EPSILON AMINOCAPROIC ACID:

The development of a laboratory assay system, and study of its absorption, distribution and excretion to permit rational dosage; and an assessment of its value as an inhibitor of fibrinolytic activity in the urinary tract

by

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PREFACE

The studies presented in this thesis were carried out in the Enzymology Division of the Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri, during my tenure of a Harkness Fellowship of the Commonwealth Fund. The project was suggested to me in broad outline on my arrival in St. Louis; and from time to time the progress of the work and its development were discussed with my colleagues there (Dr. Sherry, Dr. Fletcher & Miss Alkjaersig) in association with whom it was published*. The detailed planning of the work, its day to day evolution and its execution were my individual responsibility, and except where specifically indicated in the text, the work was entirely personally performed.

*(1) McNicol, G.P., Fletcher, A.P., Alkjaersig, N., Sherry, S. (1961). J.Lab.clin.Med., 58, 34.

(2) McNicol, G.P., Fletcher, A.P., Alkjaersig, N., Sherry, S. (1961). J.Urol.(Baltimore), 86, 829.

(3) McNicol, G.P., Fletcher, A.P., Alkjaersig, N., Sherry, S. (1962). J.Lab.clin.Med. 59, 7.

(4) McNicol, G.P., Fletcher, A.P., Alkjaersig, N., Sherry, S. (1962). J.Lab.clin.Med., 59, 15.

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SECTION 1

INTRODUCTORY

CHAPTER 1

INTRODUCTION

My personal interest in the fibrinolytic enzyme system, initially aroused by Dr. A.S. Douglas in the University Department of Medicine, Glasgow Royal Infirmary, was fostered by the opportunity of working in the Enzymology Division of the Department of Medicine, Washington University School of Medicine, St. Louis, Missouri, during my tenure of a Harkness Fellowship of the Commonwealth Fund. At the time of my arrival in St. Louis it had recently been shown that the synthetic amino acid epsilon aminocaproic acid (EACA) was a potent inhibitor of fibrinolytic activity, and my research project while in St. Louis, the study of EACA, is the subject of this thesis. In view of the potential uses of EACA in the treatment of fibrinolytic states, which may cause a severe, and sometimes fatal defect of haemostasis, knowledge about the absorption, distribution and excretion of EACA was necessary, but the appropriate investigations were hampered by the lack of a suitable assay system.

The experimental section of the thesis opens with an account of the development of a simple assay system for EACA in biological fluids, using ion exchange resin loaded paper chromatography. The method was applied to the study of the absorption, distribution and excretion of EACA/

EACA in man. It is shown that levels of EACA in the plasma, adequate to inhibit fibrinolytic activation, are readily achieved and maintained by oral or intravenous therapy. Because of rapid excretion and concentration in the urine, high levels of EACA in the urine can be produced by modest dosage schedules.

To define further the potential therapeutic role of EACA, experiments were carried out which show that it does not specifically affect thromboplastin generation or the thrombin-fibrinogen reaction. Evidence is also presented that at high concentrations EACA inhibits pepsin.

The thesis then goes on to describe experiments which explored one possible application of EACA in the investigation and treatment of disease in man, namely its use to inhibit fibrinolytic activity in the urinary tract. The observations were facilitated and extended by the availability of the assay system for EACA in biological fluids.

It has been known for many years that normal urine can digest fibrin clots, an effect due to its content of a fibrinolytic activator named urokinase. The experiments described were designed to study the possibility that physiological levels of urokinase, by promoting clot lysis, might result in impairment of haemostasis after operations in the urinary tract, and that by giving EACA to inhibit urokinase, post-operative blood loss might be reduced.

The/

The problem was approached in a controlled trial in which EACA was given to patients after transurethral prostatectomy. When given intravenously in appropriate dosage, EACA was promptly excreted in the urine in a concentration sufficient to inhibit urokinase activity. In association with the inhibition of urokinase there was fourfold reduction in post-prostatectomy blood loss in EACA treated patients as compared with control patients. Levels of EACA in the urine were measured and the relationship between EACA concentration and urokinase inhibition is discussed. A group of patients was studied after suprapubic prostatectomy (post-operative blood loss was reduced by one half) and EACA was also given, with beneficial effect to individual patients not included in the controlled trials, in whom protracted haematuria presented a major clinical problem.

The experimental results provide evidence to support the concept that physiological levels of urokinase can impair haemostasis in the urinary tract, and that EACA, by inhibiting urokinase, has a valuable place in the management of post-operative haematuria.

Plan of the thesis

The thesis is presented in two volumes; volume I contains the text and references, and volume II, figures, tables, and an account of standard methods. The tables are in two parts; those in appendix 2 are tables to which reference is made in the text, and those in/

in appendix 3 are detailed tables from which as indicated in the legends, the figures, and tables in appendix 2 were compiled.

CHAPTER 2

DEVELOPMENT OF KNOWLEDGE OF FIBRINOLYSIS

Spontaneous lytic activity of blood clots

It has been known for many years that human blood possesses fibrinolytic activity. Hunter (1794) records that in "animals killed by lightning or by electricity" or in "animals who are run very hard, and killed in such a state", the blood does not coagulate. This phenomenon was partly explained by Morawitz (1906) who found that blood from the victims of sudden death contained no fibrinogen and could destroy the fibrinogen and fibrin of normal blood. Denis (1838) and Zimmerman (1846) observed that the blood clots obtained in wet cupping redissolved in less than 24 hours. Green (1887) noted that when fibrin prepared from ox blood had dissolved when incubated in saline it could not be clotted again by thrombin. Dastre (1893), during the course of phlebotomy in dogs, observed a reduction in fibrin yield which he attributed to destruction of fibrin, a process which he named "fibrinolysis". Hedin (1904) found spontaneous proteolytic activity in the globulin fraction of ox blood, and Fleisher and Loeb (1915) demonstrated fibrinolytic activity in the tissues. Macfarlane (1937) showed that in man fibrinolytic/

fibrinolytic activity in the blood could be provoked by surgical operation.

Fibrinolytic activity induced in vitro

In parallel with observations on spontaneous lytic activity in shed blood, knowledge was accumulating on methods by which lytic activity might be induced.

Denys and Marbaix (1889) found that proteolytic and fibrinolytic activity developed in serum treated with chloroform, ether or thymol. Delezenne and Pozerski (1903) showed that the action of chloroform was probably to remove inhibitors of proteolytic activity. Nolf (1905), who induced fibrinolytic activity in dogs by injection of peptone, believed that the lysis of blood clots which he observed was the end stage of the coagulation process, and like coagulation, was due to the activity of proteolytic enzymes. Opie and Barker (1907) found that, as with spontaneous activity, the fibrinolytic activity induced by chloroform treatment of serum was in the globulin fraction.

Streptococcal fibrinolysis

Since sterile whole blood clots remain unlysed for days or weeks, the fibrinolytic activity observed in blood post-mortem, after chloroform treatment or peptone injection, and during surgical operations must, if due to enzymatic activity, be associated with an enzyme, normally inert, which can be activated under appropriate circumstances. Demonstration of such an enzyme system/

system followed the discovery of streptococcal fibrinolysis.

This major contribution to knowledge of fibrinolytic mechanisms, from which stems most subsequent work in fibrinolysis, was made by Tillett and Garner (1933) who found that culture medium of certain strains of haemolytic streptococci contained a substance (streptococcal "fibrinolysin") capable of producing rapid lysis of human blood clots. Milstone (1941) showed that this streptococcal product did not lyse highly purified human fibrin but if a small amount of the euglobulin fraction of human serum was added, lysis ensued quickly. Kaplan (1944) and Christensen (1945) found that euglobulin fraction of serum owed this effect to its content of an inactive precursor of a fibrinolytic enzyme, which was converted to an active state by the streptococcal "fibrinolysin". Christensen (1945) suggested that the streptococcal fibrinolysin be renamed streptokinase. Christensen and Macleod (1945) proposed that the inactive enzyme precursor in the serum euglobulin fraction be named plasminogen and the active enzyme to which it is converted, plasmin. Since the proteolytic action of plasmin is not confined to fibrin, this terminology is much to be preferred to the alternative usages profibrinolysin and fibrinolysin.

On the scheme for activation of fibrinolytic activity proposed by Christensen and Macleod (1945), and illustrated/

illustrated in figure 1, most subsequent work has been based, and though much elaborated, it remains essentially valid in terms of present knowledge, as discussed in the ensuing pages.

CHAPTER 3

CURRENT CONCEPTS OF THE COMPONENTS AND FUNCTIONS OF THE FIBRINOLYTIC ENZYME SYSTEM

Though of great complexity, the fibrinolytic system has four main components; plasminogen, plasmin, activators and inhibitors.

Plasminogen, a plasma globulin, inactive in its native state, is converted by activators to plasmin, a proteolytic enzyme which under suitable circumstances digests fibrin to give soluble products (figure 1). Plasminogen, a beta globulin with a molecular weight of about 140,000 (Shulman et al., 1958) is concentrated in Cohn fraction III (Cohn et al., 1946). It is stable in dilute mineral acid at pH2 (Christensen and Smith, 1950) and advantage is taken of this property in the most widely used method of preparation, that of Kline (1953), which uses Cohn fraction III as starting material.

Activation of plasminogen Plasminogen conversion to plasmin, an enzymatic reaction brought about by activators or kinases, is accompanied by the splitting off of peptides (Alkjaersig et al., 1958); the molecular weight of plasmin is probably about 108,000 (Shulman et al., 1958). Activators possess the property of splitting lysine and arginine esters and such esters act as/

as competitive inhibitors of plasminogen activation; the activation process would therefore appear to include a proteolytic step in which lysine and/or arginine bonds are split (Alkjaersig et al., 1958).

Plasminogen activators

Tissue activators Plasminogen activators are present in almost all body tissue except the liver and the placenta; high concentrations are found in the prostate, uterus, thyroid, lungs, ovary, adrenals, and lymph nodes (Albrechtsen, 1957).

Plasma activator Plasminogen activator, present in trace quantities in normal plasma, is found in increased amount after exercise, emotional stress, adrenaline injection, electric shock and administration of bacterial pyrogen (Sawyer et al., 1960). As suggested by Kwaan and associates (1957), one site of production of plasma activator is probably the walls of the veins; the histochemical studies of Todd (1959) lend support to this hypothesis. Astrup (1956a) has presented evidence which suggests that blood and tissue activators are "lysokinases" which act like streptokinase by converting proactivator to activator.

Activator in secretions Plasminogen activators are present in milk (Astrup and Sterndorff 1953), tears (Storm, 1955) saliva (Albrechtsen and Thaysen, 1955) and seminal fluid (von Kaulla and Shettles, 1953).

Urinary activator Urokinase, the physiological plasminogen activator/

activator present in normal urine, is discussed in detail in Chapter 9.

Bacterial activators Streptokinase, produced by certain strains of haemolytic streptococci, was the first activator to be identified (Tillett and Garner, 1933). It does not activate plasminogen directly, but only after reaction with a proactivator, which may be plasminogen itself (Ablondi and Hagan, 1957). Staphylococcal culture filtrates contain a plasminogen activator named staphylokinase (Gerheim et al., 1948).

Proteolytic enzymes, for example, plasmin itself (autocatalytic activation) and trypsin, are also plasminogen activators, though there is evidence to suggest that the plasmin so produced may differ in physico-chemical properties from that produced by urokinase (Alkjaersig et al., 1958).

In vitro, activation may also be produced by chloroform (Christensen, 1946) and peptone (Ungar and Mist, 1949).

Hageman Factor There is in vitro evidence to suggest that Hageman factor may play a part in plasminogen activation (Iatridis et al., 1960).

Plasmin

Plasmin is a proteolytic enzyme with wide substrate specificity. In vitro it has equal affinity for fibrinogen as for fibrin (Ratnoff, 1953). Other plasma proteins digested include glucagon, ACTH and growth hormone (Mirsky et al., 1959), factor V (Alagille and Soulier, 1956/

1956), antihaemophilic globulin (Lewis et al., 1949), and certain components of complement (Pillemer et al., 1952). Plasmin also digests casein (Remmert and Cohen, 1949).

Inhibitors of the plasminogen-plasmin system

Two categories of inhibitor exist: those acting on the activation process and those acting upon plasmin (antiplasmin).

Plasma Inhibitors of Activation Though the existence of a naturally occurring activator inhibitor in plasma has been suggested (Lewis and Ferguson, 1951; Mullertz, 1957; Jacobssen, 1955; Nilsson et al., 1961; Paraskevas et al., 1962; McNicol et al., 1963), the methods used have not adequately distinguished between antiplasmin activity and inhibition of plasminogen activator, and the separate existence of natural plasma activator inhibitors has not been proved.

Plasma antiplasmin

Plasma and serum exert a substantial inhibitory action on plasmin. Milstone (1941) found antiplasmin activity in the supernatant of the euglobulin precipitate, an observation confirmed by Macfarlane and Pilling (1946). Norman and Hill (1958) have shown that there are at least two antiplasmins in serum. One, in the alpha-2 globulin fraction, reacts quickly as a competitive inhibitor of plasmin. The other, in the alpha-1 fraction, reacts more slowly but firmly with plasmin to produce an inactive/

inactive complex. There is also antiplasmin activity in the platelets (Johnson and Schneider, 1953). According to Fletcher (1960) there is in plasma about 60 per cent more antiplasmin activity than plasminogen (i.e. than potential plasmin); according to Norman (1960), there is about 30 times more antiplasmin than plasminogen.

Other inhibitors of plasmin and plasminogen activation

Plasmin inhibitors Numerous substances have been found which inhibit the activity of plasmin. These include heparin (von Kaulla and McDonald, 1958); basic amino acids (Mullertz, 1954) and their esters (Troll et al., 1954); quaternary amines (Astrup and Alkjaersig, 1951); lauryl amine (Astrup and Alkjaersig, 1952); heavy metals (Kowalski and Latallo, 1956); pancreatic trypsin inhibitor and soya bean trypsin inhibitor (Christensen and Macleod, 1945); toxic phosphorus compounds (Mounter and Shipley, 1958); toluidine blue (Lombardo, 1958); and as discussed in Chapter 4, epsilon aminocaproic acid. The antiplasmin activity of these substances, though of theoretical interest and in some cases valuable for in vitro studies, is probably too weak to be of use in vivo in safe dosage.

Other plasminogen activator inhibitors

Mullertz (1954) observed that in vitro lysine and ornithine inhibited plasminogen activator and Alkjaersig et al. (1958) found that arginine and lysine esters are competitive/

competitive inhibitors of plasminogen activation. Alkjaersig et al. (1959a) and Ablondi et al. (1959) showed that epsilon aminocaproic acid is a potent competitive inhibitor of plasminogen activation, and their findings, together with the observation of Sjoerdsma and Nilsson (1960) on the inhibitory effects of other aliphatic amino compounds on plasminogen activation are discussed in detail in Chapter 4.

Mechanism of fibrinolysis

Sherry and his associates (Alkjaersig et al., 1959b; Sherry et al., 1959a) have provided an attractive hypothesis for the mechanism of clot lysis in vivo. According to this hypothesis, plasma and any thrombi in the vascular system respond differently to circulating activator (figure 2). In plasma, activator converts plasminogen to plasmin, but provided plasminogen conversion is not excessively rapid, antiplasmin neutralises the plasmin produced and in consequence there is no free proteolytic activity in the circulation. In thrombi on the other hand, where antiplasmin concentrations are said to be relatively low (Sawyer et al., 1961), plasminogen activator diffuses into the thrombus and activates plasminogen in close association with fibrin, which is digested. In this way substrate specificity is conferred on plasmin in vivo.

The following observations support the above hypothesis:-

(1)/

(1) Plasminogen has a strong affinity for fibrinogen and fibrin and so in normal circumstances, thrombi have a high plasminogen content (Blombäck and Blombäck, 1956). Activator is also absorbed on to fibrin in the process of clotting (Sherry, 1954).

(2) In in vitro systems, using plasminogen enriched radioactive clots as substrate, lysis is readily produced by activator in the surrounding medium, and lytic activity is a function of clot plasminogen content. Such clots, however, are resistant to lysis by plasmin in the medium (Alkjaersig et al., 1959b). It would seem probable that the function of plasma plasminogen is to endow any clots which may form with the means to mediate their subsequent lysis (Sherry et al., 1959a).

Defective fibrin polymerisation

The necessity for the mechanism described, which permits clot lysis but normally prevents the appearance of free plasmin in the circulation, has been elucidated by Sherry and his associates (Fletcher et al., 1962a; Alkjaersig et al., 1962) who have shown that when free plasmin is present in the circulation, fibrinogen is digested and fibrin polymerisation disordered. Fibrinogen-fibrin conversion takes place in three steps (figure/

(figure 3) (Scheraga and Laskowski, 1957). Thrombin, a highly specific proteolytic enzyme, forms fibrin monomer from fibrinogen by splitting off peptides; fibrin monomers condense in a polymerisation step, to form fibrin polymer which undergoes gelation to form the final visible clot. When fibrinogen is attacked by plasmin, one of the breakdown products so produced interferes with normal fibrin polymerisation. The specific fibrinogen fragment responsible for the polymerisation defect, whose electrophoretic and sedimentation characteristics have been determined, has a half-life in the circulation of about 9 hours. During fibrin polymerisation this fragment becomes incorporated in the polymer, but it differs sufficiently from fibrin monomer to result in a gross defect in polymer formation, and as a result, clot formation is delayed and defective. The abnormal friable nature of clots formed in the presence of this fibrinogen breakdown product can be seen on naked eye inspection, and the structural abnormalities, including inhibition of compound fibrin formation, partial failure of network formation and the incorporation of undifferentiated aggregates, have been demonstrated with the electron microscope (Bang et al., 1962).

Niewiarowski and Latallo (1957) and Triantaphyllopoulos (1958) have observed an anticoagulant effect of the products of fibrin proteolysis to which they attributed properties/

properties as an "antithrombin". However, as the specific fibrinogen breakdown product responsible for the coagulation defect in hyperplasminaemic states does not inhibit the enzymatic effect of thrombin on the synthetic substrate benzyl arginine methyl ester or on fibrinogen (Alkjaersig et al., 1962), it would seem likely that the apparent antithrombin effect seen was due to a defect in fibrin polymer formation rather than to interference with the action of thrombin itself.

Systemic hyperplasminaemic states

Though it is difficult to follow the complete course of naturally occurring fibrinolytic states because biochemical events may be well advanced before the problem becomes clinically apparent, the sequence of events following administration of streptokinase has been studied in detail (Fletcher et al., 1959; McNicol et al., 1962a). The biochemical effects of streptokinase infusion include acceleration of clot lysis, fall in levels of plasminogen and fibrinogen, increased thrombin clotting time and reduced levels of prothrombin, antihaemophilic globulin, factor V and to a lesser extent, Christmas factor (McNicol et al., 1962a). Though the coagulation defect induced by streptokinase is complex, with accelerated clot lysis and depletion of coagulation factors playing a part, Fletcher et al. (1962a) and Alkjaersig et al. (1962) have produced convincing evidence/

evidence to support the concept that a major factor in the genesis of the coagulation defect is conversion of plasma plasminogen to plasmin with temporary overwhelming of the physiological antiplasmin mechanism and hence the presence of free plasmin in the circulation. In consequence of this "hyperplasminaemic" state, fibrinogen is digested and defective fibrin polymerisation ensues. As proteolytic activity in the plasma, and particularly fibrinogenolysis, plays such an important part in producing this coagulation defect, Sherry et al. (1959a) have suggested that it is more appropriate to designate such states hyperplasminaemic rather than fibrinolytic. Hyperplasminaemic states are particularly liable to occur when tissues rich in plasminogen activator have been handled e.g. after thoracic surgery (Soulier et al., 1952), especially cardiac surgery with a heart-lung machine (von Kaulla and Swan, 1958; Gans et al., 1962). Such states may, however, complicate any major surgical operation (Andersson et al., 1962) and may also occur in obstetric accidents, e.g. accidental haemorrhage, amniotic fluid embolism and intra-uterine death with a retained foetus. The genesis of the coagulation defect is probably extremely complex; it is possible that in some cases at least the initial event may be release into the circulation of thromboplastin, with intravascular clotting/

clotting, the fibrinolytic activity being secondary to this (Schneider, 1959; Fletcher et al., 1962b).

Proteolytic states also occur occasionally in patients with prostatic carcinoma, especially if metastases are present (Tagnon et al., 1952), and have also been reported in association with pancreatic neoplasm (Ratnoff, 1952) and leukaemia (Mikata et al., 1959). Excessive fibrinolytic activity may also occur in patients with cirrhosis of the liver (Goodpasture, 1914; Ratnoff, 1949; Kwaan et al., 1956; Grossi et al., 1961).

Biological role of fibrinolysis

Plasma fibrinolytic activity

There is evidence to suggest that the plasminogen-plasmin system is constantly active under physiological circumstances. Sterile whole blood clots lyse spontaneously if slowly (Goodpasture, 1914) and much faster lysis is seen if plasma is diluted (Macfarlane, 1937; Fearnley and Tweed, 1953). Increased fibrinolytic activity has been found in such physiological circumstances as after exercise (Biggs et al., 1947) and in students about to take part in an examination (Truelove, 1951). Sawyer et al. (1960) have demonstrated that the fibrinolytic activity found *in vitro* in plasma from normal subjects under physiological circumstances is due to the presence of plasminogen activator in the circulation/

circulation.

A balance between coagulation and fibrinolysis was first suggested by Nolf (1908). Astrup (1956 a and b), Jensen (1956), Copley (1957) and Fearnley (1961) have been the main proponents of the attractive, though speculative, hypothesis that the fibrinolytic enzyme system has a homeostatic function, complementary to the coagulation system, in maintaining an intact patent vascular tree. The theory suggests that the two systems are in a state of dynamic equilibrium, with constant deposition by the coagulation system of a thin layer of fibrin on the endothelium and its continuous removal by the fibrinolytic system. In an extension of Duguid's hypothesis (1949) on the aetiology of atheroma, it has been proposed (Mole, 1948; Biggs and Macfarlane, 1962) that a reduction in fibrinolytic activity might permit accumulation of fibrin on the endothelium, so forming the basis of an atheromatous plaque.

Fibrinolytic activity in the tissues

The widespread occurrence of plasminogen activator in the tissues (Astrup and Permin, 1947) also suggests that the fibrinolytic system has a role in removing fibrin when it is no longer needed in processes of repair. The reported presence of a plasminogen-like pro-enzyme in the tissues (Kowalski et al., 1958) lends support to this hypothesis.

Fibrinolytic activity in other body fluids

The/

The discovery of urokinase suggested to Astrup and Sterndorff (1952) that plasminogen activators might be found in other body fluids passing through narrow excretory ducts, and as has already been reported activator was found in tears, milk and seminal fluid. Astrup (1956a) proposes that the role of these activators is, at least in part, to maintain the patency of the excretory passages by promoting the lysis of fibrinous deposits.

Other biological functions of the fibrinolytic enzyme system

There is evidence, reviewed by Ungar and Hayashi (1958) to suggest that fibrinolytic activity may be involved in the development of allergic responses. Plasmin activity may also be implicated in the production of physiologically active peptides such as bradykinin (Lewis, 1960).

Summary

In the past 15 years the components of the fibrinolytic enzyme system have been partially identified and characterised, and evidence has accumulated to support the concept that the system has a biological role in bringing about the lysis of fibrin in vivo. It has also become apparent that in certain circumstances excessive fibrinolytic activity in vivo can lead to defective haemostasis. As discussed in detail in the following chapter, the possibility of treating such fibrinolytic/

fibrinolytic states has arisen with the discovery that the synthetic amino acid, epsilon aminocaproic acid is an inhibitor of plasminogen activation.

SECTION 2

EPSILON AMINOCAPROIC ACID (EACA)

Chapter 4	Nature and actions of EACA
Chapter 5	Assay of EACA
Chapter 6	The absorption, distribution and excretion of EACA
Chapter 7	The effect of EACA on thromboplastin generation and the thrombin-fibrinogen reaction
Chapter 8	Inhibition of pepsin by EACA

CHAPTER 4

NATURE AND ACTIONS OF EACA

EACA, a white crystalline substance freely soluble in water, is a mono-amino mono-carboxylic acid with a molecular weight of 131. Its structural formula, and that of the closely related substances norleucine and lysine, is shown in figure 4.

Antifibrinolytic Activity

In 1957 a Japanese company took out a patent on the use of EACA as an anti-fibrinolytic agent; in the patent specification it is stated that the inhibitory effect of EACA is due to antiplasmin activity (Okamoto et al., 1957). It was subsequently shown by Alkjaersig et al. (1959a) and Ablondi et al. (1959) that the main action of EACA on the fibrinolytic enzyme system is as a competitive inhibitor of plasminogen activation; the findings of the two groups of workers are in quantitative agreement. Significant competitive inhibition of activation of human and bovine plasminogen by streptokinase, urokinase and tissue activator is seen at EACA concentrations of 10^{-4} Molar and higher. At concentrations above 5×10^{-2} Molar, EACA was found to be a non-competitive inhibitor of plasmin and trypsin; Alkjaersig et al. (1959a) found that in lower concentrations it enhances the/

the proteolytic activity of plasmin. EACA also, at a concentration of 3×10^{-1} Molar, non-competitively inhibits the activation of plasminogen by trypsin (Alkjaersig et al., 1959a).

Donaldson and Ratnoff (1962) have observed that EACA enhances the development of proteolytic activity in a mixture of chloroform and plasma euglobulins. No explanation of this phenomenon, or of the enhancement of plasmin activity by low concentrations of EACA, is at present apparent.

Structure of EACA and its activity

Sjoerdsma and Nilsson (1960) investigated a number of aliphatic amino compounds for activity as inhibitors of plasminogen activation and found such activity in some but not all amino acids with a carbon chain length of 4 to 8. Maximum activity was in the 6-carbon compound, EACA. The next most active were delta amino valeric acid (about half the activity of EACA) and delta amino laevulinic acid (about one third the activity of EACA). That the terminal position of the amino group is critical was shown by the observation that whereas EACA is a potent inhibitor, alpha aminocaproic acid (norleucine) is inactive. Further, gamma aminobutyric acid has significant activity, but alpha and beta aminobutyric acids do not. That the carboxyl group probably also has a function is shown because the addition of an amino group in the alpha position of EACA (alpha epsilon diamino-caproic/

caproic acid or lysine) and delta aminovaleric acid (alpha delta diamino valeric acid or ornithine) results in a considerable reduction in activity.

"Antiallergic" action of EACA Zweifach et al. (1961) have shown that systemic anaphylaxis in the mouse, acute endotoxaemia in the rat and mouse and "drum shock" (traumatic shock induced by rotation) in the rat are suppressed by EACA. EACA has also been shown to protect dogs from lethal doses of endotoxin (Spink and Vick, 1961). Johnson et al. (1962) report, without giving details, that systemic anaphylaxis in animals is inhibited at 14 hours after injection of as little as 5 mg. EACA/kg. bodyweight (i.e. 0.35 gm. for an average size adult man); and in man EACA inhibits the tuberculin reaction (Itoga and Yogo, 1959). However, Nilsson (1961) found that prior administration of EACA to a patient known to be sensitive to meprobamate failed to prevent severe anaphylactic shock when meprobamate was given. The mechanism by which EACA inhibits anaphylaxis and endotoxin shock is not known; it may be that it does so by inhibiting a possible fibrinolytic component of such states, or the protective action of EACA may be due to some quite different and unknown biochemical mechanism.

Absorption, distribution and excretion

Animal studies

Lang and Bitz (1953), who gave EACA intraperitoneally to/

to rats, found almost all the dose in the urine, and Campbell et al. (1960) report that in rabbits, 90 per cent of a single intravenous injection of EACA is recovered unchanged in the urine in 5 hours.

Observations in man

The findings of Nilsson et al. (1960) and Johnson et al. (1962) that in man EACA is rapidly absorbed from the gastro intestinal tract and largely excreted unchanged in the urine, are discussed in Chapter 6.

Metabolic effects of EACA in animals

Duesberg and Frederici (1956) observed that low concentrations of EACA (about 10^{-5} Molar) stimulated the growth of bone marrow cultures, but higher concentrations (about 10^{-2} Molar) had an inhibitory effect. Shirasaki (1950) found that EACA, in a concentration of 10^{-3} Molar, stimulated the growth of chick embryo heart fibroblasts in tissue culture. In guinea pigs EACA administration was found to increase tissue concentrations of metalloporphyrins, and to reduce plasma and urine levels (Kosaki et al., 1952). In cholesterol fed rabbits, EACA given orally for 4 - 6 weeks, resulted in an increase in high density lipoprotein as compared with a control group of animals (Siegriest and Siiteri, 1960). Lang and Bitz (1953) reported that EACA given to growing rats in a dosage of 100 mg./day for seven weeks had no effect on growth, but on scrutiny of their results it would appear that the weight gain in the EACA treated animals was/

was only 79 per cent of that found in the controls. McGinty et al. (1924) found that EACA did not substitute for lysine in growth experiments in rats fed a protein deficient diet.

Metabolism of EACA in animals

Little is known about the participation of EACA in amino acid metabolism. In dogs it does not participate in gluconeogenesis (Corley, 1929) and rat and dog liver and kidney tissue cultures do not produce oxidative deamination of EACA (Lang and Bitz, 1953).

Use of EACA in systemic fibrinolytic states

Sherry et al. (1959b) first reported on the use of EACA in man; they found that intravenous infusion of EACA inhibited the fibrinolytic activity induced by systemic administration of streptokinase, nicotinic acid and bacterial pyrogen Activity was restored in vitro to the plasma samples by removal of the EACA by dialysis. Nilsson et al. (1960) showed that oral or intravenous administration of EACA inhibited pathological fibrinolytic activity seen in a patient with cirrhosis of the liver and in another with acute leukaemia. Other investigators have reported that EACA is effective in the control of the haemorrhagic states, probably due to hyperplasminaemia, in carcinoma of the prostate (Andersson and Nilsson, 1961; Anderson 1962); surgical operations (Anderson et al., 1962; McNicol, 1962); heart surgery with extracorporeal circulation (Gans/

(Gans et al., 1962) and aplastic anaemia and leukaemia (Mikata et al., 1959). Grossi et al. (1961) found EACA to be of doubtful benefit in the treatment of the haemorrhagic state associated with cirrhosis of the liver.

Inhibition of local fibrinolytic activity

The value of EACA as an inhibitor of urokinase after prostatectomy, the subject of Section 3 of this thesis, first reported by McNicol et al. (1960), has been confirmed by Anderson and Nilsson (1961), Anderson (1962) and Sack et al. (1962).

Sata et al. (1959) have reported that EACA is valuable in the treatment of menorrhagia, presumably because of local inhibition of fibrinolytic activity in the endometrium.

Use of EACA in conditions not usually associated with fibrinolytic activity

Yokoyama and Hatano (1959) found in an uncontrolled trial that EACA was of benefit in the management of eczema and other skin conditions suspected to have an allergic basis; they attributed the beneficial effects which they observed to an anti-inflammatory, anti-allergic action of EACA.

Toxicity and side effects of EACA

Adverse effects following administration of EACA might be expected to be of two kinds:

(a) those due to inhibition of plasminogen activation.

(b)/

(b) less specific effects, due perhaps to the substitution of EACA for lysine in amino acid metabolism, or direct biochemical effects of EACA, which is not a natural amino acid.

Adverse effects due to inhibition of plasminogen activation

If it is believed that the plasminogen-plasmin system has a physiological role in maintaining the patency of the vascular tree, then the possibility must be accepted that EACA may cause vascular occlusion by inhibiting the lysis of intravascular fibrin. Patients with pre-existing vascular disease are presumably particularly at risk to this potential hazard. There are reports of two patients, both with a haemorrhagic state associated with carcinoma of the prostate and both with renal failure, in whom EACA therapy successfully controlled the haemorrhagic manifestations but was followed by multiple arterial thromboses (Anderson, 1962; Naeye, 1962); because of impaired renal function, plasma EACA levels may have remained high for an unusually long period after administration of EACA. Fletcher et al. (1962b) comment on the reluctance of physicians to report single cases of therapeutic misadventure, and report that they know of 5 unpublished cases, similar to the two reported above, of arterial thrombosis after EACA therapy. In an uncontrolled trial of EACA in patients studied after prostatectomy, Anderson (1962) reports that/

that of 50 patients given EACA post-operatively, 9 had evidence of thrombosis or embolism, and 5 of 25 control patients had post-operative thrombo-embolic complications; the incidence of such events in the two groups is almost identical.

Other side or toxic effects of EACA in man

Nilsson et al. (1960), who gave EACA in doses varying from a single intravenous dose of 1 gm. to 36 gm./day by mouth, found no changes in blood pressure, pulse rate or respiration and no subjective complaints. Of 12 patients studied by Andersson and Nilsson (1961) and Andersson (1962) and who were given EACA by mouth in doses varying from 15 - 40 gm./day, 7 had dizziness but no hypotension, and 3 had diarrhoea. Johnson (1960) found that intravenous infusion over 4 hours of 0.143 gm./kg. bodyweight produced dizziness in 5 of 16 subjects; and 6 had significant slowing of the heart rate and drop in systolic blood pressure.

Nour-Eldin and Draisey (1963) have reported that in one patient with prostatic carcinoma who was given EACA because of a haemorrhagic state and who died 32 days later, at necropsy there were scattered areas of hepatic and cardiac necrosis. Three of the patients given EACA by Nilsson et al. (1960) died respectively one day, six days, and one month after they had a dose of 8 gm. In one patient with acute leukaemia and thrombocytopenia, who died of "circulatory insufficiency and/

and broncho-pneumonia" punctate haemorrhages were found in brain and pericardium at post mortem. In the other two patients, who had respectively hepatic cirrhosis and arteriosclerosis with terminal broncho-pneumonia, there were no special features at necropsy.

Toxicity in animals Sweeney (1960) found that in rabbits single doses of EACA of 0.3 gm./kg. produce hyperthermia, bradycardia and increase in systolic blood pressure. Peck et al. (1961) claim that in dogs, rabbits and monkeys, EACA, in doses somewhat larger than those usually given to man (a minimum of 0.5 gm./kg./day), produced subendocardial haemorrhages; but Fletcher (1962) reports that extensive trials have failed to confirm these findings. Johnson et al. (1962) report in an abstract, without giving details, that EACA is teratogenic in animals.

Discussion EACA is a substance with potent biological actions. In vitro it is a competitive inhibitor of plasminogen activation and it shows promise as a therapeutic agent in the control of fibrinolytic activity in man. The mechanism of the "anti-allergic" action of EACA has not been elucidated and its potential clinical applications as an inhibitor of anaphylaxis and endotoxin shock have not been explored.

Although EACA is largely excreted in the urine, its influence on bone marrow and fibroblast tissue cultures, and on porphyrin and cholesterol metabolism, show that/

that it has important metabolic effects, some of which may be deleterious to the organism; and it has been reported to be teratogenic. Only after much more extensive use of EACA will a decision be possible as to the extent of the risk of inducing vascular occlusion with EACA. In the meantime, despite the potential hazards, EACA has such promise as an agent to control fibrinolytic activity in man that its further use seems justified where fibrinolytic activity presents a major clinical problem. To establish appropriate dosage schedules and routes of administration, a study of the absorption, distribution and excretion of EACA after its administration to man was indicated, and was made possible by the development of a suitable assay system for EACA in plasma and other biological fluids. The assay system is described in chapter 5, and its application to the study of the absorption, distribution and excretion of EACA in Chapter 6.

To further explore the pharmacological and biochemical properties of EACA, its effects on thromboplastin generation and the thrombin-fibrinogen reaction were investigated (Chapter 7) and its activity as an inhibitor of pepsin demonstrated (Chapter 8).

CHAPTER 5

ASSAY OF EACA

In this chapter is described the development of an assay system for EACA in biological fluids, using a modified chromatographic technique with ion exchange resin loaded paper.

Introduction Conventional paper chromatography of EACA in plasma, even in two dimensional systems, achieves relatively poor resolution from natural amino acids, particularly proline and valine (Smith, 1960). Sample preparation is tedious, involving ultrafiltration and desalting, and sample size is restricted, an important consideration if EACA concentrations are low.

High voltage electrophoresis has been used for the assay of EACA in urine (Sjoerdsma and Hanson, 1959) and the method is fairly satisfactory. However, resolution from the natural amino acids is not complete and the method cannot readily be adapted for assay of plasma samples because of the limitations as to sample size, the need for ultrafiltration (to remove protein which causes streaking) and the poor resolution obtained. Accordingly, a simple method for assay of EACA in plasma and other biological fluids was developed, using chromatography with ion exchange resin loaded paper.

Ion/

Ion exchange paper chromatography

Ion exchange resin loaded paper chromatography has been used in the separation and quantitation of amino acids in casein hydrolysates. Single papers (Myhre and Smith, 1958; Tuckermann, 1958; Roberts and Kolor, 1959) have been used, and two papers employed in a "tandem transfer" technique (Tuckerman et al., 1958), in which chromatography is begun on one paper and the solvent allowed to pass during the run on to another paper loaded with a different type of resin.

The method to be described involves the use of ion exchange resin loaded papers in the assay of EACA in plasma and other biological fluids. Plasma samples need only be precipitated with trichloracetic acid (TCA); the developing solvents are aqueous buffer solutions; and development takes only 1 - 2 hours. Resolution of the basic amino acids is good. The method is flexible and with variations in ionic strength and pH a variety of separations may be achieved. Because of the simplicity of sample preparation and assay technique, large numbers of samples may easily be studied.

Materials and methods

Chromatography paper The paper used was Reeve Angel grade SA2, which is loaded during manufacture with Amberlite ion exchange resin IR 120, Na + form, a strongly acidic cation exchange resin.

Developing/

Developing solvents were acetate buffers (pH 5.2, 0.2 Molar, pH 4.3, 1.0 Molar) and sodium acetate, 0.2 Molar, pH about 7. Ascending chromatography was used in all instances.

Preparation of Samples Plasma samples and tissue homogenates were prepared by precipitation with TCA (2.0 ml. plasma and 0.5 ml. 50 per cent TCA). Urine samples did not require any treatment other than dilution in some cases before assay.

Application of samples Using touch-delivery pipettes (A.S. Aloe Co., St. Louis, Mo.) and a sample applicator (Research Specialities Co., Richmond, Calif.), samples were applied to the origin on a 12" edge of 12" x 15" papers. Samples, which with plasma were usually 0.5 ml., but can be up to 1.0 ml., were applied in streaks about 2" long and parallel to the 12" margin of the paper. Streak width was kept as narrow as possible, by applying each sample in small increments, allowing full drying of the paper between each application. The sample applicator, which incorporates hot air drying of the paper, makes possible the precise application of large samples to a linear origin.

Because of the high concentration of solute applied at the sample origin, developing solvents tend to rise convexly through samples at the origin, thus producing curved bands when development is complete. By wetting the samples at the origin with about 0.2 ml. of the

first/

first solvent used, before papers were placed in the tank for development, this difficulty was overcome.

Drying and Staining After development, as described below, papers were dried at 80°C for 30 minutes and then stained by the nihydrin-copper method (appendix 4).

Chromatographic technique

The acetate buffer system, which eventually proved most satisfactory, was evolved semi-empirically. It was found that at ionic strength below 0.2 Molar, samples, perhaps due to their TCA content, ran unevenly and with streaks (figure 5). When solvent ionic strength was 0.2 Molar or higher, TCA had no effect on amino acid resolution and no significant effect on Rf values.

Figure 6 shows the mobility of the main plasma amino acids in acetate buffer, 0.2 Molar, pH 5.2. It will be seen that the amino acids have separated into three main groups; basic amino acids (arginine, histidine, lysine and ornithine) with low Rf values; aromatic amino acids (tyrosine and phenyl alanine) with Rf values about 50; and neutral and acidic amino acids with Rf values over 80. In this system EACA is poorly separated from the basic amino acids.

When 0.2 Molar sodium acetate (pH about 7) was used as developing solvent, the mobility of EACA relative to the other basic amino acids was greatly increased but separation/

separation from tyrosine and phenyl alanine was not satisfactory. With this solvent it will be seen that tyrosine and phenyl alanine can be differentiated. In order to take advantage of the attributes of each of the two solvents, a system was developed in which the papers were run initially in 0.2 Molar acetate buffer, pH 5.2, until the solvent front had travelled 8 - 10 cm.: the wet papers were then transferred for the remainder of the run, a further 15 cm. or so, to 0.2 Molar sodium acetate. Under these conditions, EACA separated cleanly from the basic amino acids: separation from the aromatic, neutral and acidic amino acids was wide (figure 7). Development of the papers in this way took less than 2 hours. Even when the fluid to be assayed, e.g. a tissue homogenate, contained high levels of naturally occurring amino acids, resolution of EACA remained acceptable; for example, chromatography of a rabbit liver homogenate is shown in figure 8. However, when sample EACA content exceeded 200 μg., trailing occurred; such samples were appropriately diluted before re-assay.

Measurement of optical density and calculation of results

Because of variation in stain intensity from paper to paper, standard EACA solutions were run on each paper, the amount of standard run being chosen to be near the level expected in the assay sample. After development and/

and staining, a strip was cut from each paper carrying the EACA band from the sample under assay and the bands from 2 standard EACA solutions. The strips were scanned with a 500 mμ. filter, in a recording integrating transmission densitometer (Beckman analytrol, Beckman Instruments Inc., Fullerton, Calif.), using a cam(B2) which linearally related optical densities on the stained strip to units of area on the densitometer tracing. The EACA content of the assay sample is calculated by proportion from the areas in the densitometer tracing of the unknown and the EACA standards. Figure 9 shows such a strip and the densitometer tracing made from it. In this case, the area under the densitometer curve from a 50 μg. standard is 140 units of area, and with a 100 μg. standard, 265 units. The area with 0.5 ml. of a plasma to which EACA had been added to give a concentration of 20 mg./100 ml., is 210 units. From a curve drawn with the standards, this indicates an EACA content in 0.5 ml. of the plasma of 78 μg. Allowing for dilution of the plasma by TCA during preparation (2.0 ml. plasma + 0.5 ml. TCA) the plasma EACA content is assayed at 19.5 mg./100 ml. as compared with the true value of 20 mg./100 ml.

Preparation of strips with clarifier (mineral oil and bromonaphthalene 4: 1) and a transparent plastic spray (Krylon Crystal Clear No. 1303; Krylon Inc., Morris-

town, Pa.), increased densitometer readings by about 10 per cent, but impaired reproducibility. It was therefore thought desirable to scan untreated papers.

Reproducibility of the assay technique

A series of plasmas with known amounts of EACA added was assayed by the technique described. The results are shown in figure 10. The correlation coefficient calculated from the data is 0.98 ($p < 0.001$); the equation for the regression line is $y = 0.93x - 0.6$. The 95 per cent confidence limits for the true concentration at various levels are shown in table 1. The coefficient of variation for the duplicate assays of EACA in plasma and urine shown throughout this thesis, excluding samples with an EACA content above 50 mg./100 ml. which were diluted before assay, is 8.98 per cent (calculated by Dr. R.A. Robb).

