Urokinase Excretion in Health and its Alteration in Certain Disease States

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Introduction

It has been known since the last century that urine would digest impure fibrin clots, an action at that time attributed to the presence of trypsin (1). However both Williams (2) and Astrup and Sterndorff (3) have shown that the lytic action of urine upon fibrin clots is due not to the presence of trypsin, but to the presence of a plasminogen activator, now named urokinase. The plasminogen, invariably present in the fibrin clot, is activated by urokinase to plasmin, and enzymatic digestion causes clot dissolution. Urokinase has recently been prepared in a highly purified form (4) and kinetic studies show that it activates plasminogen by a first order enzymatic reaction (5 and 6).

Information concerning the physiological functions of and variations in urokinase excretion is as yet scanty. Bjerrehuus (7) observed that urokinase concentrations were similar in male and female urine and also in bladder urine and that obtained from the renal pelvis. Recently von Kaulla (8) has provided evidence that urokinase activity represents, at least in part, excreted plasma plasminogen activator (referred to hereafter as plasma activator). This work raises the important possibility that determination of urokinase excretion over a period of time may serve as a simple measure of the degree of plasma fibrinolytic activity, since it is now known that this activity depends primarily upon plasma activator concentration (9).

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The present communication describes an assay method for urokinase and the variations of urokinase excretion occurring under physiological circumstances and in disease. The results indicate that urokinase excretion may be significantly altered in a variety of disease states and that these alterations are consistent with the view that urokinase activity represents, at least in part, excreted plasma activator.

Methods

Urokinase assays were made by the fibrin plate method (7). Plates were prepared by the method of Mullertz (10) except that the final fibrinogen*) concentration was 0.2%. A single plate was used for each assay, three 30 lambda drops of undiluted, 1 : 2 and 1 : 4 urine being used (occasionally, when urokinase concentration was very high the dilution factors were doubled). The plates were incubated for 16 hours at 30° C and the lysed zones defined by the addition of a drop of congo red (0.1%). The product of two perpendicular diameters was used as a measure of the lysed zone and the urokinase concentration (units/ml) determined by reference to a freshly run standard curve. The triplicate readings on each sample were averaged and the result multiplied by the urine specimen volume.

A single batch of urokinase, prepared by the method of von Kaulla (11), and stored in a series of small tubes at — 20° C was used as a standard. Fig. 1 shows the mean assay

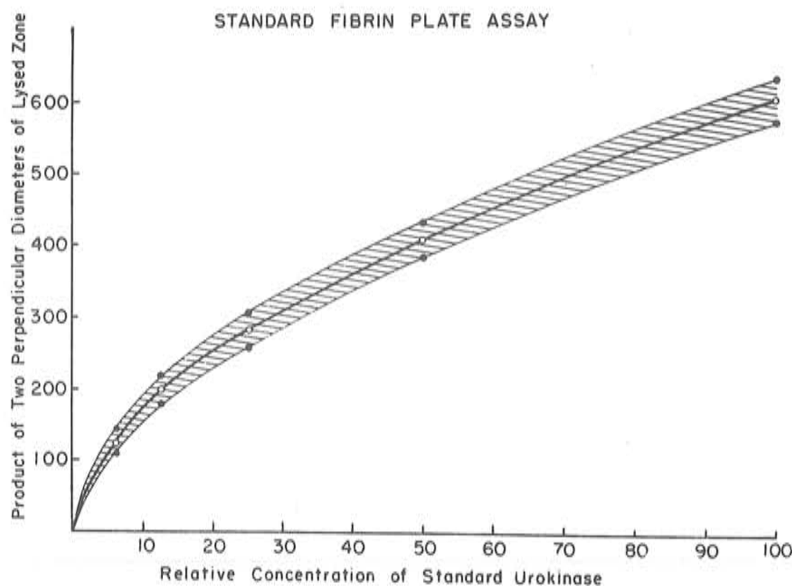

Fig. 1: Variability of standard fibrin plate assay run throughout the investigation. Center line indicates mean values, and shaded area shows standard deviation.

