Measurement of Desarginine Fibrinopeptide B in Human Blood

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ABSTRACT Thrombin converts fibrinogen to fibrin in two steps. First fibrinopeptide A and fibrin I are formed and then fibrinopeptide B (Bβ 1-14) and fibrin II. Since it is postulated that fibrin II is important in the genesis of thrombosis, it is of interest to measure fibrinopeptide B in peripheral blood samples. Previous difficulties in interpreting fibrinopeptide B immunoreactivity in plasma resulted from crossreaction of fibrinogen and of plasmin digest peptides Bβ 1-42 and Bβ 1-21 and from rapid loss of fibrinopeptide B immunoreactivity resulting from cleavage of arginine 14 by blood carboxypeptidase B. We have obviated these difficulties by removing fibrinogen from plasma by precipitation with ethanol and peptides Bβ 1-21 and Bβ 1-42 by adsorption on bentonite. Fibrinopeptide B is then converted to desarginine fibrinopeptide B, which is measured in a new specific assay. Studies of the kinetics of fibrinopeptide cleavage showed that when whole blood was allowed to clot in vitro, fibrinopeptide A was cleaved more rapidly than fibrinopeptide B. In 18 patients on an acute care medical ward, desarginine fibrinopeptide B levels were lower than fibrinopeptide A levels and did not correlate with the levels of fibrinopeptide A or Bβ 1-42. Desarginine fibrinopeptide B levels were <1 pmol/ml in all but two patients. In six patients receiving intraamniotic infusions of hypertonic saline to induce abortion, desarginine fibrinopeptide B levels increased 10-fold from the preinfusion mean level of 0.4 pmol/ml and then decreased. The pattern of changes resembled that of the fibrinopeptide A levels rather than of the Bβ 1-42 levels. On the basis of these data it is suggested that plasma desarginine fibrinopeptide B levels reflect fibrin II formation in vivo.

INTRODUCTION

The balance between thrombin and plasmin proteolysis of fibrinogen is thought to be important in determining the occurrence of thrombosis. The exact way in which this balance controls the biochemical structure of fibrin in thrombin is not known. With isolated reagents thrombin cleaves fibrinogen in two stages (1–3). In the first stage fibrinopeptide A is released and fibrin I formed. In the second stage fibrinopeptide B is released and fibrin II is formed. Plasmin initially removes the carboxy-terminal two thirds of the Aα chain and Bβ 1-42 and the residual molecule is termed fragment X (4–8). Evidence has been presented that following intrauterine infusion of hypertonic saline to induce abortion fibrinogen proteolysis occurs in a reproducible sequence (9). First, proteolysis by thrombin occurred resulting in elevated fibrinopeptide A levels. Then plasmin cleaved fibrin I to produce free Bβ 1-42 and fragment X. The extent to which fibrinopeptide B was released and fibrin II was formed in that study is not known. An index of fibrin II formation in vivo would be valuable in testing the postulate that this species determines the occurrence of thrombosis (1, 9, 10). Plasma fibrinopeptide B levels may provide a direct index of fibrin II formation in vivo. A radioimmunoassay for fibrinopeptide B has been developed, but its application has been limited to buffer solutions because blood measurements pose major problems in interpretation. The two principal problems that complicate interpretation of the fibrinopeptide B assay in blood are the presence of crossreacting peptides such as Bβ 1-42 (9) and the rapid cleavage of arginine (Bβ 14) from fibrinopeptide B with a consequent major decrease in immunoreactivity (11, 12). The solution of these technical problems and the results of initial studies on the measurement of fibrinopeptide B in clinical blood samples are reported in this paper.

METHODS

Reagents. Native human fibrinopeptide B was isolated as described by Blombäck et al. (13) or was a gift from Dr. Birger Blombäck. Desarginine fibrinopeptide B (B 1-13) was prepared by treating 1 mg native fibrinopeptide B dissolved in 1 ml 0.1 M NH_4HCO_3 with 0.06 U carboxypeptidase B for 24 h

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TABLE I

Amino Acid Analysis of Native Fibrinopeptide B and Synthetic Fibrinopeptide B Analogue after Digestion with Carboxypeptidase B

	Native FPB from sequence data (13)	CPB-treated native FPB		CPB-treated synthetic FPB analogue	
		After hydrolysis	Before hydrolysis	After hydrolysis	Before hydrolysis
Glycine	2	1.99	—	2.03	—
Valine	1	1.00	—	1.00	—
Aspartic acid	1	3.11	—	3.03	
Asparagine	2				
Glutamic acid	2	3.14	—	3.09	—
Pyroglutamic acid	1				
Phenylalanine	2	2.07	—	2.0	
Serine	1	0.93	—	0.90	
Alanine	1	0.99	—	0.95	
Arginine	1	0.98	1.01	1.00	1.03

