Metabolism of Plasminogen in Healthy Subjects: Effect of Tranexamic Acid

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ABSTRACT The metabolism of human plasminogen labeled with radioactive iodine was studied in 12 healthy men. The labeled plasminogen had a high specific activity and the same elution on Sephadex G-100 as the plasminogen activity in plasma. Immunoelectrophoresis revealed a single precipitin line. Polyacrylamide gel electrophoresis revealed six main bands, all with plasminogen properties and radioactivity. The purified plasminogen behaved as a homogeneous protein in the turnover experiments. The plasma radioactivity data were adequately approximated by a sum of two exponential terms. The metabolism of plasminogen was therefore represented by a two-compartment mammillary model.

Results in the 12 normal subjects were as follows: plasma plasminogen concentration 20.8±1.9 mg/100 ml; intravascular plasminogen pool 0.66±0.14 g; intravascular fraction 0.59±0.06; fractional catabolic rate 0.55± 0.09 of the plasma pool per day; half-life of the plasma radioactivity 2.21±0.29 days. Circulating large-molecular-weight degradation products of labeled plasminogen could not be detected by Sephadex G-100 gel filtration.

The plasminogen turnover rate was normal in a patient with Behçet's syndrome and low circulating plasminogen activator activity. This finding supports the concept that under normal conditions the primary pathway of plasminogen catabolism is not via the formation of plasmin.

The in vivo effect of tranexamic acid, a potent inhibitor of plasminogen activation, on the turnover of labeled plasminogen was studied in five normal subjects. When 1 g was administered perorally t.i.d. to three of them, one showed an increased plasminogen turnover. A 2 g dose administered t.i.d. to the other two caused markedly increased catabolism in both. This increase may be attributable to a direct reversible effect of tranexamic acid on the plasminogen molecule.

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During this study Dr. Collen and Dr. Tytgat were recipients of a grant from the Belgian National Fund for Scientific Research.

Received for publication 9 August 1971 and in revised form 13 December 1971.

INTRODUCTION

The role of intravascular coagulation and fibrinolysis in the pathogenesis of certain acquired hemorrhagic diatheses is still debated. Valid metabolic tracers for both systems make it possible to study their interaction and relative importance under normal as well as pathological conditions.

Only fibrinogen, a substrate for both thrombin and plasmin, was formerly available for metabolic studies. However, fibrinogen turnover experiments cannot distinguish between intravascular coagulation and fibrino-(geno)lysis unless the influence of anticoagulation and fibrinolytic inhibition on the fibrinogen catabolism is evaluated. The metabolism of prothrombin, the zymogen eventually converted in the coagulation system, has recently been investigated in both normal and hypocoagulable states (1–3). The metabolism of plasminogen, the zymogen eventually converted in the fibrinolytic system, has not yet been studied. A highly purified and apparently biologically intact plasminogen preparation suitable for turnover studies is now available (4).

The present experiments were designed to study the kinetics involved in the metabolism and distribution of labeled plasminogen under physiological conditions in man.

METHODS

Preparation of labeled plasminogen. Three batches of human plasminogen were prepared according to the method of Wallén and Wiman (4) starting from fresh frozen plasma. Fraction DE-IIb was further purified according to the method of the same investigators[1] by gel filtration on

[1] Wallén and Wiman. To be published.

Sephadex G-150 in 0.06 M Tris-0.06 M NaCl buffer, pH 8.5. The plasminogen-rich fractions were pooled and transferred to a DEAE Sephadex column equilibrated with the same buffer. Elution was performed with a gradient produced by mixing chambers containing 0.06 M Tris-0.06 M NaCl, pH 8.5, and 0.07 M Tris-0.22 M NaCl, pH 7.5, to eliminate partially denatured plasminogen. The specific activity, as measured by caseinolytic digestion after urokinase (UK)[2] activation, was 23–25 CTA U[3]/mg protein (extinction coefficient $A_{280nm}^{1\%} = 16.1$ [4]). The spontaneous caseinolytic activity was less than 0.5% of the maximal activity after UK activation. The preparation was labeled with Na-^{131}I or with Na-^{125}I, according to McFarlane's iodine monochloride method (5). Fraction DE-IIb (4) was labeled and further purified, as described above. The labeling efficiency was 39.1, 39.4, and 59.0% with an average ratio of 0.40, 0.48, and 0.73 atoms of iodine per molecule of plasminogen (accepted mol wt 89,000 [6]). Free iodide was removed by passage through a 1.5 × 3 cm Amberlite IRA-401 column saturated with chloride. The remaining free iodide, determined as non-TCA (trichloroacetic acid) precipitable radioactivity, was 0.7, 1.4, and 2.0% of the protein-bound radioactivity. The specific activity after labeling was 20–26 CTA U/mg protein.