Comparison with high voltage electrophoresis

EACA assays were carried out in twenty-one urine samples by the high voltage electrophoretic technique of Sjoerdsma and Hansen (1959), (appendix 4) and also by the ion exchange paper chromatographic technique; results are shown in table 2. The coefficient of variation between the pairs of assays carried out by the two techniques is 23.5 per cent (calculated by Dr. R.A. Robb). Though the chromatographic technique appears to be somewhat more sensitive in detecting low concentrations (on 4 occasions EACA was found by this method when none/

none was detected by electrophoresis), a "t" test on the differences between the values obtained shows no significant difference from zero, i.e. there is no systematic bias between the two techniques ($t = 0\ 0145$ and $p > 0.9$).

Other amino acid separations

The buffer system can be adjusted to produce other separations. For example, an initial run with 0.2 Molar acetate buffer, pH 5.2, followed by 1.0 Molar acetate buffer, pH 4.3, produced clear separation of histidine from lysine and ornithine (figure 11).

Discussion

The method described, using ion exchange resin loaded paper chromatography, permits the rapid, simple assay of EACA in plasma and other biological fluids. The technique allows for the advantage of two developers without the need for two dimensional chromatography, for the wet papers may be transferred from one buffer to another during the course of a run. The unidirectional flow of the buffers permits samples to be applied as streaks rather than spots, thus making possible the application of much larger samples and facilitating quantitation by scanning densitometry. The coefficient of variation for duplicate assays, 8.98 per cent, is acceptable.

Johnson and Skoza (1961) have described, in an abstract only, a method for the assay of EACA in plasma which involves/

involves coupling with dinitrofluorobenzene, removal of alpha amino acids by chelation with copper and removal of other reactive amines by ion exchange resin chromatography. Though details of this method are not available, it seems more complex than the present method.

The availability of this rapid simple assay system for EACA in biological fluids made possible the study described in the following chapter, of the absorption, distribution and excretion of EACA. Also made possible was the assay of EACA levels in plasma and urine of the patients reported in section 3, who were given EACA to control fibrinolytic activity in the urinary tract.

CHAPTER 6

THE ABSORPTION, DISTRIBUTION AND EXCRETION OF EACA

In this chapter is described a study of the absorption, distribution and excretion of EACA following its administration to man. Studies on EACA distribution in vivo in the rabbit, and EACA uptake by the isolated rat diaphragm are also reported.

Materials and Methods

EACA Assays were carried out as described in Chapter 5, using ion exchange resin paper chromatography.

Plasma Samples were prepared for chromatography by precipitation with TCA (2 ml. plasma + 0.5 ml. 50 per cent TCA). The anticoagulant used was potassium oxalate (10 ml. whole blood + 20 mg. potassium oxalate).

Red cell assays In red cell assays, 1 ml. of packed red cells (centrifugation at 3000 r.p.m. for 30 minutes) was mixed with 1 ml. water and 0.5 ml. 50 per cent TCA. No correction was made for trapped plasma.

Tissue assays Tissues were thoroughly washed under a running tap, blotted dry with a paper towel, and weighed. A homogenate was prepared with a Waring blendor; to 2 ml. of the homogenate was added 0.5 ml. 50 per cent TCA. After centrifugation, the EACA content/

content of the supernatant was analysed.

Urine Assays Urine samples were centrifuged at 2000 r.p.m. for ten minutes before assay; no other preparation was necessary.

Creatinine determinations in plasma and urine were carried out by the method of Bonsnes and Taussky (1945).

Human Studies

Studies on the absorption, distribution and excretion of EACA were carried out in healthy adult subjects. Where patients were used the diagnosis is given with the results.

Results

Effects of Single Doses

Single intravenous dose The effects of a single rapid injection of 10 gm. EACA intravenously into an adult male (the author) are shown in figure 12 (subject 1). It will be seen that a plasma level of 150 mg./100 ml. was observed 15 minutes after the injection; by 30 minutes the level had fallen to 51 mg./100 ml. and continued to fall off rapidly, reaching levels of 3.9 mg./100 ml. and 3.4 mg./100 ml. at 4 and 6 hours respectively; none was found 24 hours after injection. EACA promptly appeared in the urine; at the end of 1 hour, 48 per cent of the dose had been excreted, and at the end of $3\frac{1}{2}$ hours, 82 per cent had been recovered; and/

and almost complete recovery was achieved at 6 hours. The EACA urinary concentration, which reached a maximum of 15.6 mg./100 ml. in the 1 - $3\frac{1}{2}$ hour collection period, fell to 0.81 mg./ml. in the collection period 21 - 24 hours after administration. None was found in the urine 48 hours after injection.

Single oral dose In the same subject (figure 12, middle section), the administration of 10 gm. EACA orally in a single dose produced a different response. The peak plasma concentration of EACA was found 2 hours after ingestion (33 mg./100 ml.); at 4 hours the level was 16 mg./100 ml., compared with 3.9 mg./100 ml. at 4 hours after intravenous injection. By 6 hours the level (4.5 mg./100 ml.) was similar to that found after intravenous administration. None was found in the plasma 24 hours after ingestion. Renal excretion differed from that found after intravenous infusion. In 1 hour only 21 per cent of the dose had been excreted and at 3 hours 46 per cent. Seventy-seven per cent was recovered in the urine at 12 hours and 78 per cent at 24 hours.

The effect of a single 5 gm. oral dose is seen in another healthy adult subject (figure 12, subject 2). A peak plasma EACA level was again found at 2 hours after ingestion (22 mg./100 ml.); at 4 hours the level was 7 mg./100 ml. and at 6 hours 5 mg./100 ml. Urinary excretion of EACA followed a somewhat different pattern to that seen in subject 1. Only a small portion of/

of the dose was excreted in 1 hour, but 81 per cent had been excreted by 4 hours. Peak urinary concentration of EACA was 15 mg./ml. in the 2 - 4 hours collection period; and in the period from 6 - 24 hours it was 0.5 mg./ml.

In subjects 1 and 2 plasma EACA levels 30 minutes after the single oral dose were 22 mg./100 ml. and 7 mg./100 ml. respectively, demonstrating that absorption from the gastro-intestinal tract is rapid; possibly some absorption takes place from the stomach.

In table 3 are shown plasma levels found 1 and 2 hours after single doses of EACA of from 2 to 10 gm. In all cases EACA concentrations in plasma were higher at 2 hours than at 1 hour after ingestion.

Repeated oral dosage In figure 13 are shown the results of experiments in which repeated oral doses of EACA were given to 3 subjects. It will be seen that following an initial dose of 4 - 6 gm., dosage rates of 1 gm./hour produced and maintained plasma EACA concentrations of 11.5 - 18.8 mg./100 ml. ($0.9 - 1.4 \times 10^{-3}$ Molar), levels adequate to produce significant inhibition of plasminogen activator activity.

One subject was given EACA at a dosage rate of 1 gm./hour for 3 days. Plasma EACA levels found were 12.3 mg./100 ml. two hours after starting treatment; 9.5 mg./100 ml. after 6 hours; 17.2 mg./100 ml. after 20 hours and 16.4 mg./100 ml. after 72 hours.

Intravenous/

Intravenous infusion Twenty-three patients described in detail in section 3, were given an intravenous infusion of 1 gm. EACA per hour for 4 hours, followed by 0.5 gm. per hour for a further 8 hours. Mean plasma EACA level at 4 hours was 11.6 $\pm$ 3.5 mg./100 ml., and at 8 hours, 6.4 $\pm$ 1.6 mg./100 ml. (data from tables 25 and 29).

Urinary excretion of EACA

The urinary excretion of EACA was closely followed for at least 24 hours in 21 subjects receiving EACA by mouth or intravenously. Though the dosage schedule (sustained infusions, single and multiple doses) and the amount administered (5 - 24 gm.) varied considerably among the subjects, the mean urinary recovery for the entire group was 68 $\pm$ 18 per cent of the administered dose at 24 hours; and in 16 subjects followed for 72 hours, was 82 $\pm$ 7 per cent. Details are set out in table 4. In those instances where observations did not extend beyond 24 hours, significant urinary excretion probably continued beyond this time; however, even in those patients in whom observations were extended for several days, mean urinary recovery dates did not reach 100 per cent of the administered dose. Failure to identify the whole dose in the urine might be attributable to one or more of the following: prolonged excretion beyond the period of observation; deficiency in the assay technique/

nique; excretion by other routes (e.g. gastro-intestinal tract); or metabolism of a small portion of the EACA.

Renal Clearance

Renal clearance rates of EACA were estimated in 3 male subjects (figure 13 and table 5) at a time when plasma EACA levels were stable. During the same collection periods, endogenous creatinine clearance rates were also measured. It will be seen that all 3 subjects had normal creatinine clearance rates. EACA clearances, carried out when plasma EACA levels were in the range from 13.6 - 18.6 mg./100 ml., varied from 65 to 92 per cent with a mean of 75.3 ± 10.3 per cent of the creatinine clearances. The findings suggest that the kidney handles EACA primarily by filtration and reabsorption. However, an active transport mechanism may not be involved in its reabsorption, for it may be noted (subjects 2 and 3, figure 13), that EACA was still found in the urine in significant amounts when plasma levels were no longer detected (i.e. levels of less than 0.1 mg./100 ml.). It should also be noted that during the clearance periods mean urinary EACA concentrations were 67 times greater than plasma concentrations, illustrating the ease with which high urinary levels can be attained.

State of EACA in plasma

The/

The possibility that EACA might, though readily dialysable from plasma (Sherry et al., 1959b), be in part protein bound was investigated by an ultrafiltration technique. Plasma containing 1×10^{-3} Molar EACA was ultrafiltered at pH 7.4 through a 10 mμ "cellulastic" membrane (Millipore Filter Corporation, Bedford, Mass.). Assay of the protein free filtrate in duplicate demonstrated the EACA content to be 9.2×10^{-4} Molar (9.7 and 8.7×10^{-4} Molar). This recovery is quantitative within the limit of experimental error and consequently indicates that EACA is unlikely to be protein bound.

Red cell levels EACA concentrations in red blood cells were determined in the experiments illustrated in figures 12 and 13. In each case red blood cell levels were initially much less than plasma levels. Over some hours the red blood cell levels gradually increased, and when EACA administration ceased, the red cell levels of EACA fell more slowly than the plasma levels. As will be seen in figures 12 and 13, at 24 hours EACA was still present in the red cells in significant amounts when it could no longer be detected in plasma.

Animal Experiments

Distribution in the rabbit

A single intravenous dose of 5 gm. EACA was given to each of 2 Californian rabbits. One was sacrificed

8/

8 hours, the other 18 hours after medication. For each animal plasma EACA levels at 4 hours and at sacrifice were determined; and EACA levels in red blood cell, muscle (thigh), liver, spleen, myocardium, kidney and lung were determined. Values are given in table 6. Plasma EACA levels 4 hours after medication were 86.8 mg./100 ml. in the 8 hour rabbit and 97.2 mg./100 ml. in the 18 hour animal. At the time of death in the animal killed 8 hours after EACA was given, plasma levels were still quite high (22 mg./100 ml.); red cell levels were somewhat lower (18 mg./100 ml.) but tissue concentrations of EACA e.g. in muscle (42 mg. EACA/100 gm. wet weight tissue) were much higher than that found in the plasma. The very high level (73 mg./100 gm.) found in the kidney was presumably due to the content of urine. Levels in muscle and myocardium were higher than those found in liver, spleen and lung. In the animal killed 18 hours after EACA had been given, plasma levels had sharply declined (3 mg./100 ml.); but levels in muscle, myocardium, and liver were still much higher than the levels in plasma. As before, levels in the kidney exceed those found in other organs.

Rat diaphragm studies

The studies were carried out in co-operation with Dr. David Kipnis. The rat diaphragm preparations were set up by Dr. Kipnis, and the EACA assays were performed/

performed by the author.

Method

Intracellular uptake of EACA was studied using the intact rat diaphragm technique of Kipnis and Cori (1957). Healthy male rats of the Sprague-Dawley strain were used. After a 5 hour fast, the rats were killed by decapitation. An intact diaphragm preparation was made as described by Kipnis and Cori (1957) and incubated in 40 ml. Krebs-Ringer phosphate buffer, pH 6.35, with 10^{-2} Molar EACA in the buffer. Incubation was for 1 hour at 40°C, with constant oxygenation. After incubation, diaphragms were clotted, weighed and ground in 1 ml. 10 per cent TCA. After centrifugation the EACA content of the supernatant was assayed as already described.

Results

These are shown in table 7. In calculating uptake of EACA by the intracellular fluid, 17 per cent by weight of the diaphragm preparation is assumed to represent the extracellular fluid, and 57 per cent by weight intracellular fluid; EACA concentration in the extracellular fluid is assumed to be identical with that in the incubating fluid, i.e. 10^{-2} Molar. Uptake by the intracellular fluid is found by assaying EACA concentration in the supernatant after homogenisation, and subtracting the contribution from the extracellular fluid; uptake results/

results are then expressed as μM EACA/ml. intracellular fluid/hour.

Mean uptake of EACA in preparations from 5 animals was 3.1 ± 0.53μM EACA/ml. intracellular fluid/hour.

Side and toxic effects

Some subjects noted vague abdominal discomfort after oral administration, especially after large doses. One subject frequently vomited after 2 gm. oral doses. Several subjects noted conjunctival suffusion and nasal stuffiness after even a single dose of 2 gm. No other side or toxic effects were noted.

Discussion

These results indicate that EACA is rapidly and predictably absorbed from the gastro-intestinal tract; after a single oral dose peak plasma levels are achieved in about 2 hours. Since the renal clearance of this compound is high at plasma levels in the desirable therapeutic range (10^{-3} Molar or above), the greater part of the dose can be found in the urine after 12 hours. For this reason single intravenous injections are not practical for maintaining a prolonged systemic effect. Sustained plasma and urine levels of EACA can be maintained readily by repeated oral doses or by continuous intravenous infusion. Dosage rates of 1 gm./hour produce plasma levels in the range of

10/

10^{-3} Molar (13 mg./100 ml.), adequate to inhibit significantly systemic plasminogen activator activity. With oral treatment, a priming dose of 4 - 6 gm. seems desirable in order to attain rapidly a significant inhibitory level in plasma. Because of concentration in the urinary tract, effective levels of EACA in the urine may be achieved by a dosage which does not produce excessive plasma levels.

The studies on red blood cell concentrations suggest that EACA has the ability to enter and leave the intracellular compartment; this suggestion is corroborated by the distribution studies in the rabbit in vivo, and with the intact rat diaphragm in vitro. Cellular uptake of EACA was readily demonstrated in the rat diaphragm experiments. In the rabbit studies, evidence was obtained for the entry of EACA into various organ tissues when plasma levels and presumably levels in the extracellular fluid, were high; and as the plasma levels quickly declined due to renal excretion, EACA in the intracellular compartment slowly diffused back into the plasma.

As is shown in table 4, the urinary excretion of EACA following a 12 hour intravenous infusion is prolonged (significant excretion occurs over a 72 hour period) as compared with that observed following a single dose (excretion virtually ceases after 24 hours); this difference may be attributed to the more extensive extravascular/

extravascular and intracellular equilibration which occurs following sustained dosage.

The present studies also indicate that the major portion of EACA is not metabolised in vivo; a mean value of 86 per cent of the administered dose was recovered unchanged in the urine. However, since all of the EACA was not recovered in the urine, the possibility remains that a small portion may be actively metabolised.

Evidence for a metabolic action of EACA was found in other experiments carried out by Dr. Kipnis, and reported by McNicol et al. (1962b) in which EACA in the incubation medium (in a concentration of 2×10^{-2} Molar) did not affect cellular uptake of tritium tagged lysine present in a concentration of 2×10^{-2} Molar in the medium, but incorporation of lysine in TCA precipitable protein was reduced by about 50 per cent compared with control experiments. Johnson et al. (1962) also report that when C14 labelled EACA is given to man a small proportion is excreted as carbon dioxide.

The present observations differ in some respects from those of Nilsson et al. (1960) who inferred plasma EACA concentrations by comparing the inhibitory effect of test plasma on streptokinase with the effect seen with known concentrations of EACA. In Nilsson's assay/

assay system, in 4 subjects, peak plasma levels after a single oral dose of 6 gm. were seen at 1 hour (mean EACA equivalent concentration of 24 mg./100 ml.); the mean level at 2 hours was 20 mg./100 ml., and had fallen to 6 mg./100 ml. by 5 hours. A dosage rate of 36 gm./day produced plasma levels of 10 - 14 mg./100 ml., a level somewhat lower than might have been expected from the present studies. In one patient a single dose of 3 gm. apparently produced significant plasma levels for 24 hours (18 mg./100 ml. falling to 10 mg./100 ml.). Nilsson and her associates also found, as was observed in the present study, that significant amounts of EACA could be detected in the plasma within 30 minutes of a single oral dose, and that the primary means of disposal was by renal excretion.

Using the EACA assay technique of Johnson and Scoza (1961) Johnson et al. (1962) report in an abstract that 40 - 80 per cent of an intravenous infusion of EACA appears in the urine within 24 hours. With C14 labelled EACA the same authors found excretion to be primarily in the urine. Little or no EACA appeared to be bound to erythrocytes, a finding at variance with the observations in the present study.

Toxicity of EACA This has already been discussed in Chapter 4. The reported effects of EACA administration in animals have included subendocardial haemorrhages/

haemorrhages, teratogenicity, hyperthermia, bradycardia and systolic hypertension. EACA also has an effect on cells in tissue culture and on porphyrin and cholesterol metabolism. Though these animal observations suggest that EACA may also prove to have significant toxic effects in man, at the present time the major potential hazard in man appears to be the risk, inherent in the action of EACA on the fibrinolytic enzyme system, of inducing vascular occlusion by inhibiting plasma fibrinolytic activity. Presumed vascular occlusion was found in the present study in two patients after EACA therapy (Chapter 11); the problem is discussed in Chapter 13. Other side effects seen in the present study were minor - nasal stuffiness and vague abdominal discomfort. The diarrhoea and postural hypotension found by other workers, as mentioned in Chapter 4, were not seen.

The observation reported by McNicol et al. (1962b), that EACA in the medium inhibits lysine incorporation in protein in the rat diaphragm preparation, may provide part of the explanation of the teratogenic effect of EACA in animals, and EACA should not be given to women during the early months of pregnancy. Further, bearing in mind the possibility of inducing vascular occlusion with EACA, it should be given with caution to patients with pre-existing vascular disease.

CHAPTER 7

THE EFFECT OF EACA ON THROMBOPLASTIN GENERATION AND THE THROMBIN-FIBRINOGEN REACTION

To examine the possbiility that EACA might exercise its haemostatic effect in fibrinolytic states partly by an effect on thromboplastin formation or the thrombin-fibrinogen reaction, the effect of EACA on the thromboplastin generation test and the thrombin clotting time was studied.

(i) Thromboplastin generation

Method Thromboplastin generation tests, using blood from a normal subject, were carried out as described by Biggs and Douglas (1953), with the following modification. In the incubation mixture the volumes of adsorbed plasma, serum, platelet suspension and 1/40 Molar calcium chloride were 0.3 ml., and 0.3 saline, with EACA to give the desired final concentration, was also present. Plasma samples were prepared by mixing 9 ml. whole blood with 1 ml. 3.8 per cent sodium citrate.

Results are shown in table 8, where it will be seen that in concentration between 10^{-4} Molar and 10^{-1} Molar EACA did not affect thromboplastin generation.

(ii)/

(ii) Thrombin-fibrinogen reaction

Method Normal oxalated plasma was mixed 1:1 with solutions of sodium chloride, urea and EACA of varying concentrations as shown in table 9. The thrombin clotting times of 0.1 ml. aliquots of these mixtures were then estimated by the method described in appendix 4.

Results are given in table 9, where it will be seen that with sodium chloride, prolongation of the thrombin clotting time appeared when the concentration exceeded 0.2 Molar; with urea, there was also an effect, though less marked, at a concentration of 0.2 Molar, and with EACA, a slight effect appeared at a concentration of 0.5 Molar.

Discussion

In the range of concentrations which may be achieved in vivo, EACA has no effect on thromboplastin generation nor the thrombin-fibrinogen reaction. At concentrations of 0.5 Molar and higher which are never likely to be achieved in vivo (Chapter 6) EACA prolongs the thrombin clotting time of normal plasma, but to a somewhat lesser extent than do sodium chloride and urea of the same molarity. It is concluded that EACA can have no haemostatic or anticoagulant actions in vivo in virtue of its effect on thromboplastin generation or the thrombin-fibrinogen reaction.

CHAPTER 8

INHIBITION OF PEPSIN BY EACA

As EACA is known to inhibit the proteolytic enzymes plasmin and trypsin (Alkjaersig et al., 1959a) it was thought desirable to investigate the possibility that it might also be an inhibitor of pepsin.

Material and Methods

The assay system was modified from that of Northrop et al. (1948). In the assay, pepsin is incubated with haemoglobin at pH 2, and the tyrosine released during incubation is measured colorimetrically.

Preparation of Substrate The substrate used was bovine haemoglobin; 2.5 gm. pure haemoglobin (Pfanstiehl Laboratories, Waukegan, Ill.) were dissolved in 80 ml. glycine solution, 0.05 Molar, and pH was adjusted to 2.0 with about 20 ml. of 0.2 N hydrochloric acid. The substrate was freshly prepared each day it was used.

Glycine was used as a buffer. When the acid haemoglobin solution was made up without glycine, the addition of EACA raised the pH out of the range in which pepsin is active, but with glycine present as a buffer, the addition of EACA did not significantly alter the pH. Details are shown in table 10.

Pepsin/

Pepsin A three times crystallised bovine preparation (Sigma Chemical Co., St. Louis, Mo.) was used in a concentration of 0.005 mg./ml.

Method of assay To 4.0 ml. of the acid haemoglobin substrate were added 0.5 ml. of various concentrations of EACA in saline, to give the final concentrations in the assay mixture shown in figure 14. After addition of 0.5 ml. pepsin solution, the assay mixture was incubated at 37°C for 62 minutes. At 2 minutes and 62 minutes, 2.0 ml. aliquots were taken and added to 2 ml. 10 per cent sodium hydroxide, which arrests enzyme activity. After centrifugtion (2000 for 10 minutes) 1 ml. of the supernatant was added to 4.0 ml. of 0.5 N sodium hydroxide, 1 ml. 1.0 N sodium hydroxide, 1.5 ml. 5 per cent trichloracetic acid and 1.5 ml. dilute (1:2) Folin Ciocalteu reagent. After standing for 15 minutes for colour development, the optical density of the 62 minute sample was read at 650 mμ in a Beckman D.U. Spectrophotometer, with the 2 minute sample as blank.

Results These are shown in figure 14, where it will be seen that inhibition of pepsin begins at an EACA concentration of about 10^{-3} Molar (0.001 Molar); 75 per cent inhibition is seen at an EACA concentration of 10^{-2} Molar (0.01 Molar) and inhibition is virtually complete at an EACA concentration of 10^{-1} Molar (0.1 Molar).

Discussion/

Discussion

Though the concentration of EACA needed to produce inhibition of pepsin (75 per cent inhibition with 10^{-2} Molar EACA) is somewhat less than needed to inhibit plasmin and trypsin (5×10^{-2} Molar) it is unlikely that EACA levels adequate to produce significant inhibition of peptic activity in plasma or tissues can be achieved in vivo. As shown in Chapter 6, because of concentration of EACA in the urine, urinary levels of EACA adequate to inhibit urinary peptic activity (uropepsin) can readily be achieved with moderate doses. However, as the pH range of urine is 4.8 - 8.2 (Smith, 1951) and the highest pH at which gastric proteases are active is 4.5 (Buchs, 1949) it is doubtful if the potential inhibition by EACA of peptic enzymes in the urine is of any significance in vivo.

SECTION 3

USE OF EACA TO INHIBIT UROKINASE

Chapter 9	Nature and actions of urokinase
Chapter 10	Trial of EACA in transurethral prostatectomy patients
Chapter 11	Trial of EACA in suprapubic prostatectomy patients
Chapter 12	The use of EACA in individual patients with haematuria
Chapter 13	Discussion: the use of EACA to control fibrinolytic activity in the urinary tract

CHAPTER 9

NATURE AND ACTIONS OF UROKINASE

It has been known for more than three-quarters of a century that urine can digest fibrin clots, an action originally attributed to the presence of trypsin (Sahli, 1885). However Williams (1951), Astrup and Sterndorff (1952) and Sobel et al. (1952) independently demonstrated that the fibrinolytic activity of urine was due to its content of a plasminogen activator which Sobel et al. (1952) named urokinase.

Urokinase, itself a proteolytic enzyme, activates plasminogen by first order kinetics, probably by splitting lysine and/or arginine bonds (Kjeldgaard and Ploug, 1957; Alkjaersig et al. 1958). It is a colourless protein with a high degree of stability over a wide pH range; purified urokinase is stable at 50°C up to pH 10, but in urine activity is lost below pH 3, probably because of digestion by uropepsin (Ploug and Kjeldgaard, 1957).

Source of urokinase

Bjerrehuus (1952) observed that urokinase concentrations were similar in bladder urine and urine obtained from the renal pelvis, and that excretion rates were independent of sex, a finding confirmed by Smyrniotis et al./

al. (1959), who also showed that excretion rates were independent of age and urine volume.

Astrup and Sterndorff (1952) have suggested that urokinase is not a simple excretion product and have implied that it is produced locally in the kidney, a view also held by Williams (1951). However, there is evidence to suggest that urokinase may represent, in part at least, excreted plasma plasminogen activator. Von Kaulla and Swan (1958) found increased urokinase excretion in patients who developed fibrinolytic states during cardiac surgery; the increased urokinase excretion fell to normal as the intensity of the fibrinolytic state declined. Celander and Guest (1960) observed increased plasma fibrinolytic activity and increased urokinase excretion in human subjects exercising on a treadmill, and Smyrniotis et al. (1959) reported that in a group of patients studied after myocardial infarction, urokinase excretion rates were higher in patients given streptokinase than in patients treated with conventional anticoagulant drugs. On the other hand the stability of urokinase (Ploug and Kjeldgaard, 1957) is in marked contrast to the lability of plasma activator (Fearnley and Tweed, 1953), and Celander and Guest (1960) have shown in DEAE cellulose chromatography of urokinase that there are two proteins with urokinase activity. It would therefore seem possible that/

that proteins from more than one source may contribute to urokinase activity.

Proteolytic enzymes in the urine

Urine is known to contain pepsin-like enzymes (uropepsin), and the presence of small amounts of trypsin-like enzymes has also been shown (Loeper and Baumann, 1922).

Impairment of haemostasis in the urinary tract: the role of urokinase

Astrup and Sterndorff (1952) have suggested that urokinase may have a useful role by bringing about the rapid resolution of clotted blood and fibrin in the urinary system. The experiments described in the present section were designed to examine the possibility arising out of this suggested function, that urokinase, by activating clot plasminogen, might cause impairment of haemostasis in the urinary tract. The problem was studied principally in patients after transurethral prostatectomy. Such patients are excellent for investigation because of the relative uniformity of the surgical trauma and the ease of obtaining complete urine collections. The basis of this study is illustrated schematically in figure 15. After prostatectomy, urine containing urokinase flows over the clot in the raw prostatic bed; it is postulated that clot plasminogen is activated to plasmin and clot lysis and impaired haemostasis ensue. Were such a mechanism operative, the administration of EACA to patients after prostatectomy, in/

in sufficient quantity to inhibit urokinase activity, should be accompanied by reduced blood loss, as shown in the lower portion of figure 15. The experimental results to be reported were entirely consistent with this hypothesis. The administration of EACA resulted in a significant (fourfold) reduction in post-operative blood loss as compared with results obtained in a control group of patients not given EACA. A smaller group of patients was studied after suprapubic prostatectomy: post-operative blood loss in EACA treated patients was one-half that seen in controls not given EACA. EACA was also given, with benefit, to a small number of patients, not included in the controlled trials, in whom protracted haematuria presented a major problem. Advantage was taken of the administration of EACA to study further its excretion and to relate changes in urokinase activity to EACA levels.

CHAPTER 10

TRIAL OF EACA IN TRANSURETHRAL PROSTATECTOMY PATIENTS

This chapter is in four parts:

(i) Clinical data

(ii) Methods

(iii) Results

(iv) Discussion

(i) Clinical Data

Twenty-eight patients were studied. Thirteen selected at random (drawing lots) were treated with EACA and fifteen were controls. As seen in table 11, the EACA treated group and the control group were comparable as regards age, preoperative haemoglobin levels, preoperative blood urea nitrogen levels and blood pressure. Transurethral electro-resections (McCarthy resectoscope) were carried out by the urological services of Barnes and Jewish Hospitals, St. Louis.

In all patients, bladder washouts were free from macroscopic blood before the patients left the operating room and it should be emphasized that only bleeding which began post-operatively was studied. EACA infusions (see below) were commenced within ten minutes of the end of operation.

The/

The mean weight of tissue removed was in the EACA treated group 14.7 $\pm$ 8.5 gm., and in the control group 15.4 $\pm$ 7.4 gm., (tissue weighed by the pathologist).

EACA was used as a sterile 10 per cent aqueous solution. In all patients, the EACA was administered intravenously at a rate of 1 gm./hour for 4 hours, then 0.5 gm./hour for 8 hours (total dose, 8 gm.). In 7 patients the EACA was added to the routine post-operative 5 per cent dextrose intravenous infusion (usually about 1500 ml. over the 12 hour period) and in 6 patients the EACA was administered using a constant rate infusion pump (Harvard Apparatus Co., Dover, Mass.).

Histological Examination In the EACA group histological examination of the resected tissue (Surgical Pathology Departments of Barnes and Jewish Hospitals) showed 9 patients with benign hyperplasia (patients 2, 6, 12, 13, 15, 20, 22, 25 and 28), 4 of whom also showed chronic inflammation (patients 6, 12, 20 and 22), one with fibrosis (patient 10), one fibrosis and chronic inflammation (patient 21) one with leukaemic infiltration (chronic lymphatic leukaemia, patient 14), and one well differentiated adenocarcinoma of the prostate (patient 23). In the control group, two had well differentiated adenocarcinoma of the prostate, (patients 9 and 26), and the remaining 13 had benign hyperplasia, 4 of whom also showed chronic inflammation, (patients

4/

4, 5, 18 and 27).

Postoperative Urine Collections All patients had an indwelling catheter for 2 - 4 days after operation, and 24 hour collections of catheter drainage, together with return fluid from bladder lavage, were made.

Anticoagulant The anticoagulant used in collection of blood samples was potassium oxalate (10 ml. whole blood + 20 mg. potassium oxalate).

(ii) Methods

(1) Urokinase Assays

Urokinase activity was assayed by the unheated fibrin plate technique (method of Mullertz, 1952, as modified by Alkjaersig et al., 1959a).

In the fibrin plate test, a fibrinogen solution, rich in plasminogen, is clotted with thrombin in a petri dish. If a small amount of a solution containing plasminogen activator is placed on the surface of the plate, the plate plasminogen is converted to plasmin, and after incubation, the holes made in the plate where the fibrin is lysed are measured. Areas of lysis, expressed in square millimetres as the product of two perpendicular diameters of the area of lysis, are not linearlly related to activator concentration and serial dilutions of a standard urokinase preparation must be assayed with each batch of plates, activity in the test sample being read from the standard curve so produced.

Such a fibrin plate is primarily sensitive to plasminogen activator/

activator, but it is also to a lesser extent susceptible to digestion by plasmin in a test solution. Response to activator can be eliminated by heating the plate to destroy plasminogen (80°C for 45 minutes; Lassen, 1952). Response to plasmin in a heated plate is about 50 per cent of that found in a comparable unheated plate (Alkjaersig et al., 1959a).

Preparation of plates Plates were prepared by the method of Alkjaersig et al. (1959a) using Armour bovine fibrinogen (about 60 per cent fibrinogen). Square plastic petri dishes were used, internal measurement 8.9 x 8.9 cm. For each plate, 20 mg. fibrinogen were dissolved, using a magnetic stirrer, in 6.0 ml. borate buffer, 0.1 Molar, pH 8.0. After adding 2.0 ml. of 6 per cent dextran (Mead Johnstone) and 2.0 ml. of 0.7 per cent calcium chloride solution, the fibrinogen solution was poured into a petri dish and clotted with 0 3 ml. of a 50 N.I.H. units/ml. solution of thrombin (Parke Davis).

Assay Technique After leaving at room temperature for 15 minutes to allow a firm fibrin clot to form, 30 µl. aliquots of test solution were applied to the surface of the plate, using a 0.1 ml. pipette. Each plate was used for 4 tests. After sample application, plates were incubated at 30° for 16 hours and the lysed zones defined by addition of a drop of 0.1 per cent congo red solution. Typical plates are shown in figure 16.

The/

The product of two perpendicular diameters was used as a measure of the area of lysis. Urokinase concentration (units/ml.) was determined by reference to a freshly run standard curve. A single batch of urokinase, prepared by Dr. F. Smyrniotis by the method of Von Kaulla (1956) and stored in a series of small tubes at -20°C, was used as a standard. For the standard curve run with each batch of plates, the standard urokinase solution was assayed undiluted, and diluted with saline to give concentrations of 50, 25, 12.5 and 6.25 per cent. Figure 17 shows the mean values and standard deviations obtained from all the standard curves run throughout this study.

Calculation of results The standard urokinase solution was assigned an arbitrary value of 100 urokinase units/ml. and all experimental readings are expressed in these units. These units are about one eighth of the Ploug unit (Ploug and Kjeldgaard, 1957). Figure 18 shows a representative standard curve (table 46, curve 1). The serial dilutions of the standard urokinase solution gave areas of lysis of 24 x 21 mm. (100 per cent), 18 x 18 mm. (50 per cent), 19 x 13 mm. (25 per cent), 13 x 13 mm. (12.5 per cent) and 11 x 9 mm. (6.25 per cent). A urine sample (patient 1, preoperative sample) gave a zone of lysis of 15 x 11 mm. Interpolation in the standard curve as shown in the figure gave a urine urokinase activity of 12.1 per cent of the standard urokinase/

urokinase solution, that is the urokinase activity of the urine under test was 12.1 units/ml. As the urine volume was 2170 ml., 24 hour urokinase activity was calculated as 12.1 x 2170 units = 26,260 units.

Reproducibility of the Urokinase Assay Table 12 shows the results of duplicate urokinase assays of 12 samples. The standard error of a single observation is 2.414 and the coefficient of variation 7.18 per cent (figures calculated by Dr. R.A. Robb).

Urine specimens were assayed for urokinase activity before and after dialysis. Assay before dialysis was carried out overnight on the day each collection was completed. During the same night, and following day, aliquots of urine were dialysed, and were assayed for urokinase activity on the following night.

Dialysis of 5 - 10 ml. samples was carried out for 24 hours at $4^{\circ}C$ against 10 - 15 litres of constantly stirred 0.1 Molar phosphate buffer, pH 7.6, and was always associated with loss of urokinase activity. For 64 observations in the 28 subjects, loss of activity on dialysis was 34.9 ± 9.0 per cent (postoperative samples from the EACA treated patients are excluded). A similar loss of activity was seen when the standard urokinase preparation was dialysed (table 13).

The loss of activity on dialysis may be due to co-precipitation with mucoprotein. A contributory factor is/

is the slight increase in weight (4.5 ± 1.5 per cent, table 14) found after dialysis.

Effect of whole blood on the urokinase assay As many of the urine samples showed frank haematuria, the following experiment was carried out to assess the effect of whole blood on the urokinase assay system.

Method Standard urokinase solution, diluted 1:3 with (a) saline, (b) bank blood, diluted 1:49 with saline or (c) oxalated fresh blood diluted 1:49 with saline, was assayed by the fibrin plate technique.

Results are shown in table 15. It will be seen that dilute bank blood and fresh blood had no significant effect on the assay values.

(2) Urinary Blood Content

This was measured as haemoglobin by a modified cyanmethaemoglobin method. Intravascular haemolysis as a cause for haemoglobinuria was excluded by inspection of postoperative plasma specimens; in no case was haemoglobinaemia detected.

Method To 9 ml. of urine was added 1 ml. concentrated Drabkin's Solution (Fisher Scientific Co., Pittsburgh, Pa.). The specimen was centrifuged at 2,000 r.p.m. for ten minutes. If a deposit of unhaemolysed red cells was present, it was frozen and thawed and then dissolved in 0.2 ml. of urea solution (30 gm./100 ml.) and added to the supernatant. Optical density was read at 540 mμ. in/

in a Beckman D.U. spectrophotometer, using 9 ml. pooled normal urine with 1 ml. Drabkin's Solution as a blank. If urea had been used in preparing the sample, it was also added to the blank. The pooled urine which was used as a blank throughout this investigation was prepared from 10 normal urine samples. Mercurochrome, 100 mg./litre, was added as a preservative and the urine was stored at $4^{o}C$.

Urine samples were diluted with water if necessary to keep optical density readings below 0.900.

Standard curve Haemoglobin concentration (mg./ml.) was obtained by reference to a standard curve (figure 19), in which optical densities at 540 mμ., of urine treated as described, are plotted against urinary haemoglobin concentration. Two urines, one concentrated, specific gravity 1028, the other dilute, specific gravity 1010, were used to prepare the standard curve. Known concentrations of haemoglobin in urine were prepared by the addition with a microburette of fresh blood, haemoglobin level 10 gm./100 ml. The urine samples were then treated with Drabkin's Solution and optical densities at 540 mμ. estimated using the pooled normal urine as a blank. Results are shown in figure 19.

Where optical density readings were below 0.015, that is about 0.03 mg. haemoglobin/ml., or 0.2 ml. of/

of normal blood (haemoglobin 14.6 gm./100 ml.) per litre of urine, haemoglobin concentrations were recorded as zero.

The effect of EACA on urinary haemoglobin assay

In view of the possibility ahat EACA in the urine might influence urine haemoglobin estimations, the effect of EACA on the haemoglobin assay system was determined.

Method To each of 10 aliquots (10 ml.) of two normal urines was added 40 µl. fresh whole blood, haemoglobin concentration 10 gm./100 ml. EACA was then added to give pairs of samples, one from each urine, with EACA concentration of 10^{-4}, 10^{-3}, 10^{-2}, and 10^{-1} Molar. The optical density at 540 mµ of these urine samples and of two control samples from each urine to which no EACA had been added, was then determined after addition of Drabkin's Solution, using the pooled normal urine as blank.

Results are shown in table 16. It will be seen that addition of EACA in concentrations from 10^{-4} to 10^{-1} Molar did not affect the assay for haemoglobin in the urine.

Conclusion The presence of EACA in the urine did not affect the assay system for haemoglobin in urine.

(3) EACA Assays

EACA content of urine was assayed as described in Chapter 5.

(4)/

(4) Heated Fibrin Plate Tests

In heated fibrin plates, as already described, sensitivity to plasminogen activator has been eliminated by destroying the plasminogen content of the plate before assay, and fibrinolytic activity seen with heated plates is due to preformed proteolytic enzymes in the test solution. Because of partial denaturation of the fibrin, sensitivity of heated plates to plasmin is about 50 per cent of that found in comparable unheated plates (Alkjaersig et al. 1959a).

Method Fibrin plates were prepared as described earlier in this chapter, and after standing at room temperature for 15 minutes to permit firm clotting, were heated to 80°C for 45 minutes in a hot air oven. After application of test solutions (30 µl. aliquots) plates were incubated overnight at 30°C.

(5) Caseinolytic Assays

The method employed was a modification of the caseinolytic assay for plasminogen (appendix 4). Two ml. of urine was incubated at 37°C for 62 minutes with 2.0 ml. phosphate buffer, 0.1 Molar, pH 7.6, and 2.0 ml. casein solution. The tyrosine content of aliquots taken off at 2 and 62 minutes was estimated as described in the plasminogen assay (appendix 4).

(6) Urinary Proteolytic Activity at pH 2 (Uropepsin activity)

In/

In view of the property of EACA as an inhibitor of pepsin (Chapter 8) the proteolytic activity at pH 2 (uropepsin activity) of the first post operative 24 hour urine collections was studied.

Method The assay system with an acid haemoglobin substrate, described in Chapter 8, was used, 0.5 ml. urine and 0.5 ml. saline being incubated at 37°C for 1 hour with 4.0 ml. substrate. The assay thereafter was carried out as previously described, tyrosine release from the haemoglobin being estimated colorimetrically.

(7) Effect of Urine on Thrombin Clotting Times of Normal Plasma

Method The thrombin clotting time of 0.1 ml. of a 1:1 mixture of normal plasma and test urine or saline was assayed as described in appendix 4. Tests were carried out in duplicate.

(8) Plasma Plasminogen Levels

Method The caseinolytic method used is described in appendix 4. Samples from EACA treated patients were dialysed overnight at 4°C against a large volume of 0.9 per cent saline to remove EACA before assay.

(9) Fibrinogen Levels

Method The method used is described in appendix 4.

(10) Thrombin Clotting Times were estimated as described in appendix 4.

(11) Euglobulin Lysis Activity

Euglobulin/

Euglobulin lysis activity was studied by the method described in appendix 4.

(12) Plasma EACA Concentrations were assayed as described in Chapter 5.

(iii) Results

For each patient, the following were studied:

On 24 hour urine collections for 5 days after operation	(1) Urokinase activity before and after dialysis (2) Haemoglobin content (3) EACA levels*
On the first 24 hour post operative urine collection	(4) Heated fibrin plate test (5) Caseinolytic assay (6) Uropepsin assay (7) Effect on thrombin clotting time of normal plasma
On plasma samples obtained 4 hours after operation	(8) Plasminogen assay (9) Fibrinogen assay (10) Thrombin clotting times (11) Euglobulin lysis activity (in some patients only) (12) EACA concentration* (also estimated in samples taken off 8 hours after operation)

*Estimated in EACA treated patients only.

(1) Urokinase activity This activity was followed for 5 days after operation. Assays were also made before operation, and each sample was assayed after dialysis to permit comparison of true urokinase excretion rates between treated and control groups.

The data are presented in figures 20 and 21, and are summarised in tables 17 and 18.

