THE FIBRINOLYTIC ACTIVITY OF URINE.

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THE fibrinolytic activity of normal urine was first described by Macfarlane and Pilling (1947). The enzyme was found in all samples examined, usually in considerable amounts, and lysis of a fibrin clot was caused by urine dilutions of 1/2000 to 1/4000. Fibrinolytic activity of the circulating blood has also been described in anaphylaxis and peptone shock by Rocha e Silva, Andrade and Teixeira (1946) and by Ungar and Mist (1949). Macfarlane (1937), Macfarlane and Pilling (1946, 1947) and Macfarlane and Biggs (1946) found it in states of anxiety and shock, after exercise, and after the administration of adrenaline. Although exercise produced a slight increase in urinary fibrinolysin the reduction in the rate of urinary excretion made interpretation difficult.

Earlier reports on the presence in urine of proteolytic enzymes in general were made by Farnsworth, Speer and Alt (1946), who showed that the excretion of a pepsin-like enzyme was reduced in pernicious anaemia and other conditions with achlorhydria, and by Baumann and Loeper (1922), who described the excretion of pepsin, erepsin and trypsin-like enzymes and commented on the marked variability in the rate of excretion. The restricted range of pH in which gastric proteinases are active, the highest being 4·5 for cathepsin (Buchs, 1949), excludes the stomach as a source of the fibrinolytic enzyme demonstrated by Macfarlane.

The action of streptokinase on a globulin fraction of plasma (plasminogen) to form an active fibrinolysin (plasmin) was first described by Milstone (1941). The activation of plasminogen by a wide variety of different substances has since been described, including tissue suspensions (Astrup and Permin, 1947, and Lewis and Ferguson, 1950). Tagnon and Petermann (1949) and Tagnon and Palade (1950) showed that rat lung is particularly effective and that the active principle is in the mitochondria. A kinase of the same type has been demonstrated in malignant cells by a number of workers, including Fischer (1946) and Goldhaber, Cornman and Ormsbee (1947).

From this work there would appear to be three possible sources for the urinary fibrinolysin.

The enzyme may be plasmin either excreted after activation in the circulation or as its precursor plasminogen. In this case activation must take place during passage through the renal tract.

Secondly the enzyme may be trypsin which has been absorbed into the blood, combined with serum anti-trypsin and then excreted by the kidneys after separation from its antibody.

Thirdly it is possible that fibrinolysis caused by urine is due to activation of plasminogen contaminating the fibrinogen used as a substrate. The fibrinogen used by Macfarlane (1947) was purified by repeated precipitation, but it is not clear whether complete removal of plasminogen was obtained.

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This paper describes experiments designed to identify the source of urinary fibrinolysin by testing each of these hypotheses.

MATERIALS AND METHODS.

Fibrinogen.

Human fibrinogen was kindly supplied by the Blood Products Research Unit of the Lister Institute. It was used in 0·1 per cent and 0·2 per cent solutions in oxalated phosphate buffer saline, pH 7·6, and was shown to contain plasminogen (*vide infra*).

Thrombin.

Human thrombin was also supplied by the Lister Institute and was used in a buffered saline solution of 5 units/ml.

Estimation of fibrinolysis.

1. *Blood.*—Citrated plasma was examined by the technique described by Macfarlane and Pilling (1946), in which fibrinogen is added to diluted plasma and clotted with thrombin. If lysis of the clot occurred within 18 hours the test was regarded as positive.

2. *Urine.*—All samples of urine were adjusted to pH 7·5 by the addition of strong sodium hydroxide solution, using nitrazine paper as an indicator. Serial 1 : 2 dilutions, volume 0·5 ml., were made in buffered saline down to 1 : 16 and examined by the method described by Rocha e Silva and Rimington (1948) for the estimation of activated ox globulin, by the addition of 0·3 ml. of 0·2 per cent fibrinogen solution. The fibrinogen was clotted by the addition of thrombin and incubated at 37° to allow lysis to occur. Some samples of urine, chiefly concentrated ones, failed to clot unless diluted to 1 : 16. Precipitation of the fibrinogen or antithrombic activity of the urine could both be excluded as possible causes but the phenomenon remained unexplained.

The times required for fibrinolysis by urine varied from 40 minutes for undiluted urine to 24 hours for some 1 : 16 dilutions. The results were recorded as strongly positive if lysis occurred within 4 hours in any tube, positive if lysis occurred overnight in a dilution greater than 1 : 4, and weakly positive if lysis only occurred in a dilution of 1 : 4 or less overnight.

Estimation of proteolysis.

Todd's (1949) modification of the method described by Oakley, Warrack and van Heyningen (1946) was used in which azocoll, a compound between an azo-dye and powdered hide, is employed as the substrate. The colour released from the azocoll was measured in arbitrary colour units in a King single cell photo-electric colorimeter using a green tricolour filter. It was standardized in a series of 25 tests against weighed amounts of crystalline trypsin. The mean readings for each 25 tests were as follows: No trypsin, 20·9 $\pm$ 2·1; 1·6 μg. trypsin/ml., 41·2 $\pm$ 4·4; 3·1 μg. trypsin/ml., 56·8 $\pm$ 2·8; 6·25 μg. trypsin/ml.,80·1 $\pm$ 7·7; and 12·5 μg. trypsin/ml., 108·5 $\pm$ 7·1 colour units. A standard curve was prepared from these figures, the results of proteolysis estimations being expressed in μg. standard trypsin/ml.

Surgical technique.

Rabbits were anaesthetised with 0·5 ml. of veterinary Nembutal intravenously; anaesthesia was maintained with open ether. Excision of the stomach, spleen, pancreas and duodenum was performed through a left paramedian incision. The oesophagus was doubly clamped, ligated and divided, the splenic pedicle ligated and the spleen removed. Clamps were then placed on all vessels from the stomach, duodenum and pancreas. These were ligated and divided in turn. The stomach, duodenum and pancreas were removed in one piece after ligation of the upper end of the jejunum. Surgical procedures on the kidney were carried out through a lateral incision. Injection of the renal artery was performed from the anterior aspect. The nephrectomy was performed from the posterior approach, but the peritoneum was opened and the abdominal contents packed off to provide a reasonable control experiment for the procedures on the stomach.

In these cases the animals chosen were male, and urine was obtained by extraperitoneal suprapubic exposure of the bladder and aspiration with a 10 ml. syringe and a fine needle. Female animals were reserved for experiments involving catheterization.

EXPERIMENTAL.

The Relation of Proteolysis to Fibrinolysis.

The batch of azocoll in use was shown by the standardization experiments to be susceptible to trypsin. Its susceptibility to active plasmin (reported by Todd, 1949) was confirmed by the activation of plasminogen by streptokinase and the demonstration of the release of colour from azocoll by the product (Table V).

The presence of plasminogen in the fibrinogen used as a substrate was shown by the addition of 0·3 ml. streptokinase (Burroughs Wellcome & Co.) in the standard test for fibrinolysis, which occurred in 20 minutes. It was also shown that a solution of trypsin of 1·6 μg./ml. would cause complete fibrinolysis only in 80 minutes. The two reactions thus differ in sensitivity.

In any experiment the demonstration of an enzyme active against azocoll indicates the presence of trypsin, active plasmin or some similar but unidentified active enzyme. The demonstration of fibrinolytic activity must be interpreted however as showing the presence of either an active enzyme such as trypsin or plasmin or of a kinase capable of activating the plasminogen contaminating the substrate. The employment of both tests will thus show the presence of a kinase providing that active plasmin and trypsin are absent or are present in negligible amounts.

The Fibrinolytic Activity of Normal Urine.

Catheter or mid-stream specimens of urine were examined from 53 patients suffering from a variety of diseases and from 13 normal pregnant women. In each case the urine was sterile on culture and contained no excess of leucocytes or red cells in the centrifuged deposit. Fibrinolytic activity was demonstrated in all samples, but no proteolytic activity against azocoll could be found in any of the 22 samples on which the test was carried out.

The effect of variation in the rate of excretion of urine on its fibrinolytic activity was shown by collecting all urine from a normal subject at intervals, including an overnight sample, and during a diuresis induced by drinking two pints of water. The results of this experiment are shown in Table I.

TABLE I.—*The Fibrinolytic Activity of Urine from a Normal Subject.*

Time between specimens in minutes.	Volume of specimens (ml.).	Rate of excretion (ml./min.).	Time for fibrinolysis of 1 : 2 dilution (min.).
630	385	0·6	100
120	135	1·1	80
50	52	1·0	100
100	122	1·2	100
20	200	10·0	170
10	115	11·5	180
5	59	11·8	180
5	62	12·4	170

The figures show a decrease in the fibrinolytic activity of the urine with an increase in its rate of excretion. They also show that over a short period of time a water diuresis will cause a constant rate of excretion between 10 and 13 ml./minute. It is possible therefore to study the factors which influence the fibrinolytic activity of normal urine provided that a diuresis is induced and samples are taken at short intervals.

The Effect of Active Plasmin in the Blood on the Excretion of Fibrinolysin.

Man.

Water was given to normal subjects and samples of urine collected until a constant rate of excretion was established. Adrenaline hydrochloride 1 : 1000 was injected intramuscularly in a dose of 0·5 ml., and then repeated in doses of 0·2 ml. until either tachycardia, an increase in the systolic blood pressure, a marked change in the reflexes or a coarse tremor occurred. Blood was taken by venepuncture and samples of urine collected at intervals during and until 30 minutes after the injection. The blood and urine were examined for fibrinolytic activity, the urine also being examined by the azocoll technique.

In three such experiments the injection of adrenaline caused a reduction in the rate of excretion of urine, but in eight others no such change occurred. In all cases the blood showed active fibrinolysis, but no increase in the activity of the urine was observed. The results of a typical experiment are shown in Table II.

TABLE II.—*The Fibrinolytic and Proteolytic Activity of Urine after the Administration of Adrenaline in Man.*

Time in minutes.	Blood pressure (mm. Hg.).	Injection of adrenaline (ml.).	Rate of excretion of urine (ml./min.).	Fibrinolysis : urine.	Proteolysis (trypsin (μg./ml.).
0	130/78	—	—	Strongly positive	Zero
11	—	—	11·2	Positive	,,
24	160/74	1·1	10·0	,,	,,
35	138/80	—	10·1	,,	,,
60	—	—	12·0	,,	,,

Blood was taken six minutes after the injection of adrenaline and was found to show active fibrinolysis. The azocoll control tube gave a reading of 19 colour units.

Rabbit.

The examination of catheter specimens of urine from six rabbits showed marked fibrinolytic activity with no significant degree of proteolysis of azocoll.

Thus in the rabbit the same disparity of proteolytic and fibrinolytic activity has been observed as was seen in man. It was hoped to induce fibrinolytic activity in the blood of rabbits by the injection of adrenaline in order to repeat the experiments described above in man, but increasing doses, including one fatal one, caused no fibrinolysis in blood removed from an ear vein five minutes after the injections. Previous workers, including Tillett and Garner (1933) and Kaplan (1946), have commented on the resistance of rabbit plasma to fibrinolysis.

The Influence of Alterations of the Gastro-intestinal Secretion on the Excretion of Fibrinolysin.

Man.

Under the controlled conditions used for the experiments with adrenaline the effect of the administration of histamine, prostigmine and carbachol was investigated. Histamine was given in doses up to 1·0 mg. by subcutaneous injection with the production of headache and flushing, but with no influence on the excretion of fibrinolysin in any of seven experiments.

Prostigmine was given in a dose of 0·1 mg. and carbachol in a dose of 0·25 mg. each on two occasions also with no effect on the excretion of fibrinolysin in the urine.

The urine from 13 patients who had undergone subtotal gastrectomy a few days previously was examined, and compared with urine from 12 patients in the same wards who had experienced a surgical operation not on the gastro-intestinal tract. Both series showed a high and similar degree of fibrinolytic activity.

Rabbit.

Since rabbit urine shows marked fibrinolytic activity but no significant degree of proteolysis of azocoll this animal was chosen to determine the effect of the intravenous injection of trypsin on the excretion of fibrinolysin. Crystalline bovine trypsin (Armour & Co.) was dissolved in sterile saline and used for the experiments. To establish the validity of the conclusions toxicity tests were carried out by which it was shown that in rabbits doses up to 1·0 mg. intravenously produced no visible effect. A dose of 10 mg. given to one animal was rapidly fatal.

Rabbits were catheterised and samples of urine taken before and at intervals after the injection of 1·0 mg. trypsin dissolved in 0·2 ml. saline. The samples of urine were examined for fibrinolytic and proteolytic activity. The results of one of five such experiments are shown in Table III, the rate of excretion of urine remaining reasonably constant throughout.

TABLE III.—*The Fibrinolytic and Proteolytic Activity of Rabbit Urine after the Intravenous Injection of Bovine Trypsin.*

Time in minutes.	Injection of trypsin.	Fibrinolysis.	Proteolysis (trypsin μg./ml.).
0	1·0 mg. at 6 min.	Positive	Zero
14	—	,,	1·8
30	—	,,	3·0
72	—	,,	1·3

Azocoll control tube = 25 colour units.

The table shows a marked increase in the proteolytic activity of the urine within a few minutes of the injection and a decrease after 30 minutes. Direct injection into the renal artery of 2·0 mg. trypsin also produced an increase in the proteolytic activity of the urine (from zero to 1·5 μg. trypsin/ml.), but was associated with a marked reduction in the rate of excretion of urine presumably due to cortical arterial spasm and shock.

Attempts were made to influence the rate of excretion of fibrinolysin by total gastrectomy accompanied by excision of the pancreas, duodenum and spleen in rabbits. Three experiments of this type were performed. The animals were given intravenous saline at intervals during and after the operations, and were maintained under light anaesthesia until they were killed a few hours later. As a control a right nephrectomy was performed on another animal, which was allowed to survive. Samples of urine taken from these animals before, during and after the operation were examined. The results of one of these experiments and of the nephrectomy are shown in Table IV.

TABLE IV.—*The Fibrinolytic and Proteolytic Activity of Rabbit Urine after Total Gastrectomy with Excision of Pancreas, Spleen and Duodenum and after Nephrectomy.*

Rabbit.		Fibrinolysis.	Proteolysis (trypsin μg./ml.).
210/50	Before operation	Strongly positive	Zero
	At end of total gastrectomy (45 min.)	,, ,,	0·8
	After further 90 min.	,, ,,	1·2
208/50	Before operation	Positive	Zero
	At end of nephrectomy (30 min.)	,,	,,
	After further 60 min.	,,	,,

Azocoll control tube = 28 colour units.

These experiments show no appreciable change in the excretion of fibrinolysin after removal of the source of gastro-intestinal enzymes within two hours of clamping the venous drainage of the pancreas and duodenum. Since injected trypsin is mostly excreted within 30 minutes it is believed that if the fibrinolysin were derived from the pancreas a definite fall in activity of the urine would have been found in two hours.

Activation of Plasminogen by Urine in Man.

Plasma was added to urine and incubated for 15 minutes at 37° and examined for fibrinolytic and proteolytic activity. The findings were compared with the activity of plasma and urine alone and with plasma after activation with streptokinase. The results of one of several such experiments are shown in Table V.

TABLE V.—*The Activation of Human Plasminogen by Urine and Streptokinase.*

Urine (ml.).	Plasma (ml.).	Streptokinase (ml.).	Saline (ml.).	Fibrinolysis.	Proteolysis (trypsin μg./ml.).
0·5	—	—	0·5	Weakly positive	Zero
0·5	0·5	—	—	Strongly positive	4·6
—	0·5	0·5	—	,, ,,	5·2
—	0·5	—	0·5	Weakly positive	3·0
—	—	—	1·0	Negative	Zero

The citrated plasma used in these experiments was obtained from normal subjects, and showed slight spontaneous fibrinolysis with a moderate degree of activity against azocoll. The figures for proteolysis of plasma activated by urine or streptokinase are greater than for plasma alone, and this is supported by the marked difference in the degree of fibrinolysis. The presence of two sources of fibrinogen (in the tests for fibrinolysis only) complicates interpretation, but in fact the greater amount of substrate conceals an even higher fibrinolytic activity than is apparent.

DISCUSSION.

The experiments described above show that in both man and the rabbit the fibrinolytic activity demonstrable in urine is not accompanied by an equivalent degree of proteolytic activity, the level of the latter being too low to be detectable by the azocoll technique. Plasmin has been shown to be active against azocoll; hence it appears unlikely that the fibrinolysin in urine is active plasmin. It has also been shown that as far as the response to adrenaline is concerned the appearance of plasmin in the blood is not followed by a change of the level of the fibrinolytic activity in the urine.

It has further been shown that the intravenous injection of trypsin in rabbits leads to its appearance in the urine in amounts detectable by the azocoll method without an appreciable change in the fibrinolytic activity. Neither subtotal gastrectomy in man nor resection of the stomach, spleen and duodenum in the rabbit leads to a reduction in the fibrinolytic activity of the urine. It appears therefore that urinary fibrinolysin is unlikely to be derived from the proteolytic enzymes of the gastro-intestinal tract.

The presence of plasminogen in the fibrinogen used as a substrate was demonstrated by its activation when streptokinase was added. It appeared possible therefore that the fibrinolysis induced by urine was due to the activation of plasminogen by a kinase present in the urine, a hypothesis which was supported by direct experiment. It seems probable that the activating substance is derived from the renal tract.

SUMMARY.

The fibrinolytic activity of urine is confirmed in man and the rabbit.

Activation of the circulating plasminogen does not alter the rate of fibrinolysin excretion in the urine.

Urinary fibrinolysin does not appear to be derived from the gastro-intestinal tract.

The fibrinolytic activity of urine seems to be due to a kinase which is capable of activating plasminogen. The kinase is probably derived from the tissues of the renal tract and activates plasminogen which is present as a contaminant in the fibrinogen substrate.

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