Biochemical evaluation of the labeled plasminogen preparations. Sephadex G-100 gel filtration of a trace amount of labeled plasminogen in 3 ml of normal human plasma revealed a single radioactivity peak corresponding with the plasma plasminogen peak. A single precipitin line was found on immunoelectrophoresis (7) before and after labeling. Radioautography revealed a concentration of label in the precipitin line (Fig. 1). On polyacrylamide gel electrophoresis at pH 8.3 (8), six main components were observed in the purified plasminogen preparations (Fig. 2). All bands contained radioactivity as shown by radioautography and enzymatic activity as shown by enzymography by the method of Heberlein and Barnhart (9). Six bands were also detected in plasminogen in plasma by starch gel electrophoresis (4). The presence of multiple molecular forms of human plasminogen in purified preparations has already been well demonstrated (10–13).

[2] *Abbreviations used in this paper*: EACA, epsilon-aminocaproic acid; EV, extravascular; IV, intravascular; UK, urokinase.

[3] In comparison with lot No. 8 standard plasmin prepared by the Michigan Department of Public Health, Lansing, Mich.

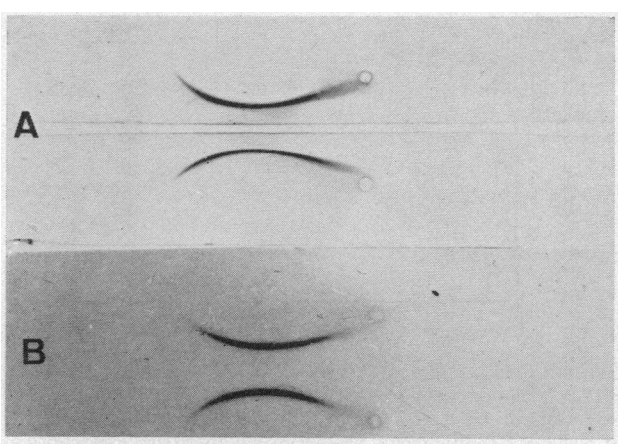

FIGURE 1 Immunoelectrophoresis of a mixture of labeled and unlabeled plasminogen in 1% agarose gel in veronal buffer, pH 8.6, according to Scheidegger's method (7). (*A*) Wells contain 3 μl plasminogen (8 mg/ml); slit: 50 μl rabbit antiserum raised against the purified plasminogen. (*B*) Radioautography of A.

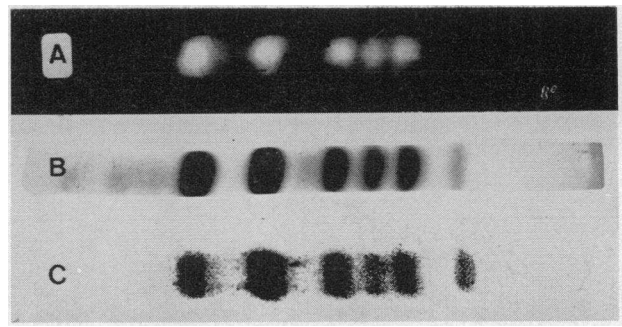

FIGURE 2 Polyacrylamide gel electrophoresis of 10 μl of a mixture of labeled and unlabeled plasminogen (8 mg/ml) in 0.4 M glycine-0.05 M Tris, pH 8.3, according to Davis's method. (*A*) Enzymography of the polyacrylamide gel according to the method of Heberlein and Barnhart (9). (*B*) Polyacrylamide gel stained with amidoblack. (*C*) Radioautogram of the polyacrylamide gel.

Metabolic studies. The 12 healthy male volunteers were given 500 mg of potassium iodide by mouth three times on the day before injection of the labeled plasminogen, which was diluted in 150 ml of 0.9% sodium chloride and infused over 30 min. This slow infusion was thought to minimize any in vivo activation of the fibrinolytic system in the event of a pyrogenic reaction. (Pyrogenicity tests carried out in rabbits were negative.) None of the study subjects developed side effects after the infusion.