Figure 20 illustrates urokinase assays performed on undialysed urine specimens obtained from the two subject groups. It will be seen that in the control subjects operation did not affect urokinase activity, which did not vary significantly from day to day throughout the period of observation. In contrast, in the EACA treated group, assayable urokinase activity, which was similar preoperatively to that of the control group ($t = 0.186$, $0.7 < p > 0.8$) was zero on the first postoperative day and a mean of 25 per cent of the mean value from the control subjects on the second day ($t = 5.988$, $p < 0.001$). Even by the third day activity in the EACA treated group had not fully returned to control levels, though the depression is not significant at the 5 per cent level. Figure 21 demonstrates that EACA administration did not alter true urokinase excretion rates, as urokinase assays on dialysed specimens from the two subject groups did not differ significantly (p values in table 18, never less than 0.5). Similarly it is apparent that in neither group did/

did urokinase excretion alter as a result of the operative procedure.

(2) Blood Loss (Urine haemoglobin content) The blood loss was studied for 5 days after operation (in one treated patient and one control patient for 4 days only, and in one control patient for three days only). Results are summarised in table 19, and are also graphically presented in figure 22, which shows that blood loss in the EACA treated patients was much reduced as compared to that of the control group. As might be expected from the extent of urokinase inhibition, the difference was most marked on the first postoperative day when there was a fourfold reduction in blood loss in the EACA treated patients as compared with that in the control group. The range in blood loss over the 5 day period in the EACA treated group was from 0.5 to 5.2 gm. haemoglobin; in the control group, from 2.5 to 45.6 gm.

Duration of bleeding The duration of bleeding in the EACA treated patients was also reduced, as is shown in figure 23, which is a comparison of the percentage of patients observed for the full 5 day period who had measurable blood loss on successive postoperative days. For the 12 EACA treated patients followed for the full 5 day period, there was blood in 36 daily samples, but no bleeding was detected in 24 samples; for the 13 control subjects, there was blood in 54 samples, and only/

only in 11 was no blood detected. The difference between the two groups is significant ($chi^2 = 7.242$, $p < 0.01$).

No correlation was observed between preoperative urokinase concentration and blood loss following surgery (for the control group $r = -0.377$, $p > 0.1$, and for the EACA treated group, $r = -0.459$, $p > 0.1$). Nor, as seen in figure 24 did a significant relationship exist between postoperative bleeding and the amount of prostatic tissue resected (for the EACA treated group, $n = 12$, $r = -0.276$ and $p > 0.1$; and for the control patients, $n = 13$, $r = +0.144$ and $p > 0.1$).

(3) Urinary EACA Content Results are summarised in table 20.

Relationship between EACA concentration and urokinase inhibition Shown in figure 25 is the relationship between EACA concentration and urokinase inhibition in various 24 hour urine collections. Samples in which urokinase inhibition was complete and in which EACA may therefore have been present in excess of the amount needed to produce complete inhibition, are excluded. Also excluded are samples with EACA concentrations below 5×10^{-4} Molar, in which inhibition was absent or negligible. It will be seen that there is a linear relationship between urokinase inhibition (units/ml.) and the logarithm of the EACA concentration ($r = +0.84$, $p < 0.01$). The regression line calculated from the data is shown ($y = 16.53x + 55.07$) (Regression coefficient and/

and equation for regression line calculated by Dr. R. A. Robb).

(4) Heated Fibrin Plate Tests Assays were carried out with aliquots of the first postoperative 24 hour urine collection from each patient in the EACA treated group and the control group. In no case was lysis of the heated fibrin plate found.

(5) Caseinolytic Assays Assays were carried out with aliquots of the first postoperative 24 hour urine collection from each patient in the EACA treated group and control group. In no case was evidence of tyrosine release, i.e. caseinolytic activity, detected.

(6) Uropepsin Assays Assays were carried out with aliquots of the first postoperative 24 hour urine collection from each patient in the EACA treated and control groups. Results are shown in tables 21 and 22. From the optical density reading obtained with each urine sample was calculated the equivalent pepsin concentration of each sample in terms of the activity in this assay system of a purified bovine pepsin preparation (see table 43: a 0.005 mg./ml. solution of the pepsin gave an optical density of 0.177). Final results are expressed in terms of pepsin activity (mg./24 hour urine collection).

It will be seen that, for the first 24 hours, "uropepsin" activity was, in the control group a mean of 2.60 $\pm$ 0.58 mg., and in the EACA treated group a mean of

1/

1.35 $\pm$ 1.26 mg.; the difference is significant ($t = 3.448$, $p < 0.01$).

(7) Effect of Urine on the Thrombin Clotting Time

The effects on the thrombin clotting times of normal plasma of urines (aliquots from the first postoperative 24 hour collections) from the EACA treated and control patients are summarised in table 23, which also shows mean values from observations made with 15 urines obtained from normal subjects (colleagues and students). All urines were also tested after overnight dialysis against a large volume of 0.9 per cent sodium chloride solution, and these results, together with control observations with saline added 1:1 to plasma, are also shown in table 23.

It will be seen that in all these groups of subjects the addition of urine to plasma before estimation of the thrombin clotting time resulted in a marked increase in the clotting time as compared with control observations with a plasma-saline mixture. However, when the urine samples were dialysed before addition to the plasma, the thrombin clotting times of the plasma-urine mixtures were identical with these of the plasma-saline mixtures.

(8) Plasma plasminogen assays were carried out on samples taken off 4 hours after operation. Results are summarised in table 24, where it will be seen that mean values and standard deviations in EACA treated and/

and control groups are identical. Further, no individual value was outwith the normal range (2 - 4 casein units/ml.).

(9) Plasma fibrinogen assays Assays were carried out on plasma samples taken off 4 hours after operation. Results are summarised in table 24, where it will be seen that the mean value obtained in the control group (300 $\pm$ 60 mg./100 ml.) does not differ significantly from that found in the EACA treated group (287 $\pm$ 63 mg./100 ml.). Further, no individual value was below the lower limit of normal (200 mg./100 ml.).

(10) Thrombin Clotting Times Assays were carried out on plasma samples taken off 4 hours after operation. Results are summarised in table 24, where it will be seen that the mean value (10.5 $\pm$ 0.9 seconds) found with control patients does not differ significantly from that found in the EACA treated group (10.3 $\pm$ 0.9 seconds). Further, no individual value was outwith the normal range (9.0 - 12.5 seconds).

(11) Euglobulin Lysis Activity

Preoperative assays were carried out on plasma samples from the patients with prostate carcinoma (patients 9, 23 and 26). In all three patients, values were normal (less than 0.1 unit). Assays were also carried out on patient 14, with leukaemia, and on patients 1, 4, 5, 6, 10 and 27, the values in all cases were normal (less than 0.1 unit).

Postoperative assays Assays were carried out in patients

1/

1 - 15 in plasma samples obtained 4 hours after operation. In all cases results were normal (less than 0.1 unit).

(12) Plasma EACA Levels Shown in table 25 are plasma EACA levels at 4 and 8 hours after operation.

Side Effects of EACA Administration No adverse effects attributable to EACA administration were seen. In particular, there was no evidence of thrombosis in the veins by which EACA was administered. There were no significant changes in body temperature or pulse rate which could not be satisfactorily explained for other reasons, e.g. chest infection or bleeding.

(iv) Discussion

The general significance of the findings in relation to inhibition of urokinase activity and reduction in blood loss, together with the results from the patients studied after suprapubic prostatectomy, and a group of individual patients, is discussed in Chapter 13. In the present discussion only specific points arising from the data in the transurethral prostatectomy patients are considered.

In approaching the problem of EACA dosage and administration, two considerations seemed important.

(1) to achieve a urinary EACA concentration sufficiently high to produce a significant inhibition of urokinase activity.

(2) to sustain such a concentration for sufficiently long periods of time.

On/

On the basis of preliminary data (Sherry et al., 1959b), an 8 gm. dose was infused intravenously over a 12 hour period. It was anticipated that during the infusion, sufficiently large amounts of EACA would be excreted to inhibit urokinase activity, and in addition, equilibration with the extravascular compartment would occur, followed by slow elimination of the body pool of EACA in a concentration sufficient to achieve a sustained inhibitory effect. The results presented show that this objective was achieved. Mean urinary EACA concentration for the first postoperative day was $2.5 \pm 1.5 \times 10^{-2}$ Molar (table 20), which produced complete inhibition of urokinase activity; and on the second day, when mean urinary EACA concentration was $6.0 \pm 5.5 \times 10^{-3}$ Molar (table 20), significant inhibition of urokinase activity was still observed. As shown in figure 25, with urinary EACA concentrations above 5×10^{-4} Molar, and where urokinase activity is not completely inhibited, there is a linear relationship between the degree of urokinase inhibition observed, and the logarithm of urinary EACA concentration. The same relationship was found in vitro by Alkjaersig et al. (1959a) in examining the effect of EACA on purified urokinase.

The data displayed in figures 20 and 21 indicate that the effect of EACA administration was to inhibit the biochemical/

biochemical action of EACA as a plasminogen activator. There is no evidence to suggest that urokinase excretion rates were altered or that urokinase was destroyed by administration of EACA. The similarity of the urokinase assay values in control subjects in both pre- and postoperative periods suggests that the contribution of prostatic secretion to urokinase levels must be minimal, an observation in accord with the absence of sex difference in urokinase excretion noted by Bjerrehuus (1952).

Effect of Blood on Urokinase Assays Expired bank blood and fresh oxalated blood when diluted 1: 49 had no effect on urokinase assay values. McNicol et al. (1963) have demonstrated that higher concentrations of plasma do however inhibit the activity of purified urokinase; approximately 50 per cent inhibition was seen in a system with 10 µl. plasma per Ploug unit of urokinase. For example, if the same quantitative relationship holds true for whole urine, in a patient with a 24 hour excretion of 50,000 units urokinase, 50 per cent urokinase inhibition might be produced by 60 ml. plasma, or say 100 ml. whole blood. (The Ploug unit of urokinase is about 8 times the unit used in the present study). It is therefore possible that in patients with heavy postoperative bleeding (perhaps over 10 gm. haemoglobin in the first 24 hours), inhibition of urokinase by plasma may have contributed to the assay values obtained.

Urinary Proteolytic Activity The heated fibrin plate tests/

tests and the caseinolytic assays demonstrate that the urines tested had no significant proteolytic activity against casein at pH 7.6 or fibrin at pH 8. These tests support the conclusion that the fibrinolytic activity found in the unheated fibrin plate assays was due to the presence of plasminogen activator and was not due to proteolytic activity in the urine.

As discussed in Chapter 8, EACA at concentrations above 10^{-3} Molar produces significant inhibition of pepsin in vitro, and as might be predicted, on the first postoperative day when mean urinary EACA concentration was $2.5 \pm 1.5 \times 10^{-2}$ Molar, there was significant inhibition of uropepsin activity. However, as the pH of the urine would never be in the range at which peptic activity is manifest, the inhibition of uropepsin is a phenomenon of little practical importance.

Effect of Urine on the Thrombin Clotting Time As discussed in Chapter 3, there is evidence to suggest that a major factor in the genesis of the coagulation defect associated with systemic fibrinolytic activity is defective fibrin polymerisation due to the presence of products of fibrinogen digestion. When such fibrinogen breakdown products are present, the clotting time of plasma in addition of thrombin (the thrombin clotting time) is prolonged, due to the abnormality in fibrin-polymer formation, and prolongation of the thrombin clotting/

clotting time is a sensitive index of defective fibrin polymerisation (Alkjaersig et al., 1962).

The postulated mechanism by which urokinase brings about impairment of postoperative haemostasis in the urinary tract is the direct effect of urokinase in promoting clot lysis. To exclude the possibility that in the postoperative phase the products of clot lysis present in the urine might further impair local haemostasis through an effect on fibrin polymer formation, the effect on the thrombin clotting time of urine from the EACA treated and control patients and from normal subjects was examined.

The finding that the urines from the normal subjects prolonged the thrombin clotting time of plasma somewhat more than urine from the prostatectomy patients, and that dialysed urines from EACA treated and control patients, and from the normal subjects, did not lengthen the thrombin clotting time, suggests that the effect of urine on the thrombin clotting time is due to its ionic strength. There is no evidence of an effect on the thrombin clotting time which might be attributed to the presence of fibrinogen breakdown products.

As would be expected from the observation (Chapter 7) that EACA does not prolong the thrombin clotting time of normal plasma unless present in a concentration of 0.5 Molar or higher, the thrombin clotting times with urines from the EACA treated patients, in whom the/

the highest EACA concentration recorded was 5.6×10^{-2} Molar (table 20), did not differ significantly from those found with urines from the control subjects.

Plasma Fibrinolytic Activity As has been discussed in Chapter 3, the manifestations of excessive systemic fibrinolytic activity (hyperplasminaemia) include accelerated clot lysis, fall in plasminogen and fibrinogen levels, and prolongation of the thrombin clotting time. These factors were therefore studied in EACA treated and control patients.

The occurrence of increased fibrinolytic activity and a haemorrhagic state in patients with prostatic carcinoma, first described by Tagnon et al. (1952) has since been recorded by many workers including Swan et al., (1957); Cohen and Kupfer (1958); Lombardo (1958); Miller et al. (1959), and Andersson (1962). Tagnon et al. (1953) found increased fibrinolytic activity in the plasma of 6 of 48 patients with prostatic carcinoma; in all six there were widespread metastases. None of the three patients with carcinoma in the present series had evidence of metastases or increased plasma fibrinolytic activity; in each, euglobulin lysis times, plasminogen and fibrinogen levels, and thrombin clotting times, were normal.

Numerous authors have reported that a rise in plasma fibrinolytic activity is common during and immediately after prostatectomy, including Scott et al. (1954); Lombardo/

Lombardo (1957); Lombardo (1959); Ladehoff and Rasmussen (1961); Andersson and Nilsson (1961); Andersson et al. (1962); and Andersson (1962).

Andersson et al. (1962) studied 23 patients during prostatectomy and found increased levels of plasma plasminogen activator in every patient. The levels of activity found in the plasma during prostatectomy were comparable with those found during cholecystectomy, and greater than those found during hernia repair or uterine curettage. In the 23 patients studied during prostatectomy there was a mean fall in fibrinogen levels of 100 mg./100 ml.; in 19 other patients, given 5 - 9 gm. EACA intravenously immediately before operation, the fall in fibrinogen levels during prostatectomy was only 30 mg./100 ml. and the increase in plasma fibrinolytic activity was much less than that observed in patients not given EACA. Andersson and his colleagues conclude therefore that the fall in fibrinogen seen in the patients not given EACA may have been partly due to plasma fibrinolytic activity.

Andersson (1962) in a study of 37 patients given EACA intravenously (0.1 gm./kg. body weight) immediately before prostatectomy, reports that in "most" of the patients plasma fibrinolytic activity had returned to preoperative levels within 3 hours of operation; in

5/

5 of the 37, plasma fibrinolytic activity was still increased 24 hours after operation.

In the present series, no evidence of increased fibrinolytic activity was found in the plasma samples taken off 4 hours after operation, when euglobulin lysis activity in all patients studied was normal. It would appear that the increased plasma fibrinolytic activity which had presumably been present during prostatectomy had passed off quickly after operation, and as thrombin clotting times were normal in all the patients, there was no evidence of previous hyperplasminaemia with fibrinogen digestion and defective fibrin polymerisation. The conclusion that fibrinolytic activity during and immediately after surgery had not been intense or associated with significant destruction of plasma proteins is reinforced by the normal plasminogen and fibrinogen levels found in all the patients.

CHAPTER 11

TRIAL OF EACA IN SUPRAPUBIC PROSTATECTOMY PATIENTS

The effect of EACA in reducing postoperative blood loss in the urinary tract was also studied in patients after suprapubic prostatectomy.

Clinical data Nineteen patients were studied; 10, selected at random (drawing lots) were treated with EACA and 9 were controls.

As seen in table 26, the EACA treated group and the control group were comparable as regards age, preoperative haemoglobin levels, preoperative blood urea nitrogen levels and blood pressures. Suprapubic prostatectomies were carried out by staff urologists of Barnes and Jewish Hospitals, St. Louis.

The mean weight of tissue removed (tissue weighed by the pathologist) was 57 ± 33 gm. in the EACA treated group and 69 ± 39 gm. in the control group; the difference is not significant ($t = 0.731$ and $0.5 < p > 0.4$).

EACA was used as a sterile 10 per cent aqueous solution. In all patients EACA was administered intravenously at a rate of 1 gm./hour for 4 hours, then 0.5 gm./hour for a further 8 hours (total dose, 8 gm.): the EACA was/

was given with the routine postoperative intravenous infusion, usually 1500 ml. of 5 per cent dextrose over the 12 hour period.

Histological examination Histological examination of the tissue removed (Surgical pathology departments of Barnes and Jewish Hospitals) showed benign nodular hyperplasia in each case. In 5 of the EACA treated patients (patients 1, 5, 8, 9 and 17) and 4 controls (patients 4, 7, 12 and 15) chronic inflammation was also seen. No evidence of carcinoma was found in any specimen.

Blood loss after operation was measured by collection of all drainage (per urethram and suprapubic) and by elution of dressings. Collection was begun as soon as the patient had entered the recovery room. Where there was much postoperative bleeding, the volume of water required to elute the dressings was large and some loss of accuracy in the haemoglobin assay was inevitable. Drainage, per urethram and suprapubic, was added to the eluate from the dressings and haemoglobin estimation was carried out after thorough mixing, by the method described in Chapter 10.

Intravascular haemolysis as a cause of haemoglobinuria was excluded by inspection of postoperative plasma specimens: haemoglobinaemia was never seen.

Anticoagulant The anticoagulant used was potassium oxalate/

oxalate (10 ml. whole blood + 20 mg. potassium oxalate).

Plasma EACA levels were assayed as described in Chapter 5.

Euglobulin lysis times were estimated as described in appendix 4.

Results

Blood loss was studied for 5 days after operation. As shown in figure 26 and table 27, blood loss in the EACA treated group was much reduced compared with the control group. As might be expected, the reduction in blood loss was most marked on the first postoperative day when there was a threefold reduction in blood loss in the EACA treated patients as compared with the controls (13.0 and 40.7 gm. respectively: $t = 2.66$ and $p < 0.02$). Over the whole 5 day period of observation blood loss in the EACA treated patients was less than one half that seen in the controls (24.7 and 57.4 gm. respectively: $t = 2.972$ and $p < 0.01$). For the first postoperative day, mean blood loss per gram of tissue resected in the EACA treated group was less than half that seen in the controls (table 28), (respectively 0.28 gm. haemoglobin and 0.65 gm. haemoglobin per gm. of tissue resected: $t = 2.936$ and $p < 0.01$).

Figure 27 shows the relationship between the weight of tissue resected and blood loss over the 5 day post-operative/

operative period. In the control group there was a positive correlation between blood loss and weight of tissue resected ($r = +0.737$, $p < 0.05$). In the EACA treated group such a correlation was not found either for the 5 day period ($r = +0.400$, $p > 0.1$) or for the first postoperative day ($r = +0.448$, $p > 0.1$).

Urinary urokinase and EACA levels Because of the dilution inevitable with elution of dressings it was not possible to measure levels of urokinase or EACA in the urine.

Plasma EACA levels Plasma EACA levels 4 and 8 hours after the start of EACA therapy are shown in table 29.

Euglobulin lysis times These were estimated for 4 patients (patients 1, 7, 10 and 19) the day before operation and in a sample taken off 4 hours after operation. In each case activity was less than 0.1 unit (within normal limits).

Side-effects of EACA administration

As in the transurethral prostatectomy group, no evidence of venous thrombosis was found following EACA infusion, and there were no significant changes in body temperature or pulse rate which could not be satisfactorily explained for other reasons, e.g. chest infection or bleeding.

Brief case reports are given of two patients who presumably/

presumably had vascular occlusion following EACA administration, though in both instances the occurrence of this accident may have been coincidental.

Patient 8 was a 74 year old man with a history of hypertension and ischaemic heart disease for 12 years. In 1950 blood pressure was 180/120 mm. Hg. In 1955 he had a myocardial infarction and because of severe intractable angina he was given a therapeutic dose of radio-active iodine in 1956.

Preoperatively (January, 1960) there was cardiac enlargement (apex beat in the anterior axillary line) and despite digitalis therapy, there was evidence of mild congestive heart failure. Blood pressure was 110/65 mm. Hg. There was a moderate hypochromic anaemia which had not responded to oral iron therapy (Hb. 9.5 gm./100 ml.). Clinically he was hypothyroid, with intolerance of cold, slow thick speech and cold dry skin. The operation and postoperative course were uneventful; the congestive heart failure improved somewhat during the postoperative period. Ten days after operation and EACA treatment he was found dead. Permission for post-mortem examination was refused, but death was presumed to have been due to vascular occlusion, cerebral, pulmonary or coronary.

Patient 13 This patient, age 64, gave a history of Parkinsonism for 17 years. At age 54 he had a transient/

ient left hemiparesis diagnosed as due to cerebral thrombosis. Because of hypertension, he had been treated with reserpine, 0.25 mg. t.i.d. for two years; preoperative blood pressure was 170/110 mm. Hg. There was cardiac enlargement (apex beat 3 cm. out-with the mid-clavicular line) and a slight Parkinsonian tremor. Cardiac enlargement was confirmed on chest X-ray, and E.C.G. showed the pattern of left ventricular hypertrophy and strain.

Operation was uneventful. EACA was given post-operatively as described above. Fifteen hours after operation, that is 3 hours after the end of the EACA infusion, he was found to be cyanosed and unresponsive. The right arm and leg were shaking, but were with-drawn in response to painful stimuli. The left arm and leg were flaccid, and there was no response to painful stimuli on the left. Head and eyes showed conjugate deviation to the right. The following day the patient had recovered consciousness and there was weakness and slight spasticity of the left arm and leg. The tendon reflexes on the left side were brisk and the left plantar response was extensor. Power gradually returned to the left side and was fully restored a week after operation, when the left plantar response was flexor.

Discussion

In this group of patients studied after suprapubic prostatectomy/

prostatectomy, the administration of EACA has resulted in a significant reduction in postoperative blood loss. That the effect of EACA is less striking than in the transurethral prostatectomy series is not surprising since in the suprapubic prostatectomy procedure, more extensive trauma is inflicted, direct visualisation of bleeding points may be difficult and reliance is placed on capsular retraction and packing to ensure haemostasis (Lowsley et al. 1956; Goodpasture and Beard, 1949). Further, the weight of prostatic tissue removed was much larger in the suprapubic prostatectomy cases (mean, 63 gm.) than in the transurethral procedure (mean, 15 gm.).

Plasma EACA levels and the two patients with presumed vascular occlusion are discussed in Chapter 13.

The significance of the results is discussed, together with the findings in the transurethral prostatectomy series, and the individual patients with prolonged haematuria, in Chapter 13.

CHAPTER 12

THE USE OF EACA IN INDIVIDUAL PATIENTS WITH HAEMATURIA

In view of the reduction in postoperative blood loss seen in the patients given EACA after both trans-urethral resection and suprapubic prostatectomy, EACA was given to a group of five patients, whose case reports follow, in whom protracted haematuria was a cause for concern.

Patients 1 - 4 were under the care of staff urologists of Barnes, Jewish or St. Luke's Hospitals, St. Louis. Patient 5 was under the care of the Ward Medical Services in Barnes Hospital.

Urinary haemoglobin assays were carried out as described in Chapter 10.

Anticoagulant The anticoagulant used was potassium oxalate (10ml. whole blood + 20 mg. potassium oxalate).

Patient 1 Bleeding during suprapubic prostatectomy in an 80 year old man (well differentiated adenocarcinoma of the prostate) was troublesome and persisted until EACA treatment was given 27 days later. Coagulation and fibrinolytic studies before EACA therapy showed no evidence of haemostatic defect or abnormal systemic fibrinolytic activity (table 30).

In/

In the period between operation and EACA therapy transfusion requirements to maintain the haemoglobin level were 30 pints.

In the 24 hours before EACA therapy blood loss in the urine was estimated at 55.7 gm. Eight gm. EACA was infused intravenously over 12 hours, then 10 gm. over the next 12 hours. Two gm. EACA was also run into the bladder with a catheter. In the 24 hours during and following EACA infusion, blood loss was estimated at 0.3 gm. haemoglobin. Seven days later bleeding recurred but was again promptly controlled with EACA, 12 gm. over 20 hours, reducing daily haemoglobin loss in the urine from 17.5 to 1.6 gm. The response to EACA administration is displayed graphically in figure 28; and figure 29 shows a sample of urine taken from the 24 hour collection immediately before EACA was given and samples from collections 2 and 24 hours after commencement of EACA therapy.

Patient 2 was a 71 year old man with severe and prolonged bleeding after suprapubic prostatectomy (benign nodular hyperplasia). During the first 31 days after operation 30 pints of blood were required to maintain his haemoglobin level. Cystoscopy on three occasions showed generalised oozing in the prostatic fossa but no definite bleeding points were seen. On his transfer to Barnes Hospital, rate of blood loss through suprapubic cystostomy/

cystostomy was estimated at 103 gm. haemoglobin/ 24 hours. Platelet count on admission was 62,000/ mm^3, and Hess' test was positive. As shown in table 30, other coagulation and fibrinolytic assays were normal. Following an intravenous infusion of EACA (12 gm. over 16 hours) blood loss was reduced to 2.9 gm. haemoglobin/24 hours, a reduction of 98 per cent.

At this time (figure 30), because rectal examination revealed a large mass in the area of the prostate, the bladder was inspected through a suprapubic incision. No bleeding points were seen and the mass was found to be a large organising haematoma, which was evacuated. Subsequent to this re-exploration, there was brisk bleeding; EACA therapy was continued and the bleeding was much reduced in 24 hours (41 gm. haemoglobin/24 hours falling to 16 gm. haemoglobin/ 24 hours) and still further reduced (3.5 gm./24 hours) at 48 hours. EACA therapy was continued for 6 days after surgery and was then withdrawn. Two days later there was a marked increase in bleeding which was again promptly controlled with EACA therapy. As can be seen in figure 30, a further recurrence of bleeding on the 19th day of observation was again controlled by EACA therapy. On the 24th day of observation reduction in EACA dosage from 3 to 2 gm./day resulted in an increase in urinary blood loss; this

was/

was controlled when dosage was increased to 3 gm./ day, and bleeding ceased on the 28th day of observation.

The platelet count in this patient on admission was 62,000/mm^3 and then gradually fell to 10,000/mm^3, at which time steroid therapy was instituted (figure 30). Apart from thrombocytopenia and moderate hypochromic anaemia, the peripheral blood picture was otherwise normal. Repeated bone marrow biopsy showed "numerous immature megakaryocytes with failure of 'budding'; the bone marrow showed marked erythropoietic activity but was otherwise normal". No final haematological diagnosis was arrived at. With prednisone therapy the platelet count rose slowly.

Patient 3, an 87 year old man with ischaemic heart disease and congestive cardiac failure, had a trans-urethral prostatectomy for benign prostatic hypertrophy (22 gm. tissue). There was some preoperative haematuria and bleeding during and after operation was troublesome. Cytoscopy on the second day after operation did not reveal any definite bleeding points but there was generalised oozing of blood from the raw surfaces of the prostatic bed. Coagulation and fibrinolytic studies at this time were within normal limits (table 30). On the fourth postoperative day, as blood loss was giving cause for concern, (4.5 gm. haemoglobin/ 24 hours) EACA therapy was instituted (20 gm. intravenously over/

over 24 hours) with almost immediate reduction in bleeding (figure 31). When EACA therapy was discontinued, bleeding recurred; cystoscopy at this time once more showed general oozing of blood from the prostatic fossa, but no definite bleeding points were seen. EACA was again given (22 gm. intravenously in 24 hours, reducing to 6 gm./24 hours) with almost immediate control of bleeding (within minutes). Recurrence of haematuria two days later was controlled by increasing the dose of EACA to 15 gm. over 24 hours. On the 18th day of observation, because of difficulty in voiding and a large residual urine volume, more prostatic tissue (10 gm.) was removed by transurethral resection under cover of EACA. Bleeding following this operation (1.95 gm. haemoglobin in 24 hours after operation) was much less than the mean blood loss in the control patients studied after transurethral prostatectomy (5.3 ± 4.9 gm. haemoglobin in the first 24 hours (Chapter 10). Bleeding thereafter was minimal and EACA therapy was discontinued 6 days after the second operation. Histological examination of the tissue removed in the second operation showed extensive necrosis.

During the postoperative period the patient's cardio-respiratory status often gave cause for concern and 36 hours after EACA therapy had been discontinued, he/

he died. At autopsy there was necrotic haemorrhagic tissue in the prostatic fossa. No evidence of arterial or venous occlusion was found. The coronary arteries were patent. There was cardiac hypertrophy, acute cardiac dilatation, pyelonephritis, and terminal hypostatic pneumonia.

Patient 4 A 57 year old man presented with a story of haematuria for about 6 weeks. Intravenous and retrograde pyelograms showed appearances suggestive of renal carcinoma on the right side, and at cystoscopy the bleeding was from the right ureter. There was no evidence of a coagulation defect or of increased systemic fibrinolytic activity (table 30). The effect of infusion of 8 gm. EACA over 24 hours is shown in figure 32, where it will be seen that in association with inhibition of urokinase activity (assayed as described in Chapter 10) was a marked reduction in haematuria. As EACA was excreted and urinary EACA levels fell, (assayed as described in Chapter 5), inhibition of urokinase passed off and urinary blood loss returned to the level seen before EACA was given. Subsequently the right kidney was removed and a transitional cell carcinoma was found, with an ulcerated surface in the renal pelvis.

Patient 5 A 22 year old man suffering from severe haemophilia (antihaemophilic-globulin level less than

1/

1 per cent of normal) was admitted with a history of haematuria for 7 days. Haematuria persisted despite fresh plasma infusions (1 litre/day for 4 days) and as a brisk febrile reaction followed each plasma infusion, it was thought desirable to try the effect of EACA. When 20 gm. EACA had been given orally over 30 hours, haematuria suddenly ceased completely and there was a simultaneous onset of right renal colic. An intravenous pyelogram at this time showed a "nephrogram" effect on the right, but no dye was seen in the right renal pelvis or ureter; the left side was normal. For the next 9 days there was intermittent renal colic and occasionally there were stringy black clots in the urine, which was otherwise clear, with no indication of fresh bleeding. An intravenous pyelogram 4 days after the onset of renal colic again showed a "nephrogram" effect on the right with no evidence of dye excretion, and intravenous pyelograms 6 days and 2 months later showed no renal function on the right side. In view of the patient's severe coagulation defect it was not thought desirable to perform retrograde ureteric catheterisation.

Discussion

In each of these patients administration of EACA was so closely related in time to marked reduction in urinary blood loss that it would seem unlikely that this/

this association was due to chance. Indeed in patients 1, 2 and 3, EACA therapy was associated with control of bleeding on 2, 3 and 3 occasions respectively. In patient 2, it may be that because of thrombocytopenia, the patient was forming mechanically poor clots which were more than usually susceptible to lysis, but were adequate in haemostasis when urokinase was inhibited by EACA. In patient 3, under cover of EACA, trans-urethral resection was carried out with only 40 per cent of the mean postoperative blood loss seen in a group of control subjects after this operation (table 19).

In case 5, after administration of EACA, perhaps blood clot, formed in the renal pelvis or ureter by the action of urine thromboplastin had a high level of EACA, and so was resistant to lysis by urokinase. Clot may then have persisted in the upper urinary tract with obstruction and consequent loss of renal function. Steiger et al. (1962) have given EACA to 3 haemophiliac patients with haematuria which had not responded to plasma infusion, and in each case haematuria cleared promptly with EACA, and without symptoms of urinary tract obstruction; Nilsson (1963) reports one similar case.

The general significance of the results, together with those from the patients given EACA after trans-urethral and suprapubic prostatectomies, is discussed in Chapter 13.

CHAPTER 13

DISCUSSION: THE USE OF EACA TO CONTROL FIBRINOLYTIC ACTIVITY IN THE URINARY TRACT

The presence in normal urine of large quantities of a plasminogen activator, urokinase, has posed problems concerning its source and possible physiological role. As discussed in Chapter 9, two views have been expressed.

(1) Urokinase represents an excretory product which reflects the dynamic state of the fibrinolytic enzyme system in vivo.

(2) Urokinase is a secretory product of the renal epithelium concerned primarily with maintaining the patency of the urinary tract, in virtue of its ability to mediate the lysis of fibrin deposits.

Regardless of these differing views, the presence of urokinase in urine makes available an enzymatic mechanism for the lysis of fibrin deposits and blood clots in the urinary tract. It is now recognised that under biological conditions significant amounts of plasminogen are incorporated into clots and fibrin deposits during their formation; the thrombi thus formed are endowed with an intrinsic enzyme system which, upon activation, results in the lysis of fibrin (Chapter 3). Under these circumstances, only the presence/

presence of a plasminogen activator in the surrounding fluid is required to mediate clot lysis; indeed the evidence suggests that activation of this intrinsic enzyme system of clots represents the major mechanism for clot lysis under physiological circumstances. It follows that the ability of urinary urokinase to promote the lysis of fresh haemostatic fibrin deposits could be a significant factor in maintaining continued bleeding in the presence of operative trauma. The present study was undertaken to determine whether, by inhibiting urokinase activity with EACA, post-operative haemostasis might be improved.

The problem was studied in patients who had undergone transurethral and suprapubic prostatectomy. In both groups the data indicate that administration of EACA was associated with a reduction in urinary haemoglobin loss. The evidence suggests that EACA produced this effect by inhibition of urokinase activity in the urinary tract, and conversely, the data also provide evidence to support the concept that urokinase, by promoting the lysis of fibrinous deposits, may play an important physiological role in maintaining the patency of the urinary tract.

As plasma levels of EACA 4 hours after operation (11.6 ± 3.5 mg./100 ml. for the transurethral and suprapubic patients combined) were at a level sufficient to produce significant inhibition of plasminogen activator activity/

activity in the plasma, the possibility has not been excluded that part of the effect of EACA was mediated through a systemic effect on the plasminogen-plasmin system. However, as in the transurethral prostatectomy series there was no evidence of significant systemic fibrinolytic activity after operation (Chapter 10), it does not seem probable that the beneficial effect of EACA administration was due to suppression of systemic fibrinolytic activity; and considerable evidence has been adduced to support the hypothesis that EACA acted locally in the urinary tract.

The observations cited also establish the effectiveness of EACA as an adjunct to conventional measures in the management of postoperative haematuria. This conclusion is supported not only by the results obtained in the controlled trials but by the observations of the beneficial effects of EACA in the individual patients with severe and prolonged postoperative haematuria. The effectiveness of EACA in controlling post-prostatectomy haematuria has been subsequently confirmed by other workers (Sack et al., 1962; Andersson, 1962). It should be emphasised that EACA therapy is only one aspect of the total management of these patients and does not replace any of the basic principles which have been established for the care of such patients.

At the present stage of investigation of EACA, certain considerations/

considerations must be borne in mind. The inhibition of fibrinolytic activity may be expected to result in more extensive deposition of fibrin; though this is the desired effect in the management of postoperative haematuria, on occasion it could result in the accumulation of undesirably large clots. In the patient with haemophilia and spontaneous haematuria, reported in Chapter 12, the administration of EACA probably resulted in a large clot in the renal pelvis, which containing EACA, was resistant to lysis. Obviously a more extensive investigational experience with EACA will be required before its full therapeutic potential and contraindications can be established. Perhaps it may ultimately prove more useful for control of bleeding in the bladder and lower urinary tract than in the upper urinary tract.

Other potential hazards remain to be evaluated. The two patients (described in Chapter 11) with presumed vascular occlusions (the diagnosis was not established) both had a background of vascular disease and the relationship with EACA therapy was possibly fortuitous. Nevertheless, large scale observations as to the incidence of vascular thrombosis in patients treated with EACA are necessary.

Further investigation of the use of EACA in the control of urinary tract bleeding appears indicated, particularly/

particularly where postoperative haematuria, not due to technical surgical deficiencies, is causing clinical concern. The benefit to the patient of control of blood loss may far outweigh the theoretical hazards of EACA therapy, and if used in appropriate circumstances, EACA would appear to have much promise in the management of postoperative haemorrhage in the urinary tract.

SECTION 4

SUMMARY AND CONCLUSIONS

CHAPTER 14 SUMMARY AND CONCLUSIONS

CHAPTER 14

SUMMARY AND CONCLUSIONS

In recent years it has become apparent that disordered fibrinolytic activity can in some circumstances give rise to defective haemostasis, and treatment of fibrinolytic states has become possible with the discovery that the synthetic amino acid epsilon aminocaproic acid (EACA) is a potent inhibitor of fibrinolytic activity.

In view of the important potential uses of EACA in the treatment of human disease, an assay system for EACA in plasma and other biological fluids was developed, and using this assay system the absorption, distribution and excretion of EACA were studied, thus making possible a rational approach to choice of dosage schedule and route of administration. The thesis also explores in some detail one application of EACA, its use to control fibrinolytic activity in the urinary tract.

The assay system for EACA in plasma was a chromatographic technique using ion exchange resin loaded paper. The advantages of this system include ease of sample preparation (only precipitation with trichloracetic acid is needed); use of aqueous solvents; speed (chromatograms were developed within 2 hours); and application of samples as streaks permitting large sample size, the/

the use of two buffers in a unidirectional system, and quantitation by strip-scanning densitometry. The system can be modified for assay of other basic amino acids e.g. histidine. The results obtained in the EACA assay are comparable with those obtained by a previously described high voltage electrophoresis technique, and the coefficient of variation in the EACA assay is acceptable (8.98 per cent).

The assay system was applied to the study of the absorption, distribution and excretion of EACA. In man, EACA was absorbed rapidly from the gastro-intestinal tract, peak plasma levels after a single oral dose being found at 2 hours. Approximately 82 per cent of a single oral or intravenous dose was recovered apparently unchanged in the urine in a 72 hour period, suggesting that only a small portion, if any, of the EACA was metabolised. Plasma levels of 10^{-3} Molar (13 mg./100 ml.), a concentration required in vitro for effective control of fibrinolytic activation, were achieved and sustained by oral administration of a priming dose of 4 - 6 gm., followed by 1 gm. of EACA per hour. At such potentially therapeutic plasma levels, the renal clearance of EACA was approximately 75 per cent of endogenous creatinine clearance, and levels of EACA in the urine were about seventy times those found in plasma. After prolonged administration, withdrawal of EACA was followed by a more prolonged period of urinary/

urinary excretion, presumably because of distribution through the extravascular compartments when plasma levels were high. Evidence was obtained that in vivo EACA readily enters human red blood cells and numerous organ tissues in the rabbit; it also entered the cells of the intact rat diaphragm preparation in vitro. EACA did not appear to be protein bound in the plasma.

To further define the potential biochemical effects of EACA, its effects on thromboplastin generation and the thrombin-fibrinogen reaction were studied. Up to a concentration of 10^{-1} Molar, EACA had no effect on thromboplastin generation in the thromboplastin generation test. An effect, presumably due to increased ionic strength, and similar to that produced by urea and sodium chloride, was found on the thrombin-fibrinogen reaction when EACA concentrations were above 0.5 Molar, a concentration never likely to be achieved in the plasma. EACA was found to inhibit pepsin, but the concentration needed (above 5×10^{-3} Molar for 50 per cent inhibition) is also unlikely to be achieved in plasma in vivo.

The thesis then goes on to give an account of one potential application of EACA in human therapeutics, its use after prostatectomy to inhibit local fibrinolytic activity in the urinary tract. Urokinase, an activator of the fibrinolytic enzyme system, is normally present in/

in urine and confers upon it the ability to lyse fibrin clots. It has been suggested that urokinase may play a role in maintaining the patency of the urinary tract by promoting the lysis of fibrinous deposit. Since such an action might also serve to impair haemostasis in the urinary tract, an investigation was undertaken of the effect on the duration and extent of bleeding observed after prostatectomy of inhibiting urokinase activity by EACA administration.

Twenty-eight patients were studied after transurethral prostatectomy; 15, selected at random, were controls, and 13 were given 8 gm. EACA intravenously over the first 12 postoperative hours. The EACA treated and control groups were comparable as regards age, pre-operative haemoglobin, blood urea nitrogen and blood pressure levels, and weight of tissue resected. In the EACA treated group, as EACA was excreted in the urine, urokinase activity was completely inhibited for 24 hours, and substantially inhibited for a further 24 hours; in some patients, there was a degree of inhibition up to 72 hours. Urinary EACA levels were assayed and were a mean of 2.5×10^{-2} Molar on the first day, 6.0×10^{-3} Molar on the second day and 8.4×10^{-4} Molar on the third day. Where urokinase activity was not completely inhibited, there was a linear relationship between the degree of urokinase inhibition and/

and the logarithm of the EACA concentration. After dialysis of the urine samples to remove EACA, urokinase activity in EACA treated and control patients was similar; it is therefore concluded that the action of EACA was to reversibly inhibit urokinase, but not to destroy it or alter its rate of production.

Associated with inhibition of urokinase activity, postoperative blood loss over a 5 day period of observation was reduced four-fold in the EACA treated patients as compared with the controls. Duration of bleeding was also significantly reduced.

Urine from all patients in both EACA treated and control groups was examined for proteolytic activity at pH 7.6 and pH 8; none was found. In urines from both groups of patients uropepsin activity (proteolytic activity at pH 2) was found, and this was significantly less in the EACA treated patients than in the controls. However, as the pH at which uropepsin activity is manifest is below the level ever found in urine, the inhibition of uropepsin activity observed in EACA treated subjects is of theoretical interest only. Urines from both EACA treated and control groups, when added to normal plasma, prolonged the thrombin-clotting time to a somewhat lesser extent than did urines from normal subjects; in all groups, ability to prolong the thrombin clotting time was lost if the urines were dialysed before assay/

assay, and it is concluded that the ability of urine to prolong the thrombin clotting time of plasma is probably due to its ionic strength.

In both EACA treated and control groups, assays were carried out for evidence of significant systemic fibrinolytic activity; no such evidence was found. Plasma EACA levels 4 and 8 hours after operation were assayed: the levels found (mean values of 11.3 ± 3.0 mg./100 ml. at 4 hours and 6.2 ± 1.8 mg./100 ml. at 8 hours) were adequate to produce significant inhibition of systemic fibrinolytic activity, but as no evidence of such activity was apparent it is not thought likely that EACA exercised its beneficial effect by inhibition of systemic fibrinolytic activity.

A controlled trial of the effect of EACA in reducing blood loss after suprapubic prostatectomy was also carried out. Nineteen patients were studied; 10, selected at random, were given 8 gm. EACA by intravenous infusion over the first 12 hours after operation, and 9 were controls. The EACA treated patients and the controls were comparable as regards age, preoperative haemoglobin, blood urea nitrogen and blood pressure levels, and weight of tissue removed. Postoperative blood loss was followed for 5 days, and over this period was reduced by one half in the EACA treated group as compared with the controls. Plasma EACA levels/

levels were estimated at 4 and 8 hours after operation and were 12.1 ± 2.7 mg./100 ml. and 6.6 ± 1.4 mg./100 ml. respectively: these levels are adequate to produce significant inhibition of systemic fibrinolytic activity. Two patients given EACA after suprapubic prostatectomy had presumed vascular occlusion; one, an episode of hemiplegia 3 hours after the end of EACA infusion; the other died suddenly 10 days after EACA treatment. These presumed vascular accidents may have been coincidental, or may have been due to inhibition of systemic fibrinolytic activity with consequent accumulation of thrombus in blood vessels.

