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C. Thomas Kisker, Ruth Rush

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Research Article

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Clinically, the method was found to provide useful information for the diagnosis and treatment of disseminated intravascular coagulation in two patients with meningococcemia, two patients with Rocky Mountain spotted fever, and three patients in whom therapeutic abortions were induced by the injection of hypertonic saline.

Detection of Intravascular Coagulation

C. THOMAS KISKER and RUTH RUSH

From The Children's Hospital, The Children's Hospital Research Foundation, and the Department of Pediatrics, University of Cincinnati, Cincinnati, Ohio 45229

ABSTRACT A method is described for the measurement of soluble thrombin-altered fibrinogen (circulating fibrin) in human plasma. This method is dependent upon the enzymatic incorporation of glycine ethyl ester-^{14}C (GEE-^{14}C) into circulating fibrin by the action of the fibrin-stabilizing enzyme, factor XIII. The mean incorporation of GEE-^{14}C into the fibrinogen of normal human plasma controls was 167 ±47 dpm/mg fibrinogen. The addition of 0.03 NIH U/ml of thrombin to normal human plasma resulted in a two to threefold increase in the incorporation of GEE-^{14}C into the fibrinogen. The addition of plasmin split products of fibrinogen to normal plasma did not increase the incorporation of GEE-^{14}C unless these products were also exposed to thrombin. The addition of plasmin split products of a fibrin clot resulted in only minimal increase in the incorporation of GEE-^{14}C (57 dpm/mg fibrinogen) at 37.5% concentration. The method was therefore sensitive to thrombin alterations of fibrinogen but insensitive to plasmin alterations of fibrinogen and fibrin.

Clinically, the method was found to provide useful information for the diagnosis and treatment of disseminated intravascular coagulation in two patients with meningococcemia, two patients with Rocky Mountain spotted fever, and three patients in whom therapeutic abortions were induced by the injection of hypertonic saline.

INTRODUCTION

The diagnosis and management of disseminated intravascular coagulation (DIC)[1] are based on often inconsistent and confusing secondary changes in various clotting factors. Were it possible to measure soluble, thrombin-altered fibrinogen (circulating fibrin), the diagnosis and management of DIC could be based on the primary abnormality. For this purpose, a method for measuring circulating fibrin was designed which depends upon the enzymatic incorporation of glycine ethyl ester-^{14}C (GEE-^{14}C) into fibrin.

Lorand and Ong (1) incorporated GEE-^{14}C into purified fibrin with activated factor XIII, a fibrin-stabilizing enzyme. In the present studies, factor XIII and GEE-^{14}C were added to an 8% ethanol fraction of human plasma and the amount of GEE-^{14}C incorporation in the clottable portion of this fraction measured. Applying this technique to in vitro and in vivo experiments revealed that an increase in incorporation occurred when thrombin-altered fibrinogen (circulating fibrin) was present, but no increase was detected when plasmin-digested fibrinogen (fibrinogen split products) were present unless the split products were also exposed to thrombin. The presence of plasmin-digested fibrin (fibrin split products) increased the incorporation only slightly at high concentrations.

METHODS

Preparation of plasma. GEE-^{14}C[2] (3.04 mCi/mmole), crude streptokinase,[3] and hirudin[4] were obtained commercially. The hirudin concentration was standardized acccording to the method of Rapaport (2). Blood for measurement of circulating fibrin was drawn by venepuncture into a plastic syringe and nine parts of blood were added to a conical 15 ml polystyrene centrifuge tube[5] containing one part of an anticoagulant mixture consisting of 2% EDTA, 10 U/ml hirudin, and 10 mg/ml soybean trypsin inhibitor (SBTI). The samples were centrifuged at 1800 *g* in an International centrifuge,[6] model PR2, for 10 min and the supernatant platelet-poor plasma recovered. Assays were

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[1] *Abbreviations used in this paper:* DFP, diisopropylfluorophosphate; DIC, disseminated intravascular coagulation; GEE-^{14}C, glycine ethyl ester-^{14}C; SBTI, soybean trypsin inhibitor.