*) Bovine fibrinogen, 92% of the nitrogen being clottable by thrombin. Kindly supplied by Dr. Kent Miller, New York State Laboratories, Albany, N. Y.

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(compiled from all standard curves run throughout the study) and its standard deviation (approximately 5%). The standard was assigned an arbitrary value of 100 urokinase units/ml and all experimental readings were expressed in these units. Assay of our standard preparation against a highly purified standard urokinase preparation**) (4) showed that the units used in this communication were 1/8 the potency of the recently described Danish unit.

The shape of the assay curve was such that the method became less sensitive at higher urokinase concentrations. For this reason and also because the agreement between triplicate assay readings was thereby improved, urine for assay was suitably diluted so that the product of measurement diameters (in mms) did not exceed 400. Small incompletely lysed zones were read as indicating zero activity.

Provided that the pH was maintained between 6—8, the urokinase activity of urine remained stable for days. Samples kept at 25° C had lost only 20% of their original activity at the end of 8 days, though a temperature of 37° C caused a loss of 50% (1/10 000 merthiolate concentration). Urine sediment from infected urine did not affect the assay nor did white cells, obtained from blood and added to urine, affect the assay readings. Urinary pathogens (B coli, coagulase-positive staphylococci, Proteus vulgaris and P. aeruginosa) incubated with aliquots of urine, for 12 hours at 37° C, did not affect the readings, nor did saphrophytic organisms alter the result. Uropepsin is known to be without activity at the assay pH used and the effect of excreted trypsin was excluded by performing similar experiments to those described by Astrup and Sterndorff (3). Though the presence of urinary materials inhibitory to urokinase could not be excluded, experiments with added urokinase yielded quantitative recoveries suggesting their absence. Thus the assay method though theoretically non-specific owing to the use of a fibrin subtrate, appeared in practice to measure only urinary plasminogen activator activity. Since the agreement between triplicate assay readings had a standard deviation of ± 7%, the method was also a reproducible one.

Most of the work was performed using hospitalized patients, though healthy laboratory personnel were also used. The „normal" group of patients comprised those admitted to the hospital for the investigation of symptoms later shown to be unimportant, those admitted prior to elective surgery, those suffering from psychosomatic disease of a mild nature, patients convalescent from fractures and a small group afflicted with osteoarthritis. Timed urine collection was made between 9 : 00 a. m. and Noon and urokinase assays were made that afternoon. Urokinase excretion figures in the text refer to the total three hour excretion period.

Results

Physiological conditions

Urokinase excretion rates appeared, at least under the conditions of this study, to be independent of the urine volume. Calculation of the correlation coefficient between urine volume and urokinase excretion showed $r = 0.12$ (not significant p greater than 0.1).

Urokinase excretion rates remain relatively constant throughout a 24 hour period. Fig. 2 illustrates the averaged data from six normal subjects in each of whom 6 hourly collections were made continuously for 5 days. Though the evening specimens 6 : 00 p. m. to Midnight tended to be lower in urokinase content than the others, statistical testing between the collection periods showed p to be greater than 0.1 in all cases.