Blood samples (20 ml) were drawn 5 min after the end of the infusion and at different intervals up to 12 days. The blood was collected in polystyrene tubes containing trisodium citrate (final concentration 0.315%), aprotinin[4] (final concentration 50 kallikrein inhibitor U/ml) and tranexamic acid[5] (final concentration 0.05 M) to prevent in vitro activation and digestion of plasminogen. In addition, 5-ml blood samples were taken on potassium oxalate (final concentration 0.25%) to determine components of the fibrinolytic system. From 12 to 21 blood samples were taken from each subject. Pooled urine samples were collected each 24 hr throughout the observation period. The 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 4 day continuous intravenous infusion of heparin on the plasminogen turnover was studied in one subject. The amount of heparin was adjusted to achieve at least a threefold increase in clotting time. The in vivo effect of tranexamic acid, a potent inhibitor of plasminogen activation (14), on the turnover of labeled plasminogen was studied in five normal men. Three of them received 1 g of the tranexamic acid t.i.d. for 4 days and two received 2 g t.i.d. for 2$\frac{3}{4}$ days. These doses were considered adequate to inhibit the fibrinolytic system in vivo (14).

[4] Trasylol, Bayer Farbewerken, Leverkusen, Germany.

[5] Cyklokapron, Kabi AB, Stockholm, Sweden.

TABLE I

Clinical Data on the 12 Healthy Subjects

Subject	Age	Weight	Plasma volume	Plasma plasminogen	
	yr	*kg*	*ml/kg*	*mg/100 ml*	*mg/kg*
D. C.	27	83	50.1	20.3	10.1
H. C.	27	78	39.3	19.4	7.6
G. G.	26	80	31.1	20.9	6.5
S. L.	28	58	50.2	21.2	10.7
L. V.	25	75	43.4	21.5	9.3
E. Q.	28	74	51.5	20.4	10.4
J. H.	42	58	54.5	20.6	11.2
C. L.	40	65	39.1	18.8	7.4
C. J.	51	72	52.3	23.6	12.4
C. G.	44	65	49.0	23.3	11.5
D. C.	52	54	49.0	16.8	7.8
D. M.	63	81	39.5	22.7	8.9
Mean	38	70	45.7	20.8	9.5
SD	12.8	10	7.1	1.9	1.9

2 ml of each of the following was pipetted into duplicate counting tubes: radioactive plasma, plasma supernate after protein precipitation with an equal volume of 10% trichloroacetic acid, and urine. The radioactivity was then measured in a well-type scintillation counter (Gamma/guard, Autowell Counting System)[6] with a sensitivity of approximately 625,000 cpm/μCi against a background of 30 cpm.

Analysis of tracer data. The tracer data were analyzed using a two-compartment mathematical model similar to that described for fibrinogen (15). (See Appendix.) This method enabled us to estimate the radioactivity distribution ratio between the extravascular (EV) and the intravascular (IV) pools, the fractional transcapillary transfer rate constant (k_{12}), and the fractional catabolic rate constant ($k_{10,p}$) (16). In the eight subjects from whom quantitative urine collections were obtained, the fractional catabolic rate constant ($k_{10,u}$) was also determined from the ratio of the daily urinary radioactivity to the corresponding mean plasma radioactivity (17), corrected for delay in urinary excretion of iodide (18).

Plasma radioactivity data versus time were fitted with a function (see legend Fig. 3)

$$x(t) = C_1e^{-a_1t} + C_2e^{-a_2t},$$

by graphic analysis on semilogarithmic paper and by nonlinear exponential approximation, by a damped Gauss-Newton procedure, using Späth's Fortran-IV program (19).

The plasma volume was obtained by dividing the total radioactivity injected by the amount in the first plasma sample. Because the radioactivity was diluted in 150 ml of 0.9% sodium chloride and infused over a 30 min period, a slight overestimation of the plasma volume was to be expected.

Laboratory procedures. The following procedures were performed: assays of plasminogen (20), antiplasmin (20), fibrinogen (21, 22), euglobulin clot lysis time (23), and euglobulin fibrinolytic activity on bovine fibrin plates (24). Plasminogen was also determined by Laurell's immunologic method (25), using a monospecific rabbit antiserum raised to purified plasminogen, in the subjects receiving tranexamic acid. Gel filtrations on Sephadex G-100 or G-200 were performed on a 2.5 × 45 cm column using 0.1 M NaCl-0.05 M Tris buffer, pH 9.0, with the addition of epsilon-aminocaproic acid (EACA) 0.1 M to prevent plasminogen activation on the column.