[2] New England Nuclear Corp., Boston, Mass., lot No. 506-160.

[3] "Varidase," Lederle Laboratories, Pearl River, N. Y.

[4] Sigma Chemical Co., St. Louis, Mo.

[5] Kimbel Products, Toledo, Ohio.

[6] International Equipment Co, Needham Heights, Mass.

TABLE I
Factor XIII Activation

Mix in this order:	
Factor XIII in 0.02 M Tris, 0.15 M NaCl, pH 7.4	0.3 ml
0.5 M cysteine, 0.1 M $CaCl_2$, 0.1 M Tris, 0.75 M NaCl, pH 7.4	0.05 ml
Thrombin (100 NIH U/ml in 0.01 M $CaCl_2$)	0.05 ml
Incubate 15 min at 37°C and then add:	
5×10^{-4} M diisopropylfluorophosphate	0.05 ml

usually done the same day as the samples were obtained; however, samples could be frozen and stored for at least 2 wk at −76°C without affecting results.

Factor XIII preparation, assay, and activation. Crude human factor XIII (fraction 5 in the method of Loewy and coworkers) was isolated as described by Loewy and coworkers (3) and its activity estimated according to their method at 800 U/mg protein. This crude fraction was used as enzyme for the incorporation of GEE-^{14}C.

Human fibrinogen,[7] deficient in factor XIII, was prepared by gradient elution chromatography of fibrinogen on diethylaminoethyl (DEAE) cellulose[8] according to the method of Finlayson and Mosesson (4). The earliest protein eluted contained fibrinogen free of factor XIII activity when tested by allowing the fibrinogen to clot for 1 hr in the presence of 2.5 NIH U/ml thrombin, 0.005 M cystine, 0.005 M calcium, 0.075 M KCL, and 0.5 mg/ml soybean trypsin inhibitor. This fibrinogen, once clotted, easily dissolved in 2% monochloracetic acid; the criterion for absent factor XIII activity.

Factor XIII was activated by incubating crude factor XIII in cysteine, calcium, and thrombin for 15 min at 37°C (see Table I). The thrombin activity was quenched at the end of the activation period by adding diisopropylfluorophosphate[9] (DFP), 5×10^{-4} M. The active factor XIII mixture was diluted in 0.02 M Tris, 0.15 M NaCl buffer, 0.05 M cysteine, 0.01 M $CaCl_2$, pH 7.4, to a concentration of 30 U/ml. This diluted activated factor XIII was used immediately in the reaction mixture for the labeling of circulating fibrin.

Labeling fibrin. An outline of the method for labeling the circulating fibrin in a plasma sample is presented in Table II. An 8% ethanol fraction of plasma was precipitated at 0°C for 1 hr from 0.8 ml of whole plasma with 0.2 ml 40% ethanol in tris buffer, pH 7.4. The ethanol precipitate was washed once with 2 ml 8% ethanol at 0°C and dissolved in 0.8 ml of tris buffer, pH 7.4; 1 mg/ml SBTI, 1 U/ml hirudin. After addition of GEE-^{14}C, active factor XIII, and 0.25 M Tris, 0.05 M NaCl buffer, pH 8.2, to the fibrinogen fraction, the mixture was incubated for 30 min at room temperature after which time 0.1 ml of 0.25 M EDTA was added to stop the action of factor XIII. The fibrinogen fraction was again precipitated from this mixture in 8% ethanol.