EACA was also given, with almost immediate control of bleeding, to 3 patients in whom post-prostatectomy haematuria was severe and protracted; haematuria was also controlled with EACA in a patient with renal carcinoma. A patient with severe haemophilia and spontaneous haematuria was given EACA; bleeding ceased, but at the same time the patient had renal colic, and permanent loss of renal function on one side. It may be that after EACA administration a large clot, which was resistant to lysis, because of its EACA content, gathered in the renal pelvis and obstructed urine flow.

It is concluded that EACA, by inhibiting local fibrinolytic activity in the urinary tract, is of value in the control/

control of postoperative haematuria not due to technical surgical deficiencies.

Demonstration of the value of EACA was a relatively straightforward problem; it was possible to carry out a controlled trial, and the haemostatic defect is uncomplicated fibrinolysis. Convincing proof of the benefit of EACA in the treatment of systemic fibrinolytic states, in which the natural history is variable and only partly elucidated, and the haemostatic defect is complex, will be more difficult to obtain and will probably depend on the evidence of well studied individual patients rather than on controlled trials. Though such individual case reports are already accumulating (Chapter 4), investigation of the pathogenesis and treatment of systemic fibrinolytic states represents an important field for further study.

The role of EACA in human therapeutics has not yet been fully defined, but the present work makes possible selection of effective dosage levels, and establishes its usefulness in promoting postoperative haemostasis in the urinary tract.

Because of potential toxicity (perhaps vascular thrombosis and teratogenicity are the most important theoretical hazards), caution must in the meantime be exercised in its use except in situations where fibrinolytic activity causes a major clinical problem.

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REFERENCES

REFERENCES

Ablondi, F.B., Hagan, J.J., (1957), Proc.Soc.exp. Biol. (N.Y.), 95, 195.

Ablondi, F.B., Hagan, J.J., Philips, M., De Renzo, E.C., (1959), Arch.Biochem., 82, 153.

Alagille, D., Soulier, J.P., (1956), Sem.Hop.Paris, 32, 355.

Albrechtsen, O.K., (1957), Brit.J.Haemat., 3, 284.

Albrechtsen, O.K., Thaysen, J.H., (1955), Acta physiol. Scand., 35, 138.

Alkjaersig, N., Fletcher, A.P., Sherry, S., (1958), J.biol.Chem., 233, 86.

Alkjaersig, N., Fletcher, A.P., Sherry, S., (1959a), J.biol.Chem., 234, 832.

Alkjaersig, N., Fletcher, A.P., Sherry, S., (1959b), J.clin.Invest., 38, 1086.

Alkjaersig, N., Fletcher, A.P., Sherry, S., (1962), J.clin.Invest., 41, 917.

Andersson, L., (1962), Personal communication, to be published as supplement 301 to Acta chir.Scand.

Andersson, L., Nilsson, I.M., (1961), Acta chir. Scand., 121, 291.

Andersson, L., Nilsson, I.M., Olow, B., (1962), Thrombos.Diathes.haemorrh.(Stuttg.), 7, 391.

Astrup, T., (1956a), Blood, 11, 781.

Astrup/

Astrup, T., (1956b), Lancet, 2, 565.

Astrup, T., Alkjaersig, N., (1951), Nature (Lond.), 165, 565.

Astrup, T., Alkjaersig, N., (1952), Nature (Lond.), 169, 314.

Astrup, T., Permin, P.M., (1947), Nature (Lond.), 159, 681.

Astrup, T., Sterndorff, I., (1952), Proc.Soc.exp. Biol. (N.Y.), 81, 675.

Astrup, T., Sterndorff, I., (1953), Proc.Soc.exp. Biol. (N.Y.), 84, 605.

Bang, N.U., Fletcher, A.P., Alkjaersig, N., Sherry, S., (1962), J.clin.Invest., 41, 935.

Biggs, R., Douglas, A.S., (1953), J.clin.Path., 6, 23.

Biggs, R., Macfarlane, R.G., (1962). Human blood coagulation and its disorders, Blackwell, Oxford; 3rd edition, p. 131.

Biggs, R., Macfarlane, R.G., Pilling, J., (1947), Lancet, 1, 402.

Bjerrehuus, I., (1952), Scand.J.clin.Lab.Invest., 4, 179.

Blombäck, B., Blombäck, M., (1956), Ark.kemi, 10, 415.

Bonsnes, R.W., Taussky, H.H., (1945), J.biol.Chem., 158, 581.

Buchs/

Buchs, S., (1949), Enzymologia, 13, 208.

Campbell, G., Siegriest, H., Siiteri, P.K., (1960), Personal communication.

Celander, D.R., Guest, M.M., (1960), Amer.J.Cardiol., 6, 409.

Christensen, L.R., (1945), J.gen.Physiol., 28, 363.

Christensen, L.R., (1946), J.gen.Physiol., 30, 149.

Christensen, L.R., MacLeod, C.M., (1945), J.gen. Physiol., 28, 559.

Christensen, L.R., Smith, D.H., (1950), Proc.Soc. exp.Biol.(N.Y.), 74, 840.

Cohen, S.N., Kupfer, H.G., (1958), New Engl.J.Med., 259, 1103.

Cohn, E.J., Strong, L.E., Hughes, W.L., Mulford, D.J., Ashworth, J.N., Melin, M., Taylor, H.L., (1946), J.Amer.chem.Soc., 68, 459.

Copley, A.L., (1957), Lancet, 2, 1119.

Corley, R.C., (1929), J.biol.Chem., 81, 545.

Dastre, A., (1893), Arch.physiol.norm.path. (Paris), 5th Series, 5, 661.

Delezenne, C., Pozerski, E., (1903), C.R.Soc.Biol. (Paris), 55, 327.

Denis, P.S., (1838), quoted by Macfarlane, R.G., Biggs, R., (1948), Blood, 3, 1167.

Denys, J., Marbaix, H.de, (1889), Cellule, 5, 197.

Donaldson, V.H., Ratnoff, O.D., (1962), J.exp.Med., 115/

115, 695.

Duesberg, R., Friederici, L., (1956), Naunyn-Schmiedeberg's Arch.exp.Path.Pharmak., 228, 347.

Duguid, J.B., (1949), Lancet, 2, 925.

Fearnley, G.R., (1961), Lancet, 1, 992.

Fearnley, G.R., Tweed, J.M., (1953), Clin.Sci., 12, 81.

Fleisher, M.S., Loeb, L., (1915), J.biol.Chem., 21, 477.

Fletcher, A.P., (1960), in N.I.H. Conference on Thrombolytic Agents, eds. Roberts, H.R., Geraty, J.D., University of North Carolina Press, Chapel Hill; p.148.

Fletcher, A.P., (1962), Personal communication.

Fletcher, A.P., Alkjaersig, N., Sherry, S., (1959), J.clin.Invest., 38, 1096.

Fletcher, A.P., Alkjaersig, N., Sherry, S., (1962a), J.clin.Invest., 41, 896.

Fletcher, A.P., Alkjaersig, N., Sherry, S., (1962b), Amer.J.Med., 33, 738.

Gans, H., Krivit, W., Runyeon, A., McAuley, M., Gans, M.A., (1962), Ann.Surg., 155, 268.

Gerheim, E.B., Ferguson, J.H., Travis, G.H., Johnston, C.L., Boyles, P.W., (1948), Proc.Soc. exp.Biol.(N.Y.), 68, 246.

Goodpasture, E.W., (1914), Bull.Johns Hopk.Hosp., 25, 330.

Goodpasture/

Goodpasture, W.E., Beard, D.E., (1949), J.Urol. (Baltimore), 62, 849.

Green, J.R., (1887), J.Physiol.(Lond.), 8, 372.

Grossi, C.E., Moreno, A.H., Rousselot, L.M., (1961), Ann.Surg., 153, 383.

Hedin, S.G., (1904), J.Physiol.(Lond.), 30, 195.

Hunter, J., (1794), A treatise on the blood, inflammation and gun-shot wounds. Nicol, London; p.87.

Iatridis, S.G., Wilson, E.G., Ferguson, J.H., Rierson, H.A., (1960), Thrombos.Diathes.haemorrh.(Stuttg.), 5, 50.

Itoga, G., Yogo, T., (1959), Keio.J.Med., 8, 299.

Jacobsson, K., (1955), Scand.J.clin.Lab.Invest., Suppl.7, 93.

Jensen, H., (1956), Exp.Med.Surg., 14, 189.

Johnson, A.J., (1960), Personal communication.

Johnson, A.J., Skoza, L., (1961), Fed.Proc., 20, 59.

Johnson, A.J., Skoza, L., Claus, E., (1962), Thrombos. Diathes.haemorrh.(Stuttg.), 7, 203.

Johnson, S.A., Schneider, C.L., (1953), Science, 117, 229.

Kaplan, M.H., (1944), Proc.Soc.exp.Biol.(N.Y.), 57, 40.

von Kaulla, K.M., (1956), Acta Haemat.(Basel), 16, 315.

von Kaulla, K.M., McDonald, T.S., (1958), Blood, 13/

13, 811.

von Kaulla, K.M., Shettles, B., (1953), Proc.Soc. exp.Biol.(N.Y.), 83, 692.

von Kaulla, K.M., Swan, H., (1958), J.thorac.Surg., 36, 519.

Kipnis, D.M., Cori, C.F., (1957), J.biol.Chem., 224, 681.

Kjeldgaard, N.O., Ploug, J., (1957), Biochim.biophys. Acta, 24, 283.

Kline, D.L., (1953), J.biol.Chem., 204, 949.

Kosaki, T., Ikeda, T., Noda, Y., (1952), J.Biochem. (Tokyo), 39, 381.

Kowalski, E., Kopec, M., Latallo, Z., Roszkowski, S., Sendys, N., (1958), Blood, 8, 436.

Kowalski, E., Latallo, Z., (1956), Sang, 27, 466.

Kwaan, H.C., Lo, R., McFadzean, A.J.S., (1957), Clin.Sci., 16, 241.

Kwaan, H.C., McFadzean, A.J.S., Cook, J., (1956), Lancet, 1, 132.

Ladehoff, A., Rasmussen, J., (1961), Scand.J.clin. Lab.Invest., 13, 231.

Lang, K., Bitz, H., (1953), Biochem.Z., 324, 495.

Lassen, M., (1952), Acta physiol.Scand., 27, 371.

Lewis, G.P., (1960), Physiol.Rev., 40, 647.

Lewis, J.H., Ferguson, J.H., (1951), Proc.Soc.exp. Biol.(N.Y.), 78, 184.

Lewis/

Lewis, J.H., Howe, A.C., Ferguson, J.H., (1949), J.clin.Invest., 28, 1057.

Loeper, M., Baumann, S., (1922), Progr.med.(Paris), 35, 205.

Lombardo, L.J., (1957), J.Urol.(Baltimore), 77, 289.

Lombardo, L.J., (1958), J.int.Coll.Surg., 30, 412.

Lombardo, L.J., (1959), J.Amer.med.Ass., 169, 102.

Lowsley, O.S., Kirwin, T.J., (1956), Clinical Urology, The Williams & Wilkins Co., Baltimore; p.447.

Macfarlane, R.G., (1937), Lancet, 1, 10.

Macfarlane, R.G., Pilling, J., (1946), Lancet, 2, 562.

McGinty, D.A., Lewis, H.B., Marvel, C.S., (1924), J.biol.Chem., 62, 75.

McNicol, G.P., (1962), Scot.med.J., 7, 266.

McNicol, G.P., Fletcher, A.P., Sherry, S., (1960), Fed.Proc., 19, 57.

McNicol, G.P., Douglas, A.S., Bayley, C., (1962a), Lancet, 2, 1297.

McNicol, G.P., Fletcher, A.P., Alkjaersig, N., Sherry, S., (1962b), J.Lab.clin.Med., 58, 34.

McNicol, G.P., Gale, S.B., Douglas, A.S., (1963), Brit.med.J., 1, 909.

Mikata, I., Hasegawa, M., Igarashi, I., Shirakura, N., Hoshida, M., Toyama, K., (1959), Keio J. Med./

Med., 8, 279.

Miller, J.M., Meisel, H.J., Jackson, D.A., Collier, C.S., (1959), J.Urol.(Baltimore), 81, 672.

Milstone, H., (1941), J.Immunol., 42, 109.

Mirsky, I.A., Perisutti, G., Davis, N.C., (1959), J.clin.Invest., 38, 14.

Mole, R.H., (1948), J.Path.Bact., 60, 413.

Morawitz, P., (1906), Beitr.chem.Physiol.Path., 8, 1.

Mounter, L.A., Shipley, B.A., (1958), J.biol.Chem., 231, 855.

Mullertz, S., (1952), Acta physiol.Scand., 26, 174.

Mullertz, S., (1954), Proc.Soc.exp.Biol.(N.Y.), 85, 326.

Mullertz, S., (1957), Ann.N.Y.Acad.Sci., 68, (i), 38.

Myhre, D.V., Smith, F., (1958), J.organic Chem., 23, 1229.

Naeye, R.L., (1962), Blood, 19, 694.

Niewiarowski, S., Latallo, Z., (1957), Bull.Acad. pol.Sci.Cl.2, 5, 219.

Nilsson, I.M., (1961), Thrombos.Diathes.haemorrh. (Stuttg.), Suppl.1, ad vol.6, p.325.

Nilsson, I.M., (1963), Personal communication.

Nilsson, I.M., Krook, H., Sternby, N.H., Soderberg, E., Soderstrom, N., (1961), Acta med.Scand., 169/

169, 323.

Nilsson, I.M., Sjoerdsma, A., Waldenstrom, J., (1960), Lancet, 2, 1322.

Nolf, P., (1905), Arch.int.Physiol., 3, 1.

Nolf, P., (1908), Arch.int.Physiol., 6, 306.

Norman, P.S., (1960), in N.I.H. Conference on Thrombolytic Agents, eds. Roberts, H.R., Geraty, J.D., University of North Carolina Press, Chapel Hill; p.108.

Norman, P.S., Hill, B.M., (1958), J.exp.Med., 108, 639.

Northrop, J.H., Kunitz, M., Herriott, R.M., (1948), Crystalline enzymes. Columbia University Press, New York; 2nd edition, p.303.

Nour-Eldin, F., Draisey, T.F., (1963), J.clin.Path., 16, 61.

Okamoto, S., Nagasawa, F., Takagi, E., Tsukada, Y., Kokoi, M., Sato, M., (1957), Refer to British Patent No.770,693, (Mitshubishi Kasei Kogyo Co. Ltd.).

Opie, E.L., Barker, B.F., (1907), J.exp.Med., 9, 207.

Paraskevas, M., Nilsson, I.M., Martinsson, G., (1962), Scand.J.clin.Lab.Invest., 14, 138.

Peck, H.M., Baer, J.E., McKinney, S.E., Wazeter, F.X., Zwickey, R.E., (1961), Personal communication.

Pillemer/

Pillemer, L., Ratnoff, O.D., Blum, L., Lepow, I.H., (1952), J.exp.Med., 97, 573.

Ploug, J., Kjeldgaard, N.O., (1957), Biochim. biophys.Acta, 24, 282.

Ratnoff, O.D., (1949), Bull.Johns Hopk.Hosp., 84, 29.

Ratnoff, O.D., (1952), J.clin.Invest., 31, 521.

Ratnoff, O.D., (1953), J.clin.Invest., 32, 473.

Remmert, L.F., Cohen, P., (1949), J.biol.Chem., 181, 431.

Roberts, H.R., Kolor, M., (1959), Anal.Chem., 31, 565.

Sack, E., Spaet, T.H., Gentile, R.L., Hudson, P.B., (1962), New Engl.J.Med., 266, 541.

Sahli, W., (1885), Pflüg.Arch.ges.Physiol., 36, 209.

Sata, S., Ishibashi, Y., Endo, T., Watanabe, T., Nakajima, K., (1959), Keio J.Med., 8, 267.

Sawyer, W.D., Fletcher, A.P., Alkjaersig, N., Sherry, S., (1960), J.clin.Invest., 39, 426.

Sawyer, W.D., Alkjaersig, N., Fletcher, A.P., Sherry, S., (1961). Arch.intern.Med., 107, 274.

Scheraga, H.A., Laskowski, M., (1957), Advanc. Protein Chem., 12, 1.

Schneider, C.L., (1959), Ann.N.Y.Acad.Sci., 75, 634.

Scott, E.V.Z., Matthews, W.F., Butterworth, C.E., Frommeyer, W.B., (1954), Surg.Gynec.Obstet., 99/

99, 679.

Sherry, S., (1954), J.clin.Invest., 33, 966.

Sherry, S., Fletcher, A.P., Alkjaersig, N., (1959a), Physiol.Rev., 39, 343.

Sherry, S., Fletcher, A.P., Alkjaersig, N., Sawyer, W.D., (1959b), Trans.Ass.Amer.Physcns., 72, 62.

Shirasaki, K., (1950), Igaku to Seibutsugaku, 16, 363. (quoted by Sweeny, W., (1960))

Shulman, S., Alkjaersig, N., Sherry, S., (1958), J.biol.Chem., 233, 91.

Siegriest, H., Siiteri, P.K., (1960), Personal Communication.

Sjoerdsma, A., Hanson, A., (1959), Acta chem.Scand., 13, 2150.

Sjoerdsma, A., Nilsson, I.M., (1960), Proc.Soc. exp.Biol.(N.Y.), 103, 533.

Smith, H.W., (1951), The kidney: structure and function in health and disease. Oxford University Press, New York; p.377.

Smith, I., (1960), Chromatographic and electrophoretic techniques. Volume I, chromatography. Heinemann, London; 2nd edition, p. 89 - 90.

Smyrniotis, F.E., Fletcher, A.P., Alkjaersig, N., Sherry, S., (1959), Thrombos.Diathes.haemorrh. (Stuttg.), 3, 257.

Sobel/

Sobel, G.W., Mohler, S.R., Jones, N.W., Dowdy, A.B.C., Guest, M.M., (1952), Am.J.Physiol., 171, 768.

Soulier, J.P., Mathey, J., Le Bollock, A.G., Daumet, P., (1952), Rev.Hemat., 7, 30.

Spink, W.W., Vick, J.A., (1961), Proc.Soc.exp.Biol. (N.Y.), 106, 242.

Steiger, B., White, J.G., Krivit, W., (1962), J-Lancet, 82, 421.

Storm, O., (1955), Scand.J.clin.Lab.Invest., 7, 55.

Swan, H.T., Wood, K.F., Daniel, O., (1957), Brit. med.J., 1, 495.

Sweeny, W., (1960), Personal communication.

Tagnon, H.J., Whitmore, W.F., Schulman, N.R., (1952), Cancer (Philad.), 5, 9.

Tagnon, H.J., Whitmore, W.F., Schulman, P., Kravitz, S.C. (1953), Cancer (Philad.), 6, 63.

Tillett, W.S., Garner, R.L., (1933), J.exp.Med., 58, 485.

Todd, A.S., (1959), J.Path.Bact., 78, 281.

Triantaphyllopoulos, D.C., (1958), Canad.J.Biochem., 36, 249.

Troll, W.S., Sherry, S., Wachman, J., (1954), J. biol.Chem., 208, 85.

Truelove, S.C., (1951), Clin.Sci., 10, 229.

Tuckerman, M.M., (1958), Anal.Chem., 30, 231.

Tuckerman/

Tuckerman, M.M., Osteryoung, R.A., Nachod, F.C., (1958), Anal.chim.Acta, 19, 251.

Ungar, G., Hayashi, H., (1958), Ann.Allergy, 16, 542.

Ungar, G., Mist, S.H., (1949), J.exp.Med., 90, 39.

Williams, J.R.B., (1951), Brit.J.exp.Path., 32, 530.

Yokoyama, K., Hatano, H., (1959), Keio J.Med., 8, 303.

Zweifach, B.W., Nagler, A.L., Troll, W., (1961), J.exp.Med., 113, 437.

Zimmerman, G., (1846), quoted by Macfarlane, R.G., Biggs, R., (1948), Blood, 3, 1167.

EPSILON AMINOCAPROIC ACID:

The development of a laboratory assay system, and study of its absorption, distribution and excretion to permit rational dosage; and an assessment of its value as an inhibitor of fibrinolytic activity in the urinary tract

by

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Volume 2

VOLUME 2

APPENDIX 1

FIGURES

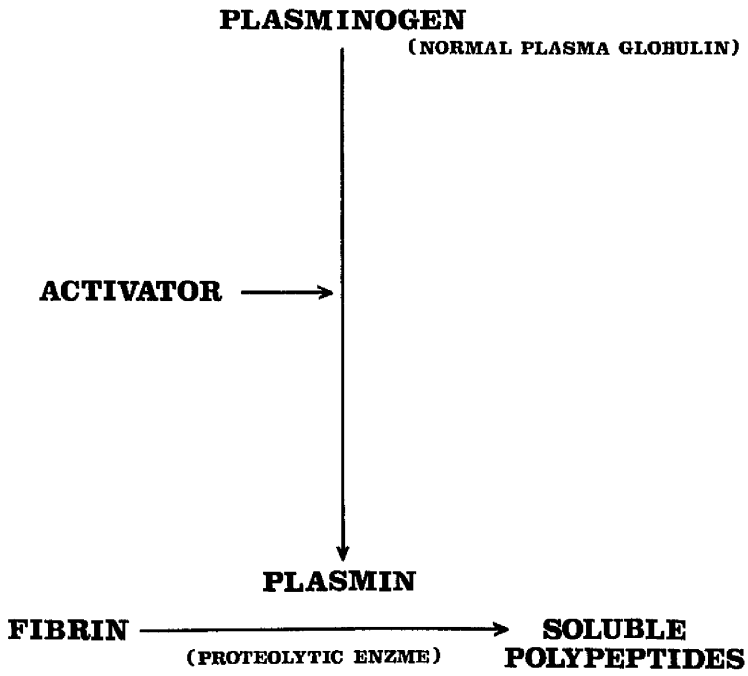

Figure 1 shows the conversion of plasminogen to plasmin under the influence of activator.

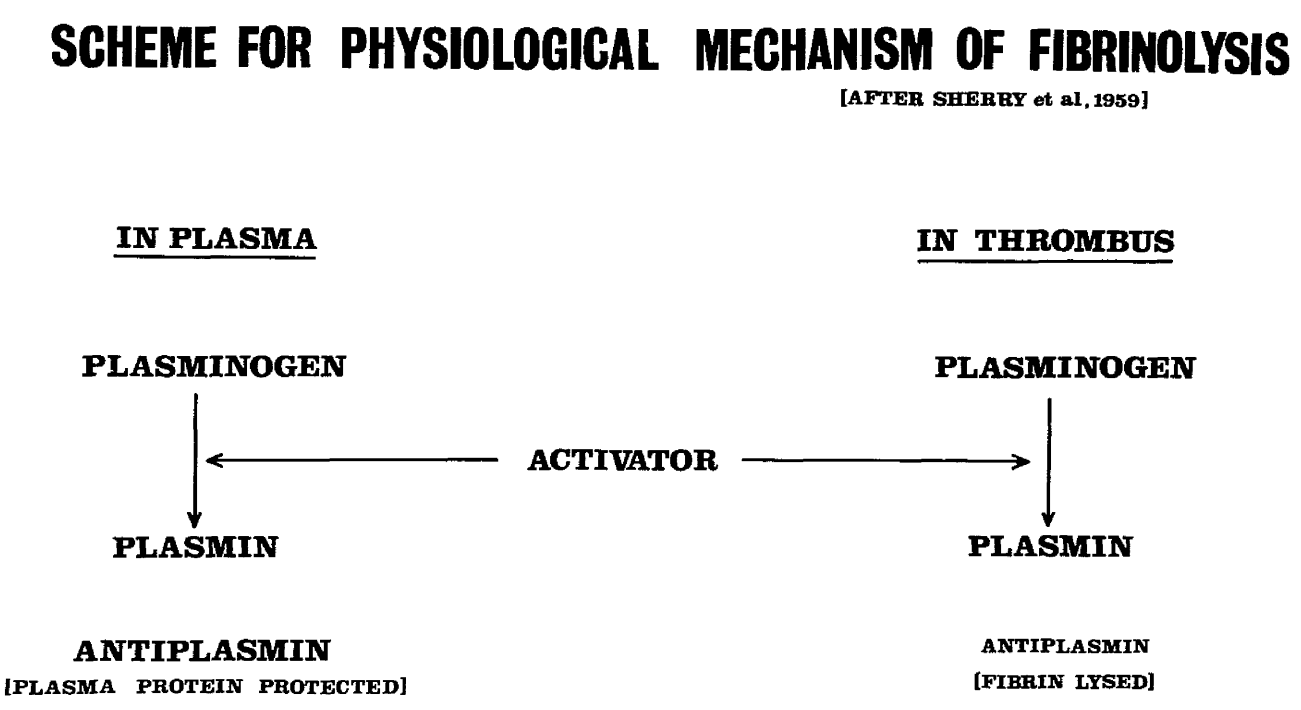

Figure 2 shows the mechanism of thrombolysis in vivo postulated by Sherry et al. (1959a).

CONVERSION OF FIBRINOGEN TO FIBRIN

PROTEOLYTIC STEP $\quad$ FIBRINOGEN $\xrightarrow{\text{THROMBIN}}$ FIBRIN + PEPTIDES

MONOMER

POLYMERISATION STEP $\quad$ n × FIBRIN MONOMER $\longrightarrow$ FIBRIN POLYMER

GELATION STEP $\quad$ m × FIBRIN POLYMER $\longrightarrow$ FIBRIN

FIBRINOGEN $\xrightarrow{\text{PLASMIN}}$ BREAKDOWN PRODUCTS

FIBRIN MONOMER + BREAKDOWN PRODUCTS $\longrightarrow$ DEFECTIVE POLYMER

Figure 3 shows how fibrinogen is converted to fibrin, and how this process is interfered with by the presence of breakdown products produced by digestion of fibrinogen by

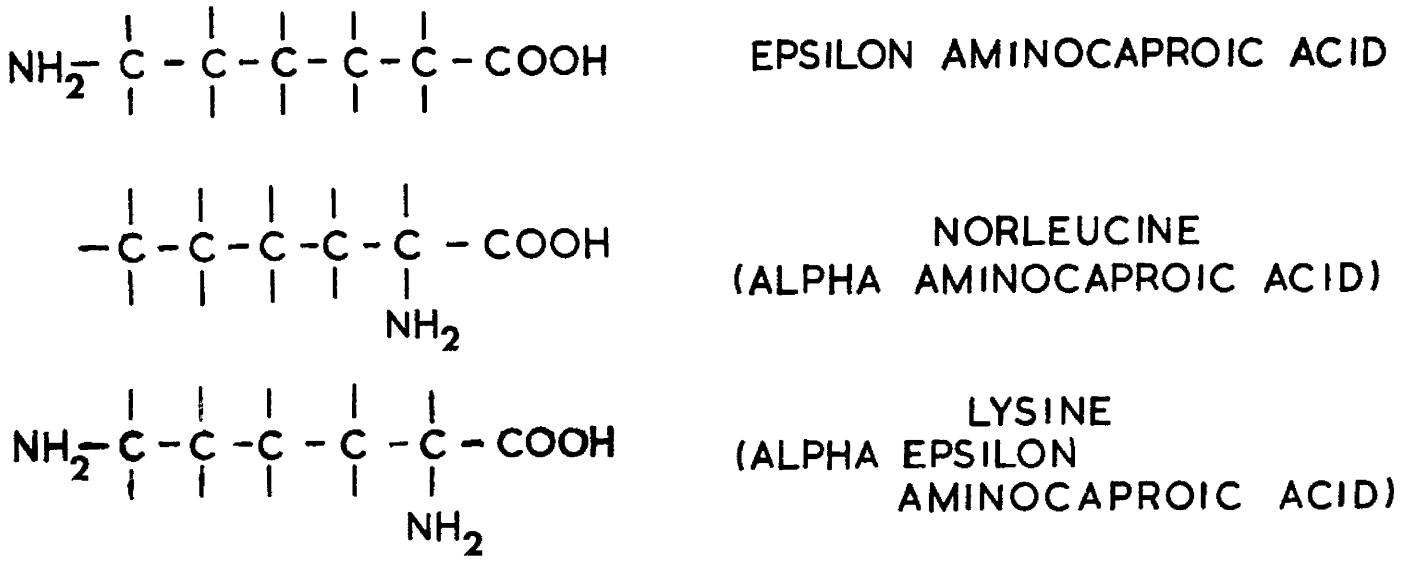

Figure 4 shows the structural formula of EACA and the closely related compounds norleucine and lysine.

Figure 5 shows the streaking when plasma was chromatographed on ion exchange resin loaded paper with acetate buffer of low ionic strength (0.05 Molar).

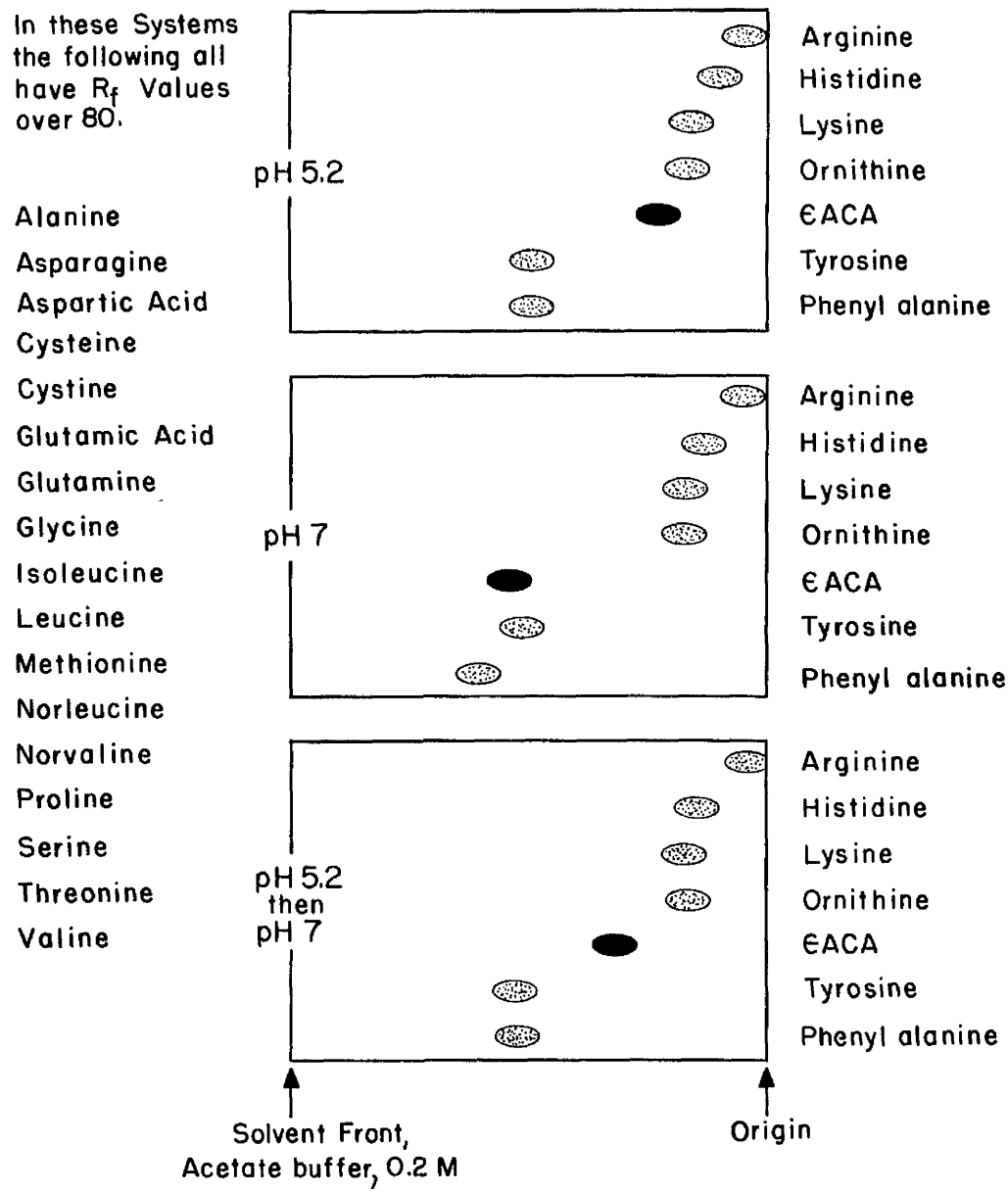

Figure 6 shows the mobilities of amino acids on Reeve Angel, grade SA2, ion exchange resin loaded paper in acetate buffer (0.2 Molar, pH 5.2), upper section, and sodium acetate (0.2 Molar, pH 7.0), middle section. In the bottom section, mobilities are illustrated when initial development is with acetate buffer followed by sodium acetate for the remainder of the run.

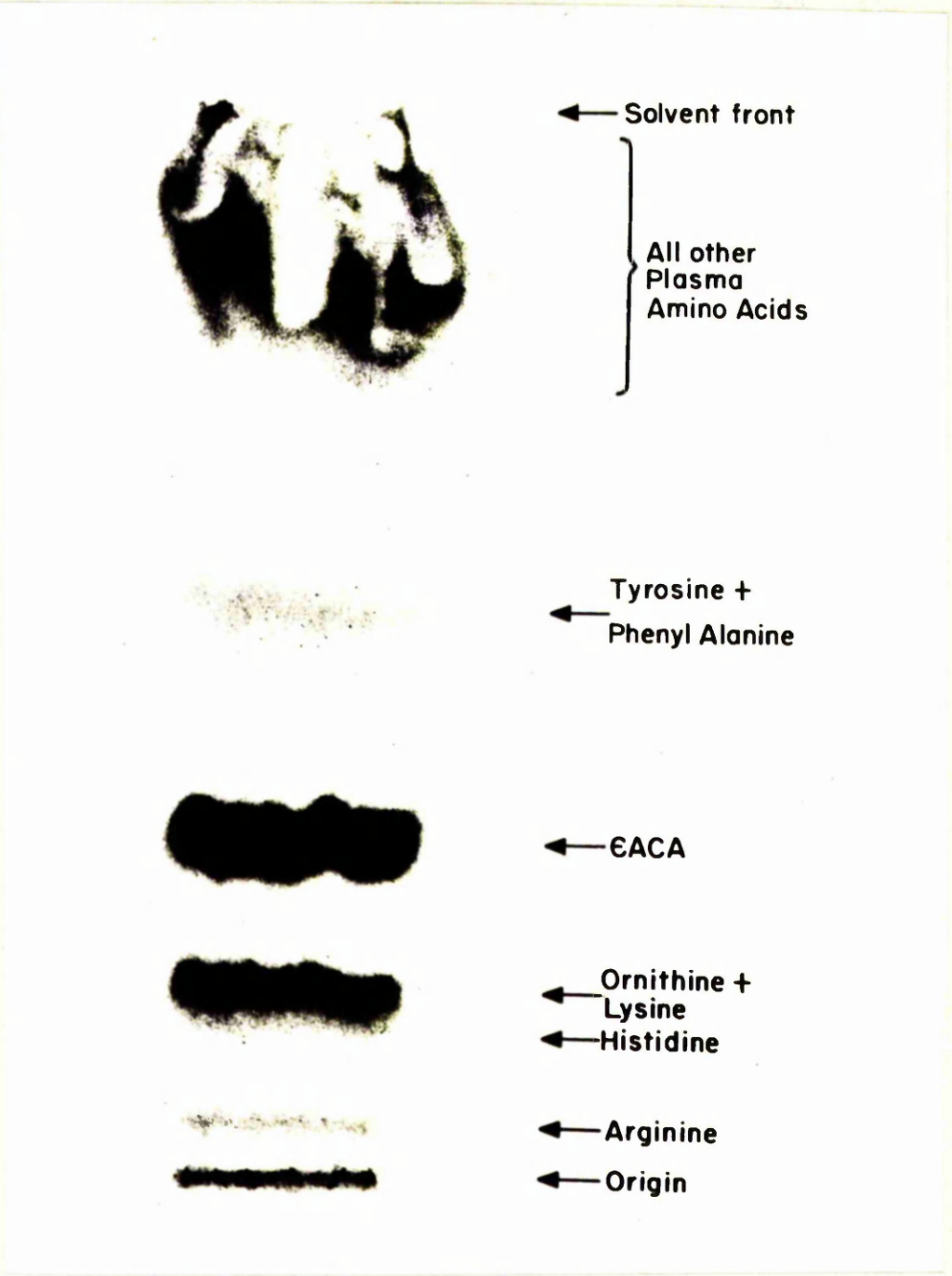

Figure 7 - Plasma amino acid chromatogram developed and stained as described in Chapter 5. Note separation of EACA from other amino acids.

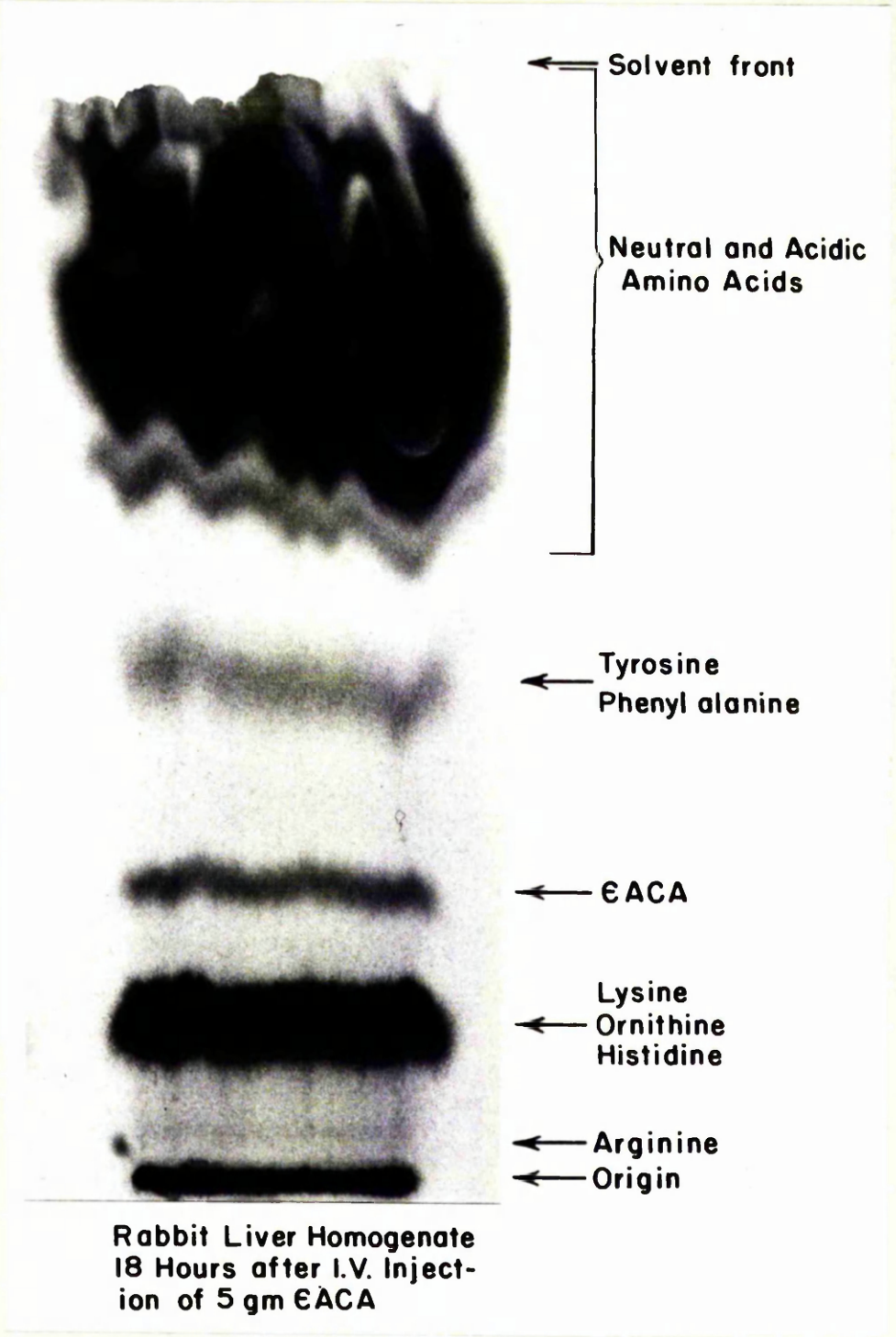

Figure 8 - Chromatogram of liver homogenate from rabbit 18 hours after intravenous injection of 5 gm. EACA.