[6] Tracerlab, Mechelen, Belgium.

RESULTS

Table I summarizes the clinical and laboratory data for the normal subjects. The mean plasma plasminogen concentration was 20.8±1.9 mg/100 ml. A conversion factor of 24.0 CTA U/mg plasminogen, as deduced from our purified preparations, was used to convert caseinolytic activity to protein concentration. Since daily fluctuations of the plasma levels of plasminogen were negligible, the subjects were assumed to be in a steady state during the experimental period.

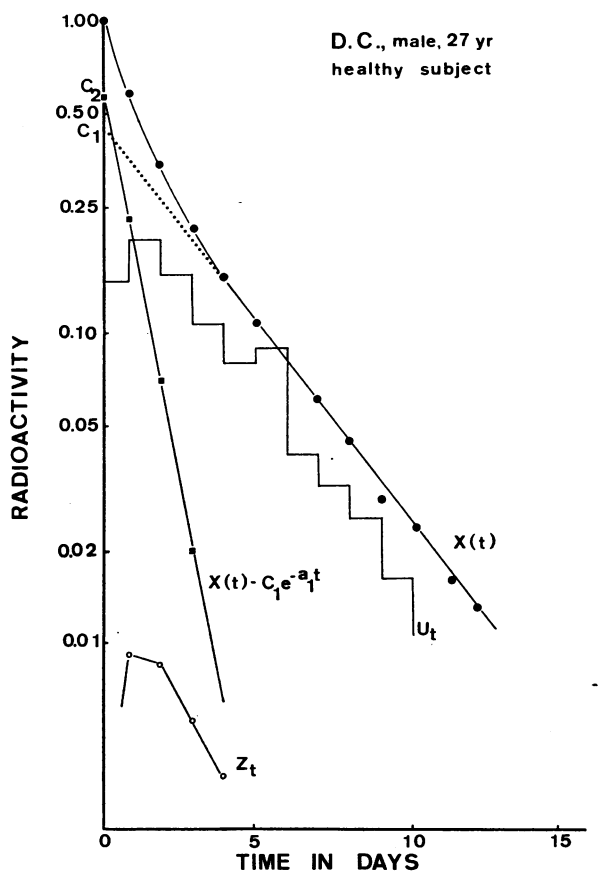

FIGURE 3 Plasminogen metabolism in a control subject. $x(t)$ = plasma radioactivity; u_t = fractional daily urinary excretion of label; z_t = non-TCA-precipitable radioactivity in plasma. Graphic curve peeling in a sum of two exponential terms. The straight linear terminal portion of the plasma radioactivity $x(t)$ 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 $x(t) - C_1e^{-a_1t}$ a new line is obtained $C_2e^{-a_2t}$ for which the slope $-a_2$ and intercept value C_2 are determined.

TABLE II

Plasminogen Tracer Data and Calculated Metabolic Parameters in Healthy Subjects

Subject	$x(t) = C_1e^{-a_1t} + C_2e^{-a_2t}$					Fractional catabolic rate constant		Absolute catabolic rate	Fractional transcapillary transfer rate constant	Activity distribution ratio	Intravascular fraction	Total body plasminogen
	C_1	a_1	C_2	a_2	$t_{\frac{1}{2}}$ for a_1	($k_{10,p}$)	($k_{10,u}$)		(k_{12})	(EV/IV)	(IV)	
								mg/kg per day				*mg/kg*
D. C.	0.44	0.29	0.56	1.16	2.40	0.50	0.56	5.2	0.27	0.74	0.57	17.7
H. C.	0.42	0.31	0.58	1.16	2.25	0.54	0.64	4.1	0.26	0.74	0.57	13.3
G. G.	0.36	0.29	0.64	1.26	2.40	0.57	—	3.8	0.34	0.97	0.51	12.8
S. L.	0.50	0.29	0.50	0.87	2.42	0.43	0.44	4.7	0.18	0.50	0.67	16.0
L. V.	0.34	0.32	0.66	1.39	2.20	0.64	0.41	6.0	0.38	1.04	0.49	18.9
E. Q.	0.37	0.26	0.53	0.75	2.65	0.39	0.36	4.1	0.15	0.50	0.67	15.7
J. H.	0.58	0.28	0.42	1.73	2.50	0.44	—	5.0	0.45	0.59	0.63	17.8
C. L.	0.43	0.35	0.56	0.99	2.00	0.55	0.54	4.2	0.15	0.59	0.63	11.7
C. J.	0.53	0.34	0.47	1.39	2.05	0.53	0.55	6.5	0.31	0.55	0.65	19.2
C. G.	0.44	0.40	0.56	1.54	1.75	0.68	0.64	7.8	0.36	0.71	0.58	19.7
D. C.	0.40	0.40	0.60	1.16	1.75	0.66	—	5.2	0.20	0.65	0.61	12.8
D. M.	0.39	0.33	0.61	1.54	2.10	0.63	—	5.6	0.44	0.92	0.52	16.8
Mean	0.44	0.32	0.56	1.25	2.21	0.55	0.52	5.2	0.29	0.71	0.59	16.0
SD	0.07	0.04	0.07	0.29	0.29	0.09	0.10	1.2	0.11	0.18	0.06	1.5