CPB, carboxypeptidase B; FPB, fibrinopeptide B.

at 37°C, and then inactivating carboxypeptidase B by adding *O*-phenanthroline (final concentration 0.01 M). Amino acid analysis of the product is shown in Table I. Synthetic fibrinopeptide B analogue was obtained from Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y. Glutamic acid replaced pyroglutamic acid in the analogue. Desarginine fibrinopeptide B analogue was prepared by treating synthetic fibrinopeptide B analogue with carboxypeptidase B exactly as described for native fibrinopeptide B. Desaminotyrosyl desarginyl fibrinopeptide B analogue was prepared by coupling desaminotyrosine to desarginyl fibrinopeptide B analogue as previously described for coupling to fibrinopeptide B analogue (3). Peptides Bβ 1-21 and Bβ 1-42 were isolated by high-pressure liquid chromatography of amino-terminal fragments of fibrinogen that had been passed over a plasmin-Sepharose column. Dr. Stephen Birken and Dr. Robert Canfield of the Department of Medicine isolated and characterized the peptides. A manuscript describing the isolation is in preparation. Peptide concentrations were determined by amino acid analysis. The development of the antisera to fibrinopeptide B has been described previously (3). Immunochemical characterization of the antisera with respect to the crossreactivity of peptides smaller than fibrinopeptide B has been described by Wilner et al. (14) and with respect to antigens larger than fibrinopeptide B by Butler et al. (15). Antisera R22, R29, and R30 were used in this study. *O*-phenanthroline (1-10 phenanthroline monohydrate) was obtained from Sigma Chemical Co., St. Louis, Mo. and was used dissolved in absolute ethanol. Bentonite (laboratory grade) was obtained from Fisher Scientific Co., Springfield, N. J., and was suspended in Tris buffer, pH 8.9 (0.1 M NaCl, 0.05 M Tris-HCl, 0.1% ovalbumin). Hirudin was obtained from Koch-Light Laboratories, Ltd., Colnbrook, Bucks, England. Streptokinase was streptodornase-streptokinase from Lederle Laboratories Div., American Cyanamid Co., Pearl River, N. Y. Carboxypeptidase B (COBC preparation of porcine pancreatic carboxypeptidase B) was from Worthington Biochemical Corp., Freehold, N. J. and was assayed by hydrolysis of hippuryl-L-arginine (Sigma Chemical Co.) immediately before use (16). The thrombin used was human α-thrombin (3,200 U/mg), generously provided by John W. Fenton, II, Division Laboratories, and Research, New York State Department of Health, Albany, N. Y. Heparin for in vitro use was obtained from Hynson, Westcott and Dunning, Inc., Baltimore, Md. and Trasylol from FBA Pharmaceuticals, New York.

Assays. Radioimmunoassays for fibrinopeptides B (17, 3) and A (18) were as previously described. Desarginine fibrinopeptide B was assayed as described for fibrinopeptide B, using as tracer desaminotyrosyl desarginine fibrinopeptide B analogue labeled with ^{125}I by the chloramine T technique as previously described for desaminotyrosyl fibrinopeptide B analogue (3). The specific activity of the preparation was 20–30 μCi/μg. Native desarginine fibrinopeptide B was used as the standard in this assay. When antiserum R29 was used desarginine fibrinopeptide B immunoreactivity was unstable in plasma or in the presence of carboxypeptidase B but was stable with antiserum R30. The R30 antiserum is more sensitive than is R29 to aspartic$_5$ and asparagine$_6$ (14). The R29 antiserum may be more sensitive to the amino acids adjacent to arginine$_{14}$ than is R30, and both the carboxypeptidase B preparation and normal plasma may digest fibrinopeptide B progressively from the carboxyterminus, thus accounting for the superiority of antiserum R30 as compared with R29 in the assay of desarginine fibrinopeptide B in whole blood samples.

