Differences in cell susceptibility to HA have been observed in other virus-red cell systems among individuals of one donor species. A slight variation was noted when chicken red blood cells were agglutinated by influenza virus(9), but a 4-fold variation occurred with mumps virus(10). These are similar to our findings that individual differences occur when human erythrocytes are agglutinated by the reoviruses. Not only do variations occur within a blood group, but significant differences occur from group to group.

Although reoviruses agglutinate A-factor erythrocytes at a higher virus dilution than non-A erythrocytes, this is not necessarily due to the Group A substance on the red cell, since erythrocytes of other blood groups are agglutinated also. Whether O-α-N-acetyl-D-galactos-aminoyl- (1→3) galactose, the substance most clearly associated with Group A specificity, as determined by inhibition of human A-isoagglutinins(11), would inhibit HA of reovirus remains to be tested. Although neuraminic acid of human red cells participates in myxovirus attachment as do sulfhydryl receptors, these same receptors are not implicated in reovirus attachment in HA tests(1,5); except that neuraminic acid is involved in attachment when Reovirus Type 3 is reacted with ox and sheep erythrocytes(12,13). Sulfhydryl groups on the viruses, rather than on the red cells, influence attachment in reovirus HA tests(1,12,13). Interaction between a glucoprotein on the surface of human erythrocytes and a glucoprotein, containing sulfhydryl groups, on the surface of the reoviruses results in hemagglutination, but the essential bond between virus and erythrocyte may not be through a sulfhydryl linkage(1). The factor or factors involved in higher HA titers when A-factor erythrocytes are reacted with reoviruses remains to be clarified.

Summary. A total of 187 human blood specimens were tested with one or several representative viruses of the reovirus group. The titers obtained with human erythrocytes containing the A grouping factor were slightly but significantly higher than those obtained with Group O or B cells.

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Evaluation of the Fibrinolytic State After Administration of Urokinase. (29131)

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During fibrinolysis, plasminogen(1,2), which is a constituent of human and animal blood, is converted into an active enzyme which lyses fibrin clots. This conversion occurs through the agency of so-called "fibrinolytic activators." A fibrinolytic activator,

urokinase(3,4), may be isolated from the urine(4-9) of many mammals. Its origin has not been established. Plasminogen activators have been obtained also from a variety of human and animal tissues(10). Several investigators have expressed the thought that these tissue activators are circulated through the blood stream and eventually are excreted in the urine as urokinase.

The present experiments were devised to study the origin of urokinase. Urokinase prepared from different species, as well as a tissue activator from normal pig heart(11,12), was administered to dogs, and its effect upon circulatory fibrinolytic activity and urokinase excretion was studied.

Experimental procedure. A. Preparation of urokinase(9). The pH of the urine was adjusted from 6.1-6.3 to 8.2 with 1 N sodium hydroxide. The mixture was kept in the refrigerator for 16 hours and centrifuged to remove any precipitate which may have formed. Crude urokinase was precipitated from the chilled solution (0°C) by addition of an equal volume of cold (—20°C) acetone. The precipitate was allowed to settle at —4°C for 16 hours, collected by centrifugation, suspended in saturated sodium borate solution and stirred for one hour at room temperature. The urokinase solution was dialyzed against 2 changes of 25 volumes of saturated sodium borate solution for 24 hours each, followed by dialysis against the same volume of 0.06 M sodium phosphate, pH 7.3, for 6 hours. Any precipitate present was removed, and the supernatant containing the concentrated urokinase solution was frozen. The urokinase yield was 60% from male human urine, 40% from rabbit urine, and 70% from dog urine.

Attempts were made to prepare urokinase from the urine of the horse and the rat. Horse urine was rather low in urokinase, containing only about 7 units/ml *vs* 50 units/ml for human urine, 20 units/ml for dog urine, and 160 units/ml for rabbit urine. Rat urine was exceptionally low in urokinase, assaying less than 3 units/ml. The recovery of rat urokinase was less than 30%, which, together with the difficulty of procuring large pools of urine from the rat, did not make it feasible to prepare urokinase from rat urine. Although the yield of urokinase from horse urine was about 40%, the low concentration in the starting urine made it an unattractive starting material.

Other methods for the preparation of urokinase from urine which have appeared in the literature were found satisfactory for processing human urine but could not be applied successfully to the isolation of urokinase from the urine of other species.

B. Preparation of plasminogen activator from pig heart. A plasminogen activator(11, 12) was isolated from the hearts of 50 freshly killed pigs. The hearts were chopped, macerated in a meat grinder, extracted with 4 v/w of 5 M urea, pH 7.0, and stirred for 2 hours, and the spent meat was removed by straining through cheesecloth. The extract was brought to 35% saturation with ammonium sulfate and allowed to settle in the cold for 16 hours. The precipitate was discarded, and the ammonium sulfate concentration was increased to 50% saturation. This precipitate was collected and restored in 0.005 M phosphate buffer at pH 7.6, and the activator concentrate was kept frozen until use.

C. Administration of urokinase. Female mongrel dogs, either unanesthetized or anesthetized (sodium pentobarbital, 35 mg/kg I.V), were restrained in a supine position, and their bladders were catheterized with a No. 12 French rubber catheter. Test materials were administered by venoclysis and blood samples were withdrawn from the contralateral limb by venipuncture. In the anesthetized state, surgical exposure of the veins was carried out.

D. Reagents. *Anticoagulant solution*—0.1 M Sodium oxalate solution.

Sodium borate buffer—Sodium borate, 1 g; sodium chloride, 9 g; q.s. to 1 l with distilled water, pH 8.9.

Barbital buffer—0.1 M Sodium diethylbarbiturate (Barbital Sodium Merck), 331 ml; 0.1 M hydrochloric acid, 169 ml; dilute with 160 ml distilled water, pH 7.8.

Fibrinogen solution for fibrin plate—Bovine fibrinogen (Armour), 0.5%, in barbital buffer was kept at —20°C. Prior to use it was thawed, centrifuged at 3°C and clarified by filtration.

Thrombin solution—Bovine thrombin (Parke, Davis), 5,000 NIH units, was dissolved in 5 ml of 50% glycerol. This concentrate was stable at 2-5°C and diluted with either borate or barbital buffer.

E. Assay for circulatory fibrinolytic activity. Coagulation of blood samples was prevented by addition of 0.12 ml of 0.1 M sodium oxalate solution per ml of blood. The plasma was separated by centrifugation at 3-5°C, diluted with 19 parts of distilled water and acidified to pH 5.3-5.4 by addition of a few drops of glacial acetic acid. In an alternate procedure, dilution and acidification were carried out as a single step, using a dilute aqueous acetic acid solution. The euglobulin precipitate was allowed to settle at 3-5°C for one hour, collected by centrifugation and restored to the original plasma volume with sodium borate buffer. The euglobulin solution (0.5 ml) was added to a solution of 0.4 ml of bovine fibrinogen containing 50 mg of fibrinogen in 20 ml of the sodium borate buffer. Addition of 0.1 ml of bovine thrombin (2 NIH units) produced clotting, and the time required for complete lysis was observed. Fibrinolytic activity was expressed in terms of a Fibrinolytic Index, which was calculated from the expression, 10,000/t, where t is the time in seconds required to lyse the fibrin clot(13).

F. Assay for urokinase activity. The urokinase activity of the urine was measured on the bovine fibrin plate. For preparation of the fibrin plates, 6 ml of fibrinogen was poured into a plastic Petri dish,* 9 cm in diameter, and clotted with 4 ml of thrombin (20 NIH units). The sample, 0.025 ml, was dropped onto the fibrin plate. The plate was incubated for 16 hours at 37°C and the lysed zone was measured. Urokinase (Leo†) was used as the primary standard, and results were evaluated by reading from a standard curve.

Results. A. Administration of homologous urokinase. Urokinase prepared from dog urine was administered intravenously (Fig. 1). Half the dose employed was given by stat injection, while the remainder was infused over a period of 30 minutes. Samples of blood were taken before administration of the drug, during infusion, and 30, 90 and 150 minutes later. Circulatory fibrinolytic activity was present at the end of the infusion and could usually be detected up to 60 minutes; however, samples of blood taken 2½ hours after administration of the urokinase showed little fibrinolytic activity which approached the preinfusion level.

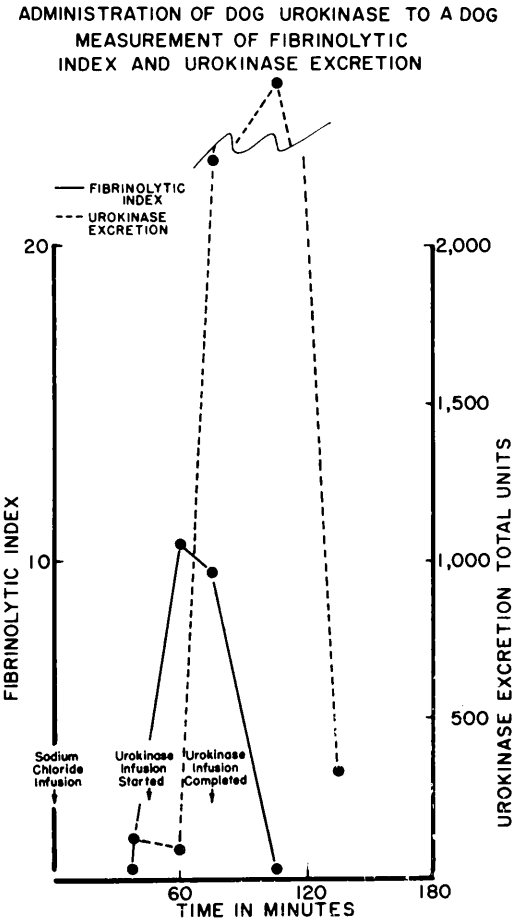

FIG. 1

Urine was collected concurrently with the blood samples. Increased urokinase excretion followed closely the pattern of euglobulin lysis. Increased amounts of urokinase were present at the end of the infusion and for an additional 1½ to 2½ hours. Approximately 10% of the exogenous administered

* Falcon Plastics, Division of B-D Laboratories, Inc.

† Leo Pharmaceutical Products, Denmark.

TABLE I. Effect of Urokinase from Different Species on the Fibrinolytic Systems of the Dog.

Exp	Source of urokinase	Amount infused in UK units/kg	Fibrinolytic index					Urokinase excretion							
			Control	During infusion	At end of infusion	30 min. after infusion	60 min. after infusion	Control		During infusion		At end of infusion		30 min. after infusion		60 min. after infusion	
								Units /ml	Total units	Units /ml	Total units	Units /ml	Total units	Units /ml	Total units	Units /ml	Total units
A	Dog	3,550	0	10.4	9.5	0	0	11	102	70	70	107	2,260	130	4,810	13	312
B	Human	4,920	0	6.7	6.2	0	0	21	357	21	357	8	96	6	92	18	288
C	Rabbit	4,146	0	10.4	7.0	5.8*	0	38	608	36	648	34	680	32	608	11	187
D	Pig heart activator	8,540	0	416	333	36.2	0	25	475	40	1,320	26	1,480	5	360	4	360

* Sample taken fifteen min. after end of infusion.

urokinase was recovered in the urine. Even more striking than the over-all recovery was the increase of urokinase concentration from a preinfusion level of 10 units/ml to 64, 70 and 130 units/ml, respectively, in 3 dogs.

B. Administration of heterologous urokinase. Administration of urokinase prepared from human or rabbit urine (Table I) produced circulatory fibrinolytic activity, but no effect on urokinase excretion was observed.

C. Administration of pig heart activator concentrate. Activator extracted from 2 pig hearts rapidly produced circulatory fibrinolytic activity. Urokinase excretion studies were not decisive. There was a slight increase in urokinase excretion which was minimal in relation to the increase in circulatory fibrinolytic activity. In repeated experiments, the same pattern was observed.

Circulatory fibrinolytic activity did not increase in control experiments in which physiological saline or phosphate-mannitol solution was infused. Increased fibrinolytic activity was observed occasionally if the euglobulin precipitates were not tested immediately, but were kept overnight in the refrigerator. Plasma samples, however, could be stored at 3°C overnight with no effect on fibrinolytic activity. Euglobulin precipitates prepared from human plasma did not undergo alteration under these conditions; other species were not tested.

Recovery of fibrinolytic activity in the euglobulin precipitate was influenced greatly by the pH at which it was precipitated. Blix(14) recommended that for maximum activity the euglobulin fraction from human plasma be precipitated at pH 6.4. In the present study, plasma from normal human subjects yielded inactive euglobulin precipitates when they were prepared at pH 5.4 and 5.9. If precipitation was carried out at the recommended pH of 6.4, the euglobulin was very active. Consequently, it was thought desirable not to adopt this recommendation but to prepare the precipitates at pH 5.4 to eliminate, if possible, false positive results.

Discussion. Urokinase, the urine plasminogen activator, has been isolated from human and animal urines, but the source of its ori-

gin is in doubt. Several theories have been proposed: 1) that urokinase originates in the pelvis, ureter or mucous membranes of the bladder(15), 2) that it derives from tissue plasminogen activator normally associated with the circulating blood(16,17), and 3) that urokinase is synthesized in the kidney, circulated through the vascular tree and is excreted ultimately by the urinary system (18).

The data presented here demonstrate that intravenous administration of homologous urokinase leads to increased urokinase excretion and to enhanced circulatory fibrinolytic activity in the dog. This supports the hypothesis that urokinase is compatible with circulating blood and that it can pass through the tubular excretory system, including probably the glomerulus.

Kjeldgard(19) injected human urokinase into dogs and humans, but did not detect increased excretion of urokinase in the urine. In our study, administration of heterologous urokinase caused increased fibrinolytic activity in the circulation only, and did not produce an increase in urokinase excretion. Thus, administration of urokinase from human sources should not be expected to produce increased urokinase excretion in the dog.

Human urokinase produces strong fibrinolytic activity when administered intravenously in humans(20). However, in a different study such procedure failed to elicit a concomitant increase in excreted activator, reported on the basis of urine concentration (units/ml)(19). From the animal studies reported herein, it may be observed (Fig. 1) that the renotrophic effect is of short duration. Therefore, urine samples should be collected in frequent serial samples to prevent dilution of the urokinase, if relative urinary concentration (units/ml) is to be used rather than the more desirable absolute excretion rate.

Our experiments in dogs indicate that urokinase passes from the blood stream into the urine, and therefore the possibility exists that blood plasminogen activator may be the origin of urokinase.

Summary. Experiments on the recovery of urokinase administered intravenously to laboratory dogs were described. Urokinase prepared from animal and human sources produced circulatory fibrinolytic activity, but only homologous urokinase administration produced increased urokinase excretion.

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