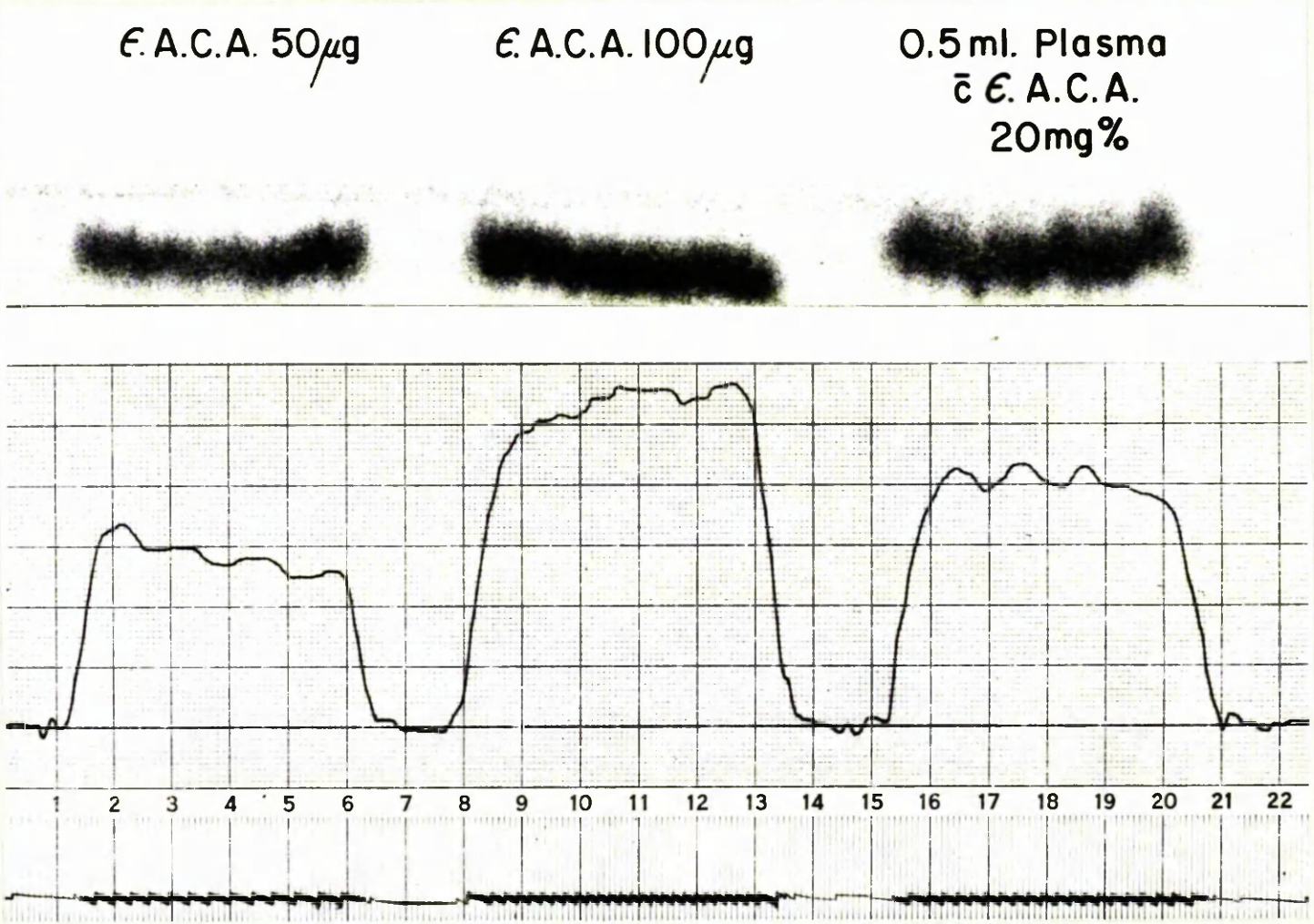

Figure 9 shows, at the top, a strip cut from a developed chromatogram, carrying the EACA band from a plasma to which EACA had been added to give a concentration of 20 mg./100 ml. (20 mg.%), and two EACA bands from a standard EACA solution, one with an EACA content of 50 μg. and one with a content of 100 μg. Also shown is the densitometer tracing obtained by scanning the strip in a recording and integrating densitometer, and at the bottom of the figure, integration pulses show units of area under each curve.

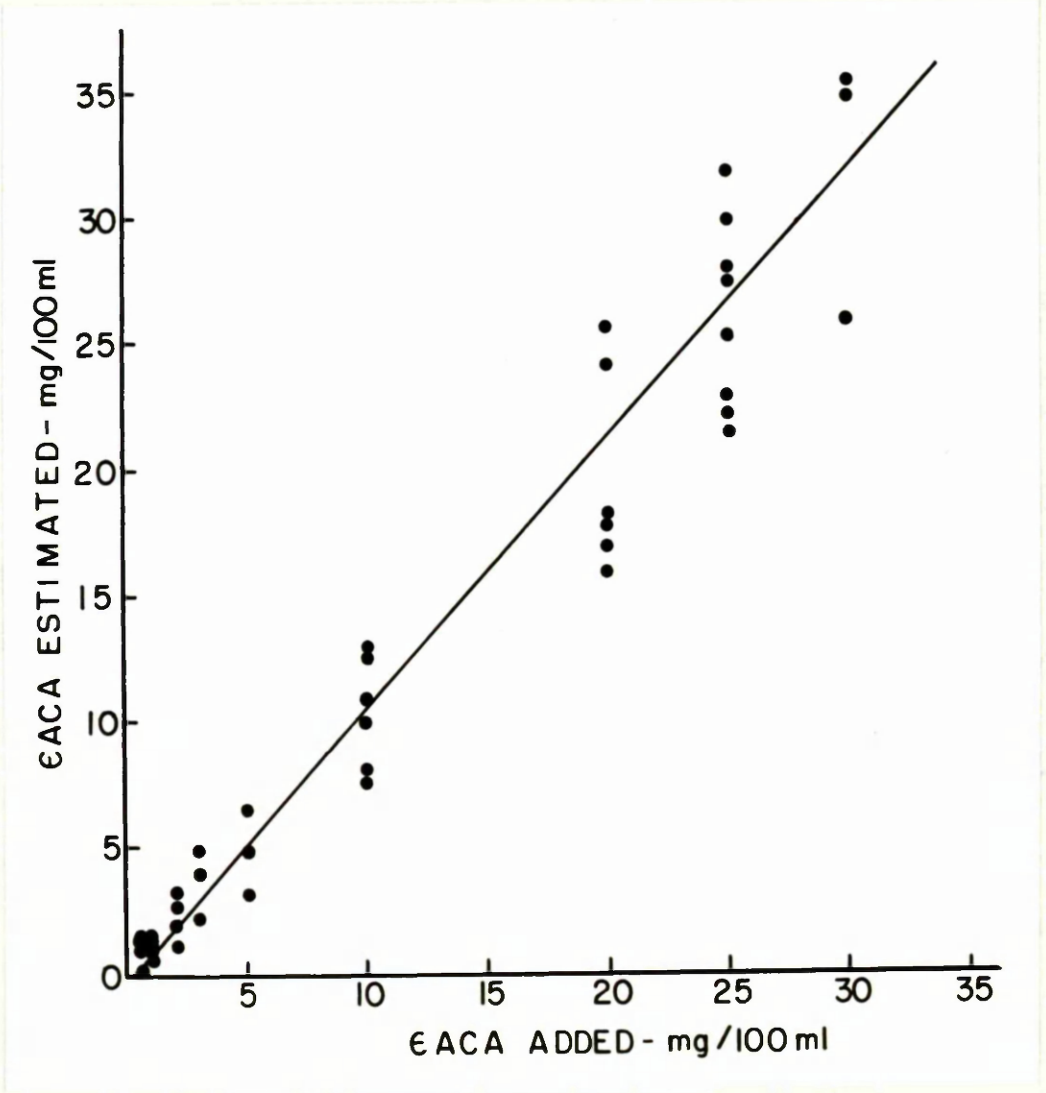

Figure 10 Relationship of known amount of EACA added to plasma to the amount assayed by the method described in Chapter 5. The regression line calculated from the data is also shown (y = 0.93x - 0.6). The figure is prepared from table 31.

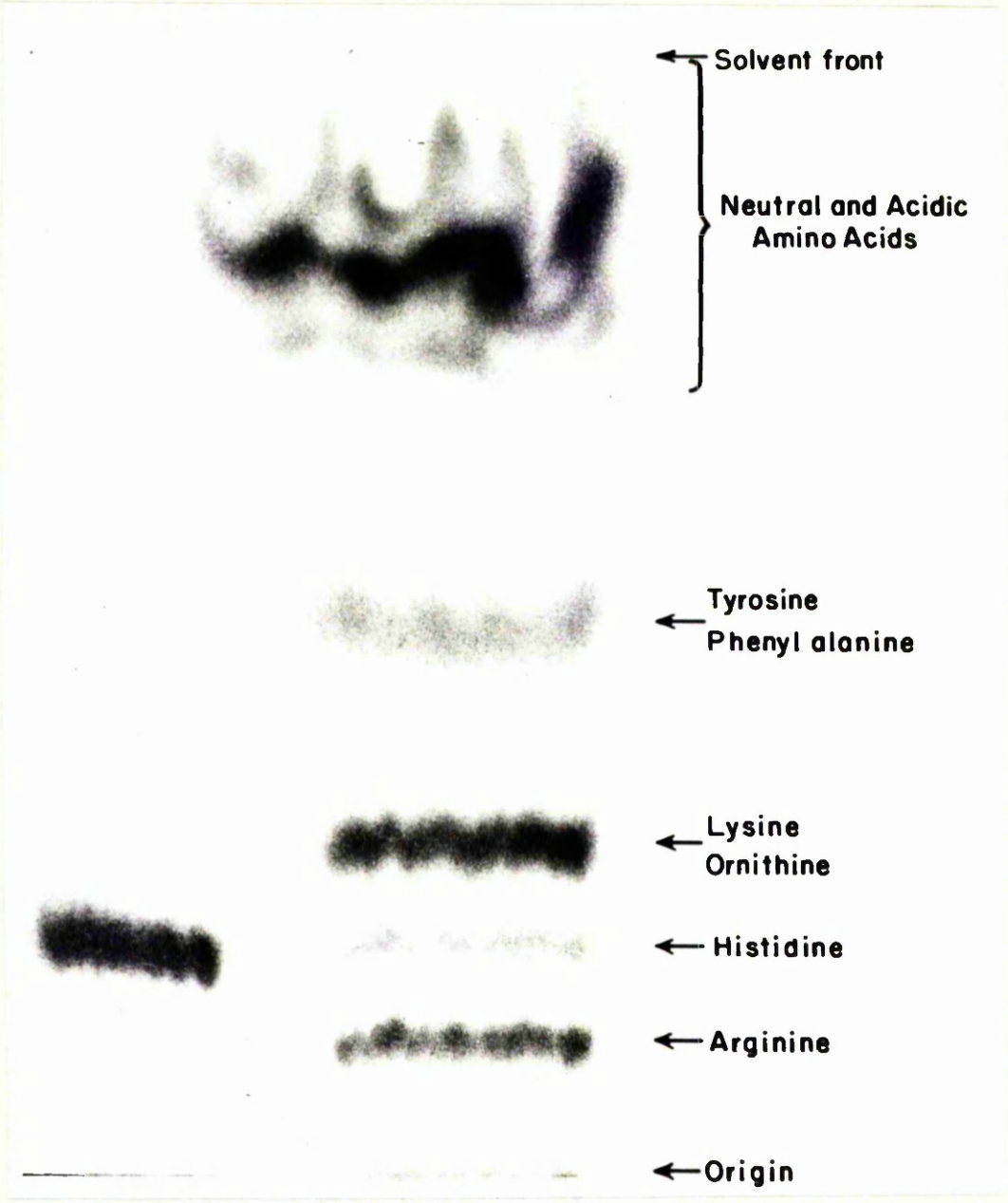

Figure 11 Application of ion exchange resin loaded paper chromatography to the separation of histidine in plasma.

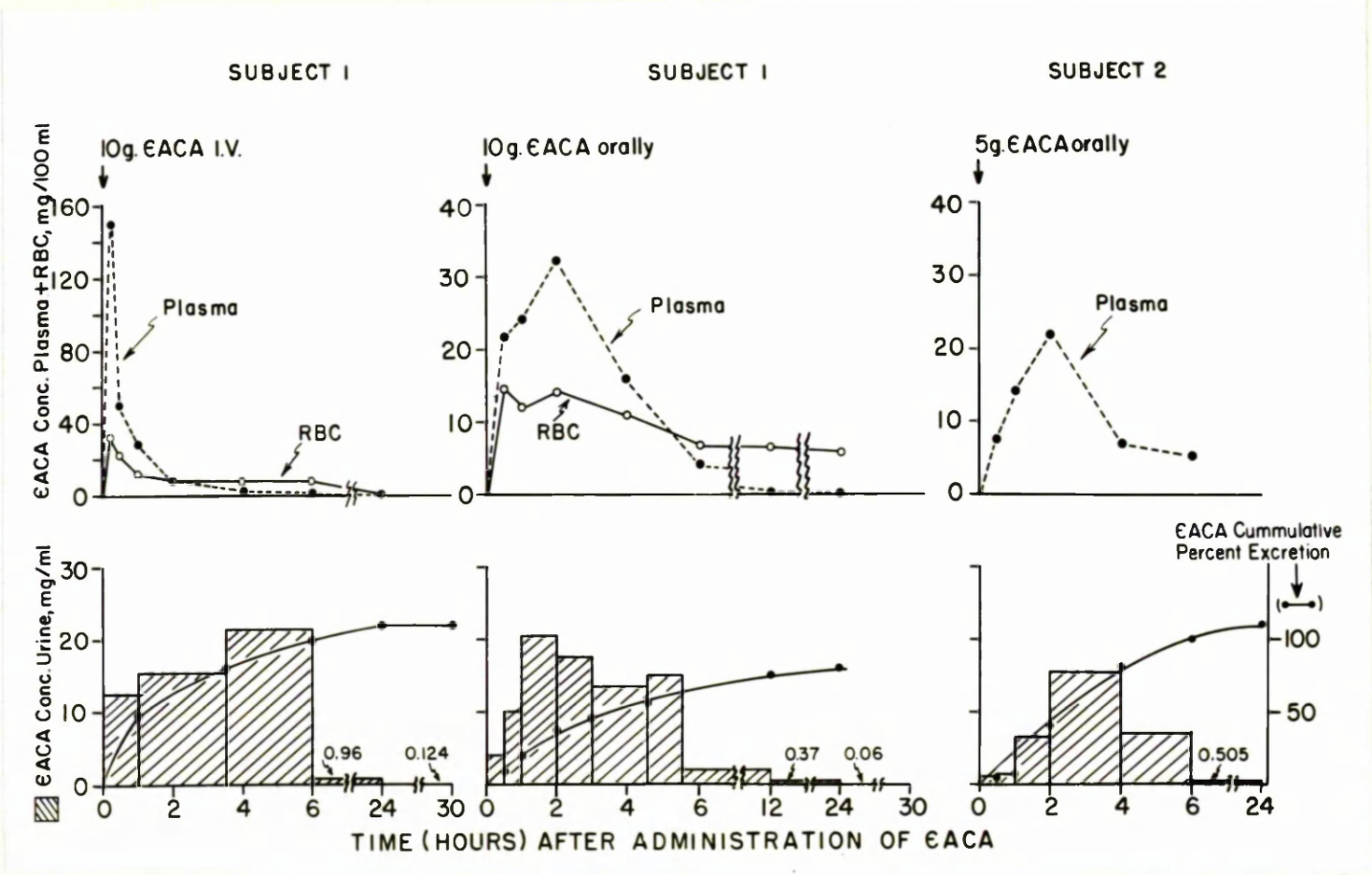

Figure 12 shows plasma and R.B.C. concentrations and urinary excretion of EACA following a single dose (oral or intravenous). The upper panels display plasma and R.B.C. levels and the lower panels illustrate urinary cumulative excretion as a function of time and urinary concentration in each specimen. Subject 1 was given EACA intravenously (left hand panel) and orally (middle panel). Subject 2 received only oral dosage. The figure is prepared from tables 32, 33 and 34.

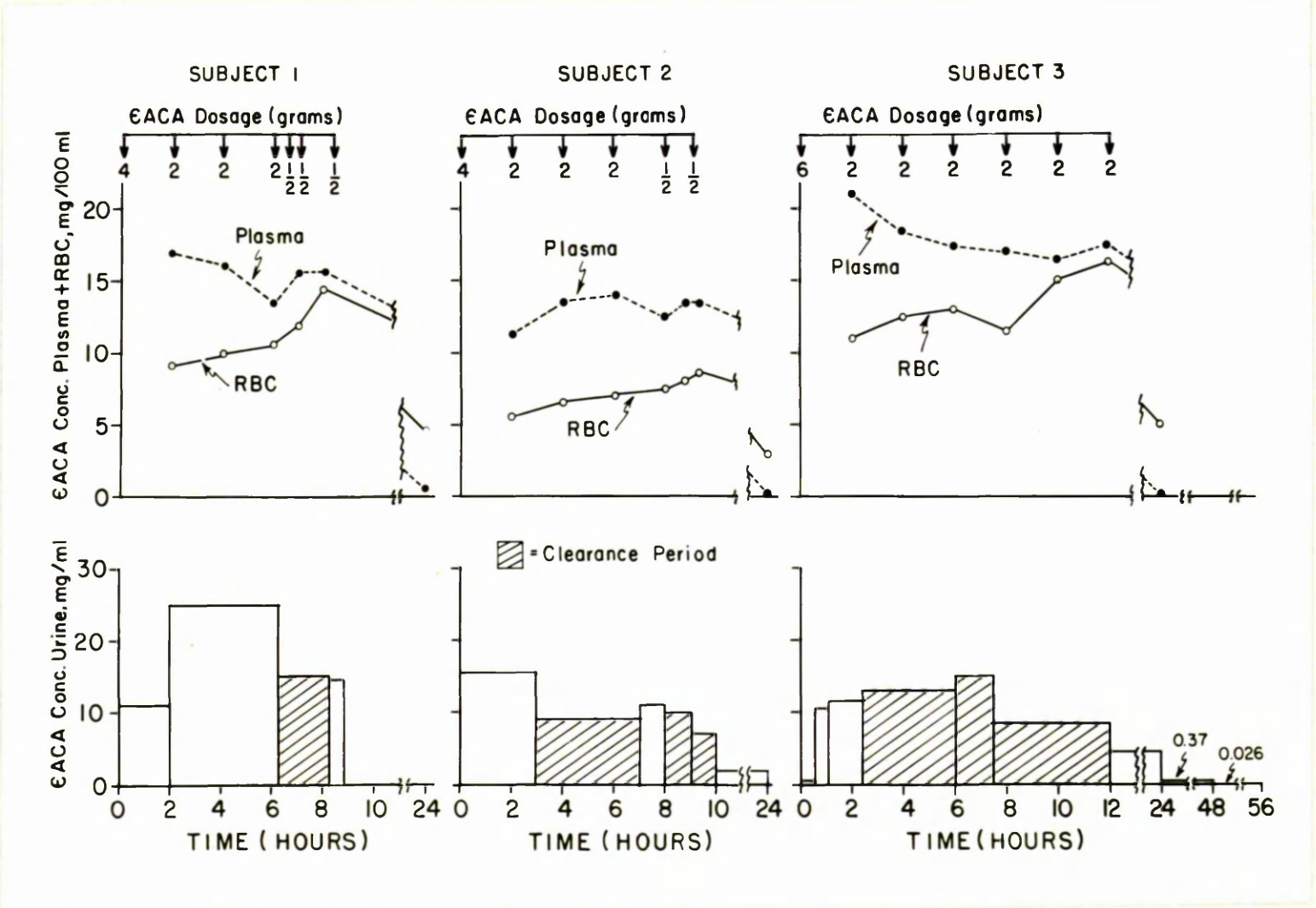

Figure 13 shows plasma, R.B.C. and urinary levels of EACA achieved by repeated oral dosage. The stability of the plasma levels achieved and the consistency in different subjects should be noted. The hatched areas (bottom section of the figure) indicate periods of creatinine and EACA clearance determination (table 5). The figure is prepared from tables 35 - 40.

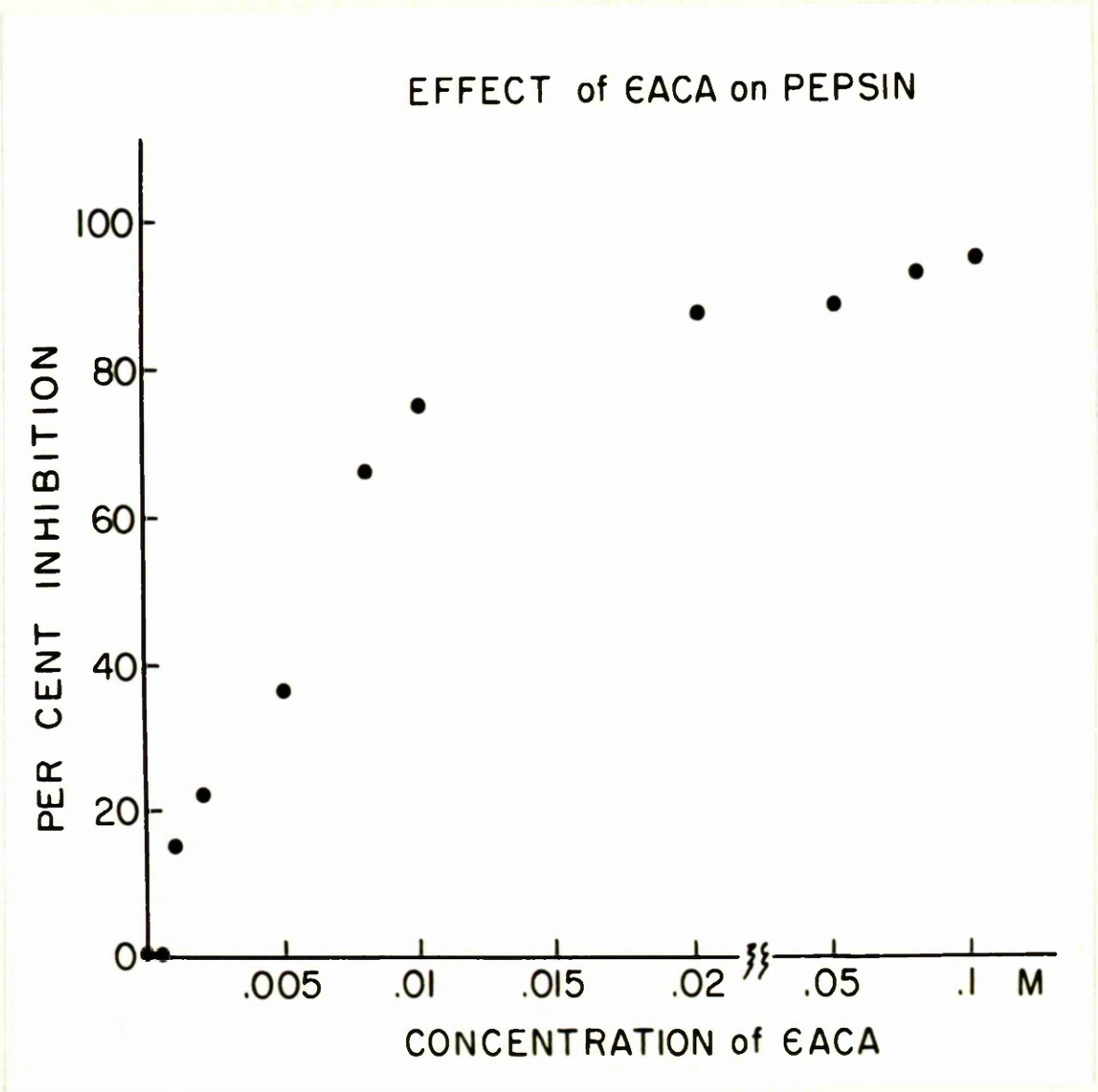

Figure 14 shows inhibition of purified pepsin by EACA, with haemoglobin buffered to pH 2 as substrate. The figure is prepared from table 43.

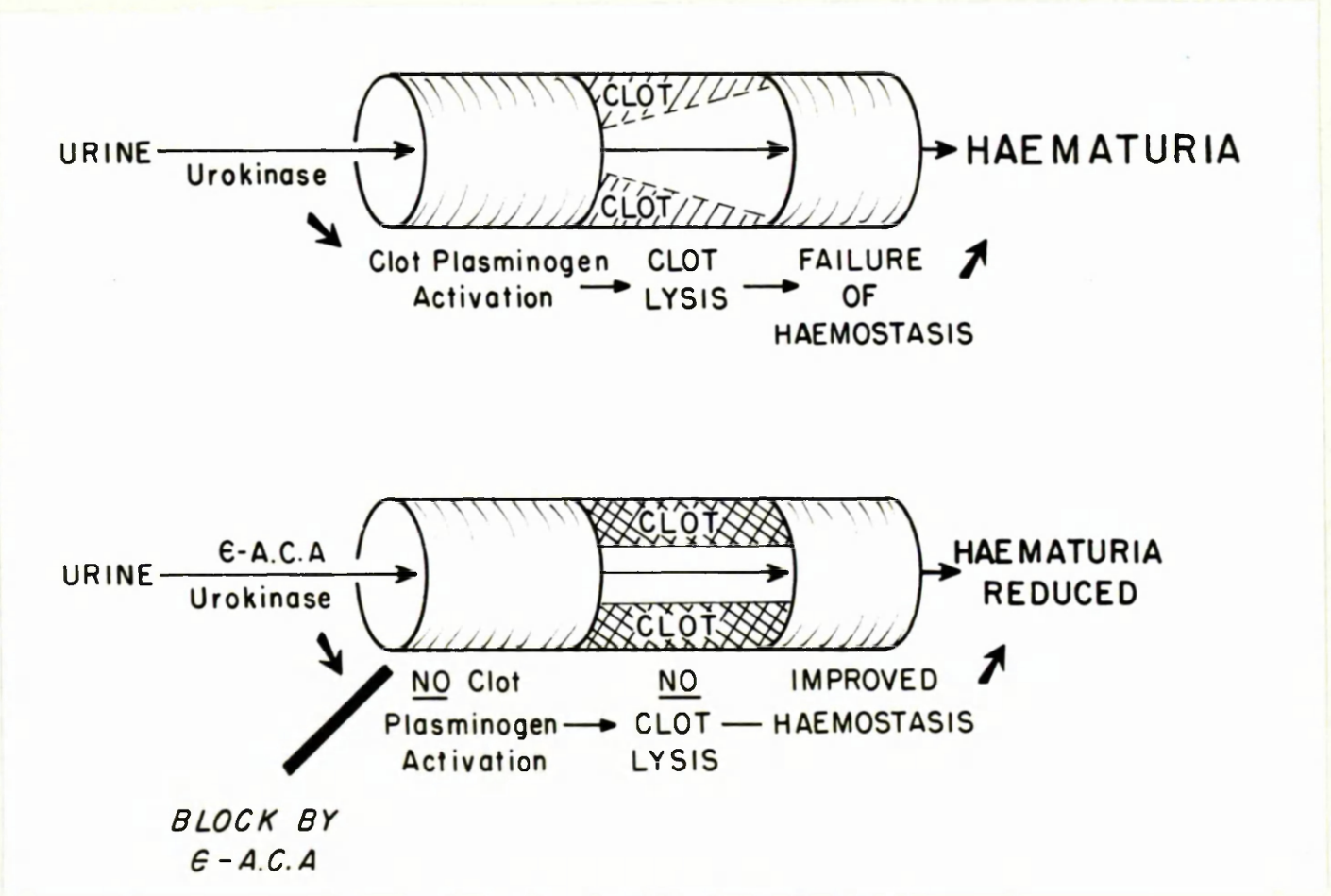

Figure 15 Investigative basis for the study of the effect of EACA in reducing blood loss after prostatectomy. The upper section illustrates the suggested mechanism by which haemostasis may be impaired following prostatectomy or other trauma to the urinary tract, and the lower section illustrates the mechanism by which EACA administration may improve haemostasis.

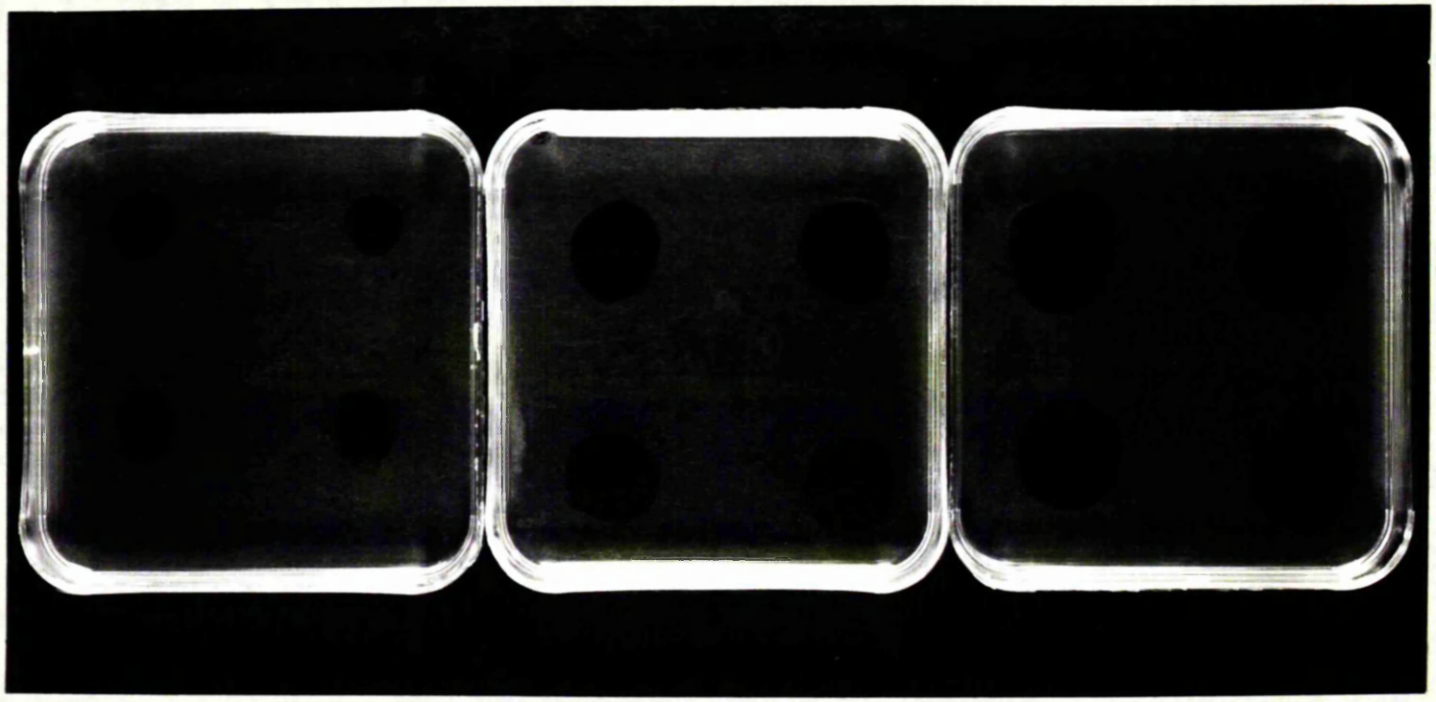

Figure 16 shows representative fibrin plates, with on each plate the areas of lysis produced by 4 30 µl. aliquots of normal urine.

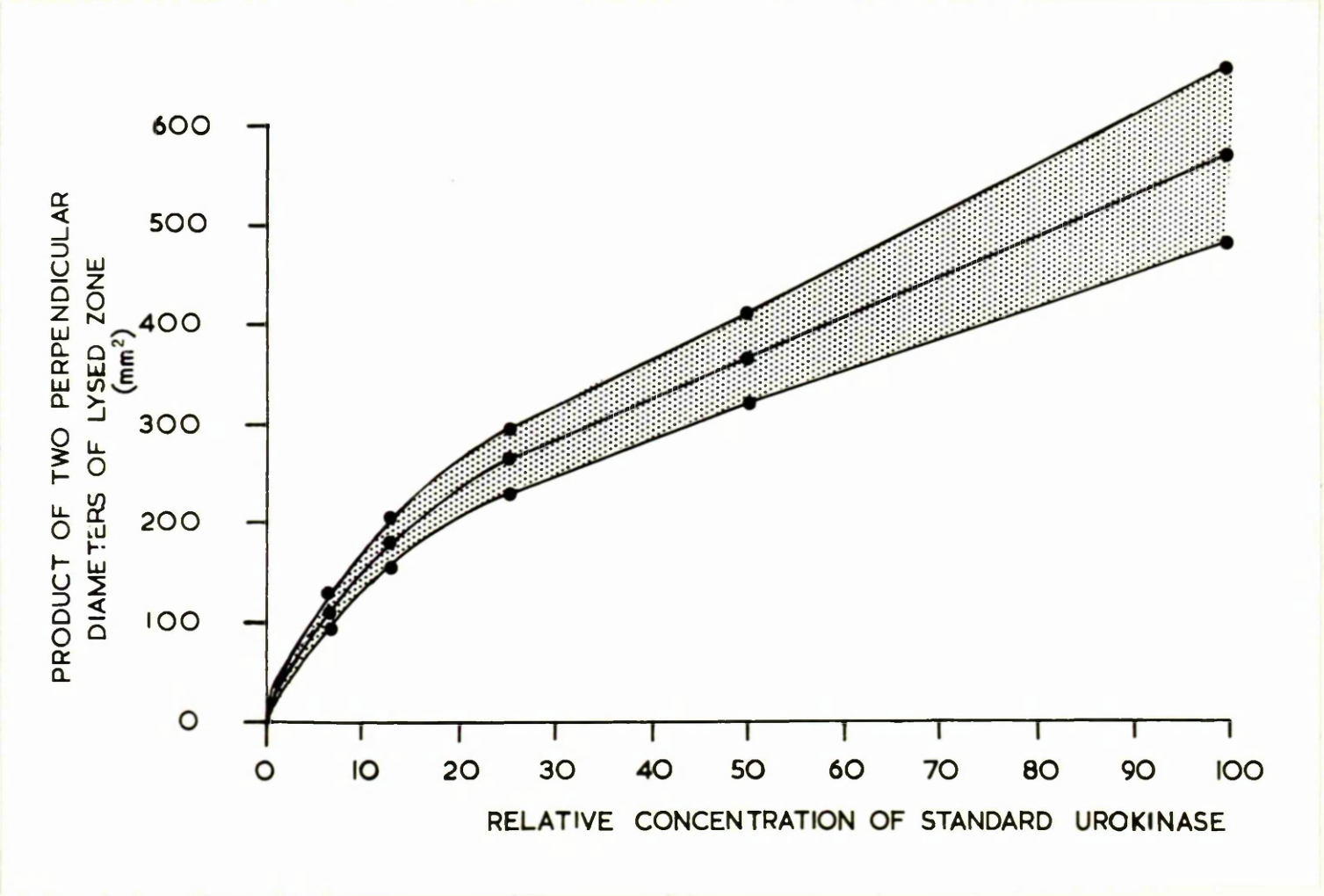

Figure 17 shows the mean value and standard deviation of the dilution curves of standard urokinase solution used throughout this study. The figure is prepared from table 46.

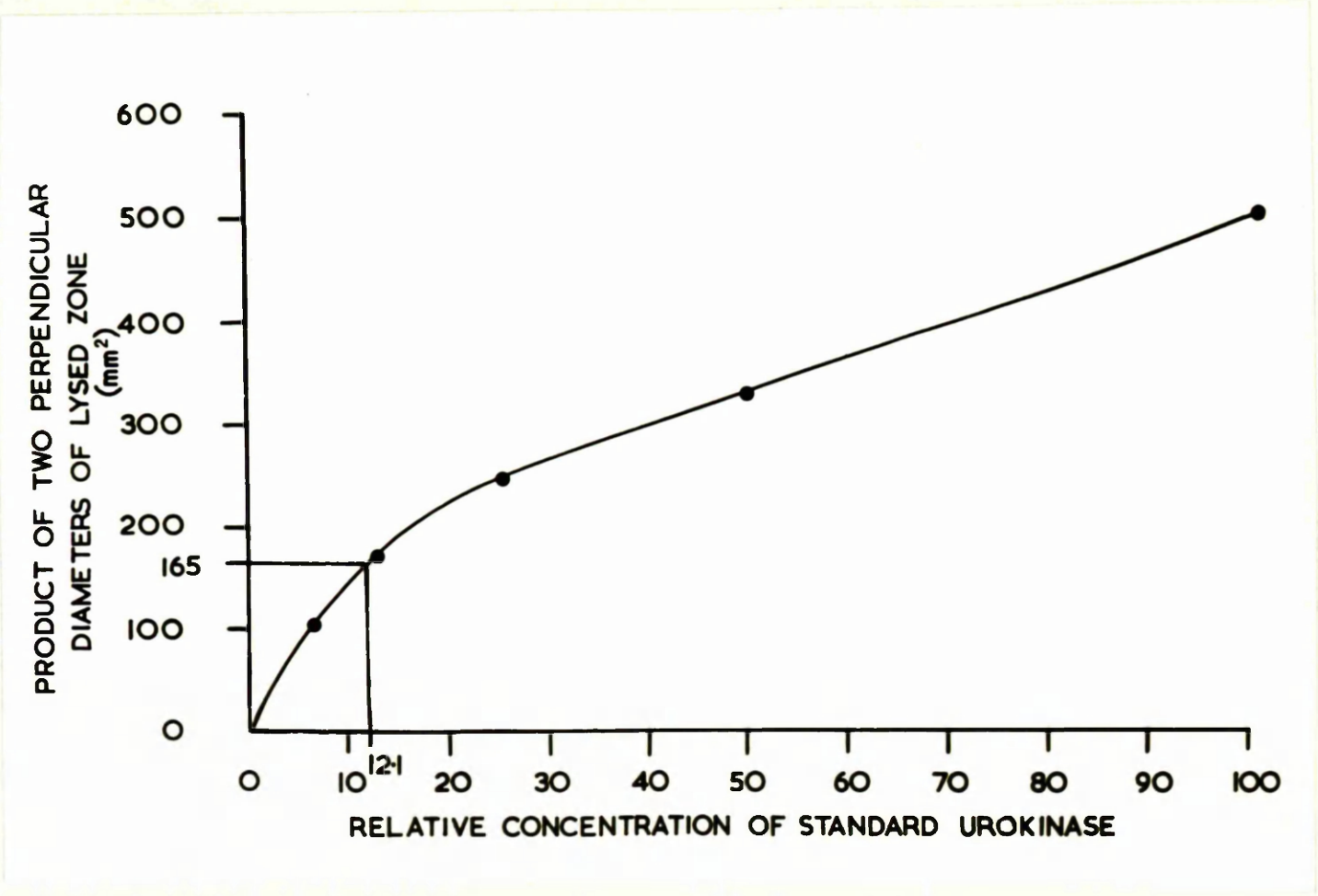

Figure 18 shows a representative dilution curve of the standard urokinase solution (table 46, curve 1). A urine sample (patient 1, preoperative sample) run at the same time gave a zone of lysis of 15 x 11 = 165 mm^2. Interpolation of this zone of lysis in the standard curve as shown in the figure gave a urine urokinase activity of 12.1 per cent of the standard urokinase solution, that is a urokinase concentration of 12.1 units/ml.

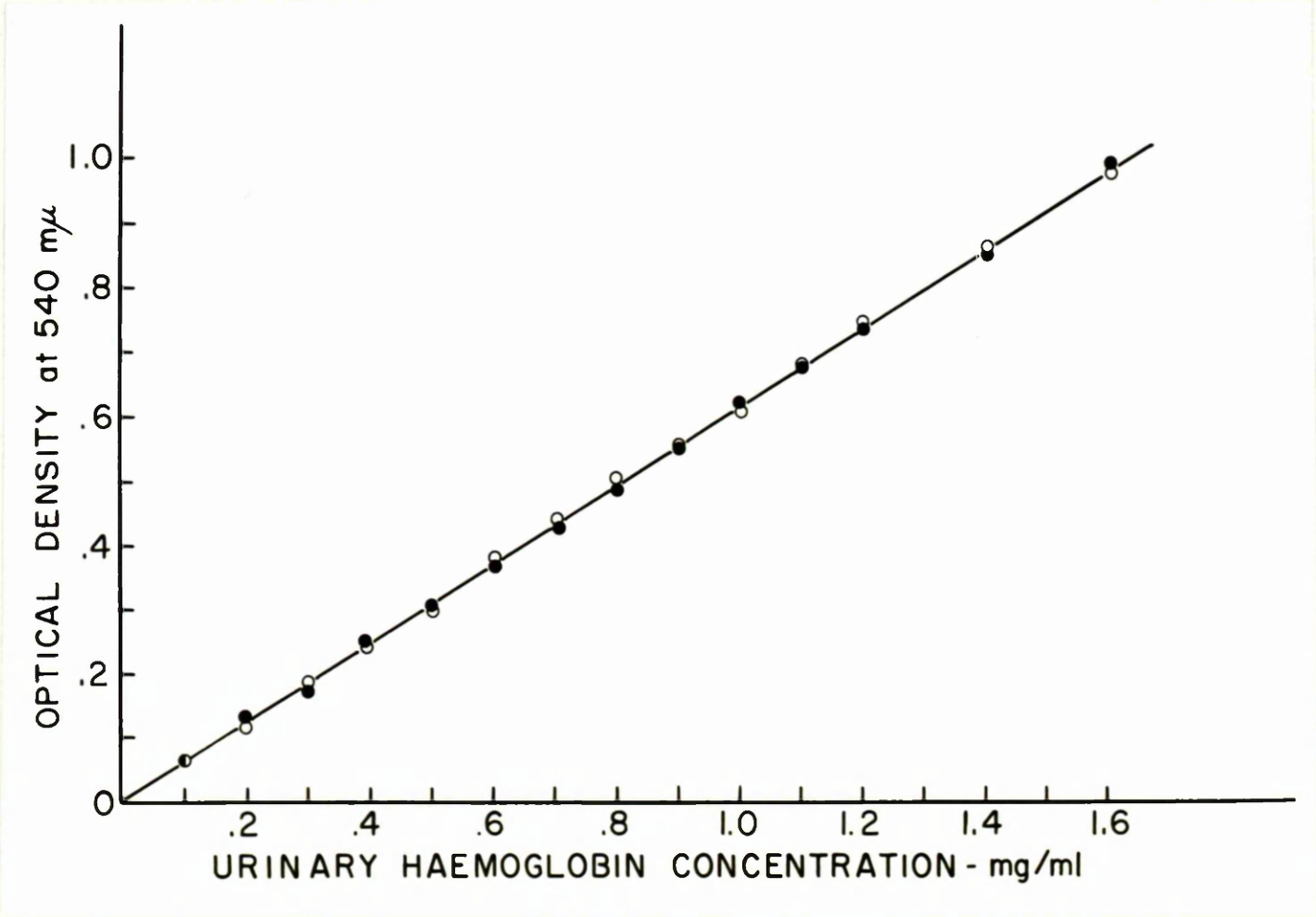

Figure 19 shows the standard curve used in the assay for haemoglobin in urine. The curve was prepared by adding known amounts of haemoglobin to a concentrated urine, SG 1028, (open circles) and a dilute urine, SG 1010 (solid circles). The optical density of each solution of haemoglobin in urine was then determined as described in Chapter 10. The figure is prepared from table 47.

