CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 22, No. 4 April 1974

Regular Articles

[Chem. Pharm. Bull. 22(4) 727—735 (1974)]

UDC 547.466.1.09 : 615.36.015.4.076.9

Metabolic Fate of Urokinase

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(Received June 13, 1973)

The metabolic fate of ^{131}I-urokinase in rats and dogs was investigated. The radioactivity in the blood of both animals after intravenous administration rapidly decreased in early stage, followed by a slow decrease of the radioactivity. When ^{131}I-urokinase was given into rats by intravenous infusion, the peak of radioactivity in the blood was found at the end of the dosing period. The radioactivity in the liver and kidneys of the rats 15 min after intravenous administration of ^{131}I-urokinase was found to be 43 and 32% of the administered radioactivity, respectively. In the rats approximately 80% of the administered radioactivity was recovered, mainly in the urine in 3 days. The radioactivity derived from high-molecular compounds in the serum of rats receiving ^{131}I-urokinase intravenously was approximately 95% of the radioactivity detected 1 min after dosing, 70% at 20 min and 40% at 60 min.

An artificial thrombus was prepared by the method of Chandler and radioactivity of the thrombus was determined by incubating with radioiodinated urokinase, plasminogen, plasmin or reference compounds. After a 6 hr incubation the ratio of the radioactivity concentration of the thrombus (cpm/mg) to that of the blood (cpm/mg) was 2 for ^{131}I-urokinase, 3 for ^{131}I-plasminogen, 3 for ^{131}I-plasmin, 0.7 for ^{131}I-human serum albumin and 0.8 for Na^{131}I. When the blood added with ^{131}I-plasminogen was employed for the preparation of an artificial thrombus, approximately 15% of the added radioactivity was detected in the thrombus.

Urokinase is a plasminogen-activator present in mammalian urine[2] and is a basic protein with a molecular weight of approximately 33000.[3] Its physiological significance is considered to be lysis of clots produced in the urinary tract and maintenance of the patency of the urinary tract.[4] Urokinase has been used widely for the treatment of thrombosis, but investigators are not all in agreement as to the dose, the method of administration, and the adverse effect of urokinase.[5] Although some data to explain the fibrinolytic mechanism of urokinase *in vivo* and *in vitro* have been presented,[5–7] more detailed studies are required. The above-mentioned clinical problems and elucidation of the fibrinolytic mechanism necessi-

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tate the elucidation of the metabolic fate of urokinase under various conditions. From this point of view only a few work have been made by Woodard, *et al.*,[8] Back, *et al.*[9] and Murakami, *et al.*[10]

We investigated the metabolic fate of urokinase by administration of ^{131}I-labeled urokinase into rats and dogs. Radioactivity of an artificial thrombus incubated with ^{131}I-urokinase, ^{131}I-plasminogen or urokinase-activated ^{131}I-plasmin was determined to examine the fibrinolytic mechanism of urokinase.

Material and Method

Materials——Urokinase was extracted and purified from normal male urine by the method of Ogawa, *et al.*[3] and lyophylized. Its specific activity was 25000 IU/mg of protein. Plasminogen was prepared from fresh human plasma by the method of Deutsch and Mertz,[11] and its specific activity was 20 CU/mg of protein. Human serum albumin was Cohn's plasma fraction V obtained from the Nutritional Biochemical Corporation. Sodium [^{131}I] iodide (dissolved in 0.1 N NaOH, carrier-free) was obtained from the New England Nuclear Corporation. The human blood samples to prepare an artificial thrombus were obtained from Nihon Seiyaku Co., Ltd. The samples were fresh human blood containing 3% sodium citrate as an anticoagulant and used within 4 days after bleeding.