The turnover study shown in Fig. 3 is similar to those obtained for all the subjects. The plasma radioactivity $x(t)$ plotted against time could readily be approximated by a sum of two exponentials

$$x(t) = C_1e^{-a_1t} + C_2e^{-a_2t}$$

either by graphic curve peeling or by computer fitting. The parameters of $x(t)$, obtained by graphic analysis are shown in Table II. The results obtained by computer analysis were practically identical.

The amount of non-TCA-precipitable label never exceeded 2% of the total plasma radioactivity which it paralleled after the 1st day.

The distribution of radioactivity in serial plasma samples was studied by gel filtration on Sephadex G-100. The radioactivity was eluted in one main peak, corresponding 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 1st day when the amount was smaller. The latter finding indicated that the initial fall in plasma radioactivity was due mainly 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 II. The plasminogen half-life was 2.21±0.29 days; the fractional catabolic rate constant, as determined from the plasma disappearance ($k_{10,p}$), was 0.55±0.09; and as determined from the urinary excretion of radioactivity ($k_{10,u}$), it was 0.52±0.10. The absolute catabolic rate, obtained by multiplying the intravascular plasminogen pool by the fractional catabolic rate constant ($k_{10,p}$), was 0.36±0.09 g/day or 5.2±1.2 mg/kg per day. The extravascular-intravascular radioactivity distribution ratio (EV/IV) was 0.71±0.18, corresponding to an intravascular fraction of 0.59±0.06.

In a patient with Behçet's syndrome 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 was in the normal range ($t_{\frac{1}{2}} = 2.05$ days).

The metabolism of plasminogen was studied during and after continuous infusion of heparin in one normal subject. There was no change in the plasma plasminogen half-life ($t_{\frac{1}{2}} = 1.95$ days) after discontinuance of the anticoagulant.

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 of whom showed no statistically significant difference in the coefficients of the regression line through the plasma radioactivity data (26) obtained during both experimental periods, whereas the other subject showed a small but significant shortening of the plasminogen half-life (from 2.5 to 2.1 days)

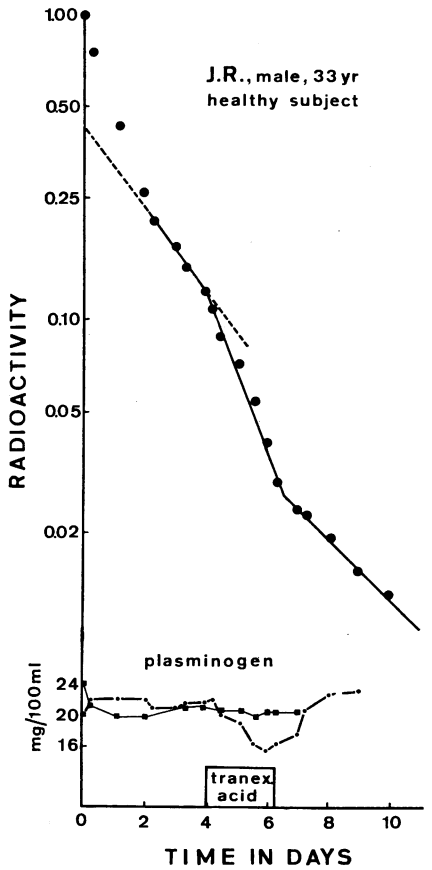

FIGURE 4 Plasminogen metabolism in a healthy subject before and during in vivo inhibition of the fibrinolytic system with 2 g tranexamic acid t.i.d. $x(t)$ = plasma radioactivity; ●—● = enzymatically determined plasminogen; ■—■ = immunoreactive plasminogen.