Blood collection and processing. Blood was collected from an antecubital vein via a 19-gauge needle into a plastic syringe and transferred into a 15 × 90-mm polystyrene tube (Electronic Space Products, Los Angeles, Calif.) containing 1/10 vol of the anticoagulant solution (heparin, 1,500 U and 1,000 IU Trasylol in buffered saline). For in vitro recovery experiments, blood was collected into a syringe containing the anticoagulant solution, immediately transferred to a 50-ml conical plastic tube kept on ice, and mixed gently. Anticoagulated blood was immediately transferred into the 15 × 90-mm polystyrene tubes containing the different mixtures of fibrinopeptide B and Bβ 1-42, and mixed gently. After mixing with anticoagulant, the blood was immediately centrifuged at 1,700 *g* at 4°C for 20 min, and the plasma pipetted off. Within 30 min of blood collection, 2 ml plasma was precipitated with an equal volume of ethanol as described for the fibrinopeptide A assay (18) and dried at 37°C in a Brinkmann concentrator (Brinkmann Instruments, Inc., Westbury, N. Y.). The plasma was reconstituted to its initial volume with distilled water. Complete conversion of fibrinopeptide B into desarginine fibrinopeptide B was assured by incubating the recon-

stituted plasma with carboxypeptidase B (0.1 U/ml final concentration) at 37°C for 5 min. The carboxypeptidase B was then removed by adsorption with bentonite. 20 mg of bentonite was suspended in 1 ml Tris-buffered saline (0.1 M NaCl, 0.05 M. Tris-HCl, 0.1% ovalbumin) pH 8.9. 2.0 ml of plasma was added, then mixed on a vortex mixer for 30 s, and then on an Ames (Ames Co., Elkhart, Ind.) aliquot mixer for 15 min at room temperature. The bentonite was sedimented by centrifugation at 5,000 rpm for 10 min at 4°C, and the supernatant plasma removed with a pipette and assayed for desarginine fibrinopeptide B. Bβ 1-42 was estimated as fibrinopeptide B immunoreactivity after thrombin treatment of the supernate of ethanol-precipitated plasma as previously described (9). The term thrombin-increasable fibrinopeptide B immunoreactivity (TIFPB)[1] represents what was measured. Most or all of this material in plasma appears to be Bβ 1-42 but the technique does not distinguish Bβ 1-42 and Bβ 1-21 (19).

Patients and normal individuals. Blood from normal individuals was donated by laboratory personnel and students who were in apparent good health. Infusion studies were made in normal young volunteers. Blood from patients with various diseases was collected on the medical service wards of the Columbia Presbyterian Medical Center. All blood samples were collected by trained laboratory personnel via the same venipuncture used to collect blood for diagnostic tests. The patients studied before and after intrauterine injection of hypertonic saline, and the infusion technique were as previously described (9). All studies in patients and normal volunteers had previously been approved by the Institutional Review Board of the College of Physicians and Surgeons. Signed, informed consent was obtained and confidentiality preserved in all cases.

Infusion of fibrinopeptide B into normal individuals. Solutions of fibrinopeptide B used for infusion were prepared by treating an antihemophilic factor, fibrinogen-containing preparation (obtained from E. R. Squibb & Sons, Princeton, N. J.) with thrombin. The technique used was as previously described (17). Assay of the infused material showed that most of the fibrinopeptide B was present in the desarginine fibrinopeptide B form, presumably reflecting the presence of traces of carboxypeptidase B in the antihemophilic factor preparation. The infused solution also contained fibrinopeptide A. The infusion technique was as previously described (18).

Treatment of plasma with streptokinase in vitro. This was as previously described (9).

RESULTS

The two principal problems in interpreting fibrinopeptide B measurements in plasma are the crossreactivity of fibrinogen, Bβ 1-42, and Bβ 1-21 in the fibrinopeptide assay and cleavage of arginine$_{14}$ from fibrinopeptide B by carboxypeptidase B, which results in major loss of immunoreactivity. Fibrinogen was completely removed from plasma by precipitation with 50% ethanol and Bβ 1-42 and Bβ 1-21 were selectively removed from the reconstituted ethanol-treated sample by adsorption with bentonite. A concentration of 10 mg bentonite/ml almost completely adsorbed Bβ 1-42 and Bβ 1-21 with <20% loss of fibrinopeptide B and desarginine fibrinopeptide B (Fig. 1). The problem of partial cleavage of arginine$_{14}$ from fibrinopeptide B was solved by completing the conversion of fibrinopeptide B to desarginine fibrinopeptide B and assaying the latter. The conversion of fibrinopeptide B to desarginine fibrinopeptide B in blood is shown in Fig. 2 and this process was ensured by incubating plasma extracts with carboxypeptidase B added in vitro. Inhibition curves for desarginine fibrinopeptide B and other materials which crossreact in the desarginine fibrinopeptide B assay are shown in Fig. 3. The assay is 10 or more times more sensitive at the 50% inhibition point to desarginine fibrinopeptide B than it is to fibrinopeptide B, Bβ 1-21, Bβ 1-42, and fibrinogen. Based on these results, the blood processing technique shown in Fig. 4 was used. With the use of this processing technique the recovery of fibrinopeptide B added to blood in vitro is shown in Table II. It is crucially important to distinguish fibrinopeptide B and Bβ 1-42 in blood and experiments were made in which mixtures of these two peptides in varying proportion were added to blood in vitro. The results of these studies are shown in Table III and indicate that each of the two peptides was measured quite accurately in the presence of the other.