Recovery of clottable protein. The labeled 8% ethanol fraction was dissolved in 0.8 ml of 0.04 M Tris, 0.15 M NaCl pH 7.4 buffer containing 1 mg/ml SBTI, 0.2% EDTA. The fibrinogen in the fraction was clotted by adding 0.1 ml of bovine thrombin (500 NIH U/ml) in 0.04 M Tris, 0.15 M NaCl, 0.25 M EDTA, pH 7.4 to the fraction and the clottable protein harvested after 1 hr of incubation with the thrombin at 37°C by removing the clot on a nicrome wire loop and drying it on filter paper. After dissolving the fibrin at 37°C in 3 ml of 2% monochloracetic acid, the protein was reprecipitated with 3 ml of 14% trichloracetic acid, washed four times with 7% trichloracetic acid to remove any unbound radioactivity, and redissolved in 3 ml of 2% monochloracetic acid. The protein soluble in the 2% monochloracetic acid solution was quantitated by the method of Lowry (5) and then converted to milligrams of fibrinogen. The radioactive contents in 1 ml of the monochloracetic acid solution were measured after its dilution in 10 ml of liquid scintillation fluid (6) and results expressed as disintegrations per minute (dpm) per milligram of fibrinogen.

Standard coagulation studies. Plasma was recovered from blood collected in disposable syringes and mixed with 1/10 vol of 0.1 M buffered citrate anticoagulant (0.1 M sodium citrate, 0.1 M citric acid, pH 5.0) in polystyrene centrifuge tubes. The prothrombin time was performed using the method of Quick (7); activated partial thromboplastin times were measured by the method of Proctor and Rapaport (8) with activated platelet factor reagent.[10] Fibrinogen concentration was determined by the method of Ratnoff and Menzie (9) and by the rapid heat precipitation method of Foster and coworkers (10). Factors V and VIII were quantitated by measuring the ability of the test plasma to correct the coagulation defect in plasma deficient in these factors (11, 12). The thrombin time was done as described by von Kaulla and von Kaulla (13) and the split products of fibrinogen in serum were detected on immunodiffusion of serum against antihuman fibrinogen[11] as described by Merskey and coworkers (14) or by the use of the red cell hemaglutination inhibition assay (14). The ethanol gel test was done by the method of Breen and Tullis (15). Thrombin used in the experiments was bovine thrombin[12] purified by chromatography on IRC-50 as described by Rasmussen (16). The specific activity of the purified preparation was 13,000 NIH U/mg protein.

RESULTS

Normal values. The mean values of amounts of GEE-^{14}C incorporated into the 8% ethanol fraction of plasma

TABLE II
Reaction Mixture for Glycine Ethyl Ester (GEE) Labeling

Mix at room temperature:	
8% ethanol fraction	0.8 ml
0.4 M GEE-^{14}C, *20 μCi/0.1 ml*	0.1 ml
0.25 M Tris, *0.05 M NaCl pH 8.2*	0.1 ml
Activated fibrinase, *30 U/ml*	0.1 ml
Incubate 30 min at room temperature, then add:	
0.25 M Tris, 0.05 M NaCl, 0.25 M EDTA, pH 8.2	0.1 ml

[7] Merck, Sharp & Dohme, West Point, Pa.
[8] Eastman Kodak Co, Rochester, N. Y.
[9] Mann Research Labs Inc., New York.
[10] Baltimore Biological Laboratories, Baltimore, Md.
[11] Hyland Laboratories, Los Angeles, Calif.
[12] Parke, Davis & Company, Detroit, Mich.

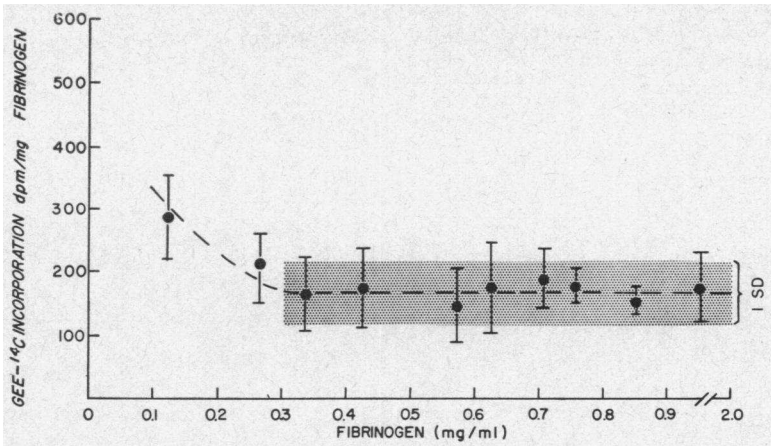

FIGURE 1 GEE-^{14}C incorporation into normal human plasma fibrinogen (range of mean [vertical bars] and 1 SD GEE-^{14}C incorporation determined from six or more plasma samples).