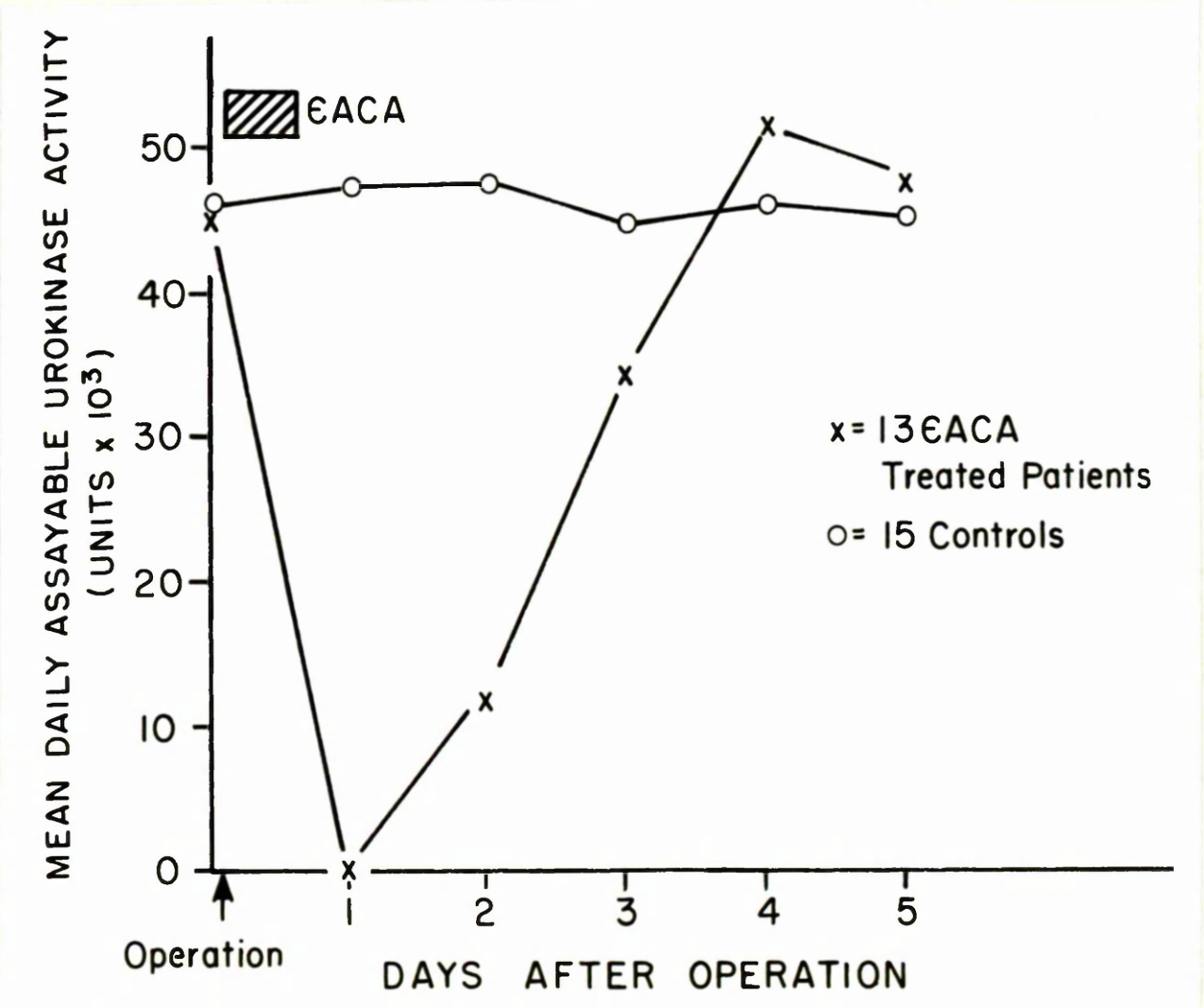

Figure 20 Mean daily assayable urokinase activity in patients studied after transurethral prostatectomy. EACA administration caused profound inhibition of assayable urokinase activity. The figure is prepared from tables 17 and 18.

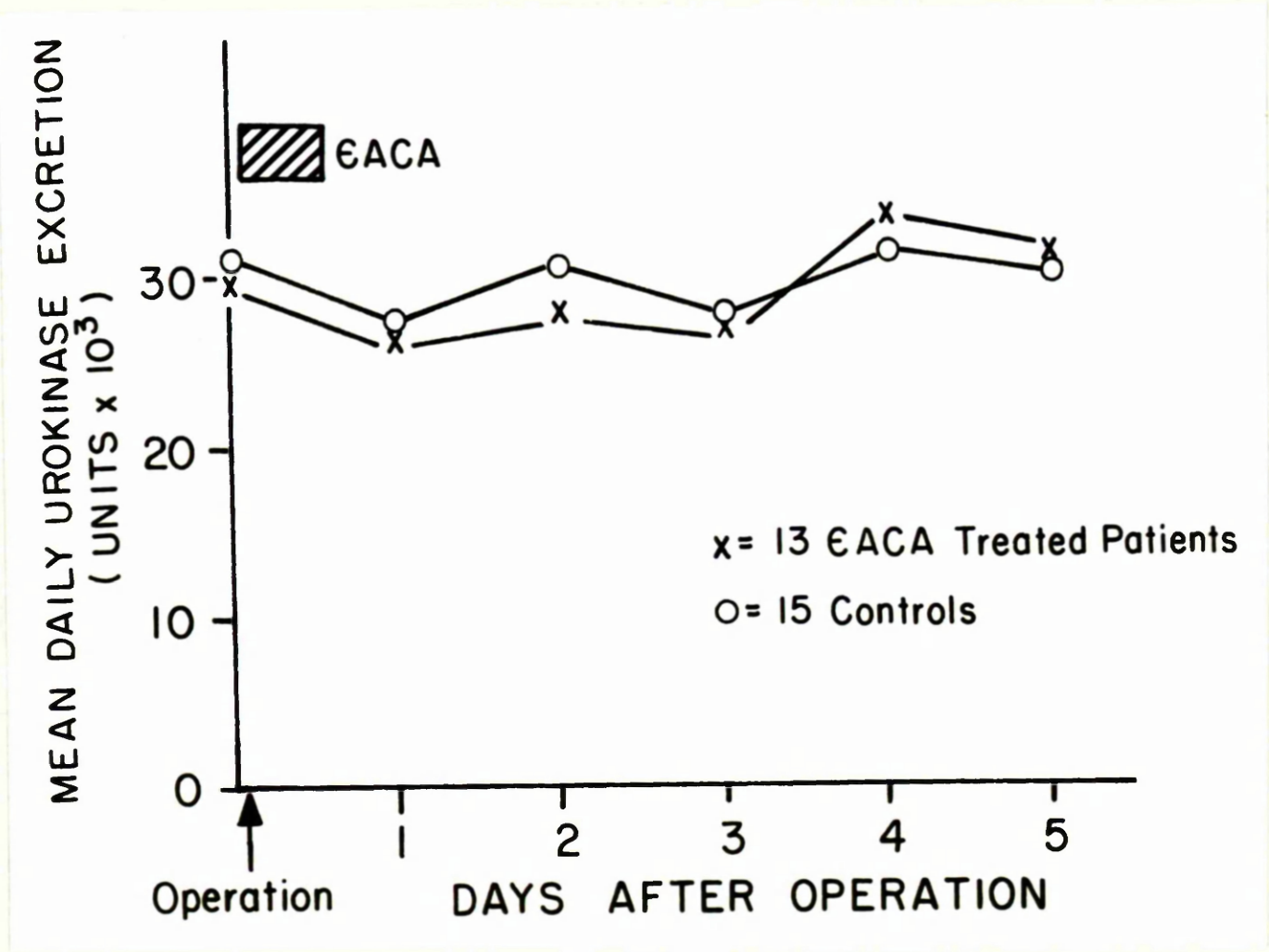

Figure 21 Mean daily urokinase activity in dialysed urine samples in patients studied after transurethral prostatectomy. The results indicate that EACA administration does not alter urokinase excretion rates. The figure is prepared from tables 17 and 18.

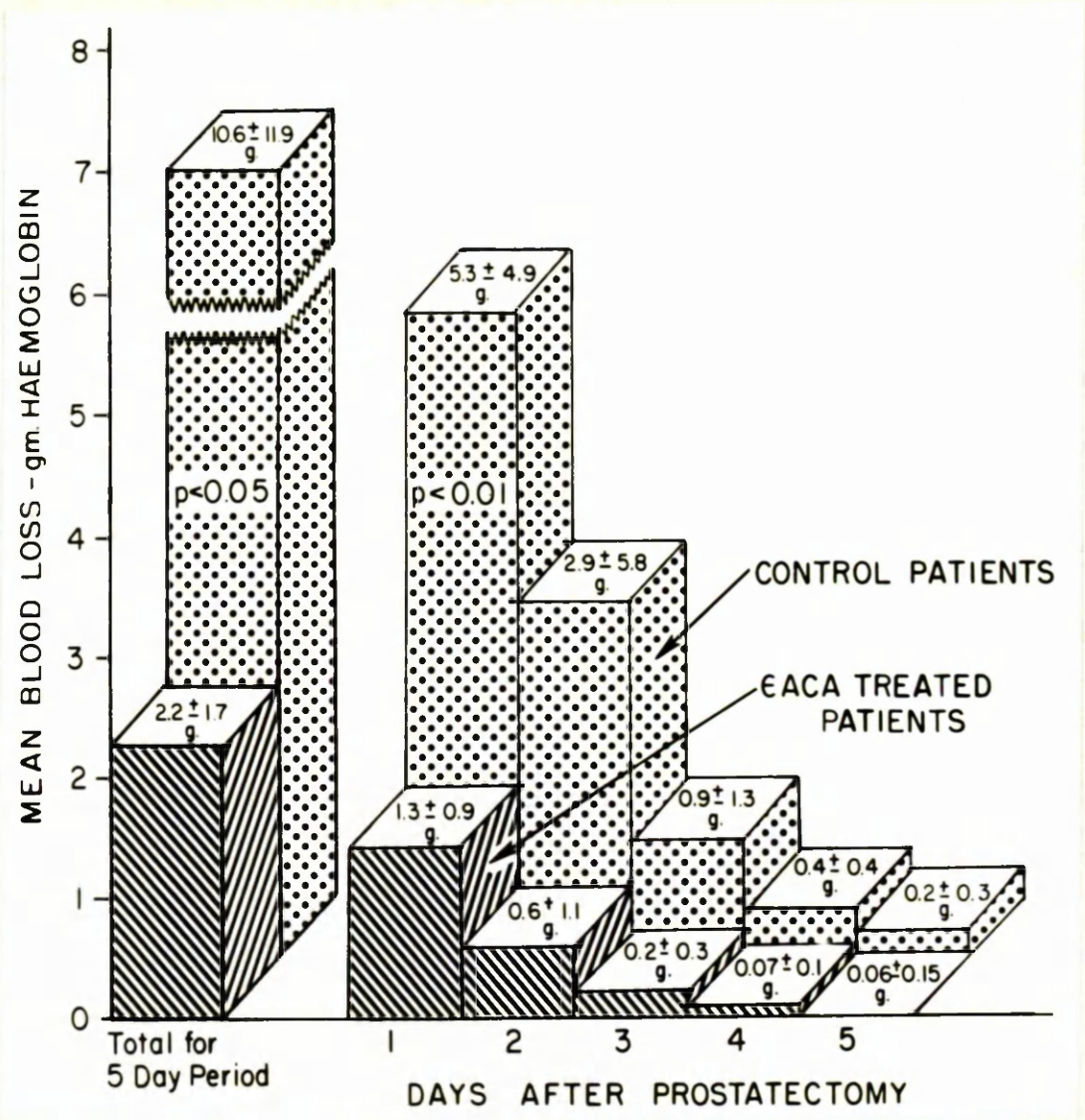

Figure 22 Daily and total (5 day period) blood loss after transurethral prostatectomy. The mean results for 13 patients treated with EACA are compared with the results obtained in 15 control subjects. EACA treatment caused a marked reduction in postoperative haematuria, particularly on the first and second postoperative days. The figure is prepared from table 19.

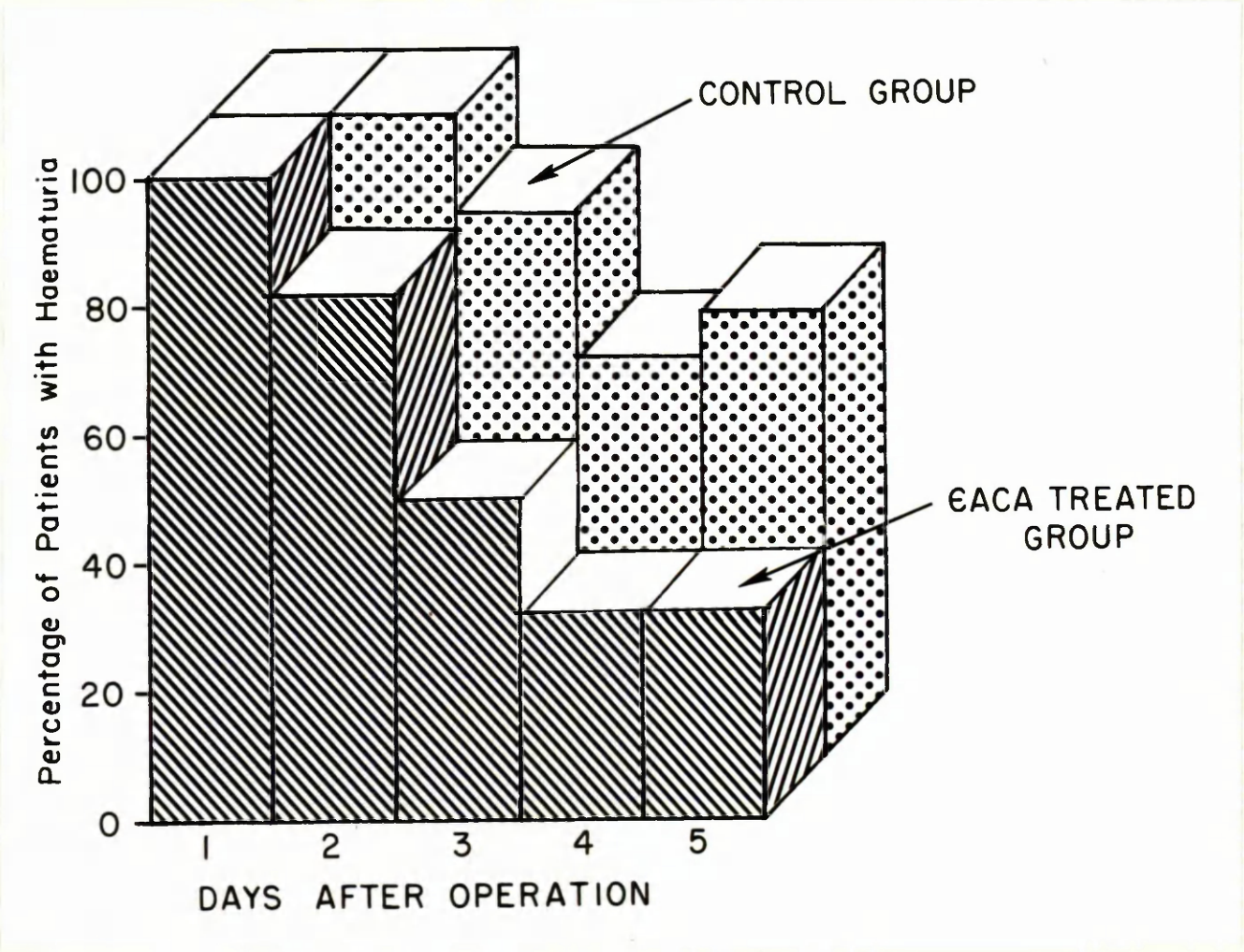

Figure 23 Effect of EACA administration on the duration of urinary bleeding after transurethral prostatectomy. Shown are the percentages of patients in the control and treated groups with blood in the urine on successive days after surgery. Significantly fewer specimens contained blood in the treated group than in the control ($chi^2 = 7.242$, $p < 0.01$). The figure is prepared from tables 64 and 65.

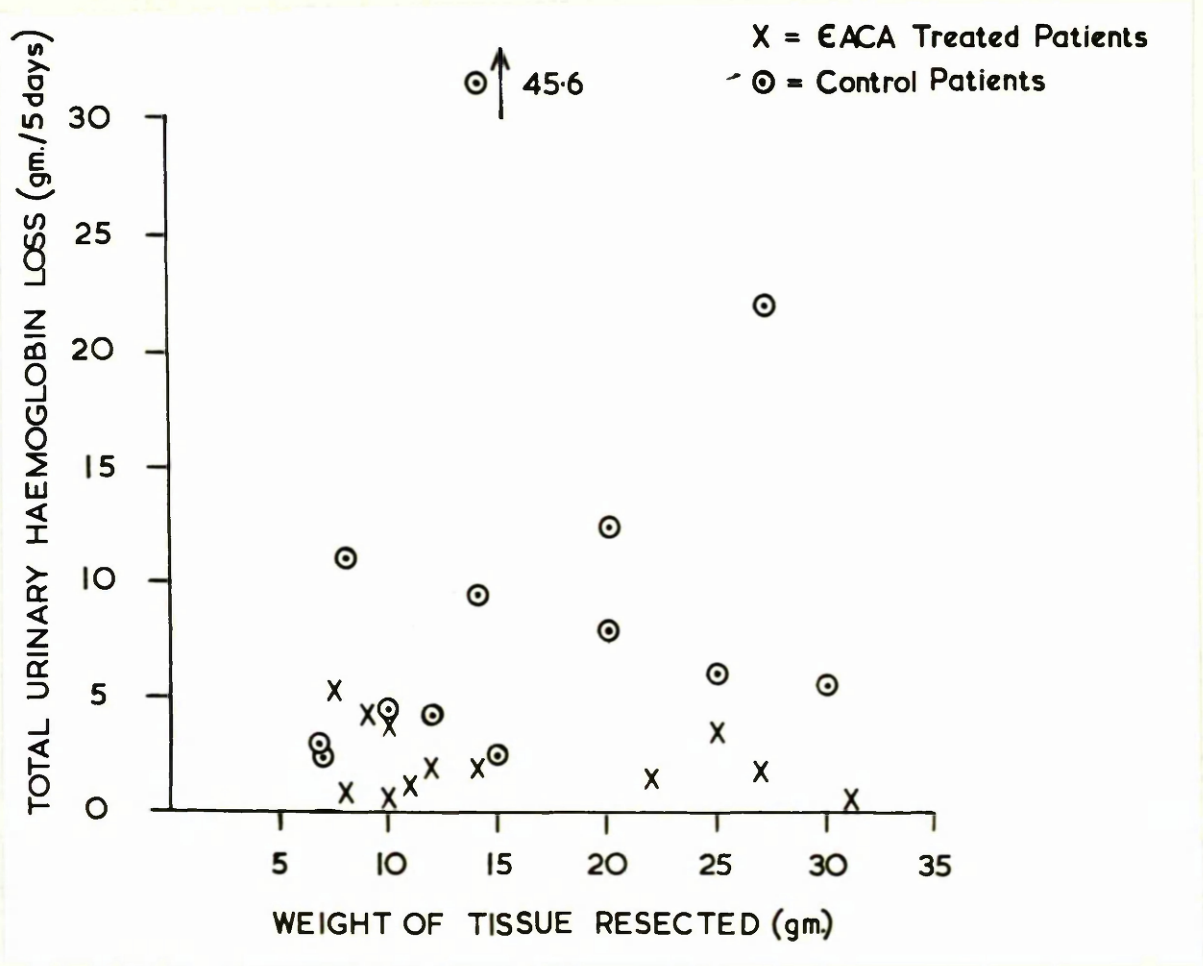

Figure 24 Transurethral prostatectomy series. Lack of relationship between total urinary haemoglobin loss (5 day period) and weight of prostatic tissue resected. For the control patients, $r = -0.377$ and $p > 0.1$, and for the EACA treated group, $r = -0.459$ and $p > 0.1$. The effect of EACA treatment can be seen in the much smaller range of blood loss in the treated patients as compared with the results obtained in the control subjects. The figure is prepared from tables 44, 45, 64 and 65.

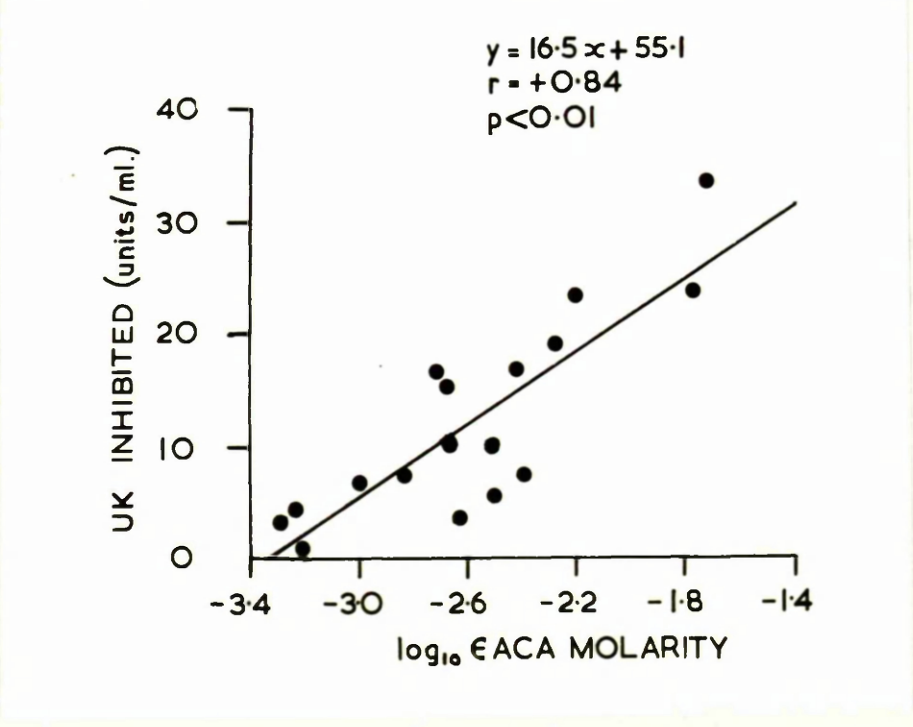

Figure 25 shows in the patients given EACA after transurethral prostatectomy, the relationship between urokinase inhibition and concentration of EACA in the urine. The regression coefficient is + 0.84, and $p < 0.01$. The equation for the regression line shown is $y = 16.53x + 55.07$. The figure is prepared from tables 68 and 73.

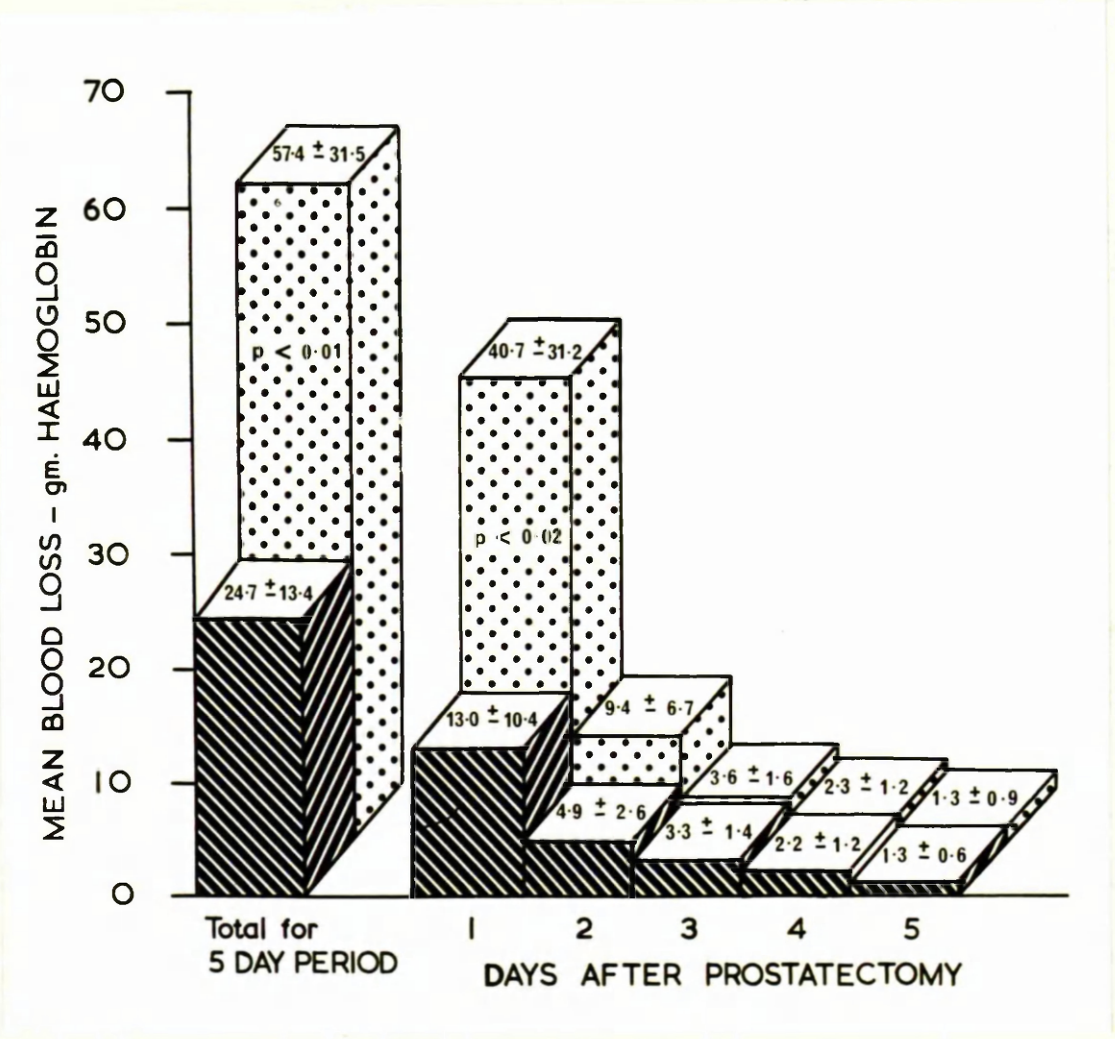

Figure 26 Daily and total (5 day period) blood loss after suprapubic prostatectomy. The mean results for 10 patients treated with EACA are compared with the results obtained in 9 control subjects. EACA caused marked reduction in postoperative haematuria, particularly on the first postoperative day. The figure is prepared from table 27.

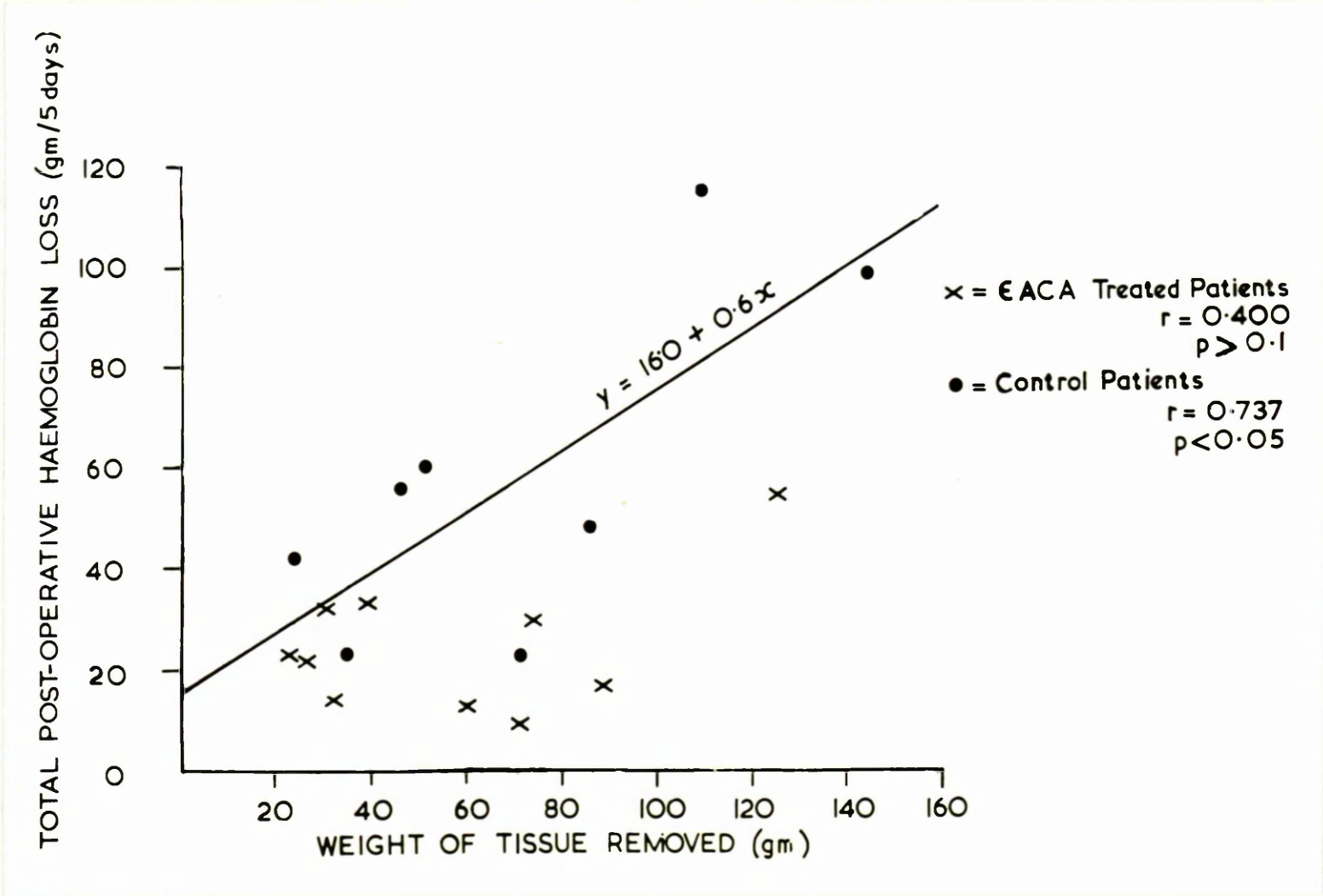

Figure 27 shows the relationship between total urinary haemoglobin loss (5 day period) and weight of prostatic tissue removed at suprapubic prostatectomy. The figure is prepared from tables 79 and 82.

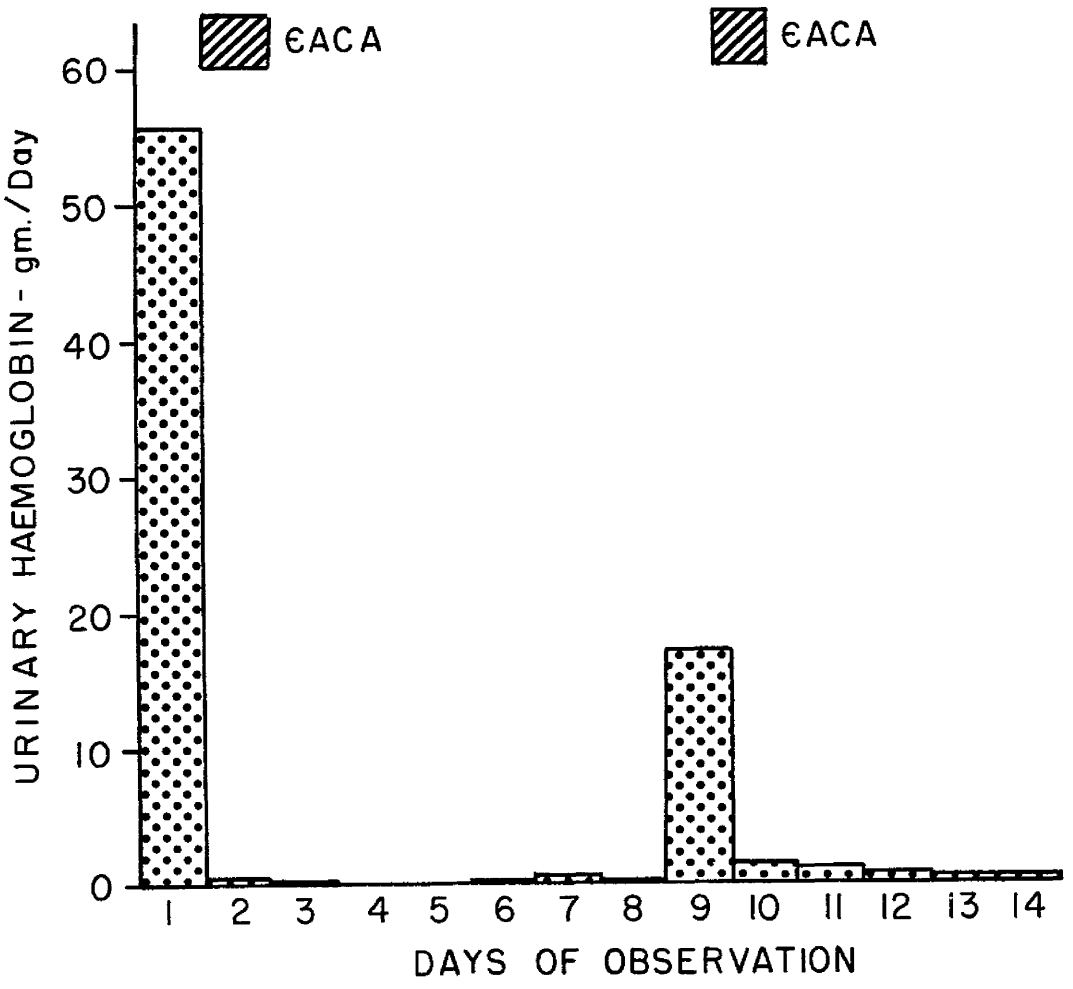

Figure 28 Effect of EACA administration on urinary haemoglobin loss in a patient with recurrent haematuria following surgery (suprapubic prostatectomy) for adenocarcinoma of the prostate. Prostatectomy was carried out 27 days before EACA was first given. (Chapter 12, patient 1). The figure is prepared from table 83.

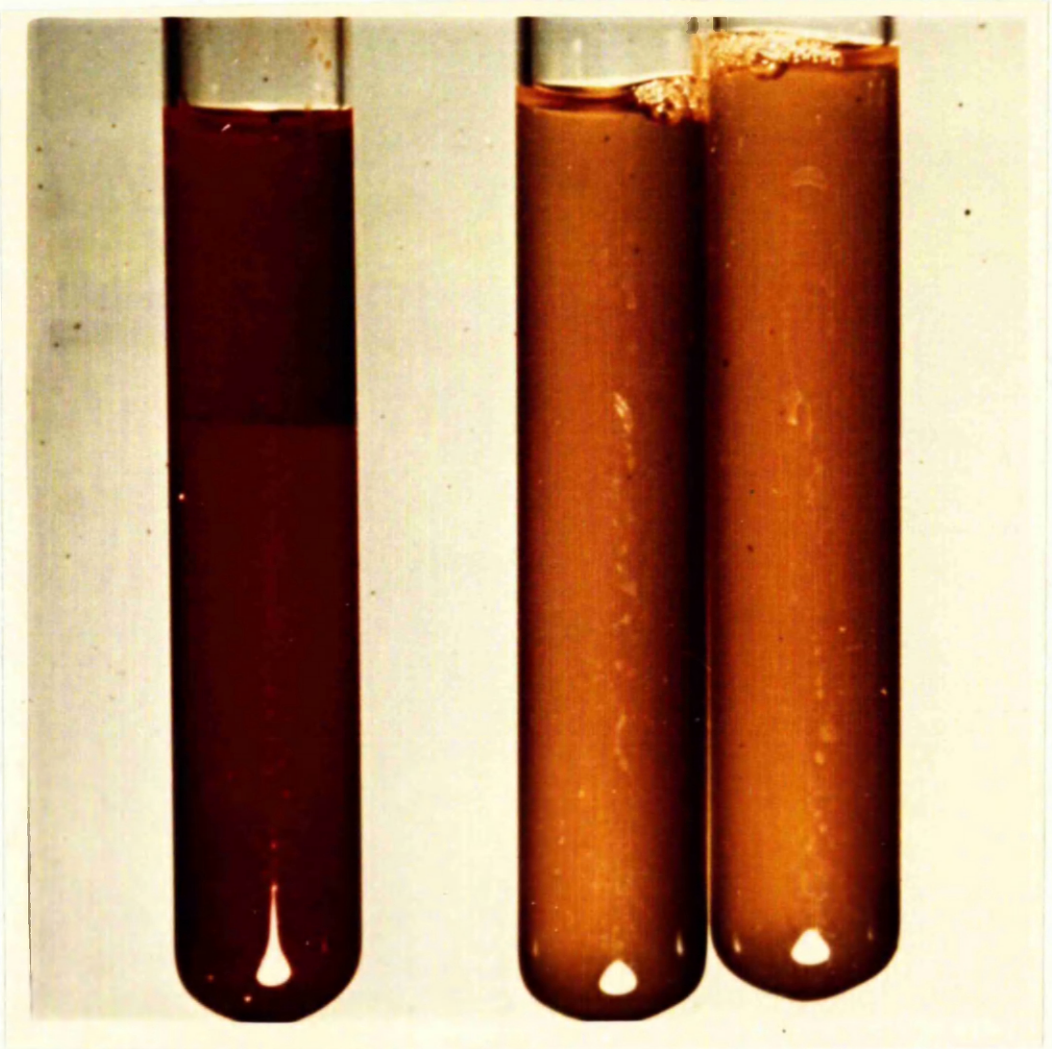

Figure 29 shows the effect of EACA administration on urinary haemoglobin loss in a patient with prolonged bleeding after suprapubic prostatectomy. The urine sample on the left is from the 24 hour collection immediately before EACA was given, the middle sample is from a collection made for the first 2 hours of EACA therapy, and the right hand sample is from the collection for the first 24 hours of therapy (Chapter 12, patient 1).

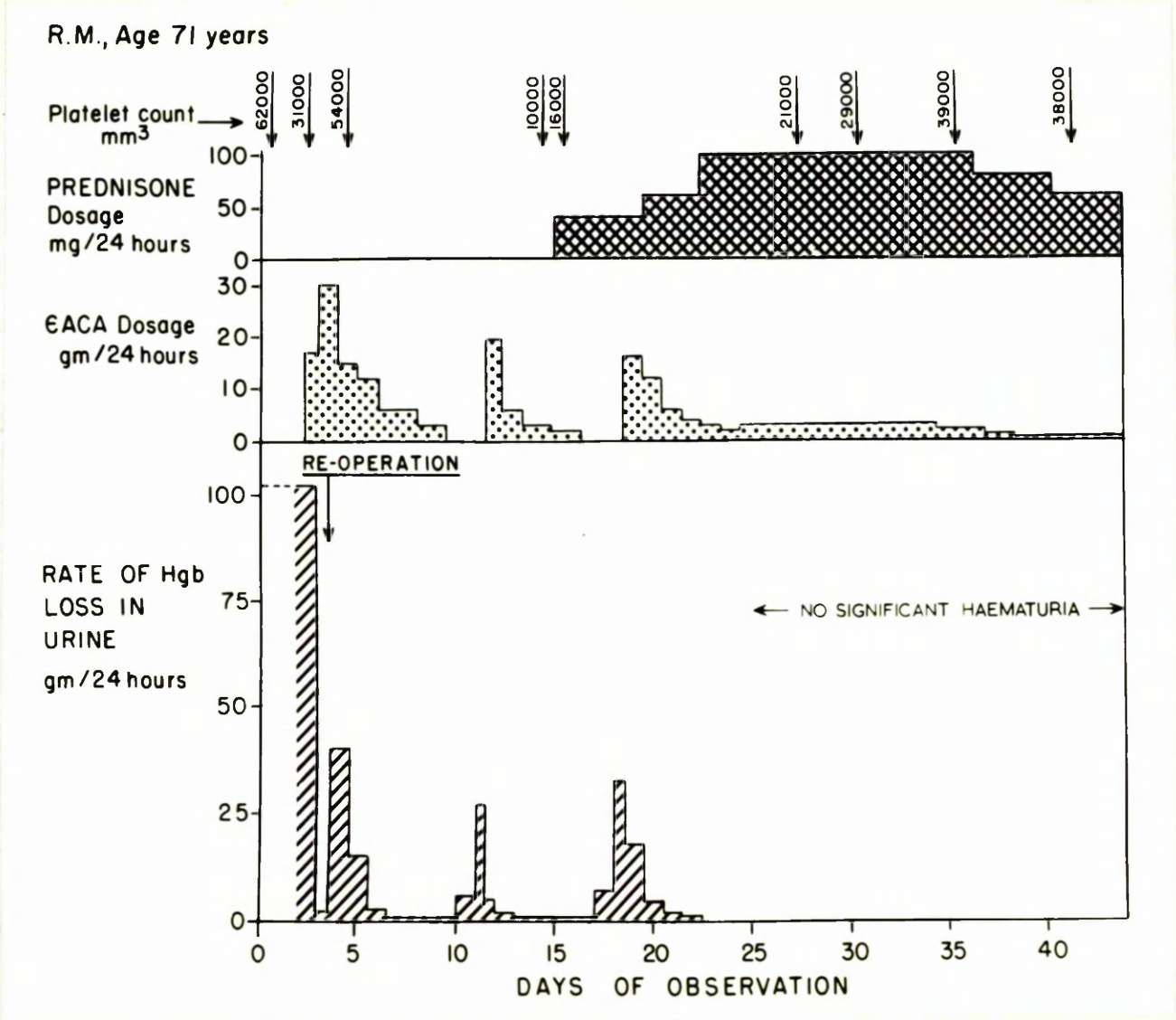

Figure 30 Effect of EACA administration on urinary haemoglobin loss in a patient with severe and protracted haematuria, and thrombocytopenia, following surgery for benign hyperplasia of the prostate (Chapter 12, patient 2). Suprapubic prostatectomy had been carried out 32 days before EACA was first given. The

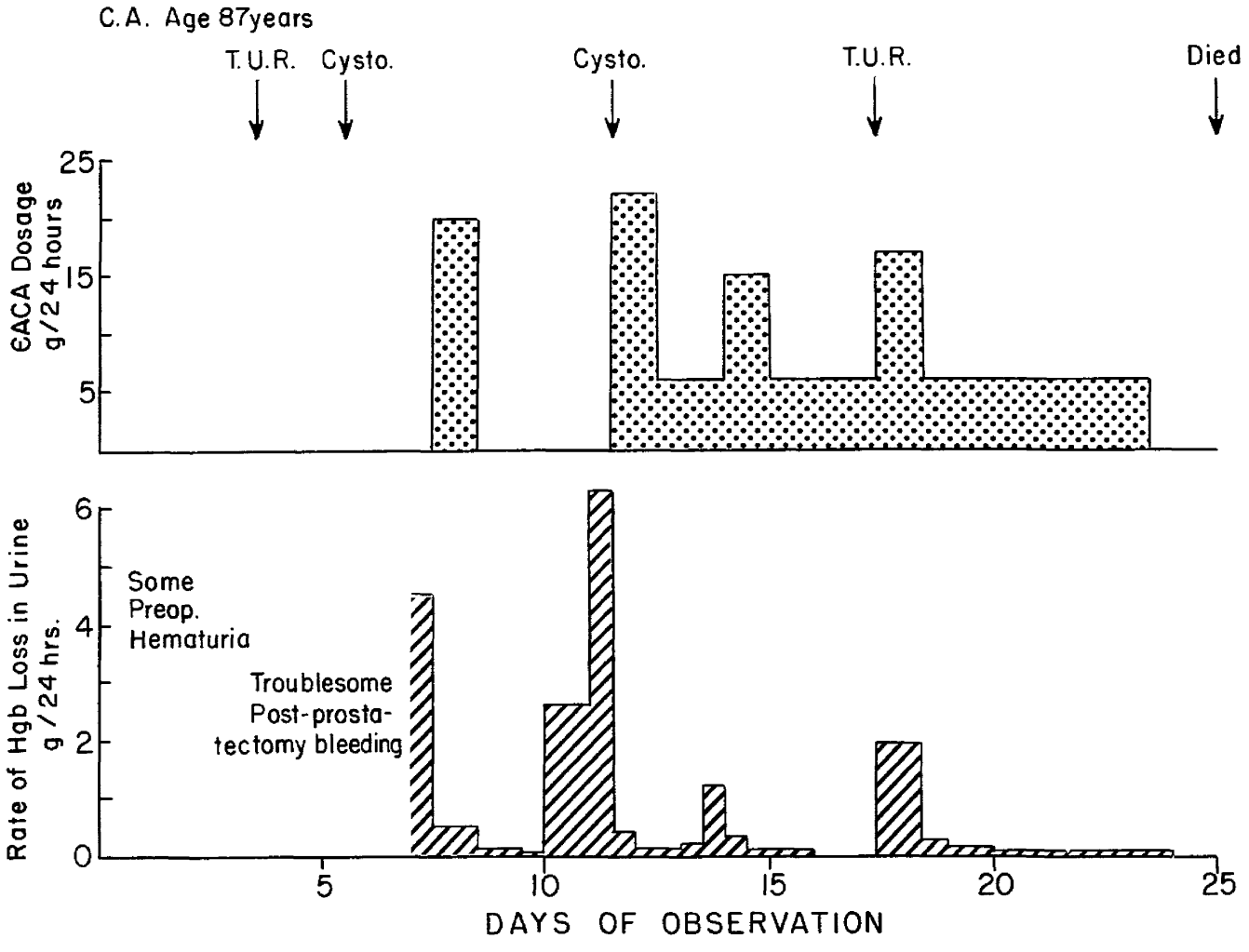

Figure 31 Effect of EACA administration on urinary haemoglobin loss in a patient with recurrent haematuria following surgery (transurethral prostatectomy) for benign hyperplasia of the prostate. (Chapter 12, patient 3). The figure is prepared from table 86.

