosis-malignancy
Controls					
Mean	34	73	13.2	39	
SD	11	12	1.6	5	

RESULTS

The turnover of labeled prothrombin was accelerated in the patient with prostatic carcinoma (fig. 1).

The plasma radioactivity disappearance curve could be described by $x(t) = 0.52e^{-0.35t} + 0.48e^{-1.54t}$ (t 1/2 : 2.0 days). The fractional catabolic rate (0.56 of the plasma pool per day) was significantly increased. The plasma prothrombin concentration was however maintained at a normal level by a slightly increased synthetic rate (2.48 mg/kg per day). Sephadex G-100 gel filtration of serial plasma samples showed elution of radioactivity in one main peak at the same volume as prothrombin activity and in a small peak at the total volume of the column. The

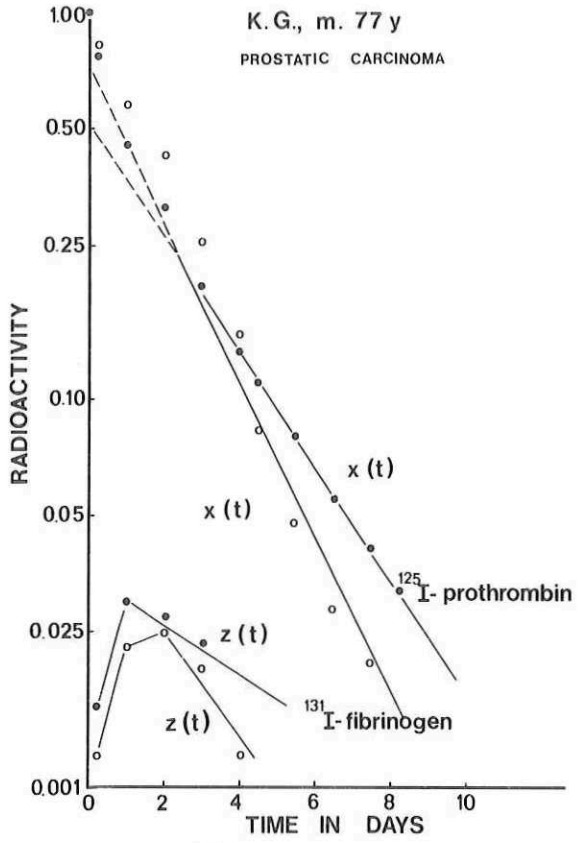

Fig. 1 – *^{125}I-Prothrombin and ^{131}I-fibrinogen metabolism in a patient with low grade intravascular coagulation secondary to metastatic carcinoma of the prostate x(t), plasma radioactivity ; z_t, non-TCA precipitable radioactivity in plasma.*

turnover of labeled fibrinogen, studied simultaneously with the prothrombin experiment, was also markedly shortened (t 1/2 : 1.50 days, controls 4.14 ± 0.51 days (1)).

The half-life of prothrombin was shortened in the patient with polycythemia (t 1/2 : 1.5 days) although her plasma prothrombin level was nearly normal (fig. 2). The plasma radioactivity disappearance curve was described by $x(t) = 0.60e^{-0.46t} + 0.40e^{-1.73t}$. The fractional catabolic rate (0.65 of the plasma pool per day) and the synthetic rate (3.16 mg/kg per day) were significantly increased.

The turnover of labeled prothrombin was accelerated in the patient with liver cirrhosis, gastric adenocarcinoma and hepatoma (fig. 3). The plasma radioactivity could be described by $x(t) = 0.61e^{-0.46t} + 0.39e^{-1.73t}$ (t 1/2 : 1.50 days). The fractional catabolic rate (0.647 of the plasma pool per day) was significantly increased. The synthetic rate (1.89 mg/kg per day) was in the normal range and the plasma prothrombin level 30-40 % of normal. Anticoagulation during 4 days