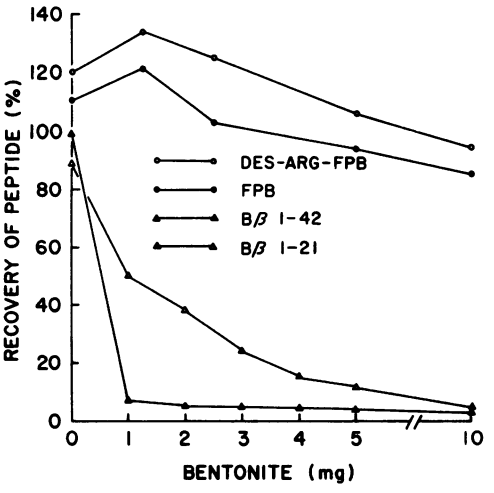

FIGURE 1 Adsorption of peptides from plasma with bentonite. The peptides were added to the supernate of plasma containing heparin and trasylol that had been precipitated with 50% ethanol and reconstituted. 1 ml plasma extract was then adsorbed with bentonite and assayed. The amounts of peptide added were: desarginine fibrinopeptide B, 64 pmol/ml, fibrinopeptide B, 64 pmol/ml, Bβ 1-21, 50–100 pmol/ml, Bβ 1-42, 50–100 pmol/ml. The results shown are the mean of three separate experiments. Desarginine fibrinopeptide B was measured in the desarginine fibrinopeptide B assay; fibrinopeptide B was measured in the fibrinopeptide B assay, Bβ 1-21 and Bβ 1-42 were treated with thrombin, 1 U/ml for 1 h at 37°C, and then measured in the fibrinopeptide B assay.

The concentration of free fibrinopeptides A and B in blood spontaneously clotting in vitro was then measured and showed rapid cleavage of both peptides, fibrinopeptide A being cleaved more rapidly than fibrinopeptide B (Fig. 5). At 30 min 1.9 mol/mol fibrinopeptide

[1] *Abbreviation used in this paper*: TIFPB, thrombin-increasable fibrinopeptide B immunoreactivity.

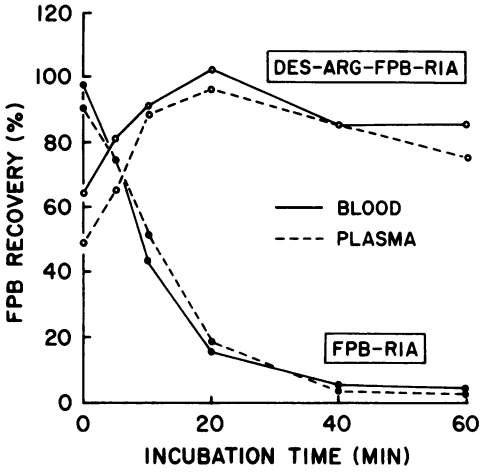

FIGURE 2 Conversion of fibrinopeptide B to desarginine fibrinopeptide B in blood and plasma. 62, 123, and 299 pmol native fibrinopeptide B were added to whole blood (containing 100 U heparin/ml) or 12.5 and 64 pmol native fibrinopeptide B were added to plasma (containing 100 U heparin/ml). The blood or plasma was maintained at room temperature and at intervals as indicated on the abscissa aliquots of blood or plasma were removed, processed by precipitation with 50% ethanol and adsorption with bentonite, and then assayed for fibrinopeptide B (FPB-RIA) or desarginine fibrinopeptide B (DES-ARG-FPB-RIA) (using antiserum R29). The data shown are the mean of three experiments.