**) Kindly supplied by Dr. J. Ploug, Leo Pharmaceutical Co., Copenhagen, Denmark.

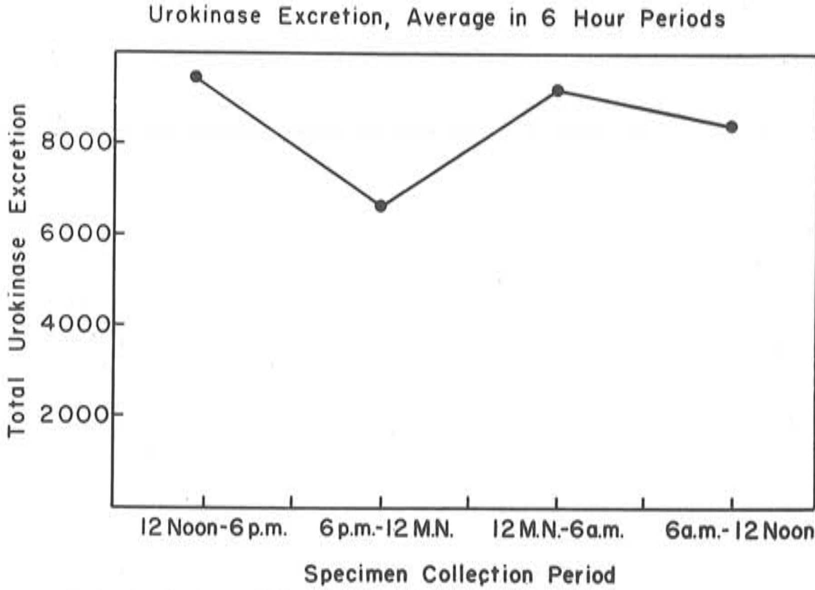

Fig. 2: Each point in the graph is the average of 30 determinations. Urine was collected 6-hourly for 5 days in 6 normal subjects.

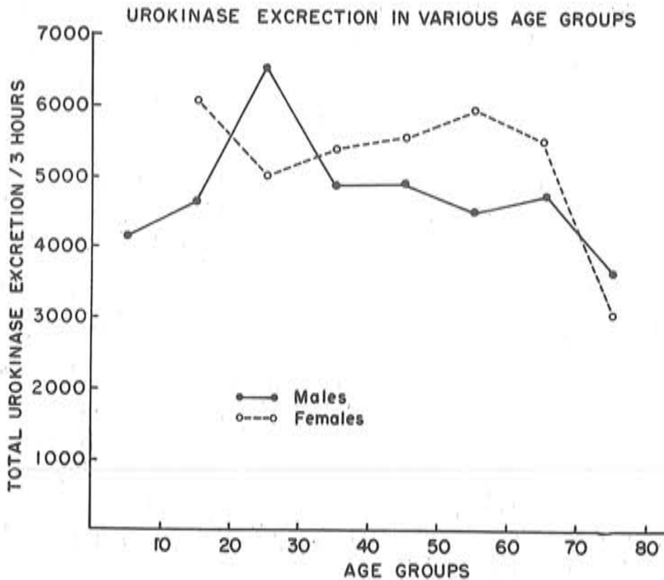

Fig. 3: Differences between male and female excretions are not statistically significant ($.05 < p < 0.1$). Differences between the various age groups are not significant ($p > 0.1$).

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Daily urokinase excretion rates, under conditions of health, tended to remain relatively constant, for instance the 24 hour excretion rate in one of us over a 10 day period was 46 000 ± 6 600 units. Similarly the variation for the 9 : 00 a. m. to Noon daily excretion over a 21 day period was small, 6590 ± 1550. Again the data illustrated in Fig. 2 had a standard deviation of mean ± 20%.

Tab. 1: Urokinase excretion in cardiac diseases

Disease State	Number of Observations	Number of Patients	Mean Urokinase Excretion and Standard Deviation	"P"
Normal subjects	165	45	5328 ± 4485	—
Myocardial*) Infarction	405	25	7345 ± 7210	< 0.001
SK Treated Myocardial Infarction	121	5	9159 ± 9219	$0.001 < p < 0.01$
Coronary**) Insufficiency	44	10	8943 ± 3976	0.02
Congestive Cardiac Failure	45	11	2369 ± 1080	$0.05 < p < 0.1$
Arteriosclerotic Heart Disease (Compensated)	87	20	4512 ± 3300	> 0.1
Rheumatic Valvular Heart Disease (Compensated)	16	4	3183 ± 3980	> 0.1
Essential Hypertension	26	5	3243 ± 3180	> 0.1

*) Within first month after infarction

**) Within first week of attack.

Thus it was apparent that a valid estimate of daily urokinase excretion, at least under conditions of health, could be obtained by the use of a timed 3 hour urine collection period (9 : 00 a. m. to Noon was adopted for convenience). In most instances three or more successive 3 hour collection periods were made at daily intervals in each patient to obviate the effect of chance variation.

Tab. 2: Urokinase excretion in renal disease

Disease State	Number of Observations	Number of Patients	Mean Urokinase Excretion and Standard Deviation	"P"
Normal subjects	165	45	5238 ± 4485	—
Pyelonephritis (without renal failure)	35	9	4512 ± 4830	> 0.1
Glomerulonephritis Chronic (2—13 age group)	27	9	3765 ± 3430	> 0.1
Multiple Myelomatosis (with renal impairment)	10	2	802 ± 1430	> 0.1
Uremia (Terminal pyelonephritis and Glomerulonephritis)	61	10	1015 ± 1150	< 0.001

Examination of the normal control data suggested that urokinase excretion rates were uninfluenced by age or sex. However this group was a comparatively small one (40 patients, 165 observations) and to reinforce this conclusion the analysis was conducted using all the observations displayed in Tables 1, 2 and 3 belonging to those disease groups that did not differ significantly from the control group ($p > 0.1$ in each case). The mean of the new group (190 patients, 702 observations) did not differ from the control mean ($p > 0.1$). The data are displayed in Fig. 3 and it is seen that males tended to run a somewhat lower excretion rate than females at all ages, but the difference did not quite reach the conventional level of statistical significance ($0.05 < p < 0.1$). Likewise analysis for age differences was not significant ($p > 0.1$). It was concluded that analysis between disease groups without regard to the sex or age composition of the groups would be valid.

Urokinase excretion in disease states

Cardiac disease: Urokinase excretion rates found in patients suffering from cardiac disease are shown in Table 1. Urokinase excretion was increased for the

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Tab. 3: Urokinase excretion in other than renal and circulatory disease

Disease State	Number of Observations	Number of Patients	Mean Urokinase Excretion and Standard Deviation	"p"
Normal subjects	165	45	5238 ± 4485	—
Carcinomatosis	94	21	2176 ± 2090	< 0.001
Acute Cholecystitis	12	3	4480 ± 2460	> 0.1
Cholecystectemy (post-operative)	21	7	5666 ± 6700	> 0.1
Hepatitis (viral)	18	5	3232 ± 3030	> 0.1
Peptic Ulcer	62	20	5628 ± 6500	> 0.1
Diabetes Mellitus (35—81 age) (av age 64)	61	15	3855 ± 2830	> 0.1
Intestinal Obstruction (medical treatment)	13	4	1928 ± 3680	> 0.1
Disseminated L. E.	5	2	3740 ± 2800	> 0.1
Rheumatoid Arthritis (exacerbation of chronic disease)	28	7	5209 ± 3180	> 0.1
Asthma and Bronchitis	18	5	4876 ± 2870	> 0.1
Pneumonia and Pneumonitis	32	10	7300 ± 7390	> 0.1
Cerebral Thrombosis (10 or more days from onset)	45	13	4666 ± 2550	> 0.1
Thrombophlebitis	16	4	7060 ± 12 800	> 0.1

month following an attack of myocardial infarction ($p < 0.001$), increased during the week following an attack of coronary insufficiency ($p = 0.02$), and probably reduced in the phase of congestive failure secondary to arteriosclerotic, hypertensive or rheumatic valvular heart disease ($0.05 < p < 0.1$). Noteworthy was the fact that patients suffering from heart disease of similar etiology, but without failure, showed normal excretion rates. This suggested that the reduced

excretion during the stage of failure was related to the haemodynamic or metabolic changes during this state rather than to the primary disease causing cardiac decompensation.

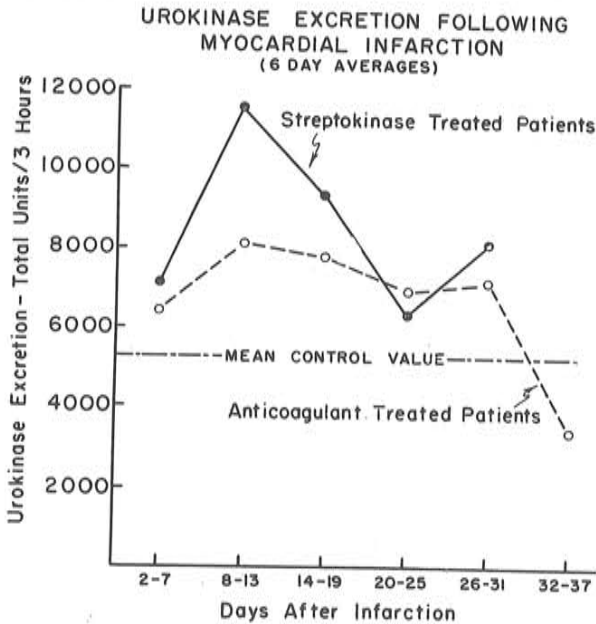

Fig. 4

Fig. 4 shows that the average urokinase excretion was raised during the week following the infarction, rose higher during the second week and thereafter declined until in the 5th week normal or below normal levels were found. An interesting and as yet unexplained difference was apparent between the group of myocardial infarction patients treated with anticoagulants (17 in number) and an apparently similar group of patients, who first received intensive therapy with streptokinase (8 in number). The streptokinase was administered by intravenous injection over a 30 hour period within 6—14 hours after the onset of the myocardial infarction (12) and the patients then treated with anticoagulants. Though studies with isotopically labelled streptokinase have shown that this material is rapidly broken down in the body (13) and biochemical studies show that the plasminogen system is apparently restored to normal soon after the cessation of the streptokinase infusion (14); the streptokinase treated patients showed much greater and faster rising urokinase

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excretion rates over the first three weeks. Statistical testing between the two groups showed a highly significant difference ($0.001 < p < 0.01$) and analysis suggested that this difference was attributable to the use of streptokinase in one group and not in the other. However it should be pointed out that this difference was not foreseen at the start of the experiment and the groups were not selected in a proper random fashion. Individual patients in the anti-coagulant treated group showed a wide pattern of urokinase excretion after myocardial infarction, most show a definite and rather transitory period of peak excretion in the second, third or fourth week and this variability with regard to the time of peak excretion accounted for the fact that the group average remained raised for the first month.

The diagnosis of coronary insufficiency was made on the basis of history, a sustained normal serum concentration of transaminase (S-GOT) and the development of signs of ischemia in the ECG without the subsequent appearance of Q waves. This group showed a statistically significant increase of urokinase excretion during the first week after the attack ($p = 0.02$). These findings indicate that the stimulus for the increased urokinase excretion found after myocardial infarction may result not from muscle death, but from the direct or indirect effects of myocardial ischemia.

Renal disease. Since urokinase is either produced or excreted by the kidney, it was expedient to investigate urokinase excretion in patients suffering from renal disease (Table 2). Patients suffering from long-standing pyelonephritis showed substantially normal excretion and though excretion in a group of children suffering from chronic glomerulonephritis (some with the nephrotic syndrome) appeared somewhat depressed, the figures did not reach the level of statistical significance. Patients in these two groups showed essentially normal blood urea nitrogen concentrations.

Two patients suffering from multiple myeloma with renal involvement showed a grossly diminished excretion, though owing to the small number of observations made, this could have been a chance finding. Patients, suffering from uremia secondary to severe grades of pyelonephritis or glomerulonephritis, showed a gross diminution of urokinase excretion, which was statistically significant when compared with the normal control group ($p < 0.001$).

Diseases other than those of renal or cardiac origin: Urokinase excretion found in a considerable variety of disease states is summarized in Table 3. A most interesting finding was the pronounced decrease of urokinase excretion found in patients suffering from carcinoma, which was highly significant ($p < 0.001$). This group of patients suffered from moderately advanced to advanced carcinoma, though none were terminal. The carcinomas were located

in the following anatomic sites; breast 4 (3 with metastases), rectum 2, cervix 1, body of uterus 2, ovary 1 (metastasis), renal 2, lung 4 (1 metastasis), esophagus 3 (2 metastases) and bladder 2 (both metastases). Diminished urokinase excretion was common to the group as a whole and did not appear to depend upon the tumor site or the presence or absence of demonstrable metastatic growth.

Though none of the other grouped data shown in Table 3 showed statistically significant differences, some points are worthy of comment. The small group of patients suffering from thrombophlebitis showed a somewhat high mean urokinase excretion rate and in some of the specimens urokinase excretion was the highest seen in any disease condition. However, the exceptionally large standard deviation associated with this mean value indicates that it would be premature to draw conclusions from these findings and urokinase excretion in this condition requires further investigation. The moderately large group of diabetic patients was somewhat elderly (average age 64) and there were indications that the collection of more data might have led to the demonstration of statistical differences between this group and the normals. Though there was insufficient data to establish the point, it appeared that the low urokinase excretion in some patients was, at least in part, due to the development of Kimmelstiel-Wilson disease. The small group of patients suffering from intestinal obstruction were in a poor state of hydration and this factor may have accounted for the somewhat low urokinase excretion observed. The group labelled pneumonia and pneumonitis were for obvious reasons being treated with antibiotics and this may have produced a substantial modification of the natural urokinase excretion pattern. This was unavoidable, but unfortunate as a study of urokinase excretion during a classic attack of unmodified labor pneumonia might be expected to produce data of great interest. The other disease conditions shown in Table 3 call for no special comment and a few random samples taken from patients with polycythemia vera, hemolytic anemia, chronic myeloid leukemia, chronic lymphatic leukemia, lymphoma and Hodgkins disease showed no obvious deviation from the normal range.

Discussion

Inspection of Tables 1, 2 and 3 shows that in disease groups whose mean did not differ significantly from the control mean, variability could be expressed as mean ± 40—80% standard deviation. This degree of variability was substantially greater than had been earlier quoted in the section on excretion under physiological circumstances — mean ± 30% standard deviation. Whereas the

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later experiments were performed under laboratory conditions, the former were made under ward conditions and the changed environment probably accounts for the difference. Ward collection was made by nurses and since catheter specimens were not taken, the presence of residual urine in bed-ridden patients may have influenced the recorded urine volumes. In any case this difficulty would tend to enhance rather than diminish the significance of the changes observed. It is surprising that females showed a somewhat higher urokinase excretion rate than males since the prostatic fluid of males contains a powerful plasminogen activator (15) and the contrary result might have been expected. However Bjerrehuus (7) also found that male and female urines were equal in urokinase content.

Interpretation of the present findings is hindered by uncertainty as to site of urokinase origin. Several authors (16 and 2) using relatively crude assay methods had previously concluded that urokinase excretion and plasma fibrinolytic phenomena were unrelated. However, recently von Kaulla (8) has provided a convincing demonstration that the increased plasma activator concentration, found in patients developing fibrinolytic states during cardiac surgery, was associated with an enhanced urokinase excretion rate and that this enhanced rate returned to normal as the intensity of the fibrinolytic state declined. This is suggestive evidence that urokinase activity represents, at least in part, excreted plasma activator.

The present data would also fit with this concept as, whilst the occurrence of myocardial infarction or the onset of myocardial insufficiency could serve to incite long-continued changes in the concentration of plasma activator, it is unlikely that such a stimulus would produce long standing changes of renal function. Similarly, while carcinomatosis may produce wide spread systemic change, these changes are not specifically renal nor indeed do they commonly occur in the kidney. Thus, provisionally at least, we believe it justifiable to attempt interpretation of the data on the basis that urokinase excretion represents, at least in part, the excretion of plasma activator.

Thus, the raised urokinase excretion demonstrated during the first month following the occurrence of myocardial infarction and for a shorter period after the onset of coronary insufficiency may reflect a raised level of plasma activator. Recent work (9) indicates that the concentration of plasma activator is the crucial determinant in the operation of in vivo thrombolytic mechanisms and consequently the finding of a raised plasma activator concentration would be an event of physiological significance. Indeed the operation of an in vivo thrombolytic mechanism, mediated by a rise in plasma activator concentration and occurring in response to the original insult, could exert a decisive influence upon the course of the disease. Presumably, since evidence of myocardial necrosis

was lacking in the patients suffering from coronary insufficiency, the inciting stimulus was related to myocardial ischemia on either a neurogenic or more likely a metabolic basis.

Similarly, the finding that patients suffering from carcinomatosis show a grossly diminished urokinase excretion may be significant in the pathogenesis of the venous thrombi that develop so frequently in this condition (17). Since investigation of coagulation moietes has failed to demonstrate sufficient cause for the thrombotic tendency (18), it is tentatively suggested that the tendency to the development of clinically apparent thromboses may reside in a failure of physiological fibrinolytic mechanisms to remove micro-thrombi rather than in an increased frequency of their production. The well known association of phlebothrombosis and its complications with congestive cardiac failure can be explained on the same basis as here too, urokinase excretion was reduced. Though renal function is also depressed in this state (19), the degree of depression was probably not sufficient to account for the decreased urokinase excretion found in our patients (vide infra).

A priori, renal disease should affect urokinase excretion and this occurred, but the data suggests that urokinase excretion is significantly diminished only in far advanced renal disease. Patients suffering from long standing pyelonephritis and chronic glomerulonephritis showed essentially normal excretion rates as compared to the control group ($p > 0.1$) and it was only at the stage of azotaemia that urokinase excretion fell to low levels ($p < 0.001$).

It is apparent that the present experimental findings raise questions of fundamental significance with regard to the role of in vivo thrombolytic mechanisms in two phases of human disease. First the findings in patients with myocardial infarction and coronary insufficiency suggest that activation of the plasminogen system with concomitant in vivo thrombolytic activity is an accompaniment of the repair process following these insults. Second the diminished urokinase excretion found in carcinoma and congestive cardiac failure, both conditions known to be frequently complicated by the development of venous thrombi, may be interpreted as indicating a diminished activity of in vivo thrombolytic mechanisms. Such physiological malfunction might fail to prevent the development of micro-thrombi into clinically evident lesions.

These hypotheses are theoretically attractive, but we would emphasize that the site of urokinase formation is still uncertain. Though the evidence can be reasonably interpreted to indicate that urokinase does, at least in part, represent excreted plasma activator, this theory cannot be adequately tested until more sensitive assay methods for plasma activator have been developed. Consequently our interpretation of the present data must, to a degree, remain hypothetical. Nevertheless, the finding of different urokinase excretion patterns, in

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systemic diseases with either minimal or absent renal involvement, must be regarded as important from both physiological and patho-physiological viewpoints.

Summary

1. Urokinase excretion rates in normal subjects were found to be independent of age, sex and urine volume.

2. Urokinase excretion rates were found to be raised following the onset of myocardial infarction ($p < 0.001$) and after an attack of coronary insufficiency ($p = 0.02$). Excretion rates were depressed in patients suffering from carcinomatosis ($p < 0.001$), congestive cardiac failure ($0.05 < p < 0.1$) and uremia ($p < 0.001$).

3. These differences suggest that significant alterations in the plasma fibrinolytic system may result as a consequence of disease states.

Résumé

1. Le taux d'excrétion de l'urokinase chez les sujets normaux est indépendant de l'âge, du sexe et du volume de l'urine.

2. Le taux d'excrétion de l'urokinase est accru après l'apparition d'un infarctus du myocarde ($p < 0.001$) et à la suite d'une insuffisance coronaire aigue ($p = 0.02$). Le taux d'excrétion est diminué chez les malades souffrants de carcinomatosis ($p < 0.001$), d'insuffisance cardiaque congestive ($0.05 < p < 0.1$) et d'urémie ($p < 0.001$).

3. Ces différences suggèrent que des altérations significatives du système fibrinolytique plasmatique peuvent être la conséquence d'états maladifs.

Zusammenfassung

1. Die Ausscheidung der Urokinase ist bei Normalpersonen unabhängig von Alter, Geschlecht und Harnmenge.

2. Die Urokinaseausscheidung ist erhöht nach dem Auftreten eines Myokardinfarktes ($p < 0{,}001$) und nach einem Anfall von Koronarinsuffizienz ($p = 0{,}02$). Die Ausscheidung ist vermindert bei Patienten mit Karzinose ($p < 0{,}001$), kardialer Stauung ($0{,}05 < p < 0{,}1$) und Urämie ($p < 0{,}001$).

3. Diese Unterschiede legen nahe, daß signifikante Veränderungen im fibrinolytischen System des Plasma als Folge dieser Erkrankungen vorkommen können.

References

(1) Sahli, W.: Über das Vorkommen von Pepsin und Trypsin im normalen menschlichen Harn. Pflügers Arch. ges. Physiol. **36:** 209 (1885).
(2) Williams, J. R. B.: The fibrinolytic activity of urine. Brit. J. exp. Path. **32:** 530 (1951).
(3) Astrup, T. and Sterndorff, I.: An activator of plasminogen in normal urine. Proc. Soc. exp. Biol. (N. Y.) **81:** 675 (1952).
(4) Ploug, J. and Kjeldgaard, N. O.: Urokinase an activator of plasminogen from human urine. I. Isolation and properties. Biochim. biophys. Acta **24:** 282 (1957).
(5) Alkjaersig, N., Fletcher, A. P. and Sherry, S.: The activation of human plasminogen. II. A kinetic study of activation with trypsin, urokinase and streptokinase. J. biol. Chem. **233:** 91 (1958).
(6) Kjeldgaard, N. O. and Ploug, J.: Urokinase an activator of human plasminogen. II. Mechanism of plasminogen activation. Biochim. biophys. Acta **24:** 289 (1957).
(7) Bjerrehuus, I.: Fibrinolytic activity of urine. Scand. J. clin. Lab. Invest. **4:** 179 (1952).
(8) v. Kaulla, K. and Swan, H.: Clotting deviation in man during cardiac by-pass: fibrinolysis and circulating anticoagulant. J. Thoracic Surg. **36:** 519 (1958).
(9) Sherry, S., Fletcher, A. P. and Alkjaersig, N.: The fibrinolysin system: some physiological considerations. Symposium on Connective Tissue, Thrombosis and Atherosclerosis. Academic Press, New York. (In press, 1958).
(10) Müllertz, S.: Fibrinolytic and fibrinogenolytic methods for estimating plasmin and trypsin. Acta physiol. scand. **26:** 174 (1954).
(11) v. Kaulla, K. N.: Methods for preparation of purified human thromboplastin and fibrinolysokinase from urine. Acta haemat. (Basel) **16:** 315 (1956).
(12) Fletcher, A. P., Alkjaersig, N., Smyrniotis, F. and Sherry, S.: The Treatment of patients suffering from early myocardial infarction with massive and prolonged streptokinase therapy. Trans. Ass. Amer. Phys. **71:** 287 (1958).
(13) Fletcher, A. P., Alkjaersig, N. and Sherry, S.: The clearance of heterologous protein from the circulation of normal and immunized man. J. clin. Invest. **37:** 1306 (1958).
(14) Sherry, S., Fletcher, A. P., Alkjaersig, N. and Smyrniotis, F. E.: An approach to intravascular fibrinolysis in man. Trans. Ass. Amer. Phys. **70:** 288 (1957).
(15) v. Kaulla, K. N. and Settles, L. B.: Relationship between human seminal fluid and the fibrinolytic system. Proc. Soc. exp. Biol. (N. Y.) **83:** 692 (1953).
(16) Macfarlane, R. G. and Pilling, J.: Fibrinolytic activity of normal urine. Nature (Lond.) **159:** 779 (1947).
(17) Bauer, G.: Diagnosis and management of peripheral venous disease. Amer. J. Med. **23:** 713 (1957).
(18) Allen, E. V., Barker, N. W. and Hine, E. A.: In Peripheral Vascular Diseases. 2nd Ed. W. B. Saunders Co., Philadelphia (1955).
(19) Heller, B. I. and Jacobson, W. E.: Renal hemodynamics in heart disease. Amer. Heart J. **39:** 188 (1950).

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