samples from normal persons was 167 ±47 dpm/mg fibrinogen when the final fibrinogen concentration in the 2% monochloracetic acid was 0.3 mg/ml or greater. Fig. 1 describes the incorporation of GEE-^{14}C by 64 plasma samples from normal persons at various fibrinogen concentrations. Low and high levels of fibrinogen were achieved by increasing or decreasing the quantity of plasma precipitated with ethanol rather than by diluting plasma before precipitation. Each point in Fig. 1 represents the mean values of six or more normal human plasma samples and the bars represent one standard deviation of the mean. When the final fibrinogen concentration was 0.3 mg/ml or greater, the mean amount of GEE-^{14}C incorporated was stable at 167 ±47 dpm/mg fibrinogen. If less than 0.3 mg/ml of fibrinogen was present, mean concentration of label was increased.

Thrombin-altered fibrinogen. The results of four in vitro studies in which soluble fibrin was formed by the addition of thrombin to plasma and changes in the incorporation of GEE-^{14}C determined are shown in Table III. To normal human plasma samples which already contained 0.2% EDTA and 1 mg/ml SBTI, purified bovine thrombin was added to give a final concentration of 0.03 NIH U/ml. The plasma thrombin mixtures were then incubated for 15 min at room temperature, and thrombin activity was then quenched by adding nine parts of plasma to one part hirudin (10 U/ml in 0.02 M Tris 0.15 M NaCl pH 7.4 buffer). Neither a visible clot nor fibrin strands appeared during incubation. In a similar experiment, hirudin (1 U/ml) was added to plasma before incubation with thrombin in order to block the enzymatic activity of thrombin on fibrinogen. After incubation of this mixture for 15 min at room temperature, there was no increase in the incorporation of GEE-^{14}C (Table III). When a plasma (No. 2, No. 4) not previously exposed to thrombin was mixed with a sample of that same plasma which had been exposed to thrombin, the increase in incorporation of GEE-^{14}C was proportional to the quantity of thrombin-treated plasma in the mixture (Table IV).

Urokinase-altered fibrinogen and fibrin. To exclude the possibility that fibrinogen or fibrin split products released by plasmin would significantly increase the incorporation of GEE-^{14}C by plasma fibrinogen, plasma-containing fibrinogen and fibrin split products was tested for its capacity to incorporate GEE-^{14}C. When normal human citrate plasma was incubated with urokinase (500 CTA U/ml final concentration) at 37°C for 18 hr, it was no longer clottable with thrombin. One-half of this plasma containing *fibrinogen* split products was then treated with thrombin (1 NIH U/ml final concentration) for 30 min. Both plasmas (thrombin-treated and untreated) containing *fibrinogen* split products were then added, nine parts of the plasma to one part SBTI, EDTA, hirudin

TABLE III
GEE-^{14}C Labeling of Thrombin-Treated Plasma

Plasma	No thrombin	Thrombin*
	GEE-^{14}C labeling	GEE-^{14}C labeling
	dpm/mg fibrinogen	
1	181	381
1 plus hirudin	199	194
2	165	474
3	153	328
4	124	482

* Thrombin was added to a final concentration of 0.03 NIH U/ml for 15 min before adding hirudin.

TABLE IV

Mixtures of Thrombin-Treated and Untreated Plasma

Per cent thrombin-treated plasma	GEE-^{14}C labeling			
	Plasma No. 2		Plasma No. 4	
	Observed	Expected	Observed	Expected
	dpm/mg fibrinogen			
0	165	—	124	—
25	272	243	165	213
50	319	322	242	300
75	442	398	336	390
100	474	—	482	—

anticoagulant. *Fibrin* split products were prepared by incubating plasma with a final concentration of 10 NIH U/ml of thrombin for 15 min at 37°C to form a plasma clot. Urokinase was then added to the clot (final concentration of urokinase 500 CTA U/ml), and after clot lysis nine parts of this plasma were added to one part of standard EDTA, SBTI, hirudin anticoagulant.