A was present in the serum and 1.3 mol/mol fibrinopeptide B. At later time periods the concentration of both fibrinopeptides A and B in serum decreased. The primary reason for measuring fibrinopeptide B in blood was to use the levels as an index of fibrin II formation in vivo. It is therefore of interest to know whether plasmin alone will generate desarginine fibrinopeptide B in blood in vitro. Desarginine fibrinopeptide B was generated at ~3% of the rate at which Bβ 1-42 was generated when streptokinase was incubated with plasma

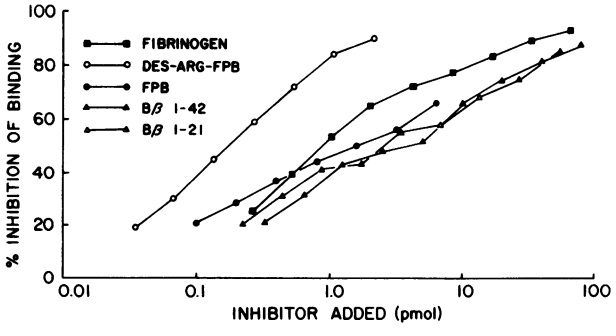

FIGURE 3 Desarginine fibrinopeptide B assay. The tracer was ^{125}I-desaminotyrosine fibrinopeptide B analogue and antiserum R29 was used. Inhibition curves are shown for desarginine fibrinopeptide B (DES-ARG-FPB), native fibrinopeptide B (FPB), Bβ 1-21, Bβ 1-42, and fibrinogen. 35% tracer was bound.

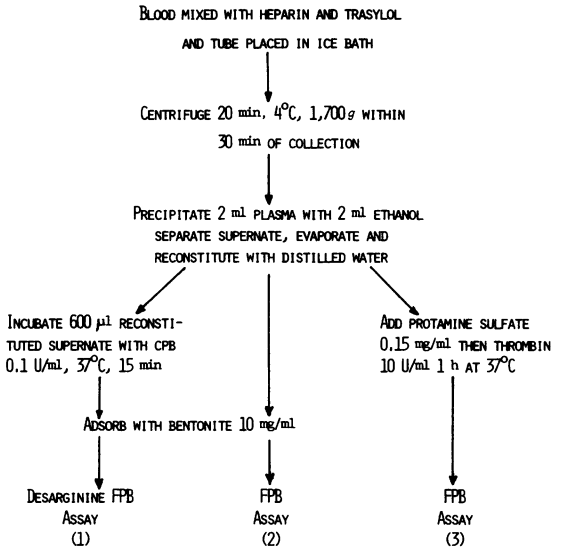

FIGURE 4 Blood collection and processing technique for assaying desarginine fibrinopeptide B and Bβ 1-42 in whole blood. (1) gives desarginine fibrinopeptide B concentration in plasma. (3) minus (2) gives Bβ 1-42 concentration in plasma. (2) is generally insignificant and can be ignored in clinical blood samples. CPB, carboxypeptidase B.

(Table IV). Gel filtration on Sephadex G-50 showed that the elution volume of the desarginine fibrinopeptide B immunoreactivity generated in streptokinase-treated plasma was similar to that of fibrinopeptide B or desarginine fibrinopeptide B immunoreactivity (data not shown). Sephadex G-50 clearly distinguishes fibrinopeptide B and Bβ 1-42, the ratio of their elution volumes being 1.3 (9).

The desarginine fibrinopeptide B assay was then applied to clinical blood samples. The level in 10 normal individuals was <0.6 pmol/ml with a mean level of 0.2 pmol/ml. The levels in 18 patients on an acute care medical ward are shown in Table V along with fibrinopeptide A and TIFPB levels. Definitive data for the normal level of TIFPB are not yet available. We currently estimate the normal range to be 1–4

TABLE II
Recovery of Fibrinopeptide B Added to Blood

Amount of FPB added to blood	FPB assayed in blood as desarginine fibrinopeptide B	Recovery
pmol/ml		%
4.3	4.9	114
8.6	8.4	98
12.9	11.7	91
17.2	18.7	109

The results are the mean of three separate experiments. FPB, fibrinopeptide B.

TABLE III
Recovery of FPB and Bβ 1-42 Added to Blood

Amount of peptide added to blood		FPB assayed in blood as desarginine fibrinopeptide B		Bβ 1-42 assayed in blood as indicated in Fig. 4 (column 3 minus column 2)	
FPB	Bβ 1-42				
	pmol/ml		*% Recovery*	*pmol/ml*	*% Recovery*
17.2	0	16.7	97	<1	
12.8	3.2	13.3	104	3.9	122
8.6	6.4	8.2	95	8.2	128
4.4	9.7	3.9	89	11.1	114
0	12.3	0.16		10.5	85

The results are the mean of three separate experiments. In each instance the basal plasma level was subtracted. FPB, fibrinopeptide B.

pmol/ml. The fibrinopeptide A level was > 1.3 pmol in 13 of the 18 patients. The desarginine fibrinopeptide B level was > 1 pmol/ml in only two patients. Serial changes in the plasma concentrations of desarginine fibrinopeptide B, fibrinopeptide A, and TIFPB after intrauterine infusion of hypertonic saline are shown in Fig. 6. The desarginine fibrinopeptide B levels rose rapidly reaching a peak 2 h after the infusion and then declined. Several infusions of desarginine fibrinopeptide B were made into normal recipients. The results of these experiments are shown in Fig. 7 and indicate a rapid clearance rate with a $t_{1/2}$ of ~1½–2 min.