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. 4 shows this increase in one of them). 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 in 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 purity of the plasminogen preparation used in this study was attested by its high specific activity, low spontaneous proteolytic activity, and homogeneity on Sephadex gel filtration and on immunoelectrophoresis. The six main bands observed on polyacrylamide gel electrophoresis were also found in fresh plasma on starch gel electrophoresis (4). Radioautography revealed a concentration of the isotope in all bands. No changes in enzymatic or physicochemical properties could be detected after labeling. Biological integrity was further evidenced 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 metabolism of plasminogen was studied in 12 healthy normal men 23–63 yr of age. The mean plasma plasminogen concentration, measured by caseinolytic digestion after activation with UK, was 20.8±1.9 mg/100 ml. This value agrees well with the data of Rabiner, Goldfine, Hart, Summaria, and Robbins (27), who obtained 20.6±0.36 mg/100 mg using a radioimmunoassay. The plasma radioactivity data were analyzed by graphic curve peeling and nonlinear exponential approximation by a damped Gauss-Newton procedure. The two methods showed comparable values for the exponents and coefficients of the sum of two exponential terms describing the plasma radioactivity data. Metabolic parameters were calculated from these function parameters, using a two-compartment mammillary model (16). The following results were obtained, using the graphically determined function parameters: fractional catabolic rate 0.55±0.09 of the plasma pool per day; plasma radioactivity half-life 2.21±0.29 days; fractional transcapillary transfer rate 0.29±0.11 of the plasma pool per day; extravascular-intravascular radioactivity distribution ratio 0.71± 0.18. The catabolic rate of plasminogen is much faster than that of many other plasma proteins, but resembles that of prothrombin (2, 3).

In view of the controversy over a continuous physiological balance between fibrin formation and fibrinolysis (28) 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 his plasminogen metabolism was normal ($t_{\frac{1}{2}} = 2.05$ days), the primary pathway 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 (14). Two of them showed no change in plasminogen turnover, but the plasma radioactivity half-life was slightly though 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 the plasminogen catabolism, which subsided when the drug was discontinued. These findings suggested a direct but reversible effect of tranexamic acid on plasminogen metabolism. Gel filtration of purified plasminogen in the presence of EACA resulted in an apparent increase in molecular weight, indicating a swelling of the plasminogen molecule.[7] Alkjaersig (29) found that the sedimentation coefficient of plasminogen decreased in the presence of EACA; Abiko, Iwamoto, and Tomikawa (30) noted a similar effect with tranexamic acid. Conformational changes in the plasminogen molecule in the presence of EACA and tranexamic acid may possibly be responsible for the enhanced catabolism. Because tranexamic acid has a direct effect on the metabolism of plasminogen, it cannot be used to test the relative importance of the activation of plasminogen on its turnover rate. Rabiner et al. (27), using an immunologic 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.

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 in normal conditions the bulk of plasminogen is catabolized neither by primary fibrinolytic activation nor by consumption secondary to intravascular coagulation. Other studies using radiolabeled fibrinogen (35) and prothrombin (2) also indicated that under physiological conditions these clotting factors are not catabolized by consumption in the coagulation system.

APPENDIX

Mathematical model for the metabolism of labeled plasminogen under physiological conditions. Berman and Schoenfeld (31) demonstrated the possibility of completely solving the n compartment mammillary model for the metabolism of plasma proteins, given the initial conditions and the constants (C_k) and coefficients (a_k) describing the plasma radioactivity $x(t)$. The results of a metabolic study consist of serial measurements of radioactivity in plasma (x_1) at different time intervals (t_1) after administration of the isotope. 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 must be estimated.

[7] Wallén and Wiman. To be published.

Tracer experiments were simulated on a high-speed digital computer (*a*) to find out whether there is a practical criterion for determining the number of exponential terms in the data-generating function, and (*b*) to estimate the influence of data error on the accuracy of the calculated metabolic parameters. Data points were generated from sums of two or three exponential terms in which the coefficients and exponents were assigned values in accordance with our experimental findings for plasminogen: $x(t) = 0.45e^{-0.30t} + 0.55e^{-1.2t}$ and $x(t) = 0.45e^{-0.80t} + 0.30e^{-0.90t} + 0.25e^{-1.5t}$. Each simulated decay curve contained 15 data points ($t = 0.01$, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0). Normally distributed random errors of 2% and 5%, calculated according to Glass and de Garreta (32), were superimposed on these data. The 5% error range corresponds to our in vivo experimental data error, arrived at by comparing the sums of deviates between the data points and their fitted values.