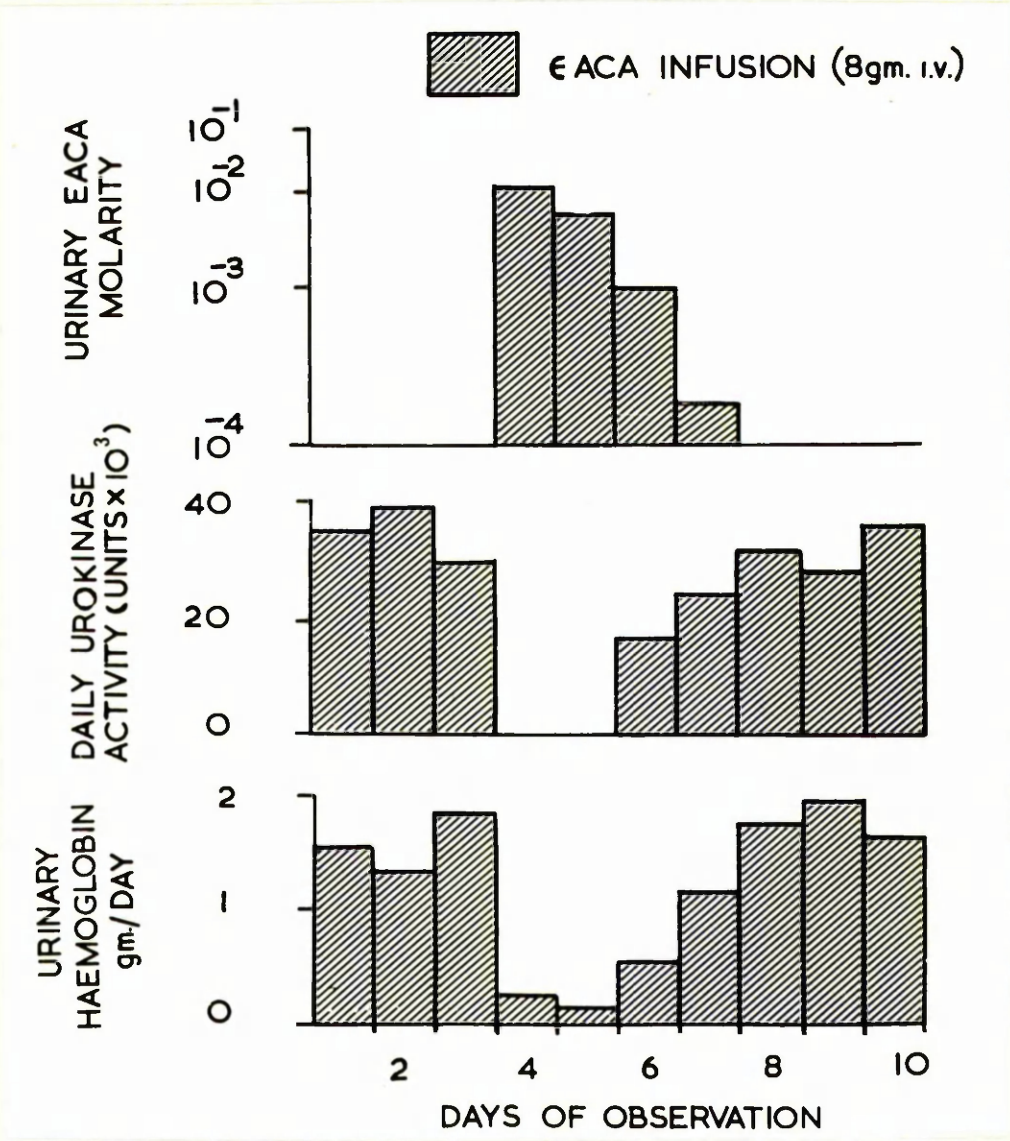

Figure 32 Effect of EACA administration on urinary urokinase activity and haemoglobin loss in a patient with renal carcinoma (Chapter 12, patient 4). Urinary EACA concentrations are also shown. The figure is prepared from tables 87 and 88.

APPENDIX 2

MAIN TABLES

Table I. Plasma EACA assay: 95 per cent confidence limits.

Concentration of EACA in plasma, mg./100 ml.	95 per cent confidence limits of the true concentration from the observed values shown in table 31.
25	25 ± 2.70
10	10 ± 2.32
1	1 ± 1.11

Shown, at various levels, are the 95 per cent confidence limits of the true concentration from the observations shown in table 31 (calculated by Dr. R.A. Robb).

Table 2. Assay of EACA in urine: comparison of high voltage electrophoresis and ion exchange resin loaded paper chromatography.

Patient Number	Day of observation	EACA concentration (mg./ml.) Electrophoresis	Chromatography
6	1	0.923	0.945
	2	0.363	0.401
	3	0	0.055
10	1	3.69	3.34
	2	0.680	0.810
	3	0	0.079
12	1	3.92	4.61
	2	0.493	0.462
	3	0.199	0.187
15	1	3.84	4.45
	2	3.61	2.28
	3	0	0.023
20	1	1.37	2.02
	2	0.601	0.410
	3	0	0.011
21	1	0.661	0.842
	2	0.970	0.524
	3	0	0
23	1	4.30	4.06
	2	0.187	0.250
	3	0.075	0.075

Comparison of the urinary EACA concentration obtained using the electrophoretic method of Sjoerdsma and Hanson (appendix 4), and the method, evolved in the present study, using ion exchange resin loaded paper chromatography. The chromatography assay values are taken from table 67.

Table 3. Plasma EACA levels 1 and 2 hours after a single oral dose.

Subject Number	Dose of EACA (gm.)	Plasma EACA concentration (mg./100 ml.) 1 hour	2 hours
1	2	-	11.7
2	2	6.8	9.2
3	2	5.2	10.7
4	2	7.3	10.1
5	2	5.0	6.3
6	2	3.1	10.1
7	2	4.8	11.4
8	2	2.8	6.6
9	2	7.2	7.8
10	4	-	12.3
11	4	-	17.1
12	5	14.8	22.2
13	6	-	21.2
14	10	24.3	35.9

Plasma EACA levels 1 and 2 hours after a single oral dose. For subjects 1-9, who were given 2 gm., the mean level at 1 hour was 5.3 ± 1.7 mg./100 ml., and 9.3 ± 2.0 mg./100 ml. at 2 hours. Subjects 1-3 and 10-14 were healthy adults; subject 4 had a duodenal ulcer, subject 5 disseminated lupus erythematosus, subject 6 thyrotoxicosis, subject 7 mitral stenosis, subject 8 an anxiety state and subject 9 ulcerative colitis.

Table 4. Urinary recovery of orally and intravenously administered EACA.

Table Number	Number of Subjects	Dosage Schedule	Total Dose (gm.)	Route	Percentage of dose recovered in urine at following times (hours) after first medication			
					24	48	72	96
70	13	1 gm./hr. for 4 hrs. then 0.5 gm./hr. for 8 hrs.	8	i.v.	61.1 ± 10.1	78.4 ± 10.1	81.5 ± 7.7	82.1 ± 6.8
41	1	2 gm. 2 hrly. for 12 doses	24	oral	54.3	76.5	85.0	-
41	1	2 gm. 2 hrly. for 12 doses	24	oral	61.2	78.6	80.4	-
38	1	11 gm. in 9 hrs.	11	oral	88.5	-	-	-
40	1	18 gm. in 12 hrs.	18	oral	76.4	81.7	-	-
34	1	5 gm. single dose	5	oral	111.6	-	-	-
33	1	10 gm. single dose	10	oral	78.2	-	-	-
32	1	10 gm. single dose	10	i.v.	111.0	-	-	-
87	1	8 gm. over 24 hrs.	8	i.v.	60.2	84.3	87.7	88.4
		Mean ± S.D.			68 ± 18	79 ± 9	82 ± 7	83 ± 7.

Shown for all subjects in whom urinary EACA assays were carried out at least 12 hours after EACA dosage had ceased, is the percentage of the EACA dose recovered in the urine.

Table 5. Simultaneous renal clearance rates of EACA and creatinine.

Subject Number	Clearance Period	Urine Sample Number	Plasma Sample Number	EACA clearance (ml./min.)	Creatinine clearance (ml./min.)
1	1	3	4, 5	105	142
2	1	2	2, 3	98	132
	2	4	5	112	124
	3	5	6	118	128
3	1	4	2, 3	81	123
	2	5	3, 4	86	127
	3	6	4,5,6	96	132

Shown for three subjects (figure 13) are renal clearance rates for EACA and creatinine, and the urine and plasma samples from which the clearance figures were calculated (tables 35-40 and 42). For the seven clearance periods shown, EACA clearance rates were 76.7 ± 10.4 per cent of creatinine clearance values.

Table 6. Distribution of injected EACA in the rabbit.

Time after EACA injection (hours)	EACA Concentration								
	mg./100 ml.		mg./ml.	mg./100 gm. Wet Tissue					
	Plasma	R.B.C.	Urine	Liver	Spleen	Muscle	Myocardium	Lung	Kidney
8	22.0	18.3	36.0	35.0	37.6	41.9	44.7	22.4	73.1
18	3.0	5.0	1.6	10.5	5.5	15.0	12.0	6.4	32.0

EACA concentrations in plasma, red blood cells, urine and tissue homogenates at 8 and 18 hours after a single intravenous injection of 5 gm. EACA (rabbits).

Table 7. Uptake of EACA by the intact rat diaphragm preparation.

Animal Number	Weight of diaphragm (mg.)	ug./ml. homogenate	ug./ml. tissue	uM./gm. tissue	uM./gm. cells	uM./ml. intra-cellular Water
1	192	93.6	0.48	3.67	1.97	3.46
2	182	76.2	0.42	3.23	1.50	2.68
3	211	66.0	0.32	2.44	0.74	1.30
4	211	114.2	0.54	4.12	2.42	4.25
5	191	73.6	0.38	2.90	1.20	2.14
					Mean ± S.D.	2.8 ± 1.1

Presented are results of 5 experiments showing uptake of EACA by the intact rat diaphragm preparation (chapter 6). EACA concentration/gm. of cellular tissue is calculated by assuming an extra cellular fluid content of 17 per cent by weight of the whole tissue, and also assuming that the extracellular fluid EACA content is the same as that of the incubation medium (i.e. 10^{-2} Molar, or 1.7 uM/gm. whole tissue). EACA concentration in the intracellular water is calculated from the concentration/gm. whole tissue by assuming that intracellular water represents 57 per cent by weight of cellular tissue.

Table 8. Thromboplastin generation test: effect of EACA

Incubation Time (minutes)	Clotting times, seconds, at varying EACA concentrations: EACA concentration (Molar)				
	Nil	10^{-4}	10^{-3}	10^{-2}	10^{-1}
1	35.5	38.0	34.5	36.0	35.0
2	14.5	16.0	14.0	13.5	15.5
3	10.5	11.5	10.5	11.0	11.0
4	11.0	11.0	11.0	11.0	10.5
5	11.0	10.5	11.0	10.5	10.5
6	10.5	11.0	11.0	11.5	10.5

The effect of varying concentrations of EACA in the thromboplastin generation test.

Table 9. Thrombin clotting time: effect of sodium chloride, urea and EACA.

Molarity of sodium chloride urea & EACA	Clotting times, seconds, with Sodium Chloride	Urea	EACA
0.1	13.5, 14	13, 13.5	13, 13.5
0.2	13, 14	14, 14.5	13.5, 13.5
0.5	18, 19	14.5, 15	14, 14
1.0	27, 28	16.5, 17	15, 15.5
2.0	48, 50	22, 22	18.5 19
Plasma/Saline control		13.5, 14	

The effect of increasing concentrations of sodium chloride, urea and EACA mixed 1 : 1 with normal plasma before assay of the thrombin clotting time. The results of duplicate assays are shown, also control observation with plasma : 0.9 per cent saline.

Table 10. Acid haemoglobin substrate: effect of glycine as a buffer.

EACA concentration (Molar)	pH of acid haemoglobin solution	
	without glycine	with glycine
1	5.7	2.2
10^{-1}	4.7	2.1
10^{-2}	2.1	2.0
10^{-3}	2.0	2.0
10^{-4}	2.0	2.0

The effect of glycine in buffering the acid haemoglobin substrate when EACA is added.

Table 11. Transurethral prostatectomy patients: clinical data.

	13 EACA treated patients	15 control patients	t	p
Age	69.2 ± 7.5	66.8 ± 9.5	0.715	>0.4
Haemoglobin (gm./100ml.)	13.5 ± 1.9	13.5 ± 1.9	0.066	>0.9
BUN (mg./100ml.)	17.8 ± 4.6	19.4 ± 6.7	0.743	>0.4
Weight of tissue resected, gm.	14.7 ± 8.5	15.4 ± 7.4	0.234	>0.8
Systolic B.P., mmHg.	140 ± 29	139 ± 30	-	-
Diastolic B.P., mmHg.	87 ± 16	84 ± 13	-	-

Shown is the similarity of age, pre-operative haemoglobin, blood urea nitrogen (BUN) and blood pressure levels, and the weight of tissue resected in the EACA and control groups (prepared from tables 44 and 45).

Table 12. Effect on urokinase activity of storage of urines at 4°C.

Urine Number	Urokinase Activity			
	Initially		After storage	
	Area of lysis (mm^2)	UK activity (units/ml.)	Area of lysis (mm^2)	UK activity (units/ml.)
1	360 384	41.0 46.3 } 43.7	323 315	42.4 40.5 } 41.5
2	324 336	32.7 35.6 } 34.2	289 272	33.5 28.8 } 31.2
3	240 252	18.2 20.2 } 19.2	210 225	17.3 20.0 } 18.7
4	144 156	7.3 8.4 } 7.9	132 143	7.8 8.7 } 8.2
5	420 441	54.4 59.2 } 56.9	380 400	55.0 60.6 } 57.8
6	361 361	41.6 41.6 } 41.6	320 336	41.3 44.6 } 43.0
Mean ± S.D.	-	33.9 ± 17.8	-	33.4 ± 17.9

The result of urokinase assays carried out with fresh normal urine samples and repeat assays carried out with the same urines after storage at 4°C for 72 hours. The standard urokinase curve run with the initial assays was curve 45, table 46, and the curve run with the assays on stored samples was curve 48. The assays were carried out in duplicate; the coefficient or variation for the 12 pairs of assays is 8.98 per cent. Mean urokinase activities before storage (33.9 ± 17.8 per cent), and after storage (33.4 ± 17.9 per cent), are almost identical ($t = 0.049$, $p > 0.9$).

Table 13. Loss of urokinase activity during dialysis.

Area of lysis, mm^2, with dialysed UK solution (25 units/ml.)	Standard urokinase dilution curve number	Urokinase activity in dialysed samples (units/ml.)	Loss of activity due to dialysis (per cent)
196	105	18.2	27
192	106	16.4	34
192	107	15.9	36
247	111	17.7	29
252	112	19.2	23
196	117	16.6	34
182	118	15.3	39
196	119	17.2	31
204	122	17.9	28
210	123	16.5	34
Mean $\pm$ S.D.			32 ± 5

Percentage loss of activity of standard urokinase solution (25 units/ml.) during dialysis. The mean value and standard deviation are also shown.

The standard urokinase dilution curves are from table 46.

Table 14. Weight increase of urine samples during dialysis.

Weight of tubing and contents before dialysis (gm.)	Weight of tubing and contents after dialysis (gm.)	Per cent increase in weight
8.862	9.321	5.2
7.197	7.487	4.0
9.915	10.512	6.0
7.334	7.667	4.5
8.581	8.953	4.3
10.244	10.899	6.4
9.606	10.111	5.3
8.123	8.215	1.1
7.867	8.233	4.5
10.198	10.553	3.5
Mean $\pm$ S.D.		4.5 $\pm$ 1.5

Shown in 10 instances is the increase in weight of dialysis tubing and its content of urine after dialysis for 24 hours. The mean value and standard deviation are also shown.

Table 15. Effect of bank blood and fresh blood on urokinase activity.

Experiment Number	Urokinase activity when diluted with: Saline Area of lysis (mm^2)	Saline Units/ml.	Bank blood Area of lysis (mm^2)	Bank blood Units/ml.	Fresh blood Area of lysis (mm^2)	Fresh blood Units/ml.
1 (45*)	288	25	285	24.5	304	28.3
2 (124*)	285	25	270	22.6	289	26.3
3 (125*)	272	25	252	21.7	256	22.4
4 (128*)	289	25	315	30.2	272	24.3
Mean		25		24.8		25.3

Results of 4 experiments in which the effects of diluting urokinase 1 : 3 with saline expired bank blood, and fresh oxalated blood were compared. The bank blood and fresh blood were diluted 1 : 49 with saline before addition to the urokinase.

*Standard urokinase dilution curve number (table 46).

Table 16. Effect of EACA on urinary haemoglobin assay.

	EACA Molarity				
	10^{-4}	10^{-3}	10^{-2}	10^{-1}	Nil
Optical Densities, Urine 1.	0.249	0.251	0.248	0.247	0.249
	0.253	0.254	0.253	0.254	0.254
Optical Densities, Urine 2.	0.252	0.254	0.257	0.255	0.254
	0.259	0.260	0.258	0.259	0.260
Mean Optical Densities	0.253	0.255	0.254	0.256	0.254

Effect of EACA on urinary haemoglobin assay. Optical densities of samples, prepared from two normal urines by the addition of a constant quantity of whole blood and varying amounts of EACA, are shown.

Table 17. Transurethral prostatectomy series: daily urokinase activity.

		Pre-Operative	Days after operation 1	2	3	4	5
EACA treated patients	UK assay ($units \times 10^3$)	± 45.06 9.92	0	± 11.62 9.16	± 34.0 14.19	± 50.86 15.32	± 47.62 7.63
	No. of pts.	12	13	13	13	13	12
Control Patients	UK assay ($units \times 10^3$)	± 46.07 16.43	± 47.17 19.24	± 47.38 19.72	± 44.67 19.30	± 45.79 14.26	± 44.92 13.18
	No. of pts.	14	15	15	15	14	13
t		0.186	-	5.988	1.628	0.891	0.618
p		>0.8	-	<0.001	>0.1	>0.3	>0.5

Shown is daily assayable urokinase activity for the day before and the five days after operation. Results are expressed in thousands of arbitrary units. Mean values and standard deviations are shown. Also shown are the numbers of patients in each group and the results of statistical comparison of the mean values for each day. The table is compiled from tables 58 and 60.

Table 18. Transurethral prostatectomy series: daily urokinase activity in dialysed urine samples.

		Pre-Operative	Days after operation 1	2	3	4	5
EACA treated patients	UK assay units x 10^3	± 29.93 ± 8.10	± 25.89 ± 6.99	± 27.81 ± 7.67	± 27.13 ± 6.34	± 33.49 ± 8.46	± 31.48 ± 6.59
	No. of pts.	12	13	13	13	13	12
Control Patients	UK assay units x 10^3	± 31.02 ± 9.51	± 26.42 ± 7.41	± 30.37 ± 11.8	± 28.00 ± 8.32	± 30.32 ± 11.82	± 29.99 ± 3.32
	No. of pts.	8	9	9	9	9	8
t		0.266	0.054	0.196	0.088	0.232	0.570
p		>0.7	>0.9	>0.8	>0.9	>0.8	>0.5

Shown is assayable urokinase activity, after dialysis of the urine samples, for the day before and the five days after operation. Results are expressed in thousands of arbitrary units; mean values and standard deviations are shown. Also shown are the numbers of patients in each group and the results of statistical comparison of the mean values for each day. The table is compiled from tables 59 and 61.

Table 19. Transurethral prostatectomy series: effect of EACA on urinary blood loss.

		Day of observation 1	2	3	4	5	Total loss 5 day period
EACA treated patients	Haemoglobin loss, gm.	1.332 ± 0.888	0.579 ± 1.059	0.214 ± 0.275	.074 ± 0.140	0.063 ± 0.139	2.230 ± 1.598
	No. of pts.	13	13	13	13	12	12
Control Patients	Haemoglobin loss, gm.	5.33 ± 4.89	2.86 ± 5.82	0.945 ± 1.320	0.372 ± 0.386	0.188 ± 0.294	10.581 ±11.85
	No. of pts.	15	15	15	14	13	13
t		2.897	1.394	1.955	2.628	1.344	2.419
p		< 0.01	> 0.1	> 0.05	< 0.02	> 0.1	< 0.05

Effect of EACA on urinary blood loss in patients after transurethral prostatectomy. Mean values and standard deviation are shown, also 't' and 'p' values. The data are displayed graphically in figure 22. (Table compiled from tables 64 and 65).

Table 20. Transurethral prostatectomy series, EACA treated patients: Urinary EACA content.

Day of observation	EACA concentration mg./ml. Mean ± S.D.	mg./ml. Range	Molar Mean ± S.D.	Mean cumulative percentage recovery ± S.D.
1	3.2 ± 2.0	0.842 - 7.28	$2.5 \pm 1.5 \times 10^{-2}$	61.1 ± 10.1
2	0.79 ± 0.73	0.250 - 2.51	$6.0 \pm 5.5 \times 10^{-3}$	78.4 ± 10.4
3	0.11 ± 0.14	0 - 0.493	$8.4 \pm 10.8 \times 10^{-4}$	81.5 ± 7.7
4	0.025	0 - 0.278	1.9×10^{-4}	82.1 ± 6.8

Shown for the first 4 days after operation are mean EACA concentrations, standard deviations and ranges. Mean cumulative percentage recovery of the 8 gm. dose administered is also shown. (Table compiled from tables 67 and 68).

Table 21. Transurethral prostatectomy series, control patients: uropepsin activity.

	Uropepsin assay		
Patient Number	Optical Density	"Pepsin" concentration (ug./ml.)	Peptic activity (mg.) per 24 hour collection
1	0.086	2.442	2.833
3	0.075	2.130	2.492
4	0.065	1.846	3.600
5	0.047	1.335	2.283
7	0.020	0.625	1.344
8	0.065	1.846	3.138
9	0.052	1.477	2.496
11	0.020	0.568	2.772
16	0.025	0.710	2.570
17	0.025	0.710	1.534
18	0.046	1.306	2.521
19	0.025	0.710	3.124
24	0.030	0.852	2.948
26	0.092	2.613	2.639
27	0.068	1.931	2.723
	Mean		2.601
	S.D.		± 0.576

Shown in the left hand column are optical densities in the assay for peptic activity of the first post operative 24 hour urine collections. In the middle column the results are expressed in terms of the activity in this assay system of a purified pepsin preparation (chapter 8). Final results, calculated from the middle column and urine volumes (table 48), are expressed in terms of peptic activity per 24 hour urine collection. The mean value and standard deviation are also shown.

Table 22. Transurethral prostatectomy series, EACA treated patients: uropepsin activity.

Patient Number	Uropepsin assay		
	Optical Density	"Pepsin" concentration (ug./ml.)	Peptic activity (mg.) per 24 hour collection
1	0.010	0.284	0.605
6	0.026	0.738	3.609
10	0.017	0.483	0.850
12	0	0	0
13	0	0	0
14	0	0	0
15	0.036	1.022	1.165
20	0.027	0.767	2.155
21	0.020	0.568	3.527
22	0.042	1.193	2.279
23	0.010	0.284	0.346
25	0.035	0.994	1.958
28	0.017	0.483	1.096
		Mean	1.353
		S.D.	± 1.261

Shown in the left hand column are optical densities in the assay for peptic activity of the first post-operative 24 hour urine collections. In the middle column the results are expressed in terms of the activity in this assay system of a purified pepsin preparation (chapter 8). Final results, calculated from the middle column and urine volumes (table 49) are expressed in terms of peptic activity per 24 hour urine collection. The mean value and standard deviation are also shown.

Table 23. Effect of urine on thrombin clotting time of normal plasma.

Subject Group	Thrombin clotting times (seconds) of plasma-urine mixture when	
	Urine not dialysed	Urine dialysed
15 normal subjects	19.5 ± 4.7	14.0 ± 0.8
13 EACA treated patients	17.0 ± 2.5	14.1 ± 0.6
15 control patients	16.3 ± 2.5	13.9 ± 0.4
Controls (plasma/saline)	13.5 - 14	

The table summarises tables 74, 75 and 76. Mean values and standard deviations are shown of thrombin clotting times of normal plasma mixed 1 : 1 with urine before assay. The thrombin clotting times of plasma/saline controls are also shown.

Table 24. Transurethral prostatectomy series: plasma plasminogen and fibrinogen levels, and thrombin clotting times.

	Plasminogen (units/ml.)	Fibrinogen (mg./100ml.)	Thrombin Time (seconds)
15 control patients	2.5 ± 0.2	300 ± 60	10.5 ± 0.9
13 EACA treated patients	2.5 ± 0.2	207 ± 63	10.3 ± 0.9
t	0	0.547	0.594
p	-	> 0.5	> 0.5

Shown for the control and EACA treated groups are mean values, standard deviations and 't' tests on plasminogen and fibrinogen levels, and thrombin time, in plasma samples taken off 4 hours after operation. (Compiled from tables 77 and 78).

Table 25. Transurethral prostatectomy patients: plasma EACA levels 4 and 8 hours after operation.

Patient Number	Plasma EACA concentration (mg./100 ml.) At 4 hours	At 8 hours
2	11.4 (12.5, 10.2)	6.3 (6.5, 6.0)
6	10.1 (11.6, 8.6)	5.6 (6.6, 4.6)
10	8.4 (9.1, 7.6)	4.8 (5.7, 3.8)
12	11.1 (13.2, 8.9)	6.2 (7.4, 4.9)
13	14.5 (17.1,11.9)	7.2 (7.3, 7.1)
14	7.2 (8.0, 6.4)	3.6 (4.0, 3.2)
15	10.4 (10.5,10.2)	5.8 (6.5, 5.0)
20	13.3 (16.2,10.4)	6.9 (7.8, 6.1)
21	15.0 (15.6,14.4)	9.1(10.0, 8.2)
22	10.7 (12.8, 8.6)	5.2 (5.6, 4.8)
23	6.3 (6.9, 5.7)	3.8 (4.7, 2.9)
25	16.4 (19.6,13.1)	10.0(11.1, 8.8)
28	12.2 (13.4,11.0)	6.5 (6.8, 6.2)
Mean	11.3	6.2
S.D.	±3.0	±1.8

Shown are plasma EACA levels 4 and 8 hours after the start of the EACA infusion. Results of duplicate assays, mean values and standard deviations are given.

Table 26. Suprapubic prostatectomy patients: clinical data.

		10 EACA treated patients	9 control patients
Age	*	66.1 ± 5.4	71.0 ± 7.7
Haemoglobin, gm./100 ml.		14.8 ± 2.2	14.6 ± 2.0
BUN, mg./100 ml.		16.9 ± 9.0	16.9 ± 5.3
Systolic B.P., mm.Hg.		143 ± 25.5	146 ± 19.7
Diastolic B.P., mm.Hg.		87 ± 12.5	89 ± 11.7
Weight of tissue, gm.	**	57.0 ± 33.5	69.1 ± 38.8

Shown are mean values and standard deviations for age, pre-operative haemoglobin, blood urea nitrogen (BUN) and blood pressure levels, and weight of prostatic tissue removed, for the EACA treated and control patients. (Compiled from table 79).

* $t = 1.620$, $p > 0.1$

** $t = 0.751$, $p > 0.4$

Table 27. Suprapubic prostatectomy patients: post-operative blood loss.

Day	Urinary haemoglobin loss following surgery (gm.). 10 EACA treated subjects	9 control subjects	t	p
1	13.0 ± 10.4	40.7 ± 31.2	2.660	< 0.02
2	4.9 ± 2.6	9.4 ± 6.7	1.948	< 0.1
3	3.3 ± 1.4	3.6 ± 1.6	0.394	> 0.6
4	2.2 ± 1.2	2.3 ± 1.2	0.254	> 0.8
5	1.3 ± 0.6	1.3 ± 0.9	0.155	> 0.8
Total, Days 1-5	24.7 ± 13.4	57.4 ± 31.5	2.972	< 0.01

Effect of EACA on urinary blood loss in patients after suprapubic prostatectomy. Mean values and standard deviation are shown, also 't' and 'p' values. The data are also displayed graphically in figure 26.

Table 28. Suprapubic prostatectomy patients: post-operative blood loss per unit weight of tissue removed.

Patient Number	Blood loss (gm. haemoglobin) per gm. prostatic tissue removed	
	Day 1	Days 1 - 5
1	0.352	0.885
5	0.339	0.852
8	0.404	0.951
9	0.169	0.444
11	0.045	0.127
13	0.884	1.091
14	0.284	0.430
16	0.092	0.209
17	0.192	0.401
18	0.093	0.183
2	0.869	1.048
3	0.246	0.561
4	0.237	0.327
6	1.021	1.772
7	0.534	0.673
10	0.460	0.681
12	0.720	0.853
15	0.964	1.237
19	0.786	1.223

Shown for each patient are blood loss on the first post operative day and for the five day period of observation, per unit weight of prostatic tissue removed.

Table 29. Suprapubic prostatectomy patients: plasma EACA levels 4 and 8 hours after operation.

Patient Number	Plasma EACA concentration (mg./ml.) at 4 hours	at 8 hours
1	15.2	7.4
5	14.0	6.5
8	15.4	7.5
9	6.9	4.4
11	10.9	6.0
13	8.8	5.0
14	13.6	8.3
16	11.6	6.4
17	11.8	8.6
18	12.5	6.0
Mean	12.1	6.6
SD	±2.7	±1.4

Shown are plasma EACA levels 4 and 8 hours after the start of EACA therapy. Mean values and standard deviation are also shown.

Table 30. Individual patients with haematuria: coagulation and fibrinolytic data.

		Patient number				
		1	2	3	4	5
Bleeding Time (mins.)	⁂	6	3	6	4	5
Clotting time (mins.)	⁂	8	6	4	7	9
Platelet Count/mm^3	⁂	197_3 x10^3	see text	280_3 x10^3	316_2 x10^2	255_3 x10^3
Hess's Test	⁂	-ve	-ve	-ve	-ve	-ve
Fibrinogen (mg./100 ml.)		230	318	276	285	244
Plasminogen (casein units/ml.)		24	2.6	2.5	2.7	2.3
Thrombin Clotting time (seconds)	Patient	11	10.5	10	11	10
	Control	10.5	10	10.5	11	10.5
Prothrombin Time(seconds)	Patient	15.5	16	14.5	15	15.5
	Control	16	16	15	15.5	15.0
Euglobulin lysis time (units)		0.1	0.1	0.1	0.1	0.1

Shown are coagulation and fibrinolytic assays of the patients reported in chapter 12.

⁂ Assays carried out by the medical or laboratory staff of Barnes, Jewish and St. Luke's Hospitals.

APPENDIX 3

SUPPORTING TABLES

Table 31. Assay of EACA in plasma: values obtained with plasmas of known EACA content.

Concentration of EACA in plasma (mg./100 ml.)	EACA assayed, (mg./100 ml.)
0.5	0.2, 0.9, 1.2, 1.4
1.0	0.5, 0.6, 1.4, 1.8, 1.9
2.0	1.1, 1.8, 2.6, 3.2
3.0	2.0, 3.9, 4.9
5.0	3.1, 4.7, 6.3
10.0	7.5, 8.0, 9.9, 10.9, 12.6, 12.9
20.0	16.7, 16.8, 17.6, 18.1, 20.5, 24.1, 25.6
25.0	21.4, 22.3, 22.9, 27.4, 27.8, 29.9, 31.7
30.0	26.0, 34.9, 35.4

Shown are values obtained in assay of plasmas with known concentrations of EACA. The data are also displayed graphically in figure 10.

Table 32. Plasma and urine concentrations of EACA after a single intravenous injection.

Time (hours) after EACA	EACA concentration mg./100 ml. Plasma	R.B.C.	mg./ml. Urine	Urine Volume (ml.)	Urine EACA content (gm.)	Percentage of dose excreted in urine
0	0	0	-	-	-	-
¼	150.0	33.6	-	-	-	-
⅓	51.4	21.2	-	-	-	-
1	25.0	14.6	12.6	380	4.788	48
2	10.8	8.9	-	-	-	-
3⅓	-	-	15.6	219	3.416	82
4	3.9	8.6	-	-	-	-
6	3.4	9.6	21.25	85	1.806	100
24	0	0	0.96	1135	1.090	111
30	-	-	0.124	360	0.045	111

Plasma, R.B.C. and urinary EACA concentrations after rapid intravenous injection of 10 gm. EACA. The data are also graphically displayed in figure 12.

Table 33. Plasma and urine concentrations of EACA after a single oral dose.

Time (hours) after EACA	EACA concentration mg./100 ml. Plasma	R.B.C.	mg./ml. Urine	Urine volume (ml.)	Urine EACA content (gm.)	Percentage of dose excreted in urine
0	0	0	-	-	-	-
$\frac{1}{2}$	21.6	14.6	4.24	210	0.890	8.9
1	24.3	12.0	10.1	124	1.252	21.4
2	35.9	14.1	20.7	75	1.553	37.0
3	-	-	17.7	48	0.850	45.5
4	15.6	10.4	-	-	-	-
$4\frac{25}{60}$	-	-	13.8	80	1.104	56.5
$5\frac{25}{60}$	-	-	14.1	42	0.592	62.4
6	4.5	6.6	-	-	-	-
12	0	6.6	2.39	585	1.398	76.4
24	0	5.3	0.37	640	0.237	78.7
30	-	-	0.06	320	0.019	79.0

Levels of EACA in plasma, red cells and urine after a single oral dose of 10 gm: cumulative urinary excretion is also shown, and the data are displayed graphically in figure 12 (subject 1).

Table 34. Plasma and urine concentrations of EACA after a single oral dose

Time (hours) after EACA	EACA concentration Plasma (mg./100ml.)	Urine (mg./ml.)	Urine volume (ml.)	Urine EACA content (gm.)	Percentage of dose excreted in urine
0	0	-	-	-	-
½	7.15	0.942	135	0.127	2.5
1	14.8	1.30	242	0.315	8.8
2	22.2	6.50	246	1.599	40.8
4	7.0	15.0	133	1.995	80.7
6	4.8	7.0	156	1.084	102.4
24	-	0.505	910	0.460	111.6

Levels of EACA in plasma and urine after a single oral dose of 5 gm.: cumulative urinary excretion is also shown, and the data are displayed graphically in figure 12. (subject 2).

Table 35. Plasma and red cell EACA concentrations with repeated oral doses.

Sample Number	Time (hours)	EACA dosage (gm.)	EACA concentration, mg./100 ml. Plasma	R.B.C.
	0	4	-	-
1	2	2	17.1	8.6
2	4	2	16.2	10.0
3	6	2	13.6	10.6
	$6\frac{30}{60}$	$\frac{1}{2}$	-	-
4	7	$\frac{1}{2}$	15.6 (17.3, 13.8)	11.8
5	8	-	15.7 (16.5, 13.9)	14.9
	$8\frac{20}{60}$	$\frac{1}{2}$	-	-
6	24	-	0.5	4.6

Plasma and red cell levels achieved by repeated doses of EACA. Urinary levels are shown in table 36. The data are also graphically displayed in figure 13 (subject 1).

Table 36. Urinary EACA concentrations with repeated oral doses.

Sample Number	Time (hours)	Urine volume (ml.)	EACA concentration (mg./ml.)	EACA content (gm.)
1	2	220	11.1	2.444
2	6.20	98	25.1	2.460
3	8.20	128	15.4 (16.3, 14.5)	1.971
4	9	32	14.4	0.461

Urinary EACA levels found after repeated oral doses. Plasma levels are shown in table 35, and the data are also graphically displayed in figure 13 (subject 1).

Table 37. Plasma and red cell EACA concentrations with repeated oral doses.

Sample Number	Time (hours)	EACA concentration (mg./100 ml.) Plasma	R.B.C.	EACA dosage (gm.)
	0	-	-	4
1	2	11.7	5.4	2
2	4	13.8 (15.0, 12.6)	6.6	2
3	6	14.3 (16.5, 12.1)	7.0	2
4	8	12.4	7.5	$\frac{1}{2}$
5	$8\frac{3}{4}$	13.6 (13.9, 13.2)	8.1	-
	9	-	-	$\frac{1}{2}$
6	$9\frac{1}{3}$	13.7 (14.7, 12.7)	8.6	-
7	24	0	2.8	-

Plasma and red cell levels achieved by repeated oral doses of EACA. Urinary levels are shown in table 38. The data are also graphically displayed in figure 13 (subject 2).

Table 38. Urinary EACA concentrations with repeated oral doses.

Sample Number	Time (hours)	Urine Volume (ml.)	EACA conc. (mg./ml.)	EACA content (gm.)
1	3	105	15.3	1.607
2	7	436	9.08 (9.98, 8.18)	3.959
3	8	120	11.1	1.332
4	9	78	10.2 (11.8, 8.6)	0.797
5	10	130	7.08 (8.68, 5.48)	0.920
6	24	720	1.55	1.116

Urinary EACA levels found after repeated oral doses. Plasma and red cell levels are shown in table 37, and the data are also graphically displayed in figure 13 (subject 2).

Table 39. Plasma and red cell EACA concentrations with repeated oral doses.

Sample Number	Time (hours)	EACA dosage (gm.)	EACA concentration, mg./100 ml. Plasma	R.B.C.
	0	6	-	-
1	2	2	21.2	11.0
2	4	2	18.6 (20.8, 16.4)	12.7
3	6	2	17.5 (19.1, 15.9)	12.9
4	8	2	17.1 (18.0, 16.1)	11.4
5	10	2	16.7 (17.0, 16.4)	15.4
6	12	2	17.7 (20.9, 14.4)	16.5
7	24	-	0	4.8

Plasma and red cell levels achieved by repeated oral dose of EACA. Urinary levels are shown in table 40. The data are also graphically displayed in figure 13 (subject 3).

Table 40. Urinary EACA concentrations with repeated oral doses.

Sample Number	Time (hours)	Urine Volume (ml.)	EACA concentration (mg./ml.)	EACA content (gm.)
1	½	92	0.330	0.030
2	1	60	10.6	0.636
3	2½	130	11.5	1.495
4	6	237	13.0 (13.9, 12.0)	3.081
5	7½	89	15.1 (15.8, 14.4)	1.344
6	12	518	8.60(9.90, 7.29)	4.455
7	24	580	4.67	2.709
8	48	2570	0.37	0.951
9	56	1000	0.026	0.026

Urinary EACA levels found after repeated oral doses. Plasma and red cell levels are shown in table 39, and the data are graphically displayed in figure 13 (subject 3).

Table 41. Urinary EACA content for 3 days after repeated oral doses.

		Day of observation		
		1	2	3
Patient 1	Urine volume (ml.)	1570	1620	1840
	EACA conc. (mg./ml.)	8.3	3.3	1.1
	EACA content (gm.)	13.032	5.346	2.024
Patient 2	Urine volume (ml.)	1710	1900	1850
	EACA conc. (mg./ml.)	8.6	2.2	0.19
	EACA content (gm.)	14.706	4.180	0.351

Urine volumes and urinary EACA concentrations and content on successive days in two subjects given EACA, 2 gm. every 2 hours to a total dose of 24 gm. The findings are summarised in table 4.

Table 42. Creatinine clearances: plasma and urine creatinine concentrations

Subject Number	Plasma Sample Number	Plasma Creatinine conc. (mg./100 ml.)	Urine Sample Number	Urine Creatinine conc. (mg./100 ml.)
1	4	1.24	3	165.4
	5	1.24		
2	2	1.11	2	209.1
	3	1.26	4	126.7
	5	1.24	5	73.0
	6	1.28		
3	2	0.98		
	3	0.94	4	104.7
	4	0.90	5	118.1
	5	0.96	6	64.7
	6	0.96		

Plasma and urine creatinine concentrations in subject given repeated oral doses of EACA (figure 13). Creatinine clearance values are shown in table 5.

Table 43. Inhibition of pepsin by EACA.

EACA concentration (Molar)	Optical densities in pepsin assay	Percentage inhibition of pepsin
0	0.176, 0.178	-
0.0001 ($1x10^{-4}$)	0.178	0
0.0005 ($5x10^{-4}$)	0.175	1
0.001 ($1x10^{-3}$)	0.150	15
0.002 ($2x10^{-3}$)	0.137	23
0.005 ($5x10^{-3}$)	0.112	37
0.008 ($8x10^{-3}$)	0.060	66
0.01 ($1x10^{-2}$)	0.045	75
0.02 ($2x10^{-2}$)	0.023	87
0.05 ($5x10^{-2}$)	0.021	88
0.08 ($8x10^{-2}$)	0.015	92
0.1 ($1x10^{-1}$)	0.010	96

Shown is the effect of increasing EACA concentrations on the action of pepsin on an acid haemoglobin substrate. The data are also shown in figure 14.