DISCUSSION

Interpretation of fibrinopeptide B immunoreactivity in clinical blood samples is made difficult by the cross-reactivity of small fibrinopeptide B-containing peptides that could be selectively removed by adsorption with bentonite (Fig. 1). Loss of immunoreactivity due to carboxypeptidase B proteolysis in plasma (11, 12) was solved by completing the conversion of fibrinopeptide B in blood to desarginine fibrinopeptide B by incubation with carboxypeptidase B added in vitro and then assaying the desarginine fibrinopeptide B. The validity and specificity of the assay system developed is shown by the recovery of fibrinopeptide B added to blood in vitro shown in Table II and by the recovery of fibrinopeptide B and Bβ 1-42 added simultaneously to blood shown in Table III.

We next considered whether desarginine fibrinopeptide B levels in plasma signified fibrin II formation or were derived by proteolysis of Bβ 1-42. The results of the experiment showed in Table IV indicate that a small amount of desarginine fibrinopeptide B is generated in plasma by streptokinase presumably as a result of plasmin formation and action. Plasmin released Bβ 1-42 from fibrinogen and then presumably cleaved

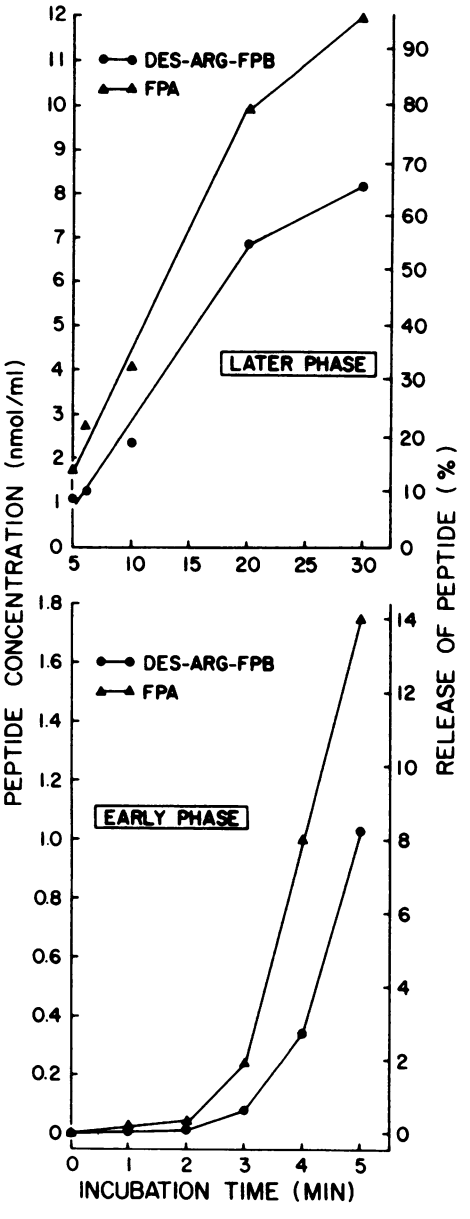

FIGURE 5 Concentration of free fibrinopeptides A and B during the spontaneous clotting of whole blood. 2 ml of blood were placed in a 12 × 75-mm glass tube maintained at 37°C. At intervals aliquots of the fluid were removed, mixed with heparin (1,500 U/ml) and Trasylol (1,000 U/ml), processed as described in Fig. 4 and the concentration of fibrinopeptide A (FPA) and desarginine fibrinopeptide B (DES-ARG-FPB) were assayed. The fibrinogen concentration of the plasma was 2.12 mg (6.25 nmol)/ml.

this to produce Bβ 1-21 and then fibrinopeptide B, which was converted to desarginine fibrinopeptide B by carboxypeptidase B. However, desarginine fibrinopeptide B formation is only 3% that of Bβ 1-42 and one would expect a 1-pmol/ml increase in desarginine fibrinopeptide B level to occur with a 33-pmol/ml in-

TABLE IV

Generation of Thrombin-increasable Fibrinopeptide B Immunoreactivity (Bβ1-42 and Bβ1-21) in Citrated Plasma Incubated with Streptokinase