Sums of exponential terms were fitted to these simulated tracer data, using Späth's Fortran-IV program in double precision (19), based on a least square approximation of the data by a damped Gauss-Newton method. Variability in estimating the main metabolic parameters was studied by analyzing for each data generating function 30 simulations differing only with respect to superimposed random error. The parameter values (C_k,a_k) of the generating function were also used as starting values in the computer program. When fitting data generated from a sum of three with a sum of two exponentials, the starting value of the second exponent in the fitting function was the mean of the second and third exponent in the generating function. The iterative procedure was interrupted after 50 steps if convergence was too slow or when the matrix of the normal equations became singular. The solution was not accepted when one of the fitted exponents or coefficients was negative or differed in order of magnitude from the starting values.

The results are summarized in Table III. The sum of the squares of differences between simulated and fitted data does not appear to permit determination of the number of exponential terms in the data-generating function. Data error influences its value to a much greater extent than systematic error introduced by underestimating the number of terms. The sum of squares of residuals therefore does not permit the estimation, in a single experimental situation, of the number of terms from which the data were generated.

When fitting data generated from a sum of two exponentials with a sum of two, the main model parameters are accurately estimated for 2 as well as for 5% data error.

When fitting data generated from a sum of three exponentials with a sum of three, convergence is obtained in only about one-half to two-thirds of the cases, whereas convergence is always obtained when approximating these data with a sum of two exponentials.

The comparative influence of systematic error and data error on the estimation of the main metabolic parameters reveals that very accurate reproducible results can be obtained for the fractional catabolic rate constant (k_{10}) under the simulated experimental conditions. Estimations of the fractional transcapillary transfer rate constant (k_{12}) and the radioactivity distribution ratio (EV/IV) are considerably less accurate. Overall estimation of the main metabolic parameters is most accurate when approximating the data with a sum of two exponential terms, regardless of the number of terms in the data-generating function. Although a two-compartment mammillary model for the metabolism

TABLE III

Analysis of Simulated Tracer Data

A. Data-generating function: $x(t) = 0.45e^{-0.30t} + 0.55e^{-1.2t}$

	Sum of squares of deviates × 10^5		$\frac{EV}{IV}$		k_{12}		k_{10}		Number of convergences
Theoretical value			0.702		0.284		0.5106		30
Fitting Function: $x(t) = C_1e^{-a_1t} + C_2e^{-a_2t}$									
Data error, %	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
2	18.0	13.0	0.711	0.041	0.280	0.016	0.5095	0.005	30
5	113.2	82.6	0.731	0.110	0.278	0.039	0.5093	0.011	30

B. Data-generating function: $x(t) = 0.45e^{-0.30t} + 0.30e^{-0.90t} + 0.25e^{-1.5t}$

	Sum of squares of deviates × 10^5		$\frac{EV}{IV}$		$k_{12} + k_{13}$		k_{10}		Number of convergences
Theoretical value			0.6667		0.280		0.5000		30
Fitting function: $x(t) = C_1e^{-a_1t} + C_2e^{-a_2t} + C_3e^{-a_3t}$									
Data error, %	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
2	11.1	10.7	0.759	0.178	0.296	0.035	0.4994	0.003	18
5	70.5	71.4	0.908	0.635	0.345	0.089	0.4973	0.008	14
Fitting function: $x(t) = C_1e^{-a_1t} + C_2e^{-a_2t}$									
Data error, %	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
0	0.5	—	0.600	—	0.269	—	0.5013	—	—
2	16.7	9.5	0.604	0.035	0.273	0.023	0.5018	0.006	30
5	100.8	57.9	0.619	0.090	0.285	0.068	0.5037	0.015	30

and distribution of plasminogen constitutes an oversimplification of the physiological system, analysis of the simulated tracer data appears to indicate that it is the best resolution available of the metabolic model, in view of the magnitude of the error inherent in biological tracer experiments.

Closely agreeing estimations of the fractional catabolic rate constant (k_{10}), as determined by the disappearance of plasma radioactivity ($k_{10,p}$) and the appearance of label in the urine ($k_{10,u}$) corrected for the delay in iodide excretion (Table II), suggests the kidneys as virtually the sole excretion sites, and no significant accumulation of isotope in the breakdown sites. The presence in the body of radioactive non-TCA-precipitable breakdown products, consisting mainly of radioactive iodide and iodothyrosine (33) and distributed in about 8 times the plasma volume (34), calls for the introduction of an additional pool in the metabolic model. The total fractional catabolic rate constant (k_{10}) is related to basic protein turnover, occurring with a rate constant k_{13} and, at least theoretically, to plasminogen consumption by in vivo activation of the fibrinolytic system (k_{15}) and in connection with intravascular coagulation (k_{16}). Our finding that the bulk of plasminogen is not catabolized by either of these systems in healthy men suggests that under normal conditions $k_{10} = k_{13}$ as k_{15} and k_{16} approach zero. The direct effect of tranexamic acid on plasminogen metabolism is probably related to the increased catabolism of a molecule with reversibly altered physicochemical characteristics and should therefore be considered as an exogenous increase of k_{13}. Fig. 5 is the simplest flow diagram of labeled plasminogen in the body, which takes these findings into account. The

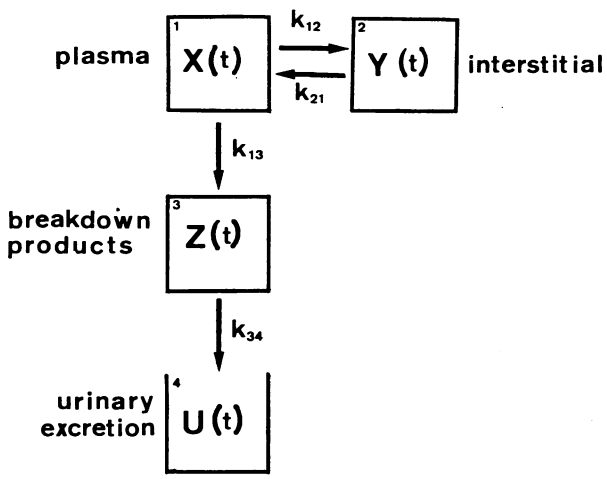

FIGURE 5 Compartment mathematical model for the metabolism of labeled plasminogen in physiological conditions. When radioactive plasminogen is injected in plasma rapid mixing occurs. The total plasma radioactivity at time t is represented by $x(t)$. Radioactivity passes into the interstitium at a rate of $k_{12}x(t)$ per day and is catabolized at a rate of $k_{13}x(t)$ per day. Interstitial radioactivity returns to the circulation at a rate of $k_{21}y(t)$ 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 $u(t)$.

description requires four differential equations:

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

$$\text{with } x(0) = 1 \text{ and } x(\infty) = 0,$$

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

$$y(0) = 0, \qquad y(\infty) = 0,$$

$$(3)\quad \frac{dz}{dt} = k_{13}x(t) - k_{34}z(t),$$

$$z(0) = 0, \qquad z(\infty) = 0,$$

$$(4)\quad \frac{du}{dt} = k_{34}z(t),$$

$$u(0) = 0, \qquad u(\infty) = 1,$$

with

$$k = k_{12} + k_{13} \text{ and } k_{10} = k_{13}.$$

The unvarying plasma plasminogen concentration under physiological conditions implies that the system is in steady state, i.e., that the fractional transfer rates are constant. The metabolic parameters can be calculated from the plasma radioactivity disappearance curve

$$x(t) = C_1e^{-a_1t} + C_2e^{-a_2t},$$

by the following formulas (16, 31):

$$k_{13} = \frac{a_1a_2(C_1 + C_2)}{a_1C_2 + a_2C_1},$$

$$k_{12} = \frac{C_1C_2(a_2 - a_1)^2}{(C_1 + C_2)(C_1a_2 + C_2a_1)},$$

$$k_{21} = \frac{a_1C_2 + a_2C_1}{C_1 + C_2},$$

$$\frac{EV}{IV} = \frac{C_2(a_2 - a_1)}{a_1C_2 + a_2C_1}.$$

ACKNOWLEDGMENTS

We wish to thank the following for their valuable help: Mr. F. DeCock for technical assistance, Dr. M. DeRoo for extending the hospitality of the Department of Nuclear Medicine, Dr. R. Piessens for cooperation in computer analysis, and Professor E. Meulepas for statistical advice, and Mrs. Elma T. Wadsworth for editorial assistance.

This work was supported by the Belgian Foundation for Medical Scientific Research (Grant No. 1216), and by the Swedish Medical Research Council (Grant No. B70-13X-635-06).

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