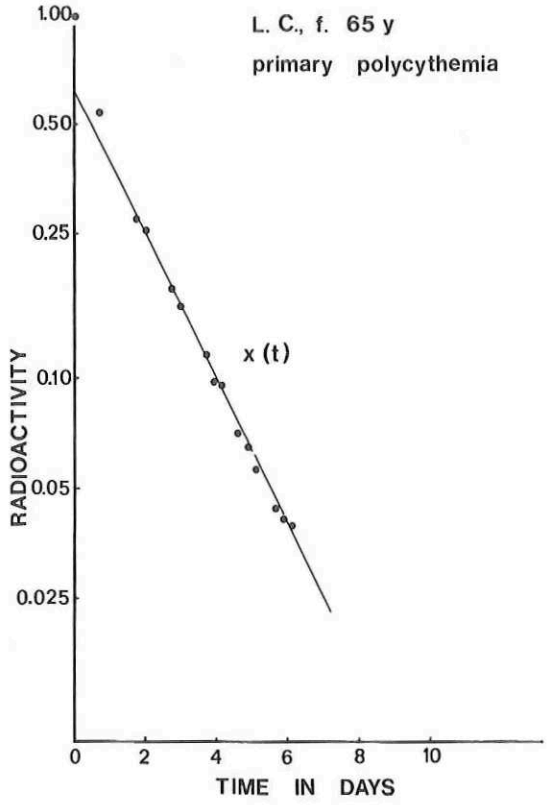

Fig. 2 – *Prothrombin metabolism in a patient with primary polycythemia. x(t) : plasma radioactivity.*

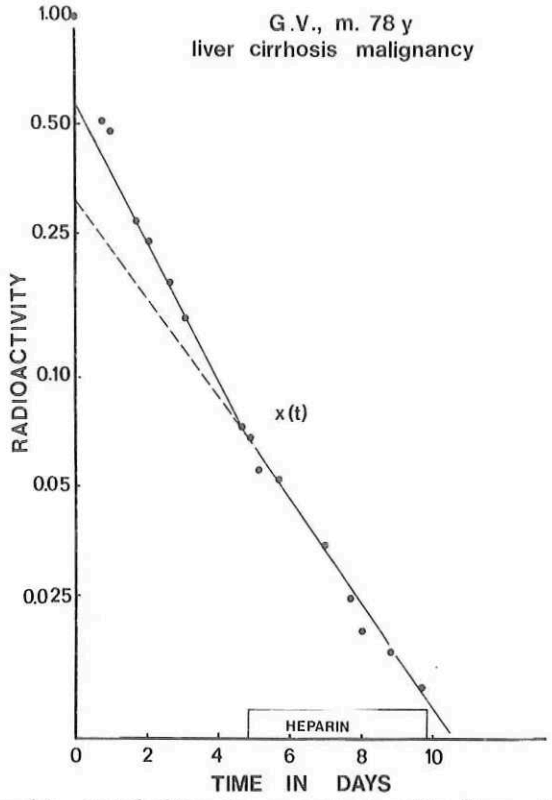

Fig. 3 – *Prothrombin metabolism in a patient with liver cirrhosis, gastric adenocarcinoma and hepatoma.*
x(t) : protein-bound radioactivity.

resulted in a prolongation of the plasma prothrombin half-life from 1.5 to 2.2 days.

The tracer data and calculated metabolic parameters in these patients are summarized in tables II and III.

DISCUSSION

The metabolism of prothrombin was studied in three patients with suspected low grade intravascular coagulation in order to demonstrate the validity of labeled prothrombin as a tracer of in vivo prothrombin consumption.

In the three patients studied we found a significantly increased turnover rate. In the two patients (KG and LC) with normal liver function, this increased turnover rate was counterbalanced by an increased synthetic rate, resulting in normal plasma prothrombin levels. In the patient (GV) with impaired liver function, the synthetic

Table II

Prothrombin tracer data

$x(t) = C_1 e^{-a_1 t} + C_2 e^{-a_2 t}$

	C_1	a_1	C_2	a_2	t 1/2 for a_1	t 1/2* for a_1
KG	0.52	0.35	0.48	1.54	2.00	
LC	0.60	0.46	0.40	1.73	1.50	
GV	0.61	0.46	0.39	1.73	1.50	2.20
Controls						
Mean	0.49	0.23	0.51	1.42	3.04	
SD	0.04	0.02	0.04	0.38	0.28	

* During anticoagulation

Table III

Calculated metabolic parameters

	Fractional catabolic rate constant	Absolute catabolic (synthetic) rate (mg/kg/day)	Fractional transcapillary transfer rate constant	Intravascular fraction
	$k_{10,p}$		k_{12}	IV
KG	0.56	2.48	0.37	0.63
LC	0.65	3.16	0.32	0.71
GV	0.65	1.89	0.31	0.71
Controls				
Mean	0.40	2.27	0.44	0.58
SD	0.40	0.34	0.18	0.05

rate was at the lower limit of the normal range, resulting in plasma prothrombin levels of about 40 % of normal. These findings demonstrate that the actual levels of prothrombin are a poor reflection of the synthesis and breakdown kinetics occurring in vivo.

In conclusion, labeled prothrombin is a sensitive tool for the quantitative determination of prothrombin kinetics in patients with suspected prothrombin consumption. Recently, Shapiro and Martinez (2) reached the same conclusion from turnover studies of labeled prothrombin and fibrinogen in patients with thrombocytosis.

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