Streptokinase concentration	Incubation time (min)							
	0′	5′	10′	15′	20′	30′	40′	60′
	Thrombin-increasable fibrinopeptide B immunoreactivity							
U/ml plasma	*pmol/ml*							
20	5.6	499	2,033	3,638	3,574	—	5,136	5,286
10	4.1	182	364	803	1,573	—	3,788	3,531
1	2.3	14.2	28.1	—	—	66.5	—	76.3
0.1	2.9	2.9	3.4	—	—	4	—	5.0
	Desarginine fibrinopeptide B							
	pmol/ml							
20	<2	10.2	39.6	73.8	98.4	—	192.9	—
10	1.3	6.6	10.8	20.4	33.0	—	120.0	111
1	<1	<1	<1	—	—	1.5	—	2.1
0.1	<1	<1	<1	—	—	<1	—	<1

crease in Bβ 1-42 level if the in vivo clearance rates of the two peptides were similar. Can elevated thrombin concentrations in the presence of elevated Bβ 1-42 levels cleave Bβ 1-42 to produce fibrinopeptide B? The plasma concentration of fibrinogen is ~2,000 times that of Bβ 1-42 in normal blood and 100 times that of the highest concentrations of Bβ 1-42 (67 pmol/ml). It seems likely that fibrinogen would competitively inhibit thrombin proteolysis of Bβ 1-42 but kinetic studies are necessary to determine the effect of excess fibrinogen on the cleavage of Bβ 1-42 by thrombin. In conclusion, it seems quite likely that some desarginine fibrinopeptide B is produced by proteolysis of Bβ 1-42 by thrombin or plasmin but that the rate of such produc-

TABLE V

FPA, DES-ARG-FPB, and Thrombin-increasable Fibrinopeptide B (TIFPB, Bβ1-42, and Bβ1-21) Levels in Patients on an Acute Care Medical Ward

No. patient	Diagnosis	FPA	DES-ARG-FPB	TIFPB (Bβ1-42 & Bβ1-21)
			pmol/ml	
	Normal range	0.1–1.3	<0.6	<4
1	Atrial flutter	0.7	0.26	2.1
2	Mitral stenosis, right leg embolus on warfarin	0.3	0.11	2.9
3	Diabetes mellitus	0.4	0.39	2.3
4	Acute pancreatitis, alcoholism	0.9	0.54	3.0
5	Bronchial asthma	0.6	0.1	2.0
6	Metastatic neoplasia	1.4	0.62	4.8
7	Syncope, acute blood loss heal injury	1.3	0.27	6.8
8	Complete heart block, heart failure	1.6	0.41	13.8
9	Nephrotic syndrome, cardiac failure	4.9	0.66	32.4
10	Pancreatic carcinoma	8.9	0.48	3.9
11	Venous thrombosis on treatment with warfarin	3.6	0.53	7.3
12	Diabetes mellitus, gastrointestinal bleeding	2.8	0.44	7.4
13	Pneumonia, cardiac and renal failure	3.7	0.57	24.9
14	Polycystic kidneys	3.8	0.45	26.3
15	Aspiration pneumonia	3.3	0.77	7.5
16	Pneumonia, alcoholism	3.6	0.75	8.9
17	Temporal arteritis	3.4	1.2	9.8
18	Acute pancreatitis, alcoholism	1.5	2.3	5.2

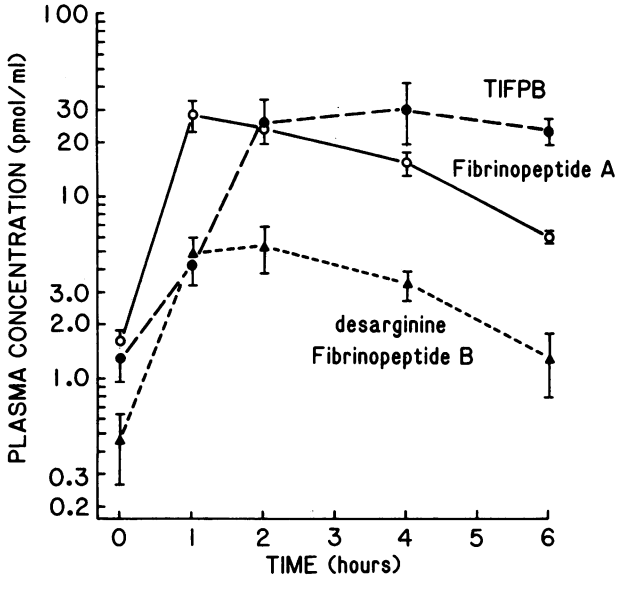

FIGURE 6 Changes in desarginine fibrinopeptide B, fibrinopeptide A, and TIFPB levels before and 1–6 h after intrauterine injection of hypertonic saline. The data plotted are the mean±SEM in six patients. The mean desarginine fibrinopeptide B level before saline infusion was 0.45 pmol/ml.