Table 44. Transurethral prostatectomy patients: Clinical data

Patient Number	Age	Hb ¤ (gm./100ml.)	BUN ¤ (mg./100ml.)	Wt. of ¤ Tissue resected (gm.)	Blood Pressure ¤ Systolic (mm.Hg.)	Diastolic (mm.Hg.)
1	66	15.0	26	10	195	100
3	41	14.8	30	20	160	90
4	72	12.3	11	7	130	80
5	75	13.3	29	25	100	70
7	69	12.0	17	30	145	80
8	67	12.7	15	10	170	105
9	69	14.3	12	12	125	75
11	79	14.7	18	27	120	80
16	62	9.3	18	8	110	70
17	72	15.2	28	14	105	75
18	62	10.8	19	15	165	100
19	62	13.7	17	14	180	105
24	70	13.2	12	7	150	85
26	79	15.0	26	12.5	135	85
27	57	16.4	13	20	100	65
Mean	66.8	13.51	19.40	15.43	139.3	84.3
S.D.	±9.54	±1.87	±6.67	±7.37	±30.1	±13.0

Shown are ages, pre-operation haemoglobin (Hb.), blood urea nitrogen (BUN) and blood pressure levels, and weight of tissue resected at prostatectomy. Mean values and standard deviations are also shown. The data are summarised in table 11.

¤ Measurements made by the medical and laboratory staff of Barnes and Jewish Hospitals.

Table 45. Transurethral Prostatectomy Series, EACA treated patients: Clinical data

Patient Number	Age	Hb ¤ (gm./100ml.)	BUN ¤ (mg./100 ml.)	Wt. of tissue resected ¤ (gm.)	Blood Pressure ¤ Systolic (mm.Hg.)	Diastolic (mm.Hg.)
2	69	13.6	14	10	110	65
6	79	16.8	15	25	180	105
10	54	14.7	17	5	160	100
12	71	12.4	14	7.5	120	80
13	65	14.1	10	27	135	95
14	61	12.7	21	14	105	65
15	69	9.3	19	31	175	100
20	74	12.8	13	8	130	90
21	60	14.7	22	9	190	110
22	77	13.9	20	22	100	60
23	68	14.3	23	10	125	85
25	77	11.0	26	11	145	90
28	75	14.6	17	12	150	85
Mean	69.15	13.45	17.77	14.73	140.4	86.9
S.D.	±7.53	±1.88	±4.56	±8.49	±29.3	±15.9

Shown are ages, preoperative haemoglobin (Hb), blood urea nitrogen (BUN) and blood pressure levels, and weight of tissue resected at prostatectomy. Mean values and standard deviation are also shown. The data are summarised in table 11.

¤ Measurements made by medical and laboratory staff of Barnes and Jewish Hospitals.

Table 46. Standard Urokinase dilution curves

Standard Curve Number	Area of lysis ($mm.^2$) with varying concentrations of standard urokinase				
	100%	50%	25%	12.5%	6.25%
1	504	324	247	169	99
2	576	360	270	182	110
3	580	361	266	182	110
4	506	320	234	154	99
5	575	360	270	170	110
6	552	361	252	170	104
7	630	399	252	196	121
8	522	323	240	156	100
9	576	361	272	180	117
10	625	400	289	196	121
11	540	336	252	169	100
12	575	324	255	165	104
13	552	360	256	169	104
14	575	361	285	180	110
15	560	378	270	182	104
16	598	357	266	192	108
17	529	324	247	156	99
18	552	361	255	169	108
19	550	360	256	180	100
20	609	391	270	195	117
21	576	378	270	182	117
22	594	399	270	195	117
23	621	396	272	196	120
24	576	340	285	169	110
25	600	380	288	195	121
26	644	399	288	210	132
27	616	380	288	196	120
28	552	324	255	182	108
29	529	324	247	169	96
30	638	400	289	196	121

Table 46. (continued)

Standard Curve Number	Area of lysis ($mm.^2$) with varying concentrations of standard urokinase. 100%	50%	25%	12.5%	6.25%
31	672	420	285	208	143
32	575	361	270	165	110
33	529	324	240	182	100
34	644	399	289	195	132
35	675	420	272	251	144
36	552	336	256	156	108
37	600	380	240	196	120
38	625	400	289	196	121
39	667	440	289	225	132
40	621	396	266	196	120
41	576	361	266	182	110
42	600	399	288	195	120
43	667	400	306	224	132
44	609	357	256	182	110
45	600	400	288	195	121
46	696	483	315	247	144
47	600	380	289	196	121
48	500	323	234	156	96
49	480	323	240	144	88
50	625	400	289	196	121
51	600	400	285	192	117
52	529	336	252	156	99
53	725	483	306	196	132
54	522	357	247	169	104
55	552	340	256	168	108
56	552	361	256	169	104
57	324	224	156	110	72
58	506	336	252	165	100
59	575	361	266	176	110
60	600	380	285	196	117

Table 46. (continued)

Standard Curve Number	Area of lysis ($mm.^2$) with varying concentrations of standard urokinase				
	100%	50%	25%	12.5%	6.25%
61	361	296	169	110	90
62	522	320	240	165	99
63	529	324	247	169	96
64	575	361	270	182	108
65	588	323	272	180	108
66	575	378	272	180	110
67	600	380	288	192	120
68	361	256	169	120	96
69	552	380	252	170	104
70	575	360	266	192	108
71	552	336	247	169	100
72	400	289	210	132	96
73	576	357	256	176	108
74	575	380	285	182	100
75	420	304	210	144	99
76	600	399	285	196	117
77	440	304	225	156	96
78	588	336	255	169	100
79	600	380	288	196	120
80	576	378	270	182	110
81	484	323	225	156	99
82	609	380	266	176	117
83	552	360	256	169	100
84	600	400	285	195	121
85	650	400	323	225	132
86	621	380	285	192	120
87	552	360	252	180	100
88	600	400	289	196	117
89	621	399	289	196	121
90	621	396	272	195	110

Table 46. (continued)

Standard Curve Number	Area of lysis (mm^2) with varying concentrations of standard urokinase				
	100%	50%	25%	12.5%	6.25%
91	576	378	272	182	110
92	625	399	288	196	120
93	725	483	306	225	132
94	609	399	266	196	108
95	575	360	266	176	108
96	600	400	289	182	121
97	675	440	306	196	121
98	615	391	272	176	117
99	529	324	234	156	99
100	529	340	240	168	99
101	552	324	255	169	110
102	696	440	306	196	121
103	576	360	272	182	110
104	575	360	266	180	108
105	625	400	289	196	121
106	624	361	288	196	117
107	575	361	256	169	110
108	624	361	285	195	120
109	496	324	225	144	90
110	552	340	255	176	104
111	486	289	240	144	88
112	644	400	289	196	121
113	575	378	270	186	110
114	648	400	289	196	121
115	588	361	272	182	110
116	550	340	256	180	104
117	625	399	234	196	120
118	576	361	272	180	110
119	560	340	270	176	108
120	525	324	234	169	108

Table 46. (continued)

Standard Curve Number	Area of lysis ($mm.^2$) with varying concentrations of standard urokinase				
	100%	50%	25%	12.5%	6.25%
121	500	323	234	156	96
122	552	360	256	176	108
123	480	323	225	156	96
124	672	440	289	195	130
125	598	400	288	196	110
126	552	340	252	168	104
127	399	275	196	132	81
128	400	256	210	132	88
129	644	432	289	196	121
130	675	450	320	240	143
131	483	315	196	143	88
132	483	306	210	121	90
133	696	483	315	247	144
134	672	441	324	210	156
135	644	432	320	225	154
136	450	320	182	132	91
137	437	304	180	120	88
138	432	289	169	121	84
139	391	270	176	117	81
Mean $\pm$ S.D.	566 ± 87	364 ± 46	262 ± 33	169 ± 26	110 ± 17

Shown are the standard urokinase dilution curves run in the fibrin plate test throughout the study. The means and standard deviations are shown graphically in figure 17.

Table 47. Standard curve for assay of haemoglobin in urine.

Volume of whole blood added (ul.)	Haemoglobin Concentration (mg./ml.)	Optical Density	
		Urine 1	Urine 2
10	0.1	0.061	0.063
20	0.2	0.112	0.132
30	0.3	0.192	0.175
40	0.4	0.247	0.259
50	0.5	0.297	0.309
60	0.6	0.388	0.368
70	0.7	0.446	0.426
80	0.8	0.510	0.487
90	0.9	0.560	0.555
100	1.0	0.610	0.630
110	1.1	0.686	0.680
120	1.2	0.755	0.741
140	1.4	0.873	0.859
160	1.6	0.987	1.00

Relationship between urine haemoglobin concentration and optical density at 540 mu after addition of Drabkin's solution, using pooled normal urine as blank. The results shown were obtained after the addition of varying amounts of whole blood, haemoglobin concentration 10 gm./100 ml., to a concentrated urine (urine 1, specific gravity 1028) and a dilute urine (urine 2, specific gravity 1010). The urine specimens were prepared as described in chapter 10. The results are displayed graphically in figure 19.

Table 48. Transurethral prostatectomy series, control patients: urine volumes.

Patient Number	Urine Volumes (ml./24 hours)					
	Day of Observation.					
	Preop.	1	2	3	4	5
1	2170	1160	2500	1540	-	-
3	1610	1170	800	2320	3300	2050
4	1890	1950	1050	2200	2360	2170
5	2260	1710	2190	1100	1950	1170
7	1310	2150	1210	1980	1610	1770
8	1910	1700	1740	2420	1140	2130
9	1340	1690	3610	4400	3400	2200
11	1780	4880	1390	2900	3200	2750
16	1420	3620	2320	1810	1720	1990
17	-	2160	2100	1690	1970	1710
18	2250	1930	1620	1440	1560	1370
19	1860	4400	2120	3810	2130	1610
24	2210	3460	2210	1670	1920	1860
26	1670	1010	1260	1410	920	-
27	1860	1410	2390	3450	2820	1440

Shown are daily volumes of urine and irrigation fluid for each patient.

Table 49. Transurethral prostatectomy series, EACA treated patients: urine volumes.

Patient Number	Urine volumes (ml./24 hours)					
	Day of Observation					
	Preop.	1	2	3	4	5
2	2750	2130	1730	1240	1860	2120
6	1720	4890	5300	3600	2900	2480
10	1800	1760	1800	2540	1380	-
12	2330	1050	2150	3260	2210	1930
13	1960	715	3550	3700	1950	2960
14	1560	780	620	960	1120	800
15	1280	1140	640	1330	1930	2210
20	1870	2810	3220	2850	2160	2310
21	1100	6210	2900	3100	2670	1110
22	2890	1910	6220	1930	2270	3640
23	-	1220	1790	1200	1390	1560
25	1540	1970	2290	1970	1880	1760
28	1360	2270	2010	1660	1310	1550

Shown are daily volumes of urine (and irrigation fluid) for each patient.

Table 50. Transurethral prostatectomy series, control patients: areas of lysis in fibrin-plate tests.

Patient Number.	Area of lysis (mm^2) in fibrin plate test					
	Day of observation					
	Preop.	1	2	3	4	5
1	165 (1)	251 (2)	144 (3)	196 (4)	-	-
3	165 (4)	196 (5)	252 (6)	132 (7)	108 (8)	176 (9)
4	252 (7)	240 (8)	336 (9)	247 (10)	225 (11)	225 (12)
5	285 (11)	336 (12)	272 (13)	360 (14)	255 (15)	336 (16)
7	396 (21)	357 (22)	437 (23)	350 (24)	376 (25)	345 (26)
8	266 (21)	285 (22)	285 (23)	234 (24)	324 (25)	272 (26)
9	357 (22)	324 (23)	182 (24)	154 (25)	216 (26)	256 (27)
11	210 (33)	130 (34)	294 (35)	156 (36)	182 (37)	192 (38)
16	225 (58)	154 (59)	204 (60)	165 (61)	225 (62)	208 (63)
17	-	210 (65)	266 (66)	234 (67)	156 (68)	234 (69)
18	299 (70)	272 (71)	306 (72)	336 (73)	380 (74)	304 (75)
19	210 (77)	117 (78)	216 (79)	130 (80)	176 (81)	252 (82)
24	225 (104)	204 (105)	251 (106)	272 (107)	256 (108)	196 (109)
26	294 (114)	361 (115)	340 (116)	289 (117)	385 (118)	-
27	270 (116)	336 (117)	247 (118)	210 (119)	204 (120)	289 (121)

Shown for each patient are areas of lysis in the fibrin plate test for urokinase concentration in successive 24 hour urine collections. The reference number of the standard urokinase dilution curve (from table 46) run with each assay is also shown in brackets.

Table 51. Transurethral prostatectomy series, control patients: areas of lysis in fibrin plate tests (dialysed urine samples).

Patient Number	Area of lysis (mm^2) in fibrin plate test					
	Day of observation					
	Preop.	1	2	3	4	5
9	270 (23)	240 (24)	154 (25)	143 (26)	144 (27)	204 (28)
11	196 (34)	108 (35)	210 (36)	132 (37)	156 (35)	182 (39)
16	196 (59)	130 (60)	90 (61)	182 (62)	204 (63)	182 (64)
17	-	176 (66)	196 (67)	108 (68)	156 (69)	225 (70)
18	234 (71)	182 (72)	304 (73)	315 (74)	266 (75)	294 (76)
19	165 (78)	91 (79)	165 (80)	77 (81)	180 (82)	196 (83)
24	204 (105)	117 (106)	182 (107)	247 (108)	132 (109)	180 (110)
26	256 (115)	252 (116)	299 (117)	234 (118)	256 (119)	-
27	216 (117)	247 (118)	132 (119)	144 (120)	117 (121)	247 (122)

Shown for each patient are areas of lysis in the fibrin plate test for urokinase concentration in successive 24 hour urine collections; urine samples were dialysed before assay. The reference number of the standard urokinase dilution curve (from table 46) run with each assay is also shown in brackets.

Table 52. Transurethral prostatectomy series, EACA treated patients: areas of lysis in fibrin plate tests.

Patient Number	Area of lysis (mm^2) in fibrin plate test					
	Day of observation					
	Preop.	1	2	3	4	5
2	234 (3)	0 (4)	154 (5)	304 (6)	324 (7)	225 (8)
6	240 (14)	0 (15)	42 (16)	117 (17)	192 (18)	210 (19)
10	336 (27)	0 (28)	182 (29)	285 (30)	462 (31)	-
12	210 (37)	0 (38)	0 (39)	110 (40)	234 (41)	252 (42)
13	266 (42)	0 (43)	0 (44)	88 (45)	336 (46)	225 (47)
14	294 (45)	0 (46)	294 (47)	285 (48)	320 (49)	484 (50)
15	285 (52)	0 (53)	256 (54)	285 (55)	270 (56)	144 (57)
20	304 (79)	0 (80)	104 (81)	216 (82)	285 (83)	304 (84)
21	294 (86)	0 (87)	88 (88)	182 (89)	210 (90)	330 (91)
22	196 (91)	0 (92)	49 (93)	225 (94)	252 (95)	182 (96)
23	-	0 (98)	104 (99)	272 (100)	285 (101)	336 (102)
25	294 (107)	0 (108)	0 (109)	90 (110)	121 (111)	320 (112)
28	266 (123)	0 (124)	0 (125)	225 (126)	234 (127)	210 (128)

Shown for each patient are areas of lysis in the fibrin plate test for urokinase concentration in successive 24 hour urine collections. The reference number of the urokinase dilution curve (from table 46) run with each assay is also shown in brackets.

Table 53. Transurethral prostatectomy series, EACA treated patients: areas of lysis in fibrin plate tests (dialysed urine samples).

Patient Number	Area of lysis (mm^2) in fibrin plate test					
	Day of observation					
	Preop.	1	2	3	4	5
2	156 (4)	192 (5)	225 (6)	225 (7)	210 (8)	196 (9)
6	180 (15)	70 (16)	80 (17)	132 (18)	165 (19)	180 (20)
10	256 (28)	240 (29)	294 (30)	240 (31)	324 (32)	-
12	165 (38)	294 (39)	154 (40)	144 (41)	196 (42)	196 (43)
13	210 (43)	272 (44)	130 (45)	154 (46)	247 (47)	144 (48)
14	169 (46)	306 (47)	266 (48)	204 (49)	285 (50)	396 (51)
15	272 (53)	210 (54)	315 (55)	225 (56)	121 (57)	156 (58)
20	255 (80)	154 (81)	165 (82)	156 (83)	252 (84)	285 (85)
21	225 (87)	56 (88)	154 (89)	117 (90)	144 (91)	272 (92)
22	176 (92)	234 (93)	56 (94)	144 (95)	196 (96)	144 (97)
23	-	176 (99)	165 (100)	247 (101)	266 (102)	266 (103)
25	252 (108)	144 (109)	182 (110)	132 (111)	225 (112)	234 (113)
28	272 (124)	195 (125)	196 (126)	176 (127)	196 (128)	247 (129)

Shown for each patient are areas of lysis in the fibrin plate test for urokinase concentration in successive 24 hour urine collections. Urine samples were dialysed before assay. The reference number of the standard urokinase dilution curve (from table 46) run with each assay is also shown in brackets.

Table 54. Transurethral prostatectomy series, control patients: urokinase concentrations.

Patient Number.	Urokinase concentration (units/ml.) Day of observation					
	Preop.	1	2	3	4	5
1	12.1	22.1	9.2	18.6	-	-
3	13.6	14.7	24.2	6.7	7.0	11.6
4	21.3	25.6	37.9	18.3	18.8	20.5
5	31.9	45.7	30.1	49.0	21.8	44.1
7	54.6	41.2	59.2	48.4	44.6	37.6
8	24.2	28.0	28.0	21.7	35.2	21.1
9	41.2	34.8	12.8	9.3	13.1	20.8
11	19.8	6.0	27.8	10.8	13.3	12.1
16	20.6	10.0	12.8	23.7	21.7	18.6
17	-	16.6	22.5	16.3	21.3	21.5
18	31.2	32.7	58.4	44.6	50.0	50.5
19	21.0	7.9	15.2	7.9	15.7	22.7
24	19.1	12.9	19.6	28.6	20.6	20.0
26	26.0	45.5	49.0	28.1	51.5	-
27	28.0	36.8	20.3	17.1	19.3	39.5

Shown for each patient are urokinase concentrations in successive 24 hour urine collections. (Calculated from table 50).

Table 55. Transurethral prostatectomy series, control patients: urokinase concentrations in dialysed urine samples.

Patient Number	Urokinase concentration (units/ml.)					
	Day of observation					
	Preop.	1	2	3	4	5
9	24.7	20.4	9.3	7.0	8.6	15.6
11	13.0	3.5	18.4	7.4	8.2	10.9
16	15.2	6.9	8.3	15.3	16.7	12.5
17	-	11.8	13.0	9.7	11.2	16.9
18	22.0	21.2	35.8	30.1	37.8	27.4
19	12.0	4.5	10.8	5.0	12.8	16.3
24	13.1	6.3	14.7	19.6	11.1	12.7
26	23.0	24.4	29.7	19.2	23.4	-
27	16.8	20.3	8.8	9.7	13.3	23.9

Shown for each patient are urokinase concentrations in successive 24 hour urine collections; urine samples were dialysed before assay. (Calculated from table 51).

Table 56. Transurethral prostatectomy series, EACA treated patients: urokinase concentrations.

Patient Number	Urokinase concentration (units/ml.)					
	Day of observation					
	Preop.	1	2	3	4	5
2	19.1	0	10.1	36.2	35.0	22.3
6	20.0	0	2.0	8.0	15.3	16.3
10	37.5	0	15.4	24.5	59.5	-
12	17.1	0	0	5.5	20.9	20.2
13	21.6	0	0	4.2	26.5	16.2
14	26.5	0	25.4	37.4	47.0	70.5
15	32.9	0	28.5	31.4	29.2	22.2
20	28.5	0	6.7	17.0	31.1	27.7
21	27.4	0	4.4	10.9	14.0	39.2
22	13.9	0	2.4	16.1	21.9	11.3
23	-	0	6.7	32.6	30.8	29.4
25	32.4	0	0	5.2	10.1	31.8
28	34.7	0	0	20.2	35.7	26.4

Shown for each patient is urokinase concentration in successive 24 hour urine collections (Calculated from table 52).

Table 57. Transurethral prostatectomy series, EACA treated patients: urokinase concentrations in dialysed urine samples.

Patient Number	Urokinase concentration (units/ml.)					
	Day of observation					
	Preop.	1	2	3	4	5
2	12.9	13.7	19.2	24.2	21.2	14.8
6	12.2	3.7	5.1	8.2	12.0	11.2
10	26.1	24.0	25.9	15.4	40.0	-
12	9.7	25.5	8.7	8.7	12.6	11.7
13	12.9	27.6	6.6	6.5	18.6	11.2
14	15.3	29.4	33.2	19.6	24.6	49.4
15	21.8	19.0	38.2	20.0	16.5	11.8
20	22.3	13.1	10.6	11.5	19.8	19.5
21	20.0	3.6	8.4	6.5	8.8	22.9
22	10.4	12.9	3.4	9.1	12.2	7.2
23	-	15.9	13.5	23.1	20.0	23.7
25	20.2	12.5	13.1	11.8	15.8	19.2
28	22.9	12.4	16.8	20.9	23.6	19.3

Shown for each patient is urokinase concentration in successive 24 hour urine collections; urine samples were dialysed before assay. (Calculated from table 53).

Table 58. Transurethral prostatectomy series, control patients: daily urokinase activity.

Patient Number	Daily urokinase activity (units x 10^3)					
	Day of observation					
	Preop.	1	2	3	4	5
1	26.26	25.64	23.00	28.64	-	-
3	21.90	17.20	19.36	15.54	23.10	23.78
4	40.26	49.92	39.80	40.26	44.37	44.49
5	72.09	78.15	65.92	53.90	42.51	51.60
7	71.53	88.50	71.63	95.83	71.81	66.55
8	46.22	47.60	48.72	52.51	40.13	44.94
9	55.21	58.81	46.21	40.92	44.54	45.76
11	35.24	29.28	38.64	31.32	42.56	33.28
16	29.25	36.20	29.70	42.90	37.32	37.01
17	-	35.86	47.25	27.55	41.96	36.77
18	70.20	63.11	94.61	64.22	78.00	69.19
19	39.06	34.76	32.22	30.10	33.44	36.55
24	42.21	44.63	43.32	47.76	39.55	37.20
26	43.42	45.96	61.74	39.62	47.38	-
27	52.08	51.89	48.52	59.00	54.43	56.88
Mean	46.1	47.2	47.4	44.7	45.8	44.9
S.D.	±16.4	±19.2	±19.7	±19.3	±14.3	±13.2

Shown for each patient are urokinase activities in successive 24 hour urine collections. (Data calculated from tables 48 and 54). Mean values and standard deviations are also shown.

Table 59. Transurethral prostatectomy series, control patients: daily urokinase activity after dialysis.

Patient Number	Daily urokinase activity (units x 10^3)					
	Day of observation					
	Preop.	1	2	3	4	5
9	33.10	34.48	29.39	30.80	29.24	34.32
11	23.14	17.08	25.58	21.46	26.24	30.00
16	21.50	24.98	19.26	27.69	28.72	24.88
17	-	25.49	27.30	16.39	22.06	28.90
18	49.50	40.92	57.97	43.34	58.97	37.54
19	22.32	19.80	22.90	19.05	27.26	26.24
24	28.95	21.80	32.49	32.73	21.31	23.62
26	38.41	24.64	37.42	27.07	21.53	-
27	31.25	28.62	21.03	33.47	37.51	34.42
Mean	31.0	26.4	30.4	28.0	30.3	30.0
S.D.	±9.5	±7.4	±11.8	±8.3	±11.9	±3.3

Shown for each patient are urokinase activities in successive 24 hour urine collections; urine samples were dialysed before assay. (Data calculated from tables 48 and 55). Mean values and standard deviations are also shown.

Table 60. Transurethral prostatectomy series, EACA treated patients: daily urokinase activity.

Patient Number	Daily Urokinase activity (units x 10^3)					
	Day of observation					
	Preop.	1	2	3	4	5
2	52.53	0	17.47	44.89	65.10	47.28
6	34.40	0	10.60	28.80	44.37	40.42
10	67.50	0	27.72	62.23	81.95	-
12	39.84	0	0	17.93	46.19	38.99
13	42.34	0	0	15.54	51.68	47.95
14	41.34	0	15.75	35.90	52.64	56.40
15	42.11	0	18.24	41.76	56.36	49.06
20	53.30	0	21.57	48.45	67.17	63.99
21	30.14	0	12.82	33.79	37.38	43.51
22	40.17	0	14.93	31.07	49.71	41.13
23	-	0	12.00	39.12	42.81	45.86
25	49.90	0	0	10.24	18.99	55.97
28	47.15	0	0	33.53	46.77	40.92
Mean	45.1	0	11.6	34.1	50.9	47.6
S.D.	9.9	0	9.2	14.2	15.3	7.6

Shown for each patient are urokinase activities in successive 24 hour urine collections. (Data calculated from tables 49 and 56.) Mean values and standard deviations are also shown.

Table 61. Transurethral prostatectomy series, EACA treated patients: daily urokinase activity after dialysis.

Patient Number	Daily urokinase activity (units x 10^3)					
	Day of observation					
	Preop.	1	2	3	4	5
2	35.48	29.18	33.22	30.01	39.43	31.38
6	20.98	18.10	27.03	29.52	34.80	27.78
10	46.98	42.24	46.62	39.12	55.20	-
12	22.60	26.78	18.71	20.36	27.85	22.58
13	25.28	19.78	23.43	24.05	36.27	33.15
14	23.87	22.93	20.58	18.82	27.55	39.52
15	27.90	21.66	24.40	26.60	31.85	26.08
20	41.70	36.81	34.13	32.78	42.77	45.05
21	22.00	22.36	24.36	20.15	23.50	25.42
22	30.06	24.64	21.15	17.56	27.69	26.21
23	-	19.32	24.12	27.72	27.80	36.97
25	31.11	24.63	30.00	23.25	29.70	33.79
28	31.14	28.15	33.77	34.69	30.92	29.92
Mean	29.9	25.9	27.8	27.1	33.5	31.5
S.D.	$\pm$8.1	$\pm$7.0	$\pm$7.7	$\pm$6.3	$\pm$8.5	$\pm$6.6

Shown for each patient are urokinase activities in successive 24 hour urine collections; urine samples were dialysed before assay. (Data calculated from tables 49 and 87). Mean values and standard deviations are also shown.

Table 62. Transurethral prostatectomy series, control patients: urinary haemoglobin concentration.

Patient Number	Urinary haemoglobin concentration (mg./ml.) Day of Observation				
	1	2	3	4	5
1	2.600 (a)	0.251	0.300	-	-
3	5.856 (c)	4.304 (b)	0.446	0.177	0.280
4	1.050	0.460	0	0	0
5	1.932 (a)	0.885	0.450	0.134	0.126
7	0.614	1.980	0.708	0.335	0.041
8	1.800 (a)	0.552	0.129	0	0.084
9	1.420 (a)	0.403	0.100	0	0
11	3.388 (b)	1.401	0.500	0.395	0.369
16	1.830 (a)	0.868	0.807	0.517	0.126
17	3.180 (b)	0.500	0.645	0.288	0.036
18	1.098	0.183	0.037	0	0
19	3.692 (b)	11.16 (d)	1.396	0.126	0.047
24	0.565	0.282	0	0	0
26	1.834 (a)	0.629	0.086	0.305	-
27	4.000 (b)	0.531	0.157	0.199	0.050

Shown is daily urinary haemoglobin concentration.

(a) Diluted 1 : 1 before assay
(b) Diluted 1 : 3 " "
(c) Diluted 1 : 7 " "
(d) Diluted 1 : 9 " "

Table 63. Transurethral prostatectomy series, EACA treated patients: urinary haemoglobin concentration.

Patient Number	Urinary haemoglobin concentration (mg./ml.) Day of Observation				
	1	2	3	4	5
2	0.812	0.449	0.413	0.181	0.211
6	0.285	0.249	0.230	0	0
10	1.199	0.510	0.060	0.039	-
12	1.306	1.802 (a)	0	0	0
13	2.682 (a)	0	0	0	0
14	0.881	0.057	0.627	0.377	0.031
15	0.413	0.039	0	0	0
20	0.225	0	0	0	0
21	0.580	0.079	0.102	0.032	0.037
22	0.458	0.060	0.808	0.030	0
23	0.185	0.050	0	0	0.154
25	0.512	0.032	0	0	0
28	0.570	0.228	0.133	0	0

Shown is daily urinary haemoglobin concentration.

(a) Diluted 1 : 1 before assay.

Table 64. Transurethral prostatectomy series, control patients: post-operative urinary haemoglobin loss.

Patient Number	Blood loss (gm. haemoglobin) Day of Observation 1	2	3	4	5	Total, 5 Day Period
1	3.016	0.628	0.462	-	-	-
3	6.852	3.443	1.035	0.584	0.574	12.448
4	2.048	0.483	0	0	0	2.531
5	3.304	1.938	0.495	0.261	0.147	6.145
7	1.320	2.396	1.402	0.539	0.073	5.730
8	3.060	0.961	0.312	0	0.179	4.512
9	2.400	1.455	0.440	0	0	4.295
11	16.533	1.947	1.450	1.264	1.015	22.209
16	6.625	2.014	1.461	0.889	0.251	11.24
17	6.869	1.050	1.090	0.567	0.062	9.638
18	2.190	0.297	0.053	0	0	2.54
19	16.244	23.663	5.320	0.268	0.076	45.571
24	1.955	0.623	0	0	0	2.578
26	1.833	0.793	0.121	0.281	-	-
27	5.640	1.269	0.542	0.561	0.072	8.084
Mean	5.330	2.860	0.945	0.372	0.188	10.581
S.D.	±4.890	±5.82	±1.320	±0.386	±0.294	±11.85

Shown is daily urinary haemoglobin loss and total loss for each patient for the 5 day period of observation. Mean values and standard deviations are also shown. (Compiled from tables 48 and 62).

Table 65. Transurethral prostatectomy series, EACA treated patients: post-operative urinary haemoglobin loss.

Patient Number	Blood loss (gm. haemoglobin) Day of Observation 1	2	3	4	5	Total, 5 day period
2	1.730	0.777	0.512	0.337	0.447	3.803
6	1.394	1.319	0.828	0	0	3.541
10	2.110	0.272	0.152	0.054	-	-
12	1.371	3.874	0	0	0	5.245
13	1.918	0	0	0	0	1.918
14	0.687	0.035	0.602	0.422	0.025	1.771
15	0.471	0.025	0	0	0	0.496
20	0.632	0	0	0	0	0.632
21	3.602	0.229	0.316	0.085	0.041	4.273
22	0.875	0.373	0.156	0.068	0	1.472
23	0.226	0.090	0	0	0.240	0.556
25	1.009	0.073	0	0	0	1.082
28	1.294	0.458	0.221	0	0	1.973
Mean	1.332	0.579	0.214	0.074	0.063	2.230
S.D.	±0.888	±1.059	±0.275	±0.140	±0.139	±1.598

Shown is daily urinary haemoglobin loss and total loss for each patient for the 5 day period of observation. Mean values and standard deviation are also shown. (Compiled from tables 49 and 63).

Table 66. Transurethral prostatectomy series, EACA treated patients: duplicate assays for urinary EACA concentration.

Patient Number	Urinary EACA concentrations (mg./ml.) (duplicate assays)							
	Day of Observation							
	1		2		3		4	
2	2.70,	2.14	0.724,	0.620	0.086,	0.054	0.015,	0.009
6	1.10,	0.790	0.443,	0.359	0.002,	0.008	0	0
10	3.60,	3.07	1.01,	0.611	0.090,	0.067	0	0
12	4.82,	4.39	0.480,	0.443	0.187,	0.186	0	0
13	7.29,	5.29	0.402,	0.378	0.130,	0.139	0	0
14	8.39,	6.17	2.10,	2.91	0	0	0	0
15	5.15,	3.75	2.71,	1.85	0.026,	0.020	0	0
20	2.25,	1.79	0.501,	0.319	0.016,	0.006	0	0
21	0.980,	0.695	0.523,	0.525	0	0	0	0
22	2.39,	2.15	0.320,	0.282	0.024,	0.017	0	0
23	4.44,	3.68	0.281,	0.219	0.087,	0.062	0	0
25	1.58,	1.18	0.913,	0.591	0.564,	0.442	0.299,	0.256
28	2.41,	1.88	0.590,	0.370	0.290,	0.270	0.045,	0.031

Shown are duplicate assay values for EACA in urine on successive days after operation. Mean values calculated from these duplicate assays are shown in table 67.

Table 67. Transurethral prostatectomy series, EACA treated patients: urinary EACA concentrations.

Patient Number	Mean urinary EACA concentration (mg./ml.)			
	Day of observation			
	1	2	3	4
2	2.42	0.672	0.070	0.012
6	0.945	0.401	0.055	0
10	3.34	0.810	0.079	0
12	4.61	0.462	0.187	0
13	6.29	0.390	0.135	0
14	7.28	2.51	0	0
15	4.45	2.28	0.023	0
20	2.02	0.410	0.011	0
21	0.842	0.524	0	0
22	2.26	0.301	0.021	0
23	4.06	0.250	0.075	0
25	1.38	0.752	0.493	0.278
28	2.15	0.480	0.280	0.038
Mean	3.2	0.79	0.11	0.025
S.D.	±2.0	±0.73	± .14	-

Shown are mean urinary EACA concentrations for successive days after surgery calculated from the duplicate assay values of table 66.

Table 68. Transurethral prostatectomy series. EACA treated patients: urinary EACA molarities.

Patient Number	Urinary EACA concentration (Molar) Day of observation			
	1	2	3	4
2	1.8×10^{-2}	5.1×10^{-3}	5.3×10^{-4}	0.9×10^{-4}
6	7.2×10^{-3}	3.1×10^{-3}	4.2×10^{-4}	0
10	2.5×10^{-2}	6.2×10^{-3}	6.0×10^{-4}	0
12	3.5×10^{-2}	3.5×10^{-3}	1.4×10^{-3}	0
13	4.8×10^{-2}	3.0×10^{-3}	1.0×10^{-3}	0
14	5.6×10^{-2}	1.9×10^{-2}	0	0
15	3.4×10^{-2}	1.7×10^{-2}	1.8×10^{-4}	0
20	1.6×10^{-2}	3.1×10^{-3}	0.8×10^{-4}	0
21	6.4×10^{-3}	4.0×10^{-3}	0.5×10^{-4}	0
22	1.7×10^{-2}	2.3×10^{-3}	1.6×10^{-4}	0
23	3.1×10^{-2}	1.9×10^{-3}	5.7×10^{-4}	0
25	1.1×10^{-2}	5.7×10^{-3}	3.8×10^{-3}	2.1×10^{-3}
28	1.6×10^{-2}	3.7×10^{-3}	2.1×10^{-3}	2.9×10^{-4}
Mean $\pm$ S.D.	$2.5 \pm 1.5 \times 10^{-2}$	$6.0 \pm 5.5 \times 10^{-3}$	$8.4 \pm 10.8 \times 10^{-4}$	1.9×10^{-4}

Shown are urinary EACA molarities, calculated from table 67, for each of the first 4 days after operation. Mean values and standard deviations are also shown.

Table 69. Transurethral prostatectomy series, EACA treated patients: urinary EACA content.

Patient Number	Urinary EACA content (gm.) Day of observation 1	2	3	4	Total, Days 1-4
2	5.155	1.163	0.087	0.022	6.427
6	4.621	2.125	0.198	0	6.944
10	5.878	1.458	0.201	0	7.537
12	4.841	0.993	0.610	0	6.444
13	4.497	1.385	0.500	0	6.382
14	5.676	1.556	0	0	7.232
15	5.073	1.459	0.031	0	6.563
20	5.732	1.320	0.031	0	7.083
21	5.227	1.519	0	0	6.746
22	4.317	1.872	0.041	0	6.230
23	4.960	0.448	0.090	0	5.498
25	2.719	1.722	0.971	0.523	5.935
28	4.881	0.965	0.465	0.050	6.361
Mean	4.889	1.377	0.248	0.046	6.567
S.D.	0.809	0.456	±0.300	-	±0.553

Shown is urinary EACA content for each of the first 4 days after operation, and the total amount of EACA recovered in the urine over this 4 day period. Mean values and standard deviations are also shown. (Calculated from tables 49 and 67).

Table 70. Transurethral prostatectomy series, EACA treated patients: cumulative recovery of EACA in urine.

Patient Number	Cumulative percentage recovery of EACA in urine. Day of observation.			
	1	2	3	4
2	64.4	79.0	80.1	80.3
6	57.8	84.3	86.8	86.8
10	73.5	91.7	94.2	94.2
12	60.5	72.9	80.6	80.6
13	56.2	73.5	79.8	79.8
14	71.0	90.4	90.4	90.4
15	63.4	81.7	82.0	82.0
20	71.7	88.2	88.5	88.5
21	65.3	84.3	84.3	84.3
22	54.0	77.4	77.9	77.9
23	62.0	67.6	68.7	68.7
25	34.0	55.5	67.7	74.2
28	61.0	73.1	78.9	79.4
Mean	61.1	78.4	81.5	82.1
S.D.	±10.1	±10.1	± 7.7	±6.8

Shown for each patient is cumulative percentage recovery of the 8 gm. dose on successive days after operation. Mean values and standard deviations are also shown.

Table 71. Transurethral prostatectomy series, EACA treated patients: inhibition of urokinase activity calculated from pre-operative levels.

Patient Number	Inhibition of urokinase activity (units/ml.) calculated from pre-operative levels			
	Day of observation			
	1	2	3	4
2	24.7 +	20.3	6.2	0
6	7.0 +	4.5	1.6	0
10	38.4 +	22.1	2.1	0
12	37.9 +	18.5 +	6.7	0
13	59.2 +	11.9 +	7.2	0
14	53.0 +	41.3	5.7	0
15	36.9 +	37.3	0.3	0
20	19.0 +	9.9	1.7	0
21	4.9 +	6.0	0	0
22	21.0 +	4.1	4.7	0
23 ✱	37.6 +	18.9	5.6	2.2
25	25.3 +	21.8 +	20.1	16.4
28	20.8 +	23.5 +	8.2	2.9
Mean	29.7	18.5	5.4	1.7
S.D.	±16.1	±11.6	±5.2	-

Shown is daily inhibition of urokinase activity, on successive days after operation, calculated from pre-operative urokinase levels.

✱ Calculated from urokinase activity on the 5th post-operative day.

+ Urokinase activity completely inhibited.

Table 72. Transurethral prostatectomy series, EACA treated patients: inhibition of urokinase activity calculated from activity after dialysis.

Patient Number	Inhibition of urokinase activity (units/ml.) calculated from activity after dialysis. Day of observation.			
	1	2	3	4
2	21.1 +	19.5	1.0	0
6	5.7 +	5.8	0	0
10	36.9 +	24.4	0	0
12	39.2 +	13.9 +	7.9	0
13	42.4 +	10.2 +	5.8	0
14	45.2 +	25.7	0	0
15	29.2 +	10.0	0	0
20	20.2 +	9.6	0.7	0
21	5.5 +	8.5	0	0
22	19.9 +	2.8	0	0
23	24.4 +	14.0	2.9	0
25	19.2 +	20.2 +	13.0	14.2
28	19.1 +	25.8 +	12.0	0.6
Mean	25.2	14.6	3.3	1.1
S.D.	±12.8	±7.8	±4.8	-

Shown is daily inhibition of urokinase activity, on successive days after operation, calculated from activity seen after dialysis (mean loss of activity due to dialysis was 35 per cent; so inhibition of activity due to EACA = $\frac{35}{65}$ x activity after dialysis).

\+ Urokinase activity completely inhibited.

Table 73. Transurethral prostatectomy series, EACA treated patients: mean inhibition of urokinase activity calculated from preoperative levels and activity after dialysis.

Patient Number	Inhibition of urokinase activity (units/ml.) Mean values calculated from activity after dialysis and preoperative levels. Day of observation			
	1	2	3	4
2	22.9 +	19.9	3.6	0
6	6.4 +	5.2	0.8	0
10	37.7 +	23.3	1.1	0
12	38.6 +	16.2 +	7.3	0
13	50.8 +	11.1 +	6.5	0
14	49.1 +	33.5	2.9	0
15	33.1 +	23.7	0.2	0
20	19.6 +	9.8	1.2	0
21	5.2 +	7.2	0	0
22	20.5 +	3.5	2.4	0
23	31.0 +	16.5	4.3	1.1
25	22.3 +	21.0 +	16.6	15.3
28	20.0 +	24.7 +	10.1	1.8
Mean	27.5	16.6	4.4	1.4
S.D.	±14.3	±8.9	4.8	-

Shown is mean daily inhibition of urokinase activity on successive days after therapy calculated from preoperation levels (table 71) and activity after dialysis (table 72). The mean values in table 71 do not differ significantly from those in table 72; for days 1, 2 and 3, respective 't' values are 0.773, 0.984 and 1.047, and p values are all greater than 0.4.

\+ Urokinase activity completely inhibited.

Table 74. Effect of normal urine on the thrombin clotting time of plasma.

Subject Number	Clotting times (seconds) Before dialysis	After dialysis
1	16.0, 16.5	13.5, 13.5
2	17.0, 17.5	14.0, 14.5
3	14.5, 15.0	13.5, 14.0
4	30.5, 32.0	15.0, 15.5
5	26.0, 27.0	14.0, 14.5
6	18.0, 18.5	15.5, 15.5
7	14.0, 14.5	13.5, 13.5
8	15.0, 15.5	13.0, 13.5
9	21.0, 22.0	13.0, 13.0
10	17.5, 18.0	14.0, 14.5
11	19.0, 20.0	14.5, 15.5
12	16.0, 17.0	14.5, 14.5
13	18.5, 19.5	13.0, 13.5
14	21.0, 22.0	13.5, 13.5
15	22.0, 23.0	13.0, 14.0
Mean	19.47	14.02
Standard Deviation	$\pm$4.65	$\pm$0.756
Saline control	13.5, 14.0	14.0, 14.0

Effect of urine from normal subjects in increasing the thrombin clotting time of normal plasma. The clotting time, on addition of thrombin, of a standard plasma, mixed 1 : 1 with urine from each subject, is shown. Duplicate assays were made before and after dialysis of the urine samples. Mean values, standard deviations, and clotting times when saline was substituted for urine, are also shown.

Table 75. Transurethral prostatectomy series, control patients: effect of urines on the thrombin clotting time of plasma.

Patient Number	Clotting time (seconds) Before dialysis	After dialysis
1	19.0, 19.5	13.5, 14.0
3	18.0, 18.5	14.0, 14.5
4	16.5, 17.0	14.6, 14.5
5	18.0, 18.5	13.5, 14.0
7	17.0, 16.0	13.5, 13.5
8	17.5, 17.0	14.0, