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Research Article

In vivo plasminogen responses to various stimuli were studied. Plasminogen-^{125}I was prepared and used first for metabolic studies of plasminogen in control dogs. The average results were: the plasma plasminogen, 29.3±4.1 (SD) mg/kg; the interstitial plasminogen, 8.79±4.47 (SD) mg/kg; the half-life of plasma plasminogen-^{125}I, 2.81±0.24 (SD) days; the fractional direct catabolic rate of plasminogen (j_3), 0.295 day^{-1}; and the catabolic (synthetic) rate of plasminogen, 8.61±1.35 (SD) mg/kg per day. Studies were then made of the plasminogen-^{125}I responses in dogs to a single injection of urokinase (*A*) and typhoid vaccine (*B*), and to vascular injury (*C*), which was produced by the damage of venous endothelium by a phenol injection. Effects of heparin were also studied in dogs given the phenol injection (*D*). Disc electrophoretic analysis of plasma showed generation of plasmin-^{125}I in all except the control experiments. The duration of plasmin-^{125}I generation was about 6 hr in *A*, 6 hr in *B*, and at least 5 days in *C*. Heparinization (*D*) shortened the duration of generation to about 6 hr. For further quantitative analysis of the tracer data, a model for coexistent plasminogen-^{125}I and plasmin-^{125}I was proposed and validated, from which some new analytical methods were derived. Using these methods, the average fractional rate of plasmin-^{125}I generation from plasminogen-^{125}I (j_4) was 0.41 day^{-1} in *A*, 0.30 day^{-1} in *B*, 0.324 day^{-1} […]

Plasminogen-^{125}I Responses in Dogs to a Single Injection of Urokinase and Typhoid Vaccine and to Vascular Injury

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ABSTRACT In vivo plasminogen responses to various stimuli were studied. Plasminogen-^{125}I was prepared and used first for metabolic studies of plasminogen in control dogs. The average results were: the plasma plasminogen, 29.3±4.1 (SD) mg/kg; the interstitial plasminogen, 8.79±4.47 (SD) mg/kg; the half-life of plasma plasminogen-^{125}I, 2.81±0.24 (SD) days; the fractional direct catabolic rate of plasminogen (j_3), 0.295 day^{-1}; and the catabolic (synthetic) rate of plasminogen, 8.61±1.35 (SD) mg/kg per day. Studies were then made of the plasminogen-^{125}I responses in dogs to a single injection of urokinase (*A*) and typhoid vaccine (*B*), and to vascular injury (*C*), which was produced by the damage of venous endothelium by a phenol injection. Effects of heparin were also studied in dogs given the phenol injection (*D*). Disc electrophoretic analysis of plasma showed generation of plasmin-^{125}I in all except the control experiments. The duration of plasmin-^{125}I generation was about 6 hr in *A*, 6 hr in *B*, and at least 5 days in *C*. Heparinization (*D*) shortened the duration of generation to about 6 hr. For further quantitative analysis of the tracer data, a model for coexistent plasminogen-^{125}I and plasmin-^{125}I was proposed and validated, from which some new analytical methods were derived. Using these methods, the average fractional rate of plasmin-^{125}I generation from plasminogen-^{125}I (j_4) was 0.41 day^{-1} in *A*, 0.30 day^{-1} in *B*, 0.324 day^{-1} in *C*, and 0.382 day^{-1} in *D*. Further mathematical consideration showed that j_3 was zero at least in *C* during plasmin generation. Plasminogen synthesis was unchanged in all experiments. The average fractional breakdown rate of plasmin-^{125}I (j_5) in *A*, *B*, *C*, and *D* was 1.19, 1.13, 1.35, and 1.11 day^{-1}, respectively, and were closely similar. These results indicate that under normal conditions a major portion of plasminogen is directly catabolized without the formation of plasmin, but that significant amounts of plasmin were generated under the conditions described, that the normal process of direct breakdown of plasminogen is abolished during plasmin generation at least in *C*, and that the potential value of j_5 determination should be further explored.

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INTRODUCTION

In vivo responses of plasminogen to various stimuli are very little understood despite their importance for further understanding of the mechanisms of thrombolysis. Studies were therefore made of the metabolism and distribution of plasminogen first in healthy dogs and then of the responses of plasminogen in dogs to a single injection of urokinase or typhoid vaccine and to vascular injury. In these studies, plasminogen-^{125}I was used as a tracer.

METHODS

Preparation of canine plasminogen. It was first necessary to prepare sufficiently pure and undenatured canine plasminogen. A method was devised and is described below: 2 liters of fresh citrated blood was obtained from dogs starved for 24 hr and spun at 5500 *g* for 30 min to remove the cellular components. To about 1 liter of plasma was added L-lysine to a concentration of 0.005 M. The plasma was then mixed with 100 ml of 4% $CaCl_2$ at 37°C by the use of a magnetic mixer and was allowed to clot. The fibrin clot was then squeezed dry in a piece of cotton gauze and was discarded. The serum was then diluted to 8 liters with cold distilled H_2O, and the pH was adjusted to 5.8 with 5% acetic acid. The mixture was left to stand at 3°C for 30 min while being gently stirred, and then it was centrifuged at 1500 *g* for 15 min, discarding the supernate. The precipitate was then homogenized in 100 ml of 0.01 M phosphate buffer (ph 8.0) at 18,000 rpm for 5 min at 3°C, using a high speed homogenizer, and was centrifuged at 1500 *g* at 3°C for 5 min. The supernate was kept, and the precipitate was rehomogenized as described above and centrifuged. The precipitate was discarded and both supernates were combined.

The supernates were then saturated 30% with $(NH_4)_2SO_4$ at 3°C with a gentle mixing for 5 min and centrifuged at 3000 *g* at 3°C for 10 min, discarding the supernate. The precipitate was then dissolved in 100 ml of 0.01 M phosphate buffer (ph 8.0), and was again saturated 30% with $(NH_4)_2SO_4$ and

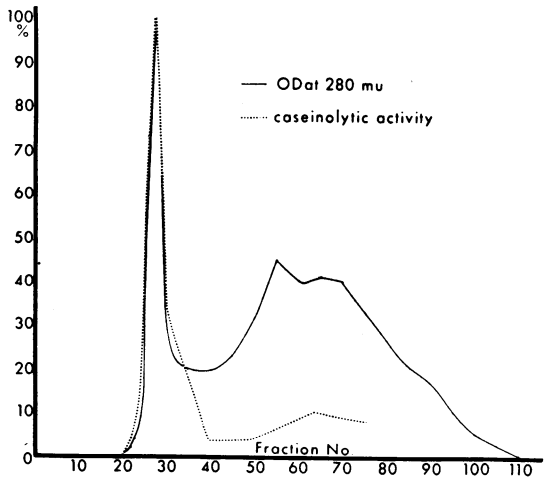

FIGURE 1 DEAE-cellulose chromatography of crude canine plasminogen. Gradient-elution method with 0.01 M phosphate buffer (pH 8.0) in 1 liter volume of mixing chamber and 1.5 liters of 0.3 M NaH_2PO_4 (pH 4.5) in the reservoir was used. The size of the column was 2.5 × 70 cm and the fraction volume, 10 ml. The flow rate was about 150 ml/hr. The fractions 20 to 35 were collected and further processed.

centrifuged as described above. The supernate was discarded and the precipitate was dissolved in 20 ml of 0.01 M phosphate buffer (ph 8.0). The crude plasminogen preparation was then dialyzed at 3°C against 8 liters of 0.01 M phosphate buffer (ph 8.0) for 4 hr. The dialysate was spun at 3°C for 5 min at 3000 *g* and the insoluble material was discarded. The supernate was then applied onto DEAE-cellulose column, 2.5 × 70 cm in size, equilibrated with 0.01 M phosphate buffer (pH 8.0). The gradient elution method with 1 liter of 0.01 M phosphate buffer (pH 8.0) in the mixing chamber and 1.5 liters of 0.3 M NaH_2PO_4 (pH 4.5) in the reservoir was used, and the volume of the mixing chamber was kept constant at 1 liter. The flow rate was about 150 ml/hr and the volume of each fraction, 10 ml. The chromatogram is shown in Fig. 1. The fractions 20–35 (Fig. 1) were collected and saturated to 30% with $(NH_4)_2SO_4$ at 3°C with gentle mixing for 5 min, and then centrifuged at 3000 *g* at 3°C for 10 min, the supernate being discarded. The precipitate was dissolved in 10 ml distilled H_2O adjusted to pH 7.0 with 1.0 N NaOH and dialyzed overnight against 8 liters of pH 7.0 H_2O at 3°C.

The dialysate was centrifuged at 3000 *g* at 3°C for 10 min to remove all insoluble material. The pH of the supernate was then adjusted to 5.2 at 3°C with 1% acetic acid, and after 10 min was centrifuged at 3000 *g* at 3°C for 5 min. The supernate was discarded and the precipitate was dissolved in appropriate volumes of pH 7.0 H_2O, and the pH was adjusted to 7.0 with 0.01 N NaOH. 5-mg portions were kept frozen at −93°C. The entire procedure takes less than 24 hr and 100–200 mg plasminogen can usually be recovered.

Physicochemical analysis of prepared plasminogens. Four different plasminogen preparations were dialyzed in Nojax casing (1 cm in diameter) against H_2O for 6 hr and lyophilized for 20 hr by a VirTis lyophilizer (VirTis Co., Inc., Gardiner, N. Y.). After further drying over P_2O_5 for 5 days, the weighed amounts of the dry plasminogens were dissolved in pH 7.0 H_2O, and the extinction coefficients were determined in a Beckman DU spectrophotometer (Beckman Instruments, Inc., Palo Alto, Calif.) at 280 mμ using 1 cm quartz cuvette. The coefficients were 14.5, 13.5, 13.9, 13.2, and averaged 13.8±0.56 (SD) for 1 g/100 ml of plasminogen solution. The specific activities of these four preparations determined by the caseinolytic assay method of Johnson, Kline, and Alkjaersig (1) were 8.5, 9.0, 8.3, 8.6, and averaged 8.6±0.56 (SD) CTA U/mg plasminogen. These preparations were then analyzed with respect to their purity by the disc electrophoresis (2) and cellulose acetate electrophoresis. The results are shown in Figs. 2*a* and 2*d*. Only a single component was demonstrated by the two analytical methods. In view of the several publications in which multiple components in pure plasminogen preparations are claimed to exist (3–5), this point was further investigated. The four plasminogen preparations were dialyzed at 3°C in Nojax casing (1 cm in diameter) against pH 8.0, 0.01 M phosphate buffer for 3 days and for 7 days, and each preparation was analyzed by the disc electrophoresis (2). The results are shown in Figs. 2*b* and 2*c*. The preparation dialyzed for 3 days showed several bands and that dialyzed for 7 days formed more bands. Thus, the multiplicity of pure plasminogen (3–5) seems to be a result of denaturation, buffer-plasminogen complex formation, or plasminogen-plasminogen complex formation of various degrees as discussed by Alkjaersig (4), which could occur during time-consuming preparative processes. A prepared plasminogen was then analyzed on Sephadex G-100 column (Pharmacia, Uppsala, Sweden) to obtain an estimate of its molecular size using the method of Andrews (6). For standards, nonenzymic protein molecular weight markers (division of Becton-Dickinson & Co., Rutherford, N. J.) were used. The results showed that the molecular weight of canine plasminogen was about 130,000.

The test of plasminogen-^{125}I. The purified plasminogens were then labeled with ^{125}I or ^{131}I in a ratio of 0.25 atoms iodine/molecule of plasminogen by the iodine monochloride method of McFarlane (7). Free ^{125}I in a preparation was removed by a passage through a Sephadex G-100 column (Pharmacia, Uppsala, Sweden) equilibrated with pH 8.0, 0.01 M phosphate buffer. The size of the column was 25 × 0.9 cm with a void volume of 5.5 ml. The flow rate was about 10 ml/hr. After a passage through the column, the free ^{125}I in a preparation was usually less than 1% of the total radioactivity. The specific activity of the plasminogens-^{125}I ranged from 10 to 30 μCi/mg of plasminogen. The radio-

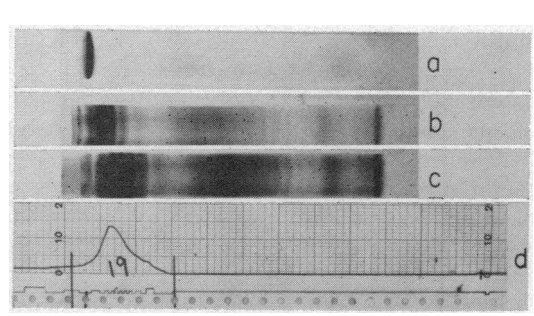

FIGURE 2 Electrophoretic analyses of purified canine plasminogens. Fig. 2*a* is the electropherogram of freshly purified canine plasminogen by the disc electrophoresis at 2 ma/gel for 3 hr. About 0.1 mg of plasminogen were applied. Fig. 2*b* and 2*c* are the electropherogram of plasminogen dialyzed for 3 days and 7 days, respectively, in 0.01 M phosphate buffer (pH 8.0). The electrophoretic conditions were the same as in Fig. 2*a*. It is seen that plasminogen which consisted of only a single component (*a*) formed multiple components after the dialysis. Fig. 2*d* is the cellulose acetate electrophoresis of fresh plasminogen at a constant voltage of 250 for 20 min. Plasminogen migrated as a β-globulin.

activity was determined in a well scintillation counter of Nuclear-Chicago (Nuclear-Chicago Corp., Des Plaines, Ill., model 8725) with the efficiency of about 34%. The plasminogen-^{125}I was then analyzed by the caseinolytic method (1) and compared with unlabeled plasminogens. It was found that the iodination of plasminogen as described above did not appreciably alter the plasmin-generating capacity of plasminogen. Next, unlabeled and labeled plasminogens were mixed and were analyzed by the disc electrophoresis (2). The gels were cut into slices of 2 mm thickness and each was counted for radioactivity to determine the distribution of plasminogen-I^{125}. For the determination of unlabeled plasminogen distribution, three to five slices of gel were analyzed by the caseinolytic method (1). It was found that the distribution of the two were very closely similar (Fig. 3*a*). Then, another mixture of unlabeled and labeled plasminogens was exposed to human urokinase (Calbiochem, San Diego, Calif.) as described in the method of Johnson, Kline, and Alkjaesig (1) and was incubated at 37.5°C for 5 min. The mixture was then immediately analyzed by the disc electrophoresis (2). The gels were then cut into slices of 2 mm thickness and analyzed for radioactivity, plasminogen, and plasmin locations as described above. The results are shown in Fig. 3*b*. It shows that both labeled and unlabeled plasminogen peaks are at gel slice 2, and both labeled and unlabeled plasmin peaks at gel slice 28. Thus, both labeled and unlabeled plasminogens behaved similarly by the disc electrophoresis (2) and there was no appreciable difference in their plasmin-generating capacities. This study also showed that plasmin can readily be separated from plasminogen by the disc electrophoresis. To make certain that the second peak (Fig. 3*b*) is of plasmin since the

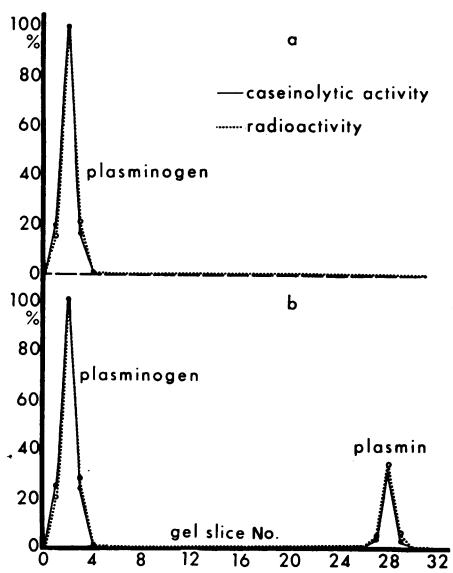

FIGURE 3 Disc electrophoresis of labeled and unlabeled plasminogens before and after activation with human urokinase. Approximately 1 mg of unlabeled plasminogen and 0.01 mg of plasminogen-^{125}I were mixed and electrophoresed before (*a*) and after activation (*b*) with urokinase. The electrophoretic conditions were the same as in Fig. 2*a*. The gels were cut into 2-mm slices and each was counted for radioactivity, and for caseinolytic activity three to five slices of gels were pooled and analyzed. It is seen that labeled and unlabeled plasminogens behaved very similarly and also that plasminogen and plasmin can readily be separated by the electrophoresis.

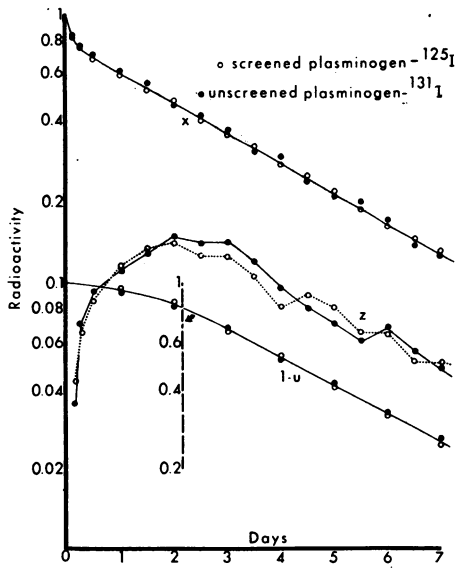

FIGURE 4 Comparative behavior of "screened" plasminogen-^{125}I and unscreened plasminogen-^{131}I in dog 1. *x* is the curve of plasma ^{131}I-labeled plasminogen, *z* is the curve of free ^{125}I or ^{131}I in the iodide space, and 1-*u* is the curve of radioactivity remaining in the body. It shows that both preparations behaved very similarly.

caseinolytic activity is not absolutely specific to plasmin, human plasmin obtained through the courtesy of Dr. Alan J. Johnson was analyzed by the disc electrophoresis. It migrated and appeared on gel slice 28. Thus, there seems no doubt that the second peak (Fig. 3*b*) is of plasmin. To estimate the reproducibility of this method, a preparation of plasminogen-^{125}I was exposed to human urokinase as described above, and the mixture of plasminogen-^{125}I and plasmin-^{125}I was analyzed 12 times by the disc electrophoresis, and the ratio of plasmin-^{125}I to plasminogen-^{125}I + plasmin-^{125}I was obtained (Fig. 3*b*). Calculation showed that the 2 coefficient of variation was 8%. Thus, this method has a reasonable degree of reproducibility, and was repeatedly used for detection and quantification of plasmin-^{125}I in later studies. For the analysis of plasma after plasminogen-I^{125} injection in later studies, volumes of plasma up to 0.4 ml were used depending on the amount of radioactivity present during the study. In these analyses, usually 100% of the radioactivity applied to the gel entered the gel and essentially all of the radioactivity was accounted for as plasminogen-^{125}I and plasmin-^{125}I. Another test was applied to the plasminogen-^{125}I. A preparation of plasminogen-^{125}I was screened in a puppy for 12 hr (8, 9) and about 50 ml of plasma containing "screened" plasminogen-^{125}I was harvested. Exactly 3 ml of the plasma was then injected into a recipient dog simultaneously with unscreened plasminogen-^{131}I and their behavior was compared. No appreciable difference was observed with respect to the curves of plasma plasminogen radioactivity (*x*),[1] the free radioactive iodine activity (*z*), and the radioactivity remaining in the body (1-*u*) between the "screened" and "unscreened" labeled plasminogens (Fig. 4). *x*, *z*, and 1-*u* were obtained as described

[1] *Abbreviations used in this paper:* j_1, j_2, j_3, fractional rate constants; $j_1\bar{x}$, $j_2\bar{y}$, $j_3\bar{x}$, plasminogen fluxes; 1-*u*, radioactivity remaining in body; *x*, plasminogen radioactivity; *z*, free radioactive iodine activity; *u*, urinary excretion of radioactivity.

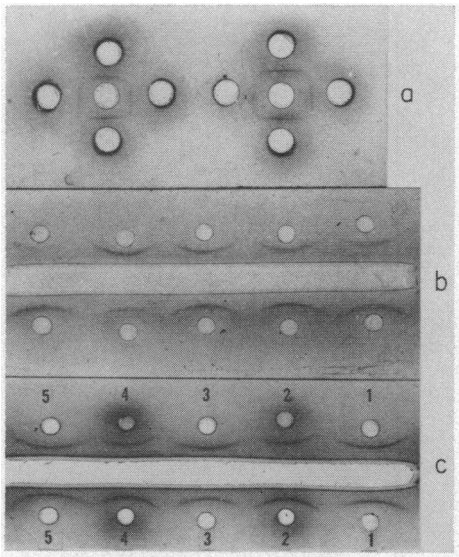

FIGURE 5 Double diffusion analyses of normal canine plasmas and purified canine plasminogens. Fig. 5*a* shows that the anticanine plasminogen formed a single precipitin arc against various normal plasmas, Fig. 5*b* shows that the antiplasminogen formed a single precipitin arc against purified plasminogen, and Fig. 5*c* also shows only a single precipitin arc against purified plasminogen (1, 3, 5) and normal plasmas (2, 4). The center wells in *a*, *b*, and *c* contained antiplasminogen.

later in details. Thus, it appears that the prepared plasminogen-^{125}I is satisfactory for metabolic studies of plasminogen in dogs.

The radial immunodiffusion method for the measurement of plasma plasminogen concentration. Anticanine plasminogen was produced in rabbits by three intramuscular injections of 100 μg of purified plasminogen as described elsewhere (9, 10), and was tested against purified canine plasminogen and normal canine plasmas by the usual double diffusion method (9). The results are shown in Fig. 5. It shows that the antiplasminogen formed a single precipitin line on the agarose plate against purified canine plasminogen and normal canine plasmas. However, this was not always true; occasionally two or three precipitin lines against the purified plasminogen and normal canine plasmas were found. This probably indicates that our canine plasminogen preparations were not absolutely pure, although there is no doubt that they were highly pure preparations (Fig. 2). In the readial immunodiffusion method for the measurement of plasma plasminogen concentration, only the antiplasminogen which produced a single precipitin line against purified plasminogen and canine plasmas was used. The details of the method are described elsewhere (9). The plasminogen standards were prepared in 1 g/100 ml human albumin solution (Albuspan, Parke, Davis & Co., Detroit, Mich.) containing ϵ-aminocaproic acid as a preservative in a concentration of 0.15 mg/ml. The standards were kept in a freezer at −93°C.

Plasminogen metabolism and distribution in healthy dogs under control conditions. Eight healthy dogs were used. They were fed on regular dry dog food mixed with horse meat and given orally about 0.5 *g* of KI daily in two divided doses to block the thyroid uptake of ^{125}I or ^{131}I. At the start of the experiment, 30–100 μCi of plasminogen-^{125}I was intravenously injected into the dogs. The interval between the completion of the preparation of plasminogen-^{125}I and its injection into the dogs was only a few minutes. The first blood sample of about 3–4 ml was obtained at 10 min after the injection of plasminogen-^{125}I, and thereafter the samples were obtained at most or all of the following times in days: $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, 1, $1\frac{1}{8}$, $1\frac{1}{4}$, $1\frac{1}{2}$, 2, $2\frac{1}{2}$, 3, $3\frac{1}{2}$, 4, $4\frac{1}{2}$, 5, $5\frac{1}{2}$, 6, $6\frac{1}{2}$, and 7. The samples were all heparinized in tubes containing 120 USP U of heparin. The plasma volume was determined from the total plasminogen-bound radioactivity initially injected divided by the counts per minute of the 10 min sample. The plasma samples were analyzed for plasminogen-bound radioactivity (x), radioactivity unbound to proteins (z), and plasma plasminogen concentration by the radial immunodiffusion method. The radioactivity unbound to proteins (z) was determined by counting 1 ml of supernate of plasma samples, which was obtained by adding 2 ml of 25% trichloroacetic acid to 1 ml of plasma and its centrifugation. 24-hr urine collections were made in every dog which was kept in a metabolism cage, and cumulative urinary excretion of radioactivity (u) was determined.

Plasma plasminogen responses to a single injection of human urokinase. This study was carried out in five healthy dogs, which were dogs 5′, 8′, 11, 23, and 24. The general methods of the experiments were the same as for the control studies. At the start of the experiment, 80–100 μCi of plasminogen-^{125}I were intravenously injected, and the plasma plasminogen-^{125}I activity (x), the radioactivity unbound to proteins (z), and the radioactivity remaining in the body ($1-u$) were determined daily for the period of 7 days. On the 3rd day from the start of the experiment, 1909 plough U of human urokinase (Calbiochem) were intravenously injected. The plasma samples were also analyzed by the disc electrophoresis (2) for the presence of plasmin-^{125}I before and after the injection of urokinase as described earlier.

Plasma plasminogen responses to a single injection of typhoid vaccine. This study was performed in six healthy dogs which were dogs 19, 20, 21, 22, 25, and 26. The general experimental procedure was the same as for the urokinase study. On the 3rd day, 1 ml of typhoid vaccine (Wyeth Laboratories, Philadelphia, Pa.) was intravenously injected, and the plasma and urine samples were analyzed as described above.

Plasma plasminogen responses to vascular injury. This study was carried out in eight dogs, which were dogs 2′, 3′, 4′, 6′, 7′, 13, 15, and 18. The vascular injury was produced as described by Sabiston, Marshall, Dunnill, and Allison (11) with some modifications. Locations 2 cm above the ankle and 5 cm above the knee were tightly occluded by a piece of cotton gauze, and the blood in the vein was removed as much as possible. Then, under pentothal sodium anesthesia, 1 ml of 90% phenol was injected into the emptied vein and then removed after 1 min. The lumen of the vein was rinsed several times with 0.9% NaCl. In some dogs a vein was exposed by incisions at a point 2 cm above the ankle and at a point about 5 cm above the knee and the vein was temporarily ligated. The same procedure was then applied as described above. In dogs 2′, 3′, and 4′ venous biopsies were performed at 24 hr after the procedure. In all three dogs, venous thrombosis was demonstrated. In dogs 2′, 3′, 4′, 6′, 7′, and 18, plasminogen-I^{131} was injected immediately after the phenol procedure, but in dogs 13 and 15 the phenol procedure was performed on the 3rd day after the intravenous injection of plasminogen-^{125}I. The general experimental procedure was the same as described for the control study. Dogs 5′, 8′, and 11 used for the urokinase study were also used as anesthesia controls. They were anesthetized by a single intravenous injection of 8 ml 2.5% pentothal sodium, and the behavior of plasma plasminogen-

^{125}I, plasma plasminogen concentration, and plasma plasmin-^{125}I was studied. No difference was observed from the results in control dogs under normal conditions, and there was no detectable degree of plasmin-^{125}I generation.

Plasminogen responses during heparin administration in dogs to which the phenol procedure was performed. In dogs 12, 14, 16, and 17, the phenol procedure without the incision described above was carried out on the 3rd day after the intravenous administration of plasminogen-^{125}I, and 1 ml of Lipo-Hepin (10,000 USP U/ml, River Laboratories, Inc., Northridge, Calif.) was given intravenously and 1 ml subcutaneously. Thereafter, 0.5 ml Lipo-Hepin subcutaneously every 6–12 hr daily was given for the period of 4 days. The Lee-White clotting time was usually greater than 1 hr during the period of study. The general experimental procedure was the same as described above. Dogs 9, 23, and 24 were also used as heparin controls. They were given the same amount of heparin as described above, and the plasma plasminogen-^{125}I and plasma plasminogen concentration were studied. No appreciable changes were found compared with the control dogs under normal conditions, and there was no evidence that plasmin-^{125}I was formed.

Analysis of the tracer data. The tracer data obtained in the control dogs were analyzed by a mathematical model (Fig. 6) similar to that described for prothrombin (9), which is later validated for plasminogen. The analysis of the tracer data by the model provides the following information: The plasma plasminogen ($\bar{x}$, milligrams/kilograms), the interstitial plasminogen ($\bar{y}$, milligrams/kilograms), the fractional transcapillary transfer rate of plasma plasminogen to interstitital space (j_1, per day), the fractional return rate of $\bar{y}$ to plasma (j_2, per day), the fractional direct catabolic rate of plasma plasminogen (j_{3p}, per day), the transcapillary flux of plasma plasminogen ($j_1\bar{x}$, milligrams/kilograms per day), the amount of interstitial plasminogen returned to plasma ($j_2\bar{y}$, milligrams/kilograms per day), and the catabolic flux of plasma plasminogen ($j_{3p}\bar{x}$, milligrams/kilograms per day) which equals in a steady state the synthesis rate of plasminogen. We calculated j_1 from $j_1 = aC_1 + bC_2 - j_3$, j_2 from $j_2 + a + b - (j_1 + j_3)$, and j_{3p} from $j_{3p} = (C_1/a + C_2/b)^{-1}$ as reported previously (9), where a, C_1, b, and C_2 are the parameters of a function ($x = C_1e^{-at} + C_2e^{-bt}$) describing the behavior of plasma plasminogen-^{125}I (x), which were obtained by the analysis of the data by the method of least squares with the use of a CDC 6400 computer. Another estimate of $j_{3p}(j_{3u})$ was obtained from $j_{3u} = [u_{2,3}/\xi_{2,3}(t_3 - t_2)] \times [(k_5 - a)/k_5]$, where $u_{2,3}$ is the urinary radioactivity excreted in the time interval t_2 to t_3 and $\xi_{2,3}$, the mean plasma plasminogen radioactivity during this time interval (12), k_5 is the daily fractional excretion rate of the breakdown products of plasminogen-^{125}I through the kidney, and is 1.2/day on average as reported previously (13). The tracer data obtained in the dogs given urokinase, typhoid vaccine, and also in the dogs with vascular injury were analyzed by the methods detailed later.

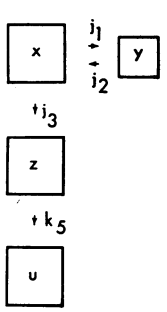

FIGURE 6 A simple model for plasminogen-^{125}I metabolism and distribution under normal conditions. x is the plasma plasminogen-^{125}I, y is the interstitial plasminogen-^{125}I, z is the free ^{125}I in the iodide space, and u is the radioactivity excreted in the urine, j_1 is the fractional transcapillary transfer rate per day of x to interstitial space, j_2 is the fraction of y returned to plasma per day, j_3 is the fractional direct breakdown rate per day of x, and k_5 is the fractional excretion rate per day of z through the kidney.

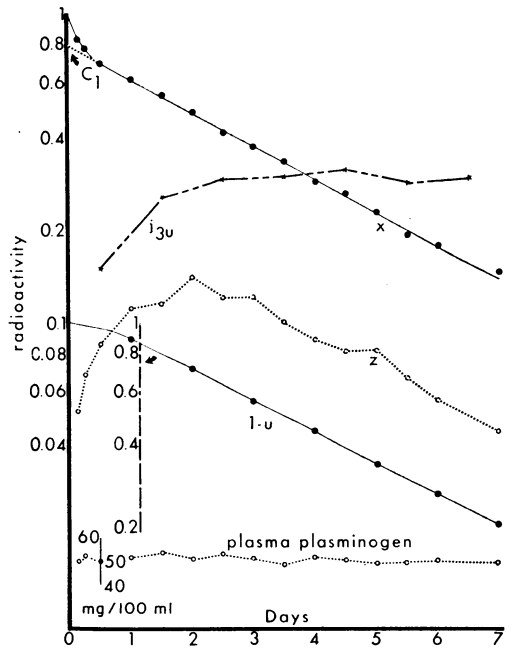

FIGURE 7 Behavior of intravenously injected plasminogen-^{125}I in a control dog. x is the curve of plasma plasminogen-^{125}I radioactivity, z is the curve of the radioactivity of free ^{125}I in the iodide space, and 1-u is the curve of the radioactivity remaining in the body. j_{3u} is the daily fractional catabolic rate calculated from x and 1-u.

RESULTS

Plasminogen metabolism and distribution in healthy dogs under control conditions

The body weight and plasma data. The average values in the eight dogs with their standard deviations are given below: body weight, 10.8±3.1 kg; plasma volume, 51.1±2.0 ml/kg; plasma plasminogen concentration, 57.2±7.2 mg/100 ml; plasma plasminogen ($\bar{x}$), 29.3 ±4.1 mg/kg; and hematocrit, 44.9±3.3. Body weight, hematocrit, and plasma plasminogen concentration showed small fluctuations about their means, the latter of which is shown in Fig. 7. Thus, it can reasonably be assumed that the animals were in a steady state during the study.

The nature of the plasma radioactivity after a single intravenous injection of plasminogen-^{125}I. It might be expected that the injected plasminogen-^{125}I is degraded

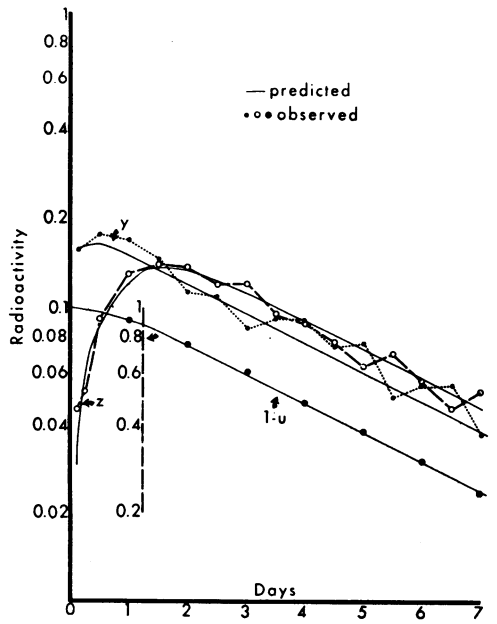

FIGURE 8 Predicted and observed values of y, z, and 1-u in a control dog. It is seen that the agreement between the predicted and observed values of 1-u is excellent and that the agreement between the predicted and observed values of y and z, respectively, is fairly good.

in vivo into several radioactive protein fractions as well as free ^{125}I. If these were present in plasma in significant amounts, it is necessary to correct them to obtain the exact behavior of plasma plasminogen-^{125}I. Therefore, in dogs 2, 3, 4, and 5, every plasma sample obtained at the times specified in the Methods section was analyzed by the disc electrophoresis (2). The radioactivity was found to be entirely confined to the plasminogen area (Fig. 3*a*), and there was no evidence that plasmin-^{125}I was formed under normal conditions. The free ^{125}I (z) is described later.

The plasma plasminogen radioactivity curve (x). The plasma plasminogen radioactivity of all samples was expressed as fractions of the 10 min sample and plotted on a semilogarithmic scale. One of the typical experiments is shown in Fig. 7. Analysis of the x curve by the computer showed that the plasma plasminogen radioactivity is closely described by a two exponential equation of the form, $x = C_1e^{-at} + C_2e^{-bt}$. The average value in the eight dogs was $x = 0.77e^{-0.24t} + 0.23e^{-7.12t}$. The half-lives ($t_{\frac{1}{2}}$) of the slower component of x ranged from 2.50 to 3.2 with the average value of 2.81±0.24 (SD) days.

The free ^{125}I-radioactivity in the iodide space (z) *and the urinary radioactivity* (u). The free ^{125}I-radioactivity in the iodide space was calculated by multiplying the free ^{125}I cpm/ml plasma by the iodide space (13), and was expressed as fractions of the total plasminogen-^{125}I initially injected. A typical behavior of z is shown in Fig. 7. The cumulative urinary excretion of radioactivity (u) in the eight dogs was expressed as fractions of the total radioactivity initially injected. Fig. 7 shows the behavior of the radioactivity remaining in the body (1-u). Analysis of the data by the computer showed that after 1 day the 1-u curve was closely described by a function $1\text{-}u = C_ue^{-a}u^t$, and averaged $1\text{-}u = 1.1e^{-0.234t}$ in the eight dogs studied. The values of a_u were not significantly different ($0.5 > P > 0.4$) from the a values of x.

Validation of the model. The model (Fig. 6) was validated by comparing the observed behaviors of the interstitial plasminogen radioactivity (y), the free radioactivity-^{125}I in the iodide space (z), and the cumulative urinary radioactivity (u) with those predicted by the model (Fig. 6), as reported previously for prothrombin (9). The observed values of y was indirectly obtained from $y = 1 - x - z - u$ (9). The results are shown in Fig. 8. It is seen that the agreement between the predicted and observed values of 1-u is excellent and that between the predicted and observed values of y and z, respectively, is fairly good. Thus, the model (Fig. 6) closely predicts the observed behavior of y, z, and 1-u.

The fractional rate constants (j_1, j_2, *and* j_3), *the plasminogen fluxes* ($j_1\bar{x}$, $j_2\bar{y}$ *and* $j_3\bar{x}$), *and the interstitial plasminogen* ($\bar{y}$). The average values in the eight dogs with their standard deviations are given below: $j_1 = 1.57\pm0.61$ day^{-1}; $j_2 = 5.51\pm1.43$ day^{-1}; $j_{3p} = 0.295\pm0.035$ day^{-1}; $j_{3u} = 0.278\pm0.037$ day^{-1}; $j_1\bar{x} = 46.6\pm23.6$ mg/kg per day; $j_3\bar{x} = 8.61\pm1.35$ mg/kg per day; $\bar{y} = 8.79\pm4.47$ mg/kg; and $\bar{y}/\bar{x} = 0.291 \pm0.110$. Since the animals were in a steady state, $j_1\bar{x} = j_2\bar{y}$ and $j_3\bar{x}$ equals the rate of plasminogen synthesis (9). Therefore, the values for $j_2\bar{y}$ and the rate of plasminogen synthesis are not separately given. There were some differences in the values of j_{3p} and j_{3u}, but these were not statistically significant ($0.4 > P > 0.3$). Daily values of j_{3u} are shown in Fig. 7.

The plasma plasminogen responses to a single injection of human urokinase

Generation of plasmin-^{125}I (v). Human urokinase was injected intravenously into the five dogs (Table I) on the 3rd day after the initial injection of plasminogen-^{125}I as described in the Methods section. As expected, analysis of the plasma by the disc electrophoresis (2) showed that significant amounts of plasmin-^{125}I were generated within 3 hr in all five dogs studied. The details of the analytical results are given in Table I. The plasmin-^{125}I radioactivity expressed as fractions of the total plasminogen-^{125}I initially injected was then calculated by multiplying the total plasma protein-bound radioactivity and the plasmin-^{125}I values in Table I, and

were plotted on a semilogarithmic scale. One of the typical results is shown in Fig. 9. The peak values of plasmin-^{125}I (Cm) were 0.094, 0.12, 0.12, 0.13, and 0.10 with the average value of 0.113±0.01 (SD) in the five dogs. The disappearance half-lives of plasma plasmin-^{125}I were 0.62, 0.62, 0.62, 0.50, and 0.55 days, and averaged 0.58±0.05 (SD) days. Thus, the behavior of plasmin-^{125}I (v) after its peak value (C_m) was reached, was $v = C_m e^{-kt}$ and averaged $v = 0.113e^{-1.19t}$ in the five dogs studied.

The behavior of plasma plasminogen-^{125}I (x). The behavior of plasma plasminogen-^{125}I was followed in the five dogs (Table I) for the period of 7 days. One of the experiments is shown in Fig. 9. These responses of plasma plasminogen-^{125}I were very consistent and similar in magnitude in all five dogs studied. The half-lives of plasma plasminogen-^{125}I during the 6 hr period after the urokinase injection were 0.42, 0.40, 0.50, 0.41, and 0.45 days with the average value of 0.44±0.04 (SD) days. Thus, the average behavior of x during the 6 hr was $x = 0.38e^{-1.57t}$ where 0.38 is the average value of plasma plasminogen-^{125}I (C_p) on the 3rd day (Fig. 9). However, despite the plasmin-^{125}I generation and the more rapid fall of plasma plasminogen-^{125}I during the 6 hr after the urokinase injection, there was no appreciable difference in the rate of decline between the plasma plasminogen-^{125}I during the 3 days before the urokinase injection and the plasma protein bound radioactivity ($x + v$) which is the sum of plasma plasminogen-^{125}I (x) and plasmin-^{125}I (v) during the period of 3–5.5 days (Fig. 9).

The behavior of the curve of the radioactivity remaining in the body (1-u), and the native plasma plasminogen concentration. One of the five experiments is shown in Fig. 9. This behavior of 1-u curve was consistent and similar in the five dogs studied. The average behavior of the 1-u curve during the 7 days was $1\text{-}u = 1.12e^{-0.243t}$. The native plasma plasminogen concentration was measured by the caseinolytic method (1), and the radial immunodiffusion method. The results showed that despite the plasmin generation there were no appreciable changes in the plasma plasminogen concentration before and after the urokinase injection.

TABLE I

Plasmin-^{125}I Generation in Plasma after Intravenous Urokinase Injection

Dog No.	Days 3	3½	3½	3½	4	4½	5	5½	6
5′	0	17	28	22	15	8	2	0.5	0
8′	0	18	31	20	14	8	2	0.5	0
11	0	15	32	22	15	9	2	0.5	0
23	0	20	31	23	17	7	1	0.5	0
24	0	16	29	19	13	7	1	0	0
Means	0	17	30	21	14	8	2	0.4	0
SD		±2	±2	±2	±1	±1	±1	±0.1	

Human urokinase was injected on the 3rd day after the initial injection of plasminogen-^{125}I. Each value for plasma plasmin-^{125}I is expressed as per cent of the total plasma protein-bound radioactivities at each specified time, which was obtained by subtracting the plasma-free ^{125}I from the total plasma radioactivity and is the sum of plasma plasminogen-^{125}I and plasmin-^{125}I. It is seen that the plasma plasmin-^{125}I reached its peak value at the 6th hr and disappeared within 2.5 days after the injection of urokinase.

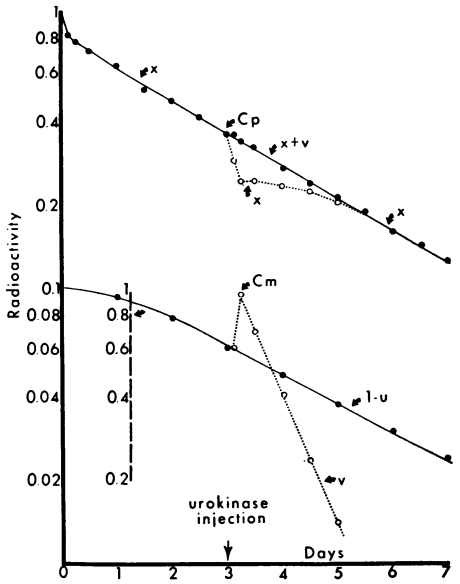

FIGURE 9 Plasminogen-^{125}I responses to a single injection of urokinase in dog 5′. It is seen that the urokinase injection on the 3rd day caused a rapid fall of plasma plasminogen-^{125}I (x) for about 6 hr, but thereafter for about 2.25 days it declined at a slower rate and then returned to the rate before the urokinase injection. However, the rate of decline of total plasma protein-bound radioactivity ($x + v$) was unchanged. C_p is the level of x on the 3rd day. It is also seen that the urokinase injection caused generation of plasmin-^{125}I (v), which reached its maximum value (C_m) of 0.094 at 6 hr and then declined at a single exponential rate. The curve of the radioactivity remaining in the body (1-u) was unchanged by the urokinase injection.

The plasma plasminogen responses to a single injection of typhoid vaccine

Generation of plasmin-^{125}I (v). 1 ml of the typhoid vaccine was intravenously injected into the six dogs (Table II) on the 3rd day after the initial injection of plasminogen-^{125}I, and the plasma samples were analyzed by the disc electrophoresis (2). The results showed that significant amounts of plasmin-^{125}I were generated within 3 hr after the vaccine injection in all six dogs studied. The details of the analysis are given in Table II. The plasmin-^{125}I radioactivity expressed as fractions of the total plasminogen-^{125}I radioactivity initially injected

TABLE II

Plasmin-^{125}I Generation in Plasma after Intravenous Injection of Typhoid Vaccine

Dog No.	Days								
	3	3½	3¾	3¾	4	4½	5	5½	6
19	0	17	20	20	15	8	2	0	0
20	0	18	21	20	13	7	1	0	0
21	0	18	19	17	11	6	1	0	0
22	0	21	21	20	9	4	1	0	0
25	0	17	35	34	18	10	1	0	0
26	0	21	33	30	27	10	3	1	0
Means	0	19	25	24	16	8	2	0	0
SD		±2	±7	±7	±6	±2	±1		

Typhoid vaccine was injected on the 3rd day. Each plasma plasmin-^{125}I value is expressed as per cent of the total plasma protein-bound radioactivities at each specified time. It is seen that the plasma plasmin-^{125}I reached its peak value at the 6th hr and disappeared within 2.5 days after the injection of the vaccine.

was then calculated as in the urokinase experiments and was plotted on the semilogarithmic scale. One of the typical experiments is shown in Fig. 10. The peak values of plasmin-^{125}I (C_m) were 0.07, 0.08, 0.07, 0.08, 0.14, and 0.14 with the average value of 0.096±0.035 (SD) in the six dogs. The disappearance half-life values of plasma plasmin-^{125}I in the six dogs were 0.63, 0.60, 0.63, 0.54, 0.63, and 0.62 days with the average value of 0.61±0.04 (SD) days, which were not significantly different ($0.4 > P > 0.3$) from that in the urokinase experiments. Thus, the behavior of plasmin-^{125}I (v), after its peak value was reached, was $v = C_m e^{-kt}$ and averaged $v = 0.096e^{-1.13t}$ in the six dogs studied.

The behavior of plasma plasminogen-^{125}I (x). The behavior of plasma plasminogen-^{125}I was followed in the six dogs (Table II) for the total period of 7 days. One of the typical experiments is shown in Fig. 10. These responses of plasma plasminogen-^{125}I were very consistent and similar in the six dogs studied. The half-lives of plasma plasminogen-^{125}I during the 6 hr were 0.52, 0.63, 0.58, 0.54, 0.34, and 0.34 with the average value of 0.49±0.12 (SD) days in the six dogs studied, and were not significantly different from those in the urokinase experiments ($0.4 > P > 0.3$). Thus, the average behavior of x was $x = 0.39e^{-1.41t}$, where 0.39 is the average value for plasma plasminogen-^{125}I levels on the 3rd day (C_p) (Fig. 10). Despite these changes in the behavior of plasma plasminogen-^{125}I due to the generation of plasmin-^{125}I, there were no appreciable changes in the rate of decline between the plasma plasminogen-^{125}I before the vaccine injection and the plasma protein-bound radioactivity ($x + v$) during the period of 3–5 days (Fig. 10).

The behavior of the curve of the radioactivity remaining in the body (1-u), and the native plasma plasminogen concentration. One of the six experiments is shown in Fig. 10. The average behavior of 1-u during the 7 days was $1\text{-}u = 1.15e^{-0.240t}$ and was very similar to the control values. The native plasma plasminogen concentration was not appreciably altered despite the generation of plasma plasmin-^{125}I.

The plasma plasminogen responses to vascular injury

Generation of plasmin-^{125}I (v). The phenol procedure described in the Methods section was performed for the six dogs (Table III) at the very start of the experiment and plasminogen-^{125}I was injected immediately thereafter, but for the two dogs on the 3rd day after the injection of plasminogen-^{125}I. Analysis of the plasma samples by the disc electrophoresis (2) showed generation of plasmin-^{125}I within 3 hr after the phenol procedure in all eight dogs studied. The details of the analytical results are given in Table III. The plasmin-^{125}I radioactivity expressed as fractions of the plasminogen-^{125}I initially injected was calculated as in the urokinase experiment and were plotted on a semilogarithmic scale. One of the six experiments is shown in Fig. 11. Graphical analysis (14) of the data showed

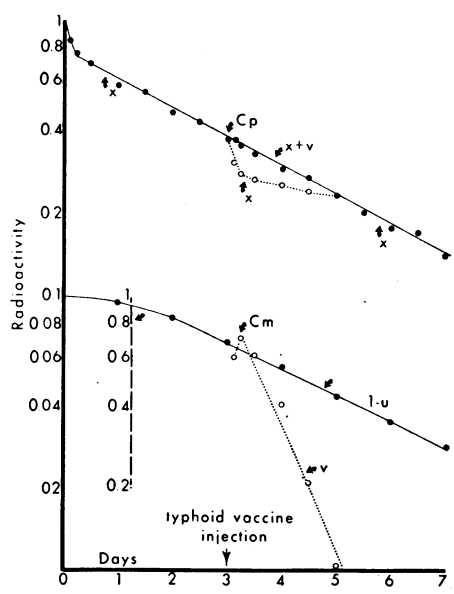

FIGURE 10 Plasminogen-^{125}I responses to a single injection of typhoid vaccine in dog 19. It shows that the vaccine injection on the 3rd day caused a rapid fall of plasma plasminogen-^{125}I (x) for about 6 hr, but thereafter it declined at a slower rate for about 2 days and then returned to the rate before the injection. Despite these changes of x, the rate of decline of total plasma protein-bound radioactivity ($x + v$) was unchanged. C_p is the level of x on the 3rd day. It is also seen that the vaccine injection generated plasmin-^{125}I (v), which reached its maximum value (C_m) of 0.07 at 6 hr and then declined rapidly at a single exponential rate. The 1-u curve was unchanged.

TABLE III

Plasmin-^{125}I Generation in Plasma after the Phenol Procedure

Dog No.	Days 0	⅛	¼	½	1	1½	2	2½	3	3⅛	3¼	3½	4	4½	5	5½	6	6½	7
2′	0	23	21	23	21	20	19	26	19			20	19	13	10	11	10	9	9
3′	0	15	25	23	17	18	18	20	21			20	22	20	15	14	15	10	15
4′	0	20	24	22	30	22	20	23	20			20	23	20	19	14	13	11	10
6′	0	11	18	30	19	27	16	23	20			21	21	12	9	9	11	10	10
7′	0	15	26	23	18	20	20	23	19			20	22	17	16	13	12	10	9
18	0	7	24	23	24	17	16	19	17			20	21	18	18	18	16	15	15
Means	0	15	23	24	22	21	18	19	19			20	21	17	13	13	13	11	11
SD		±6	±3	±3	±5	±4	±2	±4	±1			±1	±1	±5	±5	±3	±2	±2	±3
13									0	11	17	17	19	17	17	18	17	17	16
15									0	15	19	18	18	18	17	19	18	17	19
Means										13	18	18	19	18	17	19	18	17	18
SD										±3	±1	±1	±1	±1	±0	±1	±1	±0	±2

In dogs 2′, 3′, 4′, 6′, 7′, and 18 the phenol procedure was carried out immediately before and in dogs 13 and 15 on the 3rd day after the intravenous injection of plasminogen-^{125}I. Each value for plasma plasmin-^{125}I is expressed as per cent of the total plasma protein-bound radioactivities at each specified time. It is seen that in the six dogs the plasma plasmin-^{125}I reached its peak value in 6–12 hr, thereafter remained relatively constant for about 5 days and then somewhat decreased, but that in the end of 7 days there still remained considerable amounts of plasmin-^{125}I. In the two dogs the behavior of plasma plasmin-^{125}I was similar except that the time scale is shifted to the right by 3 days.

that the behavior of plasmin-^{125}I (v) can be approximated by a two exponential equation of the form $v = C_3e^{-at} + C_4e^{-Bt}$, where $C_3 + C_4 = 0$. The values of C_3 were 0.19, 0.23, 0.20, 0.17, 0.25, and 0.17 with a mean of 0.20±0.032 (SD). The values of a were 0.219, 0.238, 0.281, 0.231, 0.287, and 0.297, and averaged 0.259±0.033 (SD) day^{-1}. Statistical analysis showed that these were not significantly different ($0.5 > P > 0.4$) from the a values in the control dogs. The values of B ranged from 7.2 to 13.8 with a mean of 10.6±2.1 (SD). Thus, the average behavior of v in the six dogs was $v = 0.20e^{-0.259t} - 0.20e^{-10.6t}$ for the 5 day period. One of the two experiments in dogs 13 and 15 is also shown in Fig. 12. Graphical analysis (14) also showed that v can be approximated by $v = C_3'e^{-at} + C_4'e^{-Bt}$, where $C_3' + C_4' = 0$. The average v in the two dogs was $v = 0.06e^{-0.260t} - 0.06e^{-10.3t}$.

The behavior of plasma plasminogen-^{125}I (x). These were followed in the eight dogs (Table III) for the period of 7 days. One of the experiments in the six dogs is shown in Fig. 11. Graphic analysis (14) of the data showed that the behavior of plasma plasminogen-^{125}I (x) can be closely expressed by a two exponential function of the form $x = C_1e^{-at} + C_2e^{-bt}$, where $C_1 + C_2 = 1.0$. The values of C_1 were 0.66, 0.71, 0.68, 0.69, 0.73, and 0.74 with a mean of 0.70±0.03 (SD), and were significantly less ($0.02 > P > 0.01$) than the C_1 values in the control dogs. The a values were 0.219, 0.238, 0.281, 0.231, 0.287, and 0.297 with a mean value of 0.259±0.033 (SD), and were not significantly different ($0.5 > P > 0.4$) from those in the control dogs. The values of b were 5.2, 4.6, 6.9, 8.3, 10.3, and 8.3 with a mean of 7.3±2.1 (SD), and were also not significantly different ($0.7 > P > 0.6$) from those in the control dogs. Thus, the average behavior of x in the six dogs for the 5 day period was $x = 0.70e^{-0.259t} + 0.30e^{-7.3t}$. One of the experiments in the two dogs is also shown in Fig. 12. The graphical analysis (14) of the data showed that the behavior of plasma plasminogen-^{125}I (x) can be approximated by a two exponential function $x = C_6e^{-at} + C_7e^{-At}$, where $C_6 + C_7 = C_p$ which is the level of x on the 3rd day (Fig. 12). The average x in the two dogs was $x = 0.25e^{-0.260t} + 0.07e^{-15.0t}$. Despite this altered behavior of plasma plasminogen-^{125}I the total plasma protein-bound radioactivity ($x + v$) (Figs. 11 and 12) behaved very similarly to that of plasma plasminogen-^{125}I in control dogs (Figs. 7, 11, and 12).

The behavior of the curve of the radioactivity remaining in the body (1-u), and the native plasma plasminogen concentration. One of the experiments in the six dogs is shown in Fig. 11. This behavior of 1-u was also very similar in the two dogs in which the phenol procedure

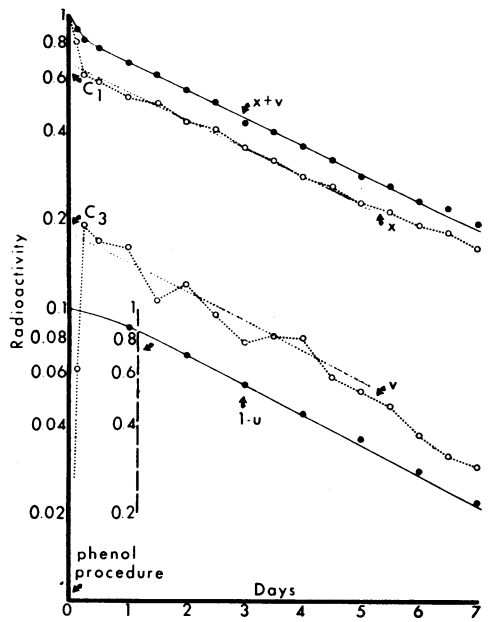

FIGURE 11 Plasminogen-^{125}I responses to vascular injury in dog 18. It shows the behavior of plasma plasminogen-^{125}I (x) when the phenol procedure was carried out at zero time. x was closely expressed by a two-exponential function, $x = C_1e^{-at} + C_2e^{-bt}$, where $C_1 + C_2 = 1.0$, for the period of 5 days, but thereafter it declined more slowly. The total plasma protein-bound radioactivity ($x + v$) behaved very similarly to x under control conditions (Fig. 7). It is also seen that the phenol procedure caused generation of plasmin-^{125}I (v), which reached its maximum value at 6 hr and then declined at a rate closely similar to that of x. v was approximated by a two-exponential function, $v = C_3e^{-at} + C_4e^{-Bt}$ for the period of 5 days, where $C_3 + C_4 = 0$, but thereafter it appeared to decline more rapidly. The 1-u curve was unchanged by the phenol procedure.

was carried out on the 3rd day (Fig. 12). The average behavior of 1-u during the 7 day period was $1\text{-}u = 1.2e^{-0.254t}$ in the eight dogs studied and was not significantly different ($0.5 > P > 0.4$) from the control values. The native plasma plasminogen concentration showed no appreciable changes during the study.

Plasma plasminogen responses during heparin administration in dogs subjected to the phenol procedure

Generation of plasmin-^{125}I (v). This study was carried out in four dogs (Table IV). On the 3rd day after the initial injection of plasminogen-^{125}I the phenol procedure was carried out and immediately thereafter heparin was given as described in the Methods section. The plasma samples were analyzed by the disc electrophoresis (2) and considerable amounts of plasmin-^{125}I were again found to be generated despite the heparin administration. The details of the analytical results are given in Table IV. The plasmin-^{125}I radioactivity expressed as fractions of the total plasminogen-^{125}I initially injected was also calculated as in the urokinase experiments. One of the experiments is shown in Fig. 13. This behavior of plasmin-^{125}I was consistent in the four dogs studied (Table IV). The peak values of plasmin-^{125}I (C_m) were 0.073, 0.073, 0.08, and 0.09 with a mean of 0.079±0.008 (SD) in the four dogs. The disappearance half-lives of plasmin-^{125}I were 0.60, 0.73, 0.58, and 0.61 days with the average value of 0.63±0.07 (SD) days which were very similar to those in the urokinase and typhoid vaccine experiments. Thus, the behavior of plasmin-^{125}I (v), after its peak value (C_m) was reached was $v = C_me^{-kt}$, and averaged $v = 0.079e^{-1.11t}$.

The behavior of plasma plasminogen-^{125}I (x). The behavior was studied in the four dogs (Table IV) for the total period of 7 days. One of the experiments is shown in Fig. 13. This behavior of plasma plasminogen-^{125}I was very consistent and similar in the four dogs studied. The half-lives of x during the 6 hr were 0.54, 0.42, 0.42, and 0.55 with a mean of 0.48±0.06 (SD) days.

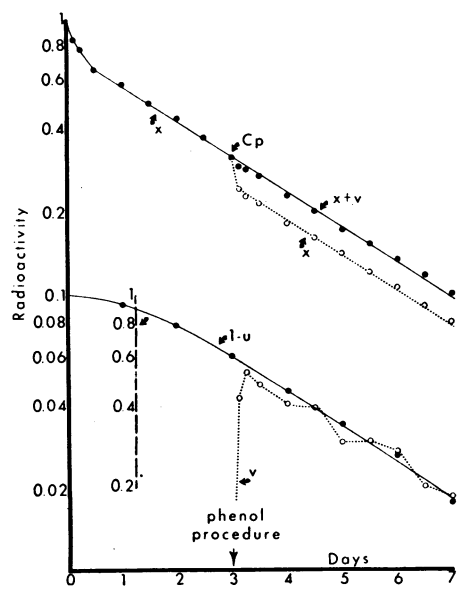

FIGURE 12 Plasminogen-^{125}I responses to vascular injury in dog 15. It shows that the phenol procedure on the 3rd day caused a rapid fall of plasma plasminogen-^{125}I (x) for about 6 hr, but that thereafter it declined at a rate closely similar to that of x before the phenol procedure. The entire x curve after the phenol procedure was expressed by a two-exponential function of the form, $x = C_6e^{-at} + C_7e^{-At}$, where $C_6 + C_7 = C_p$ which is the level of x on the 3rd day. Despite these changes of x, the total plasma protein-bound radioactivity ($x + v$) did not change its rate of decline compared with that of x before the phenol procedure. It is also seen that the phenol procedure caused generation of plasmin-^{125}I (v), which reached its peak value of 0.052 at 6 hr and then declined at a rate close to that of x. The entire behavior of v was approximated by a two-exponential function, $v = C_3'e^{-at} + C_4'e^{-Bt}$, where $C_3' + C_4' = 0$. The 1-u curve was unchanged.

Thus, the average behavior of x during the same period in the four dogs was $x = 0.38e^{-1.44t}$, where 0.38 is the average value for the plasma plasminogen-^{125}I levels on the 3rd day (C_p). Despite this altered behavior of plasma plasminogen-^{125}I the total protein-bound radioactivity in plasma ($x + v$) behaved very similarly with that of plasma plasminogen-^{125}I in control dogs (Fig. 7).

The behavior of the curve of the radioactivity remaining in the body (1-u), and the native plasma plasminogen concentration. These were studied in the four dogs (Table IV) for the period of 7 days. Fig. 13 shows that the 1-u curve was not appreciably altered by the phenol procedure, compared with that in control dogs (Fig. 7). This was true for all four dogs studied. The average behavior of 1-u during the 7 days was 1-u $= 1.09e^{-0.240t}$. The native plasma plasminogen concentration showed no appreciable changes before and after the phenol procedure with heparin injection.

The model for coexistent plasminogen-^{125}I and plasmin-^{125}I analysis and interpretation of the data

The model. The data presented for the urokinase, typhoid vaccine, vascular injury, and heparin experiments show that both plasminogen-^{125}I and plasmin-^{125}I coexisted at least for some time during the total period of 7 days, and the model shown in Fig. 6 does not apply to these data, since it does not account for generation and catabolism of plasmin-^{125}I. Thus, a more complete model is shown in Fig. 14, where x, y, z, u, j_1, j_2, j_3, and k_5 are the same as defined earlier for the model shown in Fig. 6. v is plasmin-^{125}I, j_4 is the fractional rate per day of plasmin-^{125}I generation from plasminogen-^{125}I (x), and j_5 is the fractional catabolic rate per day of plasmin-^{125}I (v). All j's and k_5 are assumed constants at least for some time during the study. In the Appendix the methods are derived to calculate these constants and the model (Fig. 14) is validated.

TABLE IV

Plasmin-^{125}I Generation in Plasma Under the Influence of Heparin in Dogs to Which the Phenol Procedure was Performed

Dog No.	Days									
	3	3½	3¾	4¼	4	4½	5	5½	6	6½
12	0	18	30	16	7	6	4	1	0	0
14	0	19	20	18	12	9	4	1	0	0
16	0	18	27	26	24	13	6	3	1	0
17	0	13	28	30	24	20	6	5	1	0
Means	0	17	26	23	17	12	5	3	1	0
SD		±3	±4	±7	±8	±6	±1	±2	±0	

The phenol procedure was carried out on the 3rd day after the initial injection of plasminogen-^{125}I and heparin was given immediately thereafter. The Lee-White clotting time was maintained greater than 1 hr throughout the experiment. Each plasma plasmin-^{125}I value is expressed as per cent of the total plasma protein-bound radioactivities at each specified time. It is seen that the plasma plasmin-^{125}I reached its peak value at the 6th hr and completely disappeared within about 2.5 days after the phenol procedure.

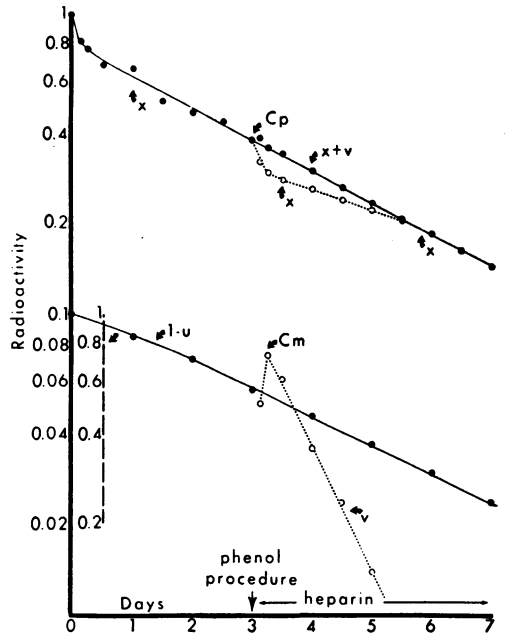

FIGURE 13 Plasminogen-^{125}I responses under heparinized conditions in dog 14 to which the phenol procedure was performed. It is seen that despite the heparinization the phenol procedure on the 3rd day caused a rapid fall of plasma plasminogen-^{125}I (x) for about 6 hr, but thereafter it declined more slowly for about 2.25 days and then returned to the rate before the phenol procedure. Despite these changes of x, the rate of decline of total plasma protein-bound radioactivity ($x + v$) was unchanged from that of x before the phenol procedure. C_p is the level of x on the 3rd day. It also shows that the phenol procedure caused generation of plasmin-^{125}I (v) despite the heparinization, which reached its peak value (C_m) of 0.072 at 6 hr and then declined rapidly at a single exponential rate. The 1-u curve was unchanged during the entire period.

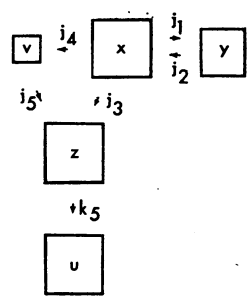

FIGURE 14 The model for coexistent plasminogen-^{125}I and plasmin-^{125}I. See Fig. 6 for x, y, z, u, j_1, j_2, j_3, and k_5. v is plasmin-^{125}I, j_4 is the fractional rate per day of plasmin-^{125}I generation from x and j_5 is the fractional breakdown rate per day of v.

Analysis and interpretation of the data. In the urokinase experiments in which urokinase was given on the 3rd day (Fig. 9), j_4 (Fig. 14) may be reasonably assumed to have ceased to exist at 6 hr because of the steep decline of the plasma plasminogen-^{125}I during the 6 hr after the urokinase injection and the sudden change to a slower decline thereafter, in addition to the steep decline of v after 6 hr (Fig. 9). The values of j_4 during this period and j_5 (Fig. 14) can be calculated using equations 12 and 17 in the Appendix and experimentally obtained values of x, v, and 1-u. The average x, v and 1-u in the five dogs as given earlier were $x = 0.38e^{-1.57t}$, $v = 0.113e^{-1.19t}$, and 1-$u = 1.12e^{-0.243t}$. The calculated values for j_4 and j_5 were $j_4 \doteqdot 0.41$ day^{-1} and $j_5 = 1.19$ day^{-1}. During the period of 6 hr to 2.5 days after the urokinase injection in which the plasma plasminogen-^{125}I declined at a slower rate than that in the control dogs (Fig. 9), it is obvious that $j_4 = 0$ and j_3 is now operating. The j_3 value during this period was calculated from the mean plasma plasminogen-^{125}I value of 0.281 and the average 1-u of 1-$u = 1.12e^{-0.243t}$ in the five dogs, using equation 13 (Appendix). The average j_3 was 0.30 day^{-1}. In the period thereafter, it is clear that the catabolic state of plasminogen-^{125}I returned to that in the control dogs (Fig. 9). Thus, upon urokinase injection plasmin was generated at a rate of $j_4 \doteqdot 0.41$/day for the 6 hr period with $j_3 = 0$, thereafter, $j_4 = 0$ and plasma plasminogen was directly catabolized at a rate of $j_3 \doteqdot 0.30$ day^{-1} for the period of 2.25 days, and then returned to the normal state of $j_4 = 0$ and $j_3 = 0.295$ day^{-1}. Considering experimental and analytical errors, it appears that $j_3 + j_4 \doteqdot 0.30$ day^{-1} during the total experimental period of 7 days. The synthesis rate of plasminogen during the experiment did not change since the plasma plasminogen concentration and $j_3 + j_4$ did not differ much from the control values (15).

In the experiments in which typhoid vaccine was injected on the 3rd day (Fig. 10), the responses of plasminogen-^{125}I were very similar to those in the urokinase experiments (Fig. 9). The average behaviors of x, v, and 1-u in the six dogs as given earlier were $x = 0.39e^{-1.41t}$, $v = 0.096e^{-1.13t}$, and 1-$u = 1.15e^{-0.240t}$. Calculating in the same way as for the urokinase experiments, $j_4 \doteqdot 0.3$ day^{-1} and $j_3 = 0$ during the 6 hr period after the vaccine injection, and $j_5 = 1.13$ day^{-1}. During the next 2.25 days $j_4 = 0$ and $j_3 \doteqdot 0.36$ day^{-1} using the mean plasma plasminogen-^{125}I of 0.261. Thus, it appears that $j_3 + j_4$ was constant at approximately 0.30 day^{-1} during the total experimental period of 7 days. The rate of plasminogen synthesis also stayed at the control level, since the plasminogen concentration and $j_3 + j_4$ did not change appreciably during the entire experimental period (15).

The tracer data in the six vascular injury experiments (Fig. 11) are analyzed in the Appendix. The results showed that the phenol procedure caused generation of plasmin at a rate of $j_4 = 0.324$ day^{-1} with $j_3 \doteqdot 0$ and that this state persisted for the 5 day period after the procedure. The data in the two dogs (Fig. 12) in which the phenol procedure was carried out on the 3rd day were analyzed by equations 11, 14, and 16 (Appendix), using the average values of $x = 0.25e^{-0.260t} + 0.07e^{-15.0t}$, $v = 0.06e^{-0.260t} - 0.06e^{-10.3t}$ and 1-$u = 1.2e^{-0.254t}$, which were given earlier. The results were $j_3 + j_4 = 0.332$ day^{-1}, $j_4 = 0.330$ day^{-1} and $j_5 = 1.38$ day^{-1}. The similar value of j_4 with $j_3 + j_4$ again supports the above conclusion that j_3 was probably zero during the study. Thus, the results were very similar in the two types of vascular injury experiments (Figs. 11 and 12). The synthesis rate of plasminogen did not change appreciably during the study, since plasma plasminogen concentration and $j_3 + j_4$ did not vary much from those in the control dogs.

The tracer data obtained in the dogs subjected to the phenol procedure and injected with heparin (Fig. 13) were analyzed as in the urokinase and the typhoid vaccine experiments. As given earlier $x = 0.38e^{-14.4t}$, $v = 0.079e^{-1.11t}$, and 1-$u = 1.09e^{-0.240t}$ on average during the 6 hr period after the phenol procedure. The calculated value of j_4 during this period was 0.382 day^{-1}, and j_5 was 1.11 day^{-1}. During the next 2.25 days $j_4 = 0$ and $j_3 = 0.352$ day^{-1}, using the mean plasma plasminogen-^{125}I activity of 0.260. Thereafter, it is obvious that the normal state of $j_4 = 0$ and $j_3 = 0.295$ day^{-1} was reached (Fig. 13). Thus, the phenol procedure with periodic injections of heparin did not inhibit the generation of plasmin during the 6 hr period after the procedure, j_4 being 0.283 day^{-1} which is as much as that in the vascular injury experiments without heparin injection (Figs. 11 and 12). However, the heparin injection caused abolition of j_4 at 6 hr whereas, in the vascular injury experiments without heparin injection, j_4 persisted at $j_4 = 0.324$ day^{-1} for the 5 day period (Fig. 11).

DISCUSSION

The method described here for purification of plasminogen is simple and rapid and the entire procedure can be completed within 24 hr. Several physiochemical analysis of the products (Figs. 2 and 3) showed only a single component, although this does not necessarily mean that they are absolutely pure. As a matter of fact, antibodies against them occasionally produced two or 3 precipitin arcs against normal canine plasmas, probably indicating that the products contained small amounts of impurities. However, an absolute purity of plasminogen preparations is not essential for metabolic studies of plasminogen. In the previous metabolic studies of albumin (16, 17), fibrinogen (18, 19), and prothrombin (9, 20), we have successfully used 95% pure albumin,

96% pure fibrinogen, and about 98% pure prothrombin. What is more important is that the plasminogen-^{125}I used as a tracer is not appreciably denatured. Iodination of various proteins could denature the protein molecules (21), but it has been reported that the levels of iodination of one atom iodine per molecule of albumin (21–23), of 0.5 atom iodine per molecule of fibrinogen (8, 15, 18) and of 0.25 atom iodine per molecule of prothrombin (9, 20) do not cause an appreciable degree of denaturation. In the present studies, the canine plasminogen was labeled with ^{125}I in a ratio of about 0.25 atom iodine per molecule of plasminogen (mol wt 130,000). The in vitro and in vivo tests of the plasminogen-^{125}I (Figs. 3 and 4) showed that this level of iodination did not cause an appreciable degree of alteration of plasminogen molecule. This is further supported by another in vivo finding that j_{3u} calculated daily (Fig. 7) was much lower during the first day, since denatured plasminogen-^{125}I would give a greater value of j_{3u} during the 1st day than during the later period (24).

Metabolic studies of plasminogen using tracer-labeled plasminogen have not been reported previously. The present studies in helathy dogs under control conditions showed that plasminogen is synthesized and catabolized at a rate of 8.61 mg/kg per day. The analysis of plasma by the disc electrophoresis (2) after the intravenous injection of plasminogen-^{125}I showed only the plasminogen-^{125}I peak, and no plasmin-^{125}I was found at any time during the study. Thus, under normal conditions a major portion of plasminogen, if not all, must be catabolized directly without the formation of plasmin. This is further supported by the finding that the model (Fig. 6) in which plasminogen is assumed to be directly catabolized without the formation of plasmin closely predicted the observed behaviors of plasminogen-^{125}I (Fig. 8). Thus, these studies defined the plasminogen metabolism under normal conditions, but very little is known of plasminogen metabolism under abnormal conditions. This is the reason for the studies of the effects of urokinase, typhoid vaccine, and vascular injury on plasminogen metabolism. However, to analyze the complex tracer data, it was necessary to develop some new mathematical methods as detailed in the Appendix.

In all studies, considerable amounts of plasmin-^{125}I were found to be generated (Figs. 9–13). However, there were some differences in the duration of plasmin-^{125}I generation. Thus, in the urokinase and typhoid vaccine experiments (Figs. 9 and 10), the effects of their injection on plasmin generation lasted only for about 6 hr, but in the vascular injury experiments (Figs. 11 and 12) the effects lasted for at least 5 days (Table VI). The heparin injection (Fig. 13) only shortened the duration of plasmin generation to 6 hr. The average rate of plasmin generation (j_4) during the 6 hr was 0.41 day^{-1} in the urokinase experiments, 0.30 day^{-1} in the typhoid vaccine experiments, and 0.382 day^{-1} in the heparin experiments. Thereafter, for the period of about 2.25 days plasminogen was directly catabolized at the average rate of $j_3 \doteqdot 0.30$ day^{-1} in the urokinase study, at the rate of $j_3 \doteqdot 0.36$ day^{-1} in the typhoid vaccine study, and at the rate of $j_3 \doteqdot 0.352$ day^{-1} in the heparin experiments. Thereafter, in these studies the state of plasminogen metabolism returned to that under normal conditions with $j_3 = 0.295$ day^{-1}. Considering the experimental errors and the theoretical approximation of equation 13, (Appendix), the differences in the j_3 values in the three experiments during 2.25 days from that in the control dogs do not appear significant. Thus, it seems that after the cessation of plasmin generation at 6 hr the catabolic state of plasminogen returned to that in the control dogs. The question is what happened to j_3, the rate of direct breakdown of plasminogen, during the 6 hr in which plasmin was being generated in the urokinase, typhoid vaccine, and heparin experiments. The values of j_4 given above were calculated on the assumption that $j_3 = 0$ using equation 12 (Appendix). The validity of this assumption could not be tested by the data obtained in the urokinase, typhoid vaccine, and heparin experiments (Figs. 9, 10, and 13), but it was possible by the data in the eight vascular injury experiments (Figs. 11 and 12). It is shown in the Appendix that in the vascular injury experiments, the rate of direct breakdown of plasminogen (j_3) was zero or negligibly small during the 5 day period in which plasmin was being generated at a constant rate of $j_4 = 0.324$ day^{-1}. There is no proof that j_3 was zero during the 6 hr in the urokinase, typhoid vaccine, and heparin experiments, but it seems unlikely that plasminogen behaves differently in such an essential way in the urokinase, typhoid vaccine, and heparin experiments. Thus, in all experiments it appears that $j_3 = 0$ when j_4 is operating and vice versa, and that $j_3 + j_4 \doteqdot 0.30$ day^{-1} which is at the control level during the entire period of the study. In other words, the total catabolism of plasminogen ($j_3 + j_4$) was not appreciably altered during any of the studies. Only change was that during plasmin generation j_3 was abolished and replaced by similar values of j_4. This and plasma plasminogen concentrations similar to those under control conditions led to the conclusion that the plasminogen synthesis was not appreciably altered during any of the studies (Figs. 9–13). These findings raise another question whether greater amounts of urokinase or typhoid vaccine could alter the value of j_4 or merely prolong the duration of plasmin generation above 6 hr. The same question may be asked if more extensive vascular injury or thrombosis or greater amounts of heparin could alter the j_4 value or duration of plasmin generation, or both. A complete answer to

these questions awaits future study. The latter question is particularly difficult to answer since the effects of heparin on plasminogen activators are not clearly understood (25). However, there is no doubt that the plasmin generation in the present studies (Figs. 11–13) is at least a result of the vascular injury.

Although the metabolic studies of plasmin can better be done in a separate study, the present studies were able to determine the catabolic rate of plasmin (j_5) as shown earlier. Using the different methods in the Appendix (Equations 15–17) devised for different experimental conditions, the calculated average j_5 for the urokinase experiments (Fig. 9), typhoid vaccine experiments (Fig. 10), the vascular injury experiments (Figs. 11 and 12), and the heparin experiments (Fig. 13) were 1.19, 1.13, 1.32, 1.38, and 1.11 day^{-1}, respectively. These values are very close and the differences between them seem small enough to be within experimental and analytical errors. Thus, the average j_5 under the various experimental conditions is 1.23±0.11 (SD) day^{-1}. In view of the close similarity of j_5 values under these conditions, the studies of j_5 do not appear to be of much value. However, it might be altered in more extensive vascular injury or thrombosis, and this also remains to be seen.

APPENDIX

The model shown in Fig. 14 can be described by the following sets of differential equations with their initial and final conditions:

$$dx/dt = j_{2}y - (j_1 + j_3 + j_4)x \qquad x(0) = 1,\ x(\infty) = 0 \quad (1)$$

$$dy/dt = j_1x - j_2y \qquad y(0) = 0,\ y(\infty) = 0 \quad (2)$$

$$dv/dt = j_4x - j_5v \qquad v(0) = 0,\ v(\infty) = 0 \quad (3)$$

$$dz/dt = j_3x + j_5v - k_5z \qquad z(0) = 0,\ z(\infty) = 0 \quad (4)$$

$$du/dt = k_5z \qquad u(0) = 0,\ u(\infty) = 1. \quad (5)$$

The solutions of these equations were obtained by the Laplace transform and only the pertinent are listed below:

$x = C_1e^{-at} + C_2e^{-bt}$, where $C_1 + C_2 = 1.0$ for the time interval 0 to t in which all the j's and k's are constants. $C_2e^{-bt} \rightarrow 0$ after some time t because $b \gg a$. Therefore, (6)

$x = C_1e^{-at}$ after some time t. Given that $x = C_1e^{-at}$ after some time t, (7)

$v = C_3e^{-at} + C_4e^{-Bt}$, which is $v = C_3e^{-at}$ after some time T, because $B \gg a$, and is valid for the time interval T to t in which all the j's and k's are constants. $C_3 = j_4C_1(j_5 - a)^{-1}$ and $C_4 = -C_3$. (8)

$1\text{-}u = C_5e^{-at}$ after some time t_1, and is valid for the time interval t_1 to t in which all j's and k_5 are constants. $C_5 = k_5(j_3C_1 + j_5C_3)[a(k_5 - a)]^{-1}$. (9)

$j_3 + j_4 = (C_1/a + C_2/b)^{-1}$ (12, 15), and when assumed that $j_3 = 0$ (10)

$j_4 = (j_5 - a)(k_5 - a)u_{1,2}[j_5k_5\xi(t_2 - t_1)]^{-1}$, where $u_{1,2}$ is the urinary radioactivity excreted in the time interval t_1 to t_2 and ξ is the mean plasma plasminogen-^{125}I activity during the same interval (12). Equation 11 can be approximately expressed by the following equation, although some over-estimates of j_4 may result: (11)

$j_4 \doteqdot u_{1,2}/\xi(t_2 - t_1)$, and when $j_4 =$ (12)

$j_3 \doteqdot u_{1,2}/\xi(t_2 - t_1)$. This is the "degradation rate" by Berson, Yalow, Schreiber, and Post (21) or "metabolic rate" by McFarlane (26). Another method can be derived to calculate $j_3 + j_4$ when given that $x = C_6e^{-at} + C_7e^{-At}$, where $C_6 + C_7 = C_p$ which is the level of plasma plasminogen-^{125}I on the 3rd day when the phenol procedure was performed (Fig. 12). The sum of plasma plasminogen-I^{125} which could be converted to plasmin-^{125}I and which could be directly catabolized must equal to C_p. Thus, $(j_3 + j_4)\int_0^\infty x dt = C_p$. Therefore, (13)

$j_3 + j_4 = C_p[C_6/a + C_7/A]^{-1}$. In addition to what have been described above, a solution for j_5 is also required for the validation of the model (Fig. 14). This is possible only on the assumption that $j_3 = 0$ for the time interval 0 to t (Fig. 14). When $j_3 = 0$, x will be all converted to v, which, in turn, will be completely broken down in the course of time. Thus, the total amount of v broken down will equal to 1.0. This applies when the phenol procedure was carried out at zero time (Fig. 11). Thus, $j_5\int_0^\infty v dt = 1.0$, where $v = C_3e^{-at} + C_4e^{-Bt}$ and $C_3 + C_4 = 0$. Therefore, (14)

$j_5 = (C_3/a + C_4/B)^{-1}$. In a similar way j_5 may be calculated when $v = C_3'e^{-at} + C_4'e^{-Bt}$ and $j_3 = 0$ after some time t, where $C_3' + C_4' = 0$ and when the level of plasma plasminogen-^{125}I is C_p. In this case the total amount of v which will be broken down must equal to C_p. This was the case when the phenol procedure was carried out on the 3rd day (Fig. 12). Thus, $j_5\int_0^\infty v dt = C_p$ and therefore, (15)

$j_5 = C_p(C_3'/a + C_4'/B)^{-1}$. In the same way j_5 can be obtained when $v = C_me^{-kt}$ and $j_4 = 0$ for the time interval t_1 to t_2, which was the case during the urokinase, typhoid vaccine, and heparin experiments (Figs. 9, 10, and 13). C_m is the maximum value of v and it is evident that the total amount of v which could be catabolized is C_m. Thus, $j_5\int_0^\infty v dt = C_m$ and therefore, (16)

$j_5 = k$. It should be noted that the model (Fig. 14) reduces itself to that shown in Fig. 6, when $j_4 = 0$ which actually occurred during some periods of the experiments. (17)

Validation of the model. Equations 6–10 and 14 hold true whether j_3 and j_4 were coexistent or one of the two was zero, but equations 11, 12, 15, and 16 are valid only when $j_3 = 0$. The important question is then whether or not the assumption $j_3 = 0$ is valid when j_4 is operating at some constant level. The most complete data for the test of this assumption and the validity of the model (Fig. 14) without j_3 are provided by the six vascular injury experiments in which the phenol procedure was carried out at zero time (Fig. 11). It was given earlier that

$$x = 0.7e^{-0.259t} + 0.3e^{-7.3t} \quad (18)$$

$$v = 0.2e^{-0.259t} - 0.2e^{-10.0t} \quad (19)$$

$1\text{-}u = 1.2e^{-0.354t}$. From these and equations 10, 11, and 15, $j_3 + j_4$, j_4, and j_5 were calculated, which were $j_3 + j_4 = 0.364$, $j_4 = 0.324$ and $j_5 = 1.32$ day^{-1}. Then, using the calculated values of $j_4 = 0.324$ and $j_5 = 1.32$ as well as $k_5 = 1.2$ day^{-1} (13), the values of C_3 and C_5 in equations 8 and 9 were calcu- (20)

lated, which were $C_3 = 0.213$ and $C_5 = 1.3$, and were very close to the observed value of 0.2 in equation 19 and 1.2 in equation 20, respectively. Thus, given that $x = 0.7e^{-0.259t} + 0.3e^{-7.3t}$ the model without j_3 (Fig. 14) closely predicts the qualitative and quantitative behaviors of v and 1-u. Another support for the validity of the model without j_3 (Fig. 14) comes from the fact that the calculated $j_3 + j_4$ and j_4 given above were very close, the difference being only 0.04 per day. This could be a true difference or due to the experimental error, but the latter seems more likely because errors in urine collection can readily occur. At any case, the maximum value for j_3 under the experimental condition is 0.04 day^{-1} and is negligibly small.

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