tion is trivial in comparison with that resulting from thrombin proteolysis of fibrin I in most circumstances. This conclusion needs to be tested by studies of kinetics of enzyme action on fibrin I and on Bβ 1-42, by studies of plasma desarginine fibrinopeptide B

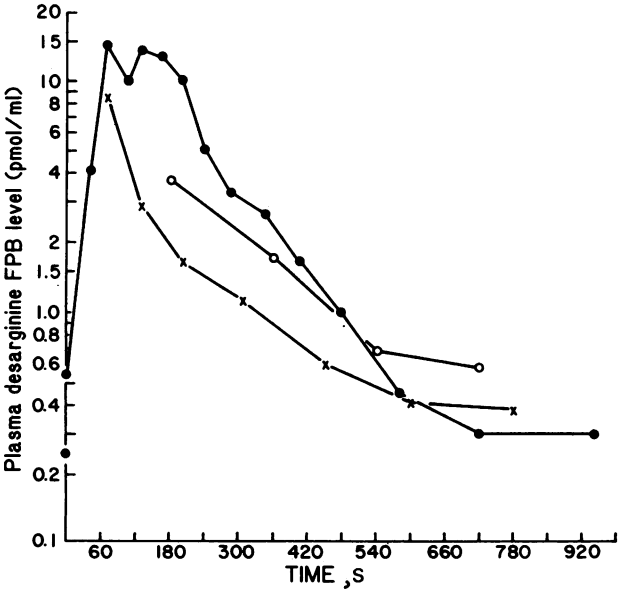

FIGURE 7 Desarginine fibrinopeptide B levels in the plasma of a normal recipient infused with fibrin clot supernate containing ●, 160 nmol desarginine fibrinopeptide B; × and ○, 48 nmol.

levels in thrombosis and of the detailed biochemical composition of fibrin in thrombi. In unusual circumstances such as proteolysis by enzymes other than thrombin or plasmin, increased desarginine fibrinopeptide B levels may not reflect fibrin II formation.

When whole blood clots rapidly in vitro, it is evident that fibrin I is rapidly transformed to fibrin II (Fig. 5). The incomplete recovery of fibrinopeptide B in the serum (only 1.3 pmol/ml instead of 2 mol/mol) is unexplained. Preliminary experiments suggest that the loss of immunoreactivity is enzyme-mediated, and the putative enzyme is more effective or more concentrated in serum than in plasma since desarginine fibrinopeptide B is stable in plasma over 60 min (12). In thrombosis, locally produced fibrinopeptide B may be degraded as if it were in serum and the clearance rate obtained by infusion of fibrinopeptide B may give a falsely high value. It will therefore be important to clarify the mechanism and quantify the rate of degradation of fibrinopeptide B in serum. Blood flow and the presence of plasmin may affect the amount of fibrin II formed. Measurable desarginine fibrinopeptide B levels in normal plasma imply the occurrence of fibrinogen proteolysis and fibrin II formation at a low level in normal individuals. These data should prompt studies to identify fibrin II in plasma. When the data on the patients in an acute care medical ward was considered there was no evident correlation between the concentrations of desarginine fibrinopeptide B and Bβ 1-42, suggesting that the former is not derived by proteolysis of the latter. Desarginine fibrinopeptide B levels > 1 pmol/ml occurred in two patients only; one with acute pancreatitis and one with temporal arteritis. In the patient with pancreatitis the desarginine fibrinopeptide B level exceeded the fibrinopeptide A level consistent with a trypsinlike effect (3). Following intrauterine infusion of hypertonic saline desarginine fibrinopeptide B levels increased ~10-fold 2 h after infusion (mean level 5 pmol/ml) over the preinfusion level (0.45 pmol/ml). The pattern of changes in desarginine fibrinopeptide B levels resembles that of fibrinopeptide A rather than of Bβ 1-42, suggesting that the desarginine fibrinopeptide B reflects fibrin II formation.

The results of the infusion studies show that desarginine fibrinopeptide B is cleared from the blood more rapidly than fibrinopeptide A (18). Data on the distribution volume, clearance rate, and removal of $arginine_{14}$ from fibrinopeptide B will be necessary to calculate the rate of fibrin II formation in vivo from plasma fibrinopeptide B levels. The clearance rate of fibrinopeptide B may not be identical with that of desarginine fibrinopeptide B.

In conclusion, the availability of this technique now permits measurement of fibrin II formation simultaneously with fibrin I (18) and fragment X (9) formation in vivo.

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