Preparation of Radioiodinated Urokinase, Plasminogen and Human Serum Albumin——Urokinase was radioiodinated by a modification of the method of Greenwood, *et al.*[12] To 1 mg of urokinase dissolved in 1 ml of 0.05 M phosphate buffer, pH 8.0, NaI (20 μg) in 0.1 ml of 0.5 M phosphate buffer, pH 8.0 and 3 mCi of Na^{131}I solution were added while cooling in an ice bath. Immediately thereafter Chloramine-T (360 μg) in 0.1 ml of 0.5 M phosphate buffer was added. After each addition, the mixture was stirred for 1 min in an ice bath. Then 0.1 ml of $Na_2S_2O_5$ solution (4 mg/ml 0.5 M phosphate buffer) was added to stop the reaction. At this stage any reduction of fibrinolytic activity of urokinase was not observed. Separation of ^{131}I-urokinase from the reaction mixture was carried out by gel-filtration over a column of Sephadex G-100. Equilibration of the gel and elution from it was carried out with 0.9% NaCl solution. The radiochemical purity of the resultant ^{131}I-urokinase was 94.1—99.6% and the specific radioactivity was 1—3 mCi/mg of protein. Its fibrinolytic activity increased to 46000 IU/mg of protein by the gel-filtration. Radioiodination of plasminogen was carried out by the method of McConahey and Dixon,[13] and the resultant preparation was dialyzed against 0.1 M glycine buffer, pH 2.9, over night at 4° to remove unbound iodide. Human serum albumin was radioiodinated by the method of Greenwood, *et al.*,[12] followed by an overnight dialysis against 0.9% NaCl solution at 4° to remove unbound iodide. Caseinolytic activity of plasminogen, determined by the method of Johnson, *et al.*,[14] decreased by approximately 30% in the course of radioiodination.

Distribution and Excretion of Radioactivity after Administration of ^{131}I-Urokinase——Rat: There were three to five male Wistar rats weighing 250±10 g in each group. ^{131}I-Urokinase in a dose of 1800 IU/kg (3.5×10^6 cpm/rat) made up in a constant volume of 0.5 ml for single intravenous injection and 5 ml for intravenous infusion with 0.9% NaCl solution was given into the femoral vein. Intravenous infusion was carried out at the rate of 5 ml/hr by means of a KN-201 microinfuser (Natsume Seisakusho Co., Ltd.). After dosing, blood samples were taken from the carotid artery at determined intervals. The rats were killed by exanguination and the objective tissues were isolated. The blood, serum and tissues were assayed for radioactivity. For the determination of radioactivity in excreta the rats were placed in individual metabolism cages designed for the separate collection of urine and feces. Excreta were collected in 24 hr fractions, and assayed for radioactivity. Bile was collected by inserting cannula into the common bile duct for up to 72 hr after administration of ^{131}I-urokinase and assayed for radioactivity.

Dog: Two male beagle dogs weighing 15 and 12 kg were employed. ^{131}I-Urokinase in doses of 100 IU/kg (2×10^7 cpm/dog) or 500 IU/kg (9×10^7 cpm/dog) prepared in 10 ml volume of 0.9% NaCl solution was given into the right femoral vein. Before the injection, the dogs were placed in a dorsal position and cannula was inserted into the left femoral artery. For up to 6 hr after dosing, bleeding was made from the cannula by placing the dogs in a dorsal position, and then they were placed in individual cages to collect urine and feces separately. The blood, urine and feces were assayed for radioactivity.

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Analysis of Radioactive Compounds in Serum, Urine and Bile of Rats——Male Wistar rats weighing 250±10 g were given intravenously 1800 IU/kg (3.5×10^6 cpm/rat) of ^{131}I-urokinase prepared in 0.5 ml of 0.9% NaCl solution into the femoral vein. Analysis of radioactive compounds was made on the serum obtained 1, 20 and 60 min after dosing, on the urine in the first 24 hr fraction and on the bile in the first 2 hr fraction.

Gel-filtration: Gel-filtration of 0.5 ml of the rat serum was made on a column (1×50 cm) of Sephadex G-100. Equilibration of the gel and elution from it was carried out with 0.9% NaCl solution. An optical density of the eluate was determined at 280 nm with 034 UV-VIS Effluent Monitor (Hitachi Ltd.). Each fraction was assayed for radioactivity.

Dialysis: Cellulose tubes (8/32, Union Carbide Corp.) containing 1 ml of the serum, urine or bile were dialyzed against 300 ml of 0.9% NaCl solution for 48 hr with continuous stirring in a refrigerator. The saline was changed at 2, 4, 6, and 24 hr after the onset of dialysis. The whole tube was assayed for radioactivity and this radioactivity was considered to be derived from nondialyzable compounds.