The immunoelectrophoretic mobility of fibrinogen antigenic determinants in these plasmas was determined by immunoelectrophoresis on 2% agar (Fig. 2). In this Figure wells *1* and *2* contained normal plasma. The third well contained the plasma treated with urokinase and the fourth well contained the plasma clot treated with urokinase. After electrophoresis, immunofixation was done with rabbit antihuman fibrinogen antibody [11] rendered monospecific by adsorption with human serum. Precipitin bands representing fibrinogen with altered mobility are seen in the urokinase-treated plasmas containing fibrinogen and fibrin split products. The untreated plasma samples (wells *1* and *2*) contain single fibrinogen precipitin bands of identical net charge.

Plasma containing *fibrinogen* split products, which had not been exposed to thrombin, was mixed with normal human plasma. The incorporation of GEE-^{14}C into the fibrinogen in these mixtures was no greater than that in normal plasma alone (Fig. 3). However, the mixture of normal plasma with 37.5% *fibrinogen* split products that had also been exposed to thrombin incorporated more GEE-^{14}C (108 dpm/mg fibrinogen above the normal plasma alone). The GEE-^{14}C incorporation into the fibrinogen of normal plasma with 37.5% *fibrin* split products increased only 57 dpm/mg fibrinogen above the normal plasma. Therefore, plasmin-digested fibrinogen increased the incorporation of GEE-^{14}C in normal plasma only after being hydrolyzed by thrombin, and plasmin-digested fibrin clot in normal plasma increased GEE-^{14}C incorporation only minimally and only at high concentrations.

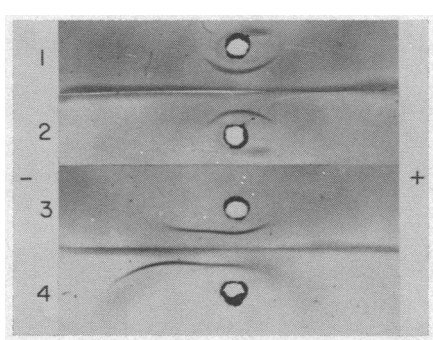

FIGURE 2 Agar electrophoresis with immunofixation against rabbit antihuman fibrinogen antibody. Well *1*, normal human plasma; well *2*, normal human plasma; well *3*, normal human plasma treated with 500 CTA U/ml urokinase; well *4*, normal human plasma clot treated with 500 CTA U/ml urokinase.

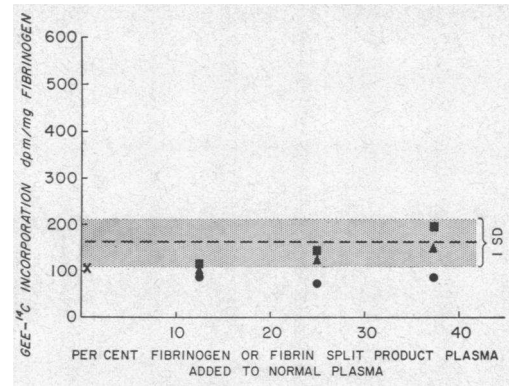

FIGURE 3 Mixtures of normal human plasma with plasma containing plasmin digestion products of fibrinogen and fibrin. ×, normal human plasma; ●, normal human plasma mixed with *fibrinogen* split products; ■, normal human plasma mixed with thrombin-treated *fibrinogen* split products; ▲, normal human plasma mixed with *fibrin* split products.

Per cent recovery. As the concentration of fibrinogen or fibrin split products added to normal plasma was increased, the amount of clottable protein which could be recovered from the mixtures decreased. When the concentration of fibrin split products was increased to 37.5, the per cent of fibrinogen recovered was only 18% of that expected. With concentrations greater than 37.5%, the 8% ethanol fraction was no longer clottable and recovery was therefore zero. However, provided the over-all recovery was 0.3 mg/ml of protein in the final 3 cc of 2% monochloracetic acid solution, variation in the recovery of fibrinogen from plasma was not related to changes in incorporation of GEE-^{14}C.

Dogs infused with thrombin. To evaluate the method for measuring circulating fibrin in vivo, two mongrel dogs were anesthetized with Penthrane [11] and the saphenous veins of the hind limb and fore limb were cannulated with No. 19 scalp vein sets. Blood samples were withdrawn from the saphenous vein of the fore limb at

varying time intervals before, during, and after continuous intravenous infusion of bovine thrombin into the hind limb. In one experiment factors V and VIII were measured on the samples using plasma from humans severely congenitally deficient in each factor as substrate for determining the ability of the dog plasma to shorten their clotting times (11, 12). Fibrinogen concentrations were determined by the method of Ratnoff and Menzie (9) and serum was tested for split products of fibrinogen by immunodiffusion (14). When given an infusion of 1 NIH U/kg per min of bovine thrombin for 20 min, an early increase in the ability of the plasma from two dogs to incorporate GEE-^{14}C into fibrinogen occurred and persisted for 1 hr after cessation of the infusion (Fig. 4). Concentrations of factors V, VIII, and fibrinogen in the plasma of dog No. 4 decreased and split products were present toward the end of the 20 min thrombin infusion (Table V).

Clinical observations. Two patients with bacteriologically proven meningococcemia were found to have circulating fibrin in association with other evidence suggesting disseminated intravascular coagulation (DIC). The first, patient A, was a 3$\frac{11}{12}$ yr old girl admitted to the hospital with a temperature of 104°F, blood pressure 86/50, and a generalized petechial rash. The prothrombin time of her plasma was prolonged (15 sec; normal, 12 sec), but the partial thromboplastin time was normal (37 sec), as was the fibrinogen concentration measured by the heat precipitation method (190 mg/100 ml), and the number of platelets appeared normal on the blood smear. The ethanol gel test was negative. Circulating fibrin, however, was present, as indicated by a GEE-^{14}C incorporation of 344 dpm/mg fibrinogen. 11 hr after the initiation of heparin therapy the level of GEE-^{14}C incorporation had returned to normal, 188 dpm/mg fibrinogen. Heparin was discontinued and the patient recovered.

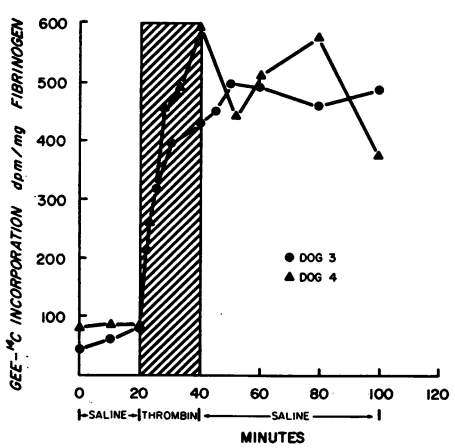

FIGURE 4 Dogs given intravenous thrombin, 1 U/kg per min for 20 min. Venous blood samples withdrawn at timed intervals before, during, and after infusion and GEE-^{14}C incorporation measured.