Radioactivity of an Artificial Thrombus incubated with ^{131}I-Urokinase, ^{131}I-Plasminogen or ^{131}I-Plasmin——An artificial thrombus was prepared by the method of Chandler.[15] One milliliter of human blood was drawn into a polyvinyl tube (26.7 cm in length and 0.3 cm in internal diameter). Since the blood contained 3% sodium citrate as an anticoagulant, 50 μl of 6% $CaCl_2\cdot2H_2O$ was added. The two ends of the tube were then brought together to form a circle. The circular tube was placed on a turntable tilted to an angle of 28° and rotated at 17 rpm at 37°. After a 10 min rotation, 50 μl of a test sample was added into the tube and rotation was continued for a determined period. The blood and thrombus in the circular tube were separately weighed and assayed for radioactivity. The ratio of the radioactivity concentration (cpm/mg) of the thrombus to that of the blood was calculated. If the ratio was higher than 1, there was a preferential localization of radioctivity in the thrombus. Test samples were as follows. (1) ^{131}I-Urokinase which was added into the tube to a final concentration of 1, 4 or 8 IU/ml, corresponding to 0.025, 0.1 or 0.2 μg as protein and 2.0×10^4, 1.1×10^5 or 2.2×10^5 cpm as radioactivity. (2) ^{131}I-Plasminogen which was added into the tube to a final concentration of 0.008 CU/ml. (3) ^{131}I-Plasmin which was derived from the activation of ^{131}I-plasminogen described in (2) with 4 IU of urokinase. (4) ^{131}I-Human serum albumin whose protein content was 0.1 μg. (5) Na^{131}I whose radioactivity was 1.1×10^5 cpm.

Further, 1 ml of the blood was drawn into a tube, followed by an addition of ^{131}I-plasminogen and 50 μl of 6% $CaCl_2\cdot2H_2O$. The concentration of ^{131}I-plasminogen after its addition was approximately 0.008 CU/ml. The tube was rotated for exactly 10 min at 37° similarly as above and radioactivity of the thrombus formed was determined.

Measurements——Fibrinolytic activity was determined by the method of Ploug, *et al.*,[16] protein by the method of Lowry, *et al.*[17] and radioactivity by using a well-type scintillation spectrometer, JDC-207 13/4″$\phi\times2$″ (Japan Radiation and Medical Electronics, Inc.).

Result

I. Distribution and Excretion of Radioactivity after Administration of ^{131}I-Urokinase

Radioactivity in Blood——By assuming that the volume of rat blood is one-fifteenth of its total body weight, disappearance of radioactivity after intravenous administration of 1800 IU/kg of ^{131}I-urokinase into rats is shown in Fig. 1. Radioactivity in the blood fell rapidly after injection of ^{131}I-urokinase and 37% of the administered radioactivity remained in the blood after 7.5 min. The radioactivity gradually diminished and that in the blood at 4 hr was found to be 9.5% of the administered radioactivity. As shown in Fig. 2, in the case of intravenous infusion in the rats, radioactivity in the blood 30 min after the beginning of ^{131}I-urokinase administration was 25.4% of the administered radioactivity, which corresponds to 12.7% of the total radioactivity to be administered. The radioactivity in the blood reached the maximum 34.6% at the end of the dosing period, and reduction of radioactivity thereafter was almost similar to that obtained later than 7.5 min after dosing in the intravenous injection. Radioactivity in the blood 4 hr after the end of administration was 7.3% of the administered radioactivity. In both intravenous administration and intravenous infusion, approximately 70% of the radioactivity in the blood was present in the serum, and the percentage remained unchanged throughout the course of the experiment. When 100 or 500

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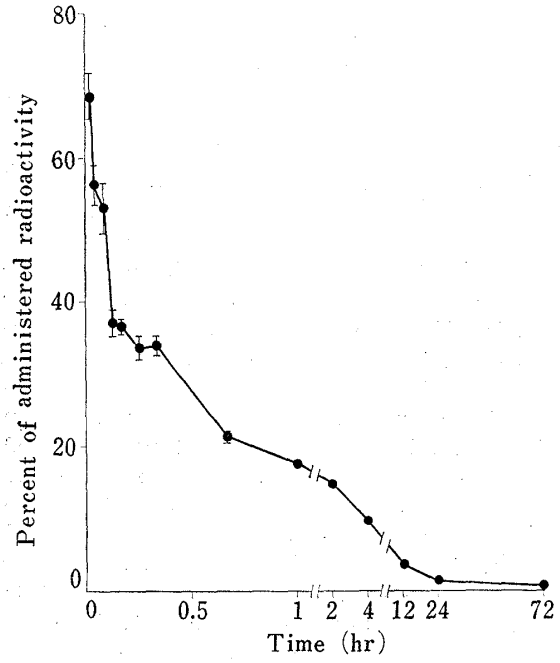

Fig. 1. Radioactivity in Blood of Rats after Intravenous Injection of ^{131}I-Urokinase

Each rat received 1800 IU/kg (3.5×10^6 cpm/rat) of ^{131}I-urokinase. The volume of rat blood was assumed to be 1/15 of its total body weight. Each point represents the mean ±S.E. of five rats.

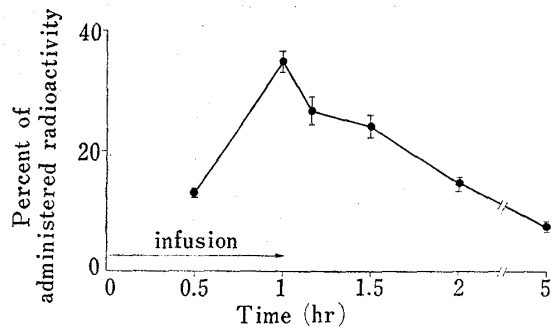

Fig. 2. Radioactivity in Blood of Rats from the Beginning of Intravenous Infusion of ^{131}I-Urokinase

Each rat received 1800 IU/kg (3.5×10^6 cpm/rat) of ^{131}I-urokinase. The volume of rat blood was assumed to be 1/15 of its total body weight. Each point represents the mean ±S.E. of four rats.

IU/kg dose of ^{131}I-urokinase was administered into dogs, the radioactivity in blood was determined at different intervals (Fig. 3). Due to a difference in the administered dose of ^{131}I-urokinase into dogs and rats, it is difficult to compare these data strictly, but the radioactivity in the blood of the dogs decreased more rapidly than that of the rats. At 10 min after dosing, radioactivity in the blood of the dogs appeared to be 47% of the administered radioactivity. Because only one dog was employed for one dose, strict comparison cannot be made but any significant discrepancy was not found in the values of (total radioactivity in blood/administered radioactivity)×100 between the dogs receiving 100 and 500 IU/kg of ^{131}I-urokinase.

Table I. Distribution of Radioactivity in Organs after Intravenous Injection of ^{131}I-Urokinase

	Percent of administered radioactivity in organs					
	1 min	5 min	15 min	20 min	40 min	12 hr
Liver	21.53±0.42	38.20±1.07	43.58±0.96	38.49±1.24	8.82±0.22	0.95±0.08
Kidney	8.37±0.80	20.68±1.97	32.28±1.06	21.29±0.48	9.33±0.22	0.65±0.06
Heart	0.52±0.04	0.42±0.04	0.37±0.02	0.51±0.06	0.34±0.02	0.06±0.01
Lung	0.92±0.07	0.88±0.14	0.62±0.02	0.86±0.04	0.57±0.01	0.13±0.01
Spleen	0.37±0.03	0.72±0.06	0.93±0.11	0.93±0.07	0.45±0.04	0.05±0.00
Brain	0.14±0.01	0.10±0.01	0.10±0.01	0.12±0.01	0.08±0.00	0.04±0.01
Adrenal	0.04±0.00	0.05±0.00	0.06±0.00	0.04±0.00	0.02±0.00	—a)
Thymus	0.07±0.01	0.07±0.01	0.08±0.00	0.12±0.02	0.13±0.01	0.03±0.00

Each value represents the mean ±S.E. of five rats.
Each rat received 1800 IU/kg (3.5×10^6 cpm/rat) of ^{131}I-urokinase.
a) less than 0.01% of the administered radioactivity

Tissue Distribution——The tissue distribution of radioactivity after administration of ^{131}I-urokinase into rats is shown in Tables I and II, and in Fig. 4. In both intravenous injection and intravenous infusion a considerably large amount of radioactivity was found in the liver and kidneys. In intravenous administration the maximum radioactivity in the liver and kidneys was obtained 15 min after dosing; 43 and 32% of the administered radioactivity, respectively. In intravenous infusion, the maximum radioactivity in the liver and kidneys was obtained at the conclusion of the infusion period. The disappearance curves of radioac-

tivity in the liver and kidneys are shown in Fig. 4, when ^{131}I-urokinase was given intravenously. The disappearance curves of radioactivity in other tissues were almost identical with those in Fig. 4. In intravenous infusion the changes of radioactivity in tissues well reflect those in blood. Although data are not shown in this table, radioactivity was found in the testis, seminal vesicle, prostate, hypothalamus and bladder, but the activity did not exceed 0.5% of the administered radioactivity nor was there any storage of radioactivity in these tissues.

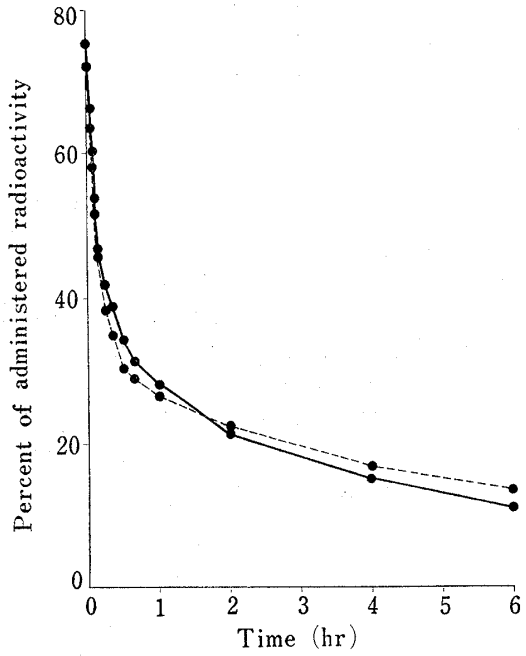

Fig. 3. Radioactivity in Blood after Intravenous Injection of ^{131}I-Urokinase in a Dog

Blood samples were taken from the femoral artery of one animal for one dose. The administered radioactivity was 2×10^7 cpm/dog (100 IU/kg) —●—; and 9×10^7 cpm/dog (500 IU/kg) ---●---. The volume of dog blood was assumed to be 1/15 of its total body weight.

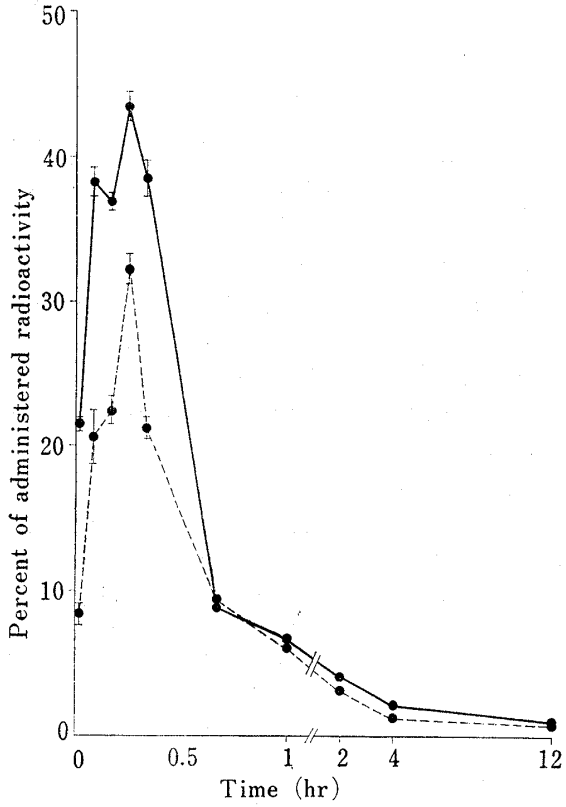

Fig. 4. Distribution of Radioactivity in Liver and Kidney after Intravenous Injection of ^{131}I-Urokinase

Each point represents the mean S.E. of five rats.
—●—: liver, ---●---: kidney

TABLE II. Distribution of Radioactivity in Organs after Intravenous Infusion of ^{131}I-Urokinase

	Percent of administered radioactivity in organs				
	30 min	60 min	70 min	90 min	5 hr
Liver	13.51±1.19	30.63±1.71	23.02±2.24	16.52±2.13	2.64±0.89
Kidney	10.01±0.54	21.22±1.58	19.67±2.78	11.76±2.36	2.87±1.56
Heart	0.18±0.02	0.35±0.02	0.32±0.03	0.37±0.04	0.10±0.02
Lung	0.22±0.02	0.59±0.01	0.64±0.09	0.55±0.07	0.24±0.05
Spleen	0.56±0.05	0.99±0.04	0.73±0.10	0.48±0.05	0.14±0.05
Brain	0.06±0.01	0.12±0.01	0.10±0.01	0.12±0.01	0.03±0.01
Adrenal	0.01±0.00	0.02±0.00	0.02±0.00	0.02±0.00	—a)
Thymus	0.04±0.00	0.12±0.02	0.14±0.02	0.13±0.02	0.05±0.01

Each value represents the mean ±S.E. of four rats.
Each rat received 1800 IU/kg (3.5×10^6 cpm/rat) of ^{131}I-urokinase.
Time shows the period after beginning of infusion.
a) less than 0.01% of the administered radioactivity

Radioactivity was detected in the stomach, small intestine and their contents from 40 min to 4 hr after dosing.

Table III. Urinary and Fecal Excretion of Radioactivity after Intravenous Injection of ^{131}I-Urokinase in Rats

Collection period (hr)	Percent of administered radioactivity	
	Urine	Feces
0—24	62.9±1.6	1.4±0.3
24—48	9.3±1.1	2.0±0.2
48—72	3.9±0.3	2.0±0.2
Total	76.1±0.8	5.4±0.5

Each value represents the mean ±S.E. of five rats.
Each rat received 1800 IU/kg (3.5×10^6 cpm/rat) of ^{131}I-urokinase.

Excretion——The excretion of radioactivity in urine and feces of rats is shown in Table III. Within 24 hr, 63 and 1.4% of the administered radioactivity were recovered in the urine and feces, respectively, and the total excretion of radioactivity in the urine and feces reached 81% within 72 hr. The excretion of radioactivity in the bile was not marked; 1.4% of the administered radioactivity recovered in the first 1 hr fraction and 1.0% in the second 1 hr fraction. Then the radioactivity excreted into the bile was reduced and the total excretion of radioactivity in the bile reached 7% of the administered radioactivity within 72 hr. The excretion of radioactivity in urine and feces of dogs receiving ^{131}I-urokinase is shown in Table IV. Almost all the radioactivity was found to be excreted into the urine and the rate of excretion was little slower than that in the rats.

Table IV. Urinary and Fecal Excretion of Radioactivity after Intravenous Injection of ^{131}I-Urokinase in Dogs

Collection period (day)	Percent of administered radioactivity			
	Exp. I		Exp. II	
	Urine	Feces	Urine	Feces
0— 2	44.0	1.4	61.2	0.3
2— 4	37.0		10.9	
4— 5	19.2		8.1	
5— 6	3.9		7.4	
6— 7	0.8		3.7	
7— 9	2.5		4.4	
9—14	—		4.7	
Total	107.4	1.4	100.4	0.3

Exp. I : A dog which received 100 IU/kg (2×10^7 cpm/dog) of ^{131}I-urokinase.
Exp. II: A dog which received 500 IU/kg (9×10^7 cpm/dog) of ^{131}I-urokinase.

Gel-filtration and Dialysis of the Serum, Urine and Bile of Rats Receiving ^{131}I-Urokinase——The rat serum obtained 1, 20 and 60 min after intravenous administration of ^{131}I-urokinase was subjected to gel-filtration and the result is shown in Fig. 5. Radioactivity in the serum 1 min after dosing appeared in the ^{131}I-urokinase fraction (Fraction numbers 21—30) and in the higher-molecular compound fraction (Fraction numbers 13—20) in 1:1 ratio, and radioactivity in the high-molecular compound fraction involving ^{131}I-urokinase fraction (Fraction numbers 13—30) was 96.1% of the applied radioactivity. The radioactivity in the ^{131}I-urokinase fraction gradually diminished with time, with increasing the radioactivity in the low-molecular compound fraction but 42.2% of the applied radioactivity was still found

in the high-molecular compound fraction 1 hr after dosing. Results of dialysis of the serum are shown in Table V. The portion of radioactivity bound with non-dialyzable compounds was approximately similar to that of the radioactivity in the high-molecular compound fraction involving the ^{131}I-urokinase fraction in gel-filtration. The results of dialysis of urine in the first 24 hr fraction and bile in the first 2 hr fraction are also shown in Table V. Non-dialyzable radioactivity was found to be 0.9% in the urine and 2% in the bile.

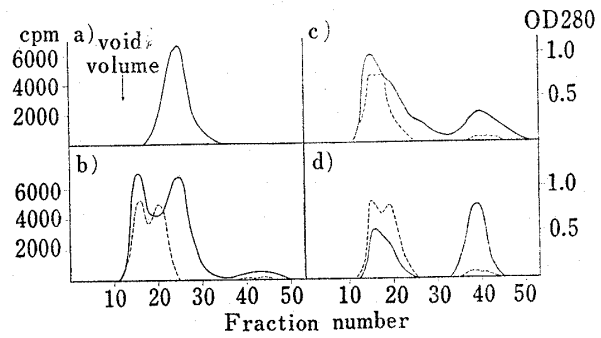

Fig. 5. Gel-filtration of Rat Serum on a Sephadex G-100 Column after Intravenous Injection of ^{131}I-Urokinase

Elution was made with 0.9% NaCl and 0.8 ml fraction were collected.
a) ^{131}I-urokinase
b) rat serum 1 min after injection of ^{131}I-urokinase
c) rat serum 20 min after injection of ^{131}I-urokinase
d) rat serum 60 min after injection of ^{131}I-urokinase
——: radioactivity, ------: optical density

TABLE V. Nondialyzable Radioactive Compounds in Serum, Urine andBile of Rats Received ^{131}I-Urokinase

Percent of initial radioactivity				
Serum obtained at			Urine[a)]	Bile[b)]
1 min	20 min	60 min		
97.4	74.7	55.9	0.9	2.3

a) first 24 hr fraction, *b*) first 2 hr fraction

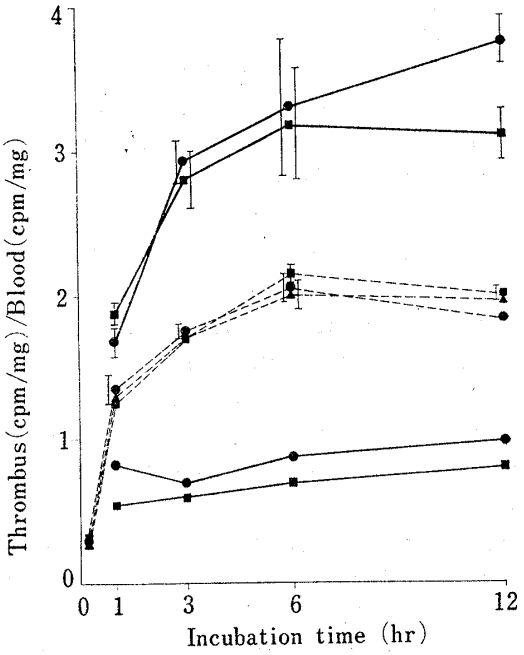

Fig. 6. Distribution of Radioactivity to the Thrombus by Incubation with ^{131}I-Urokinase, ^{131}I-Plasminogen, ^{131}I-Plasmin, ^{131}I-Human Serum Albumin or Na^{131}I

—●—: ^{131}I-plasminogen
—■—: ^{131}I-plasmin
---●---: ^{131}I-urokinase 1 IU/ml
---■---: ^{131}I-urokinase 4 IU/ml
---▲---: ^{131}I-urokinase 8 IU/ml
—●—: Na^{131}I
—■—: ^{131}I-human serum albumin

II. Radioactivity of an Artificial Thrombus incubated with ^{131}I-Urokinase, ^{131}I-Plasminogen or ^{131}I-Plasmin

The radioactivity of an artificial thrombus was determined by adding ^{131}I-urokinase to the blood in which a thrombus had been prepared. As shown in Fig. 6, radioactivity of the artificial thrombus increased with time when the thrombus was in contact with ^{131}I-urokinase. The ratio of the radioactivity per mg of the thrombus (cpm/mg) to the radioactivity per mg of blood (cpm/mg) reached a plateau after a 6 hr incubation, irrespective of the concentration of ^{131}I-urokinase. The ratio at the plateau was found to be approximately 2. This was significantly higher than the ratio obtained with Na^{131}I or ^{131}I-human serum albumin. The radioactivity of an artificial thrombus incubated with ^{131}I-plasminogen or urokinase-activated ^{131}I-plasmin was also determined (Fig. 6). After a 6 hr incubation, the ratio described above was approximately 3 with either of ^{131}I-plasminogen or urokinase-activated ^{131}I-plasmin. Furthermore, when an artificial thrombus was prepared by using the blood containing ^{131}I-plasminogen followed by the addition of $CaCl_2$, 15% of the added radioactivity was observed in the thrombus and the ratio mentioned above was found to be 8.

Discussion

Urokinase has been radioiodinated by the potassium iodide nitrite method[8,9] or by McFarlane's monochloride method.[10] In the present work urokinase was radioiodinated by the method of Greenwood, *et al.*,[12] using Chloramine-T. We investigated the amounts of Chloramine-T and nonradioactive NaI as a carrier and accepted the method described in the text. Since specific radioactivity of the resultant ^{131}I-urokinase was 1—3 mCi/mg of protein and no loss of its fibrinolytic activity was shown, ^{131}I-urokinase prepared by this method was employed as a tracer to investigate the metabolic fate of urokinase.

Radioactivity in the blood of rats receiving 1800 IU/kg of ^{131}I-urokinase rapidly fell up to 7.5 min after dosing, followed by a comparatively slow disappearance of the radioactivity up to 72 hr. Woodard, *et al.*[8] investigated disappearance of radioactivity in blood by an intravenous injection of 20—2000 CTA units/rat of ^{125}I-urokinase into rats. The disappearance curves obtained by them are in agreement with ours, except that their radioactivity in the blood were lower than that in our experiment.