TABLE V
Infusion with Thrombin, 1 U/kg per min, Dog 4

Time	Infusion	Fibrinogen	Split products	Factor V	Factor VIII
min		*mg/100 ml*		%	%
0	saline	664	Negative	100	100
10	saline	702	Negative	100	88.5
20	saline	680	Negative	100	154
23	thrombin	578	Negative	80	—
28	thrombin	279	Negative	86	59
33	thrombin	455	Negative	86	46
40	thrombin	325	Positive	85	32
50	saline	222	Positive	60	48
70	saline	358	Positive	53	49
90	saline	433	Positive	56	53
110	saline	452	Positive	54	48

The second patient with meningococcemia, a 7 month old girl, was admitted to the hospital in a moribund condition with a temperautre of 102°C; blood pressure, 80/40; generalized petechiae and ecchymoses. She had received 250 U of heparin at another hospital before her admission to The Children's Hospital. Her fibrinogen GEE-^{14}C incorporation was increased, 585 dpm/mg fibrinogen, and the ethanol gel test was positive. Heparin treatment was continued and 12 hr after admission GEE-^{14}C incorporation had returned to normal, 129 dpm/mg fibrinogen. However, the patient had a cardiac arrest and died 16 hr after admission.

Two patients with Rocky Mountain spotted fever were studied. Plasma from both had prolonged prothrombin times (27.6 sec and 22.9 sec), prolonged partial thromboplastin times (70 sec and 67.2 sec), less than 50 mg/100 ml of fibrinogen by the heat precipitation method and low platelet counts (7,900 and 12,000/mm^3). Neither patient had a positive ethanol gel test. In plasma from both patients, increased amounts of GEE-^{14}C were incorporated (499.2 and 358.7 dpm/mg fibrinogen). Both patients recovered after therapy with antibiotics and heparin, and when GEE-^{14}C incorporation was repeated 24 hr and 1 wk after admission, the amounts were within the normal range (222 and 129 dpm/mg fibrinogen).

Three patients, E, F, and G, were studied in whom therapeutic abortions were induced by the intrauterine injection of hypertonic saline. Details of these patients have been presented in a separate publication (17). All three patients developed circulating fibrin after the intrauterine injection of saline as measured by GEE-^{14}C incorporation. One patient (E) with a retained dead fetus had increased GEE-^{14}C incorporation before the injection of hypertonic saline. All three patients had

TABLE VI
Saline-Induced Abortion

Patient	Time	GEE-^{14}C incorporation	Factor V	Factor VIII	Fibrinogen*	Ethanol gel test	Fibrin split products
		dpm/mg fibrinogen	%	%	*mg/ml*		
E	Preinjection	289	78	110	391	Negative	2.9 ug/ml‡
	2 hr postinjection	400	100	93	280	Negative	36.0 ug/ml‡
	6 hr postinjection	551	40	40	280	Negative	72.5 ug/ml‡
	35 hr postinjection	232	86	102	260	Negative	72.5 ug/ml‡
F	Preinjection	237	107	165	280	Negative	Negative§
	3 hr postinjection	469	96	96	190	Negative	Negative§
	9.5 hr postinjection	808	93	52	150	Negative	Negative§
	50 hr postinjection	233	234	182	280	Negative	Positive 1:8§
G	Preinjection	227	63	109	230	Negative	Not done
	2 hr postinjection	427	73	108	190	Negative	Not done
	6 hr postinjection	494	36	78	150	Negative	Not done
	74 hr postinjection	196	78	175	210	Negative	Not done

* Method of Ratnoff and Menzie.
‡ Mersky red cell hemaglutination inhibition assay.
§ Fi test, Hyland Laboratories fibrin split products assay.

changes in other coagulation factors consistent with the diagnosis of disseminated intravascular coagulation (Table VI).

Patients with other acute illnesses have also been studied, but did not have circulating fibrin as measured by GEE-^{14}C incorporation. Included in this group were two patients with prosthetic heart valves and thrombocytopenia, a patient with bacteriologically proven meningococcal meningitis without septicemia, and three patients with the hemolytic-uremic syndrome.

DISCUSSION

Diminished concentrations of clotting factors known to be consumed during clotting plus evidence of fibrinolytic activity may permit a presumptive diagnosis of intravascular clotting. Increased consumption of a clotting factor may be inapparent, however, when the rate of synthesis or degree of activation of a clotting factor exceeds its rate of consumption. The present study was designed to evaluate a method for direct measurement of an effect of thrombin by measuring that portion of fibrinogen in plasma which has been converted to fibrin but remained soluble (circulating fibrin). Lorand and Ong (1) originally demonstrated the enzymatic incorporation of GEE-^{14}C into fibrin. The method described in this manuscript is based upon theirs in which GEE-^{14}C is incorporated into fibrin by the action of factor XIII.

In the method described, EDTA, hirudin, and SBTI were added to the plasma and the 8% ethanol fraction before and during the enzymatic incorporation of GEE-^{14}C into fibrinogen to prevent any further proteolytic alterations of the fibrinogen once it had been removed from the patient. Since only the clottable portion of the 8% ethanol fraction of plasma was tested for incorporation of GEE-^{14}C, it is not known whether other nonclottable proteins in this fraction may also incorporate the label. Under the conditions described, some incorporation occurred in the clottable protein portion of the 8% ethanol fraction in normal plasma (167 ±47 dpm/mg fibrinogen), as if there may be circulating fibrin in normal plasma. However, these studies do not exclude the possible incorporation of some GEE-^{14}C by unaltered fibrinogen. When the amount of normal plasma was decreased so that the final yield of fibrinogen was less than 0.3 mg/ml (0.9 mg total), there was a progressive increase in the incorporation of the label. This finding suggests that increased incorporation of GEE-^{14}C in unaltered fibrinogen will occur at high enzyme to substrate concentrations. If the final yield of fibrinogen was 0.3 mg/ml or greater, no significant variation in labeling was found even though the over-all recovery of fibrinogen was only 18%. Though some incorporation may occur with unaltered fibrinogen, the presence of thrombin-altered fibrinogen (circulating fibrin) markedly increased the incorporation of GEE-^{14}C. This effect was clearly evident when thrombin was added to normal plasma in vitro (Tables III and IV) and in animal studies in which dogs were given continuous intravenous infusions of thrombin (Fig. 4).

The ethanol gel test of Breen and Tullis (15) proposed as a specific measure for the presence of circulating fibrin was apparently not reliable in that it was often

negative in the face of evidence that circulating fibrin was present as measured by GEE-^{14}C incorporation. Moreover, plasma from the two patients with Rocky Mountain spotted fever and all three women after induced abortion had alterations of other clotting factors, including GEE-^{14}C incorporation, consistent with the diagnosis of disseminated intravascular coagulation although the ethanol gel test was negative in all instances.

The possibility that plasmin-altered fibrinogen or fibrin might increase GEE-^{14}C incorporation was considered. Urokinase-induced split products of unclotted fibrinogen did not significantly increase the incorporation of the label when mixed with normal plasma unless these products were exposed to thrombin. Urokinase-induced split products from a fibrin clot when present at 37.5% concentration in normal plasma increased the labeling by only 57 dpm/mg fibrinogen. The appearance of split products in the serum of the dog infused with thrombin and in patients with DIC did not correlate with changes in GEE-^{14}C incorporation. Thus the method is apparently insensitive to plasmin alterations of fibrinogen and fibrin.

The potential usefulness of the method for measuring circulating fibrin was shown in the patients studied. The first patient with meningococcemia had circulating fibrin as measured by GEE-^{14}C. With heparin therapy, the GEE-^{14}C labeling of fibrinogen returned to normal, suggesting that the patient suffered an episode of disseminated intravascular coagulation which improved on therapy with heparin. Other changes in clotting factor concentrations suggestive of disseminated intravascular coagulation were not, however, present in this patient. The three patients in whom intrauterine injection of hypertonic saline was used for the induction of therapeutic abortion all had circulating fibrin as measured by GEE-^{14}C incorporation. The usual changes in coagulation factor concentrations associated with disseminated intravascular coagulation also occurred in these patients (Table VI). The diagnosis of disseminated intravascular coagulation could therefore have ultimately been made by conventional methods, although increased GEE-^{14}C incorporation preceded other changes. The method thus provides a sensitive tool for identifying circulating fibrin in DIC and should therefore aid in our understanding of this condition and its therapy.

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