Two beagle dogs were given 100 or 500 IU/kg of ^{131}I-urokinase and radioactivity in blood was determined at different intervals. Due to an insufficient number of animals employed, strict evaluation cannot be made but a rapid decrease of radioactivity in the blood was observed at the early stage after dosing, followed by a slow disappearance of the radioactivity as in the case of rats. On the other hand, marked discrepancy was not found in the values between two doses of ^{131}I-urokinase given to the dogs in (total radioactivity in blood/administered radioactivity) $\times 100$ at any time after dosing. This implies that radioactivity in blood was in proportion to the administered radioactivity. Back, *et al.*[9] reported similar results to those of ours by investigating disappearance of radioactivity in blood of dogs receiving ^{131}I-urokinase.

As shown in Fig. 5, the radioactivity derived from high-molecular compounds in the blood of rats receiving ^{131}I-urokinase decreased with time. Therefore, careful consideration should be given to assume a half-life of urokinase from that of radioactivity in the blood of animals receiving ^{131}I-urokinase.

Radioactivity derived from the high-molecular compounds involving ^{131}I-urokinase in serum was determined by gel-filtration and dialysis, by taking blood samples from the rats receiving ^{131}I-urokinase (Fig. 5, Table V). At 1, 20 and 60 min after administration, the radioactivity was approximately 95, 70 and 40%, respectively. As is evident from the results of gel-filtration in Fig. 5, the radioactivity in the serum appeared in the ^{131}I-urokinase fraction decreased at the early stage after administration, with increasing radioactivity in the higher-molecular compound fraction and the low-molecular compound fraction. We had observed that the radioactivity had been distributed to the serum proteins by incubating ^{131}I-urokinase with human serum.[18] On the other hand, under the same conditions as above, distribution of the radioactivity derived from Na^{131}I to human serum proteins was not observed. These results suggest that the administered ^{131}I-urokinase in the blood may exist both in a free and serum protein-bound type at the early stage, and then the metabolites of low-molecular weight begin to appear with time.

Fifteen minutes after the administration of ^{131}I-urokinase into rats, 43 and 32% of the administered radioactivity was found in the liver and kidneys, respectively. Murakami, *et al.*[10] assumed that urokinase might have an affinity for kidneys, because when ^{131}I-urokinase was given to human, its distribution in the kidneys was quite similar to that of ^{203}Hg-neohydrin which had been known to localize preferentially in the kidneys. Woodard, *et al.*[8] noted that liver might help to regulate the amount of circulating urokinase by relating the slow disappearance of the radioactivity in the blood later than 16 min after dosing to the radioac-

18) N. Ogawa, J. Ishiguro, R. Nonaka, M. Kurita and S. Tanaka, unpublished data.

tivity localized in the liver after administration of ^{131}I-urokinase into rats. However, further investigation may be required in order to elucidate the physiological significance of the localization of urokinase in liver and kidneys after administration of urokinase.

The radioactivity derived from the high-molecular compounds was scarcely found in the urine and bile (Table V). On the basis of this observation, ^{131}I-urokinase might be metabolized for the most part into low-molecular compounds in the body and then excreted mainly into the urine.

An artificial thrombus prepared by the method of Chandler[15] was incubated with ^{131}I-urokinase, ^{131}I-plasminogen or ^{131}I-plasmin, and the formed thrombus was assayed for radioactivity. It is suggested from Fig. 6 that urokinase, plasminogen and plasmin have an affinity for the thrombus. When a thrombus was prepared by employing the blood containing ^{131}I-plasminogen, incorporation of plasminogen into the thrombus in the course of the thrombus formation was suggested. On the basis of these results, there may possibly be the following three mechanisms by which urokinase causes clot lysis. The dissolution of *in vivo* clots is considered to proceed by the cooperation of these three mechanisms. (1) Plasminogen incorporated into a thrombus in the course of its formation is activated by urokinase to plasmin which causes clot lysis. (2) Plasminogen existing in blood is activated by urokinase, and the resultant plasmin dissolves clots. (3) Urokinase and plasminogen are incorporated into blood clots, and the resultant urokinase-activated plasmin in the clots dissolves the clots.

The urokinase concentration of 1 IU/ml employed in this *in vitro* experiment corresponds to the urokinase concentration in the blood of man weighing 50 kg, given intravenously 100 IU/kg of urokinase. The administered urokinase is assumed to be distributed equally into the whole blood soon after injection. Though the urokinase concentration in the tube was 1 IU/ml, the ratio of the radioactivity concentration of the throumbus (cpm/mg) to that of the blood (cpm/mg) was higher than 1. This result is of great interest in relation to a clinical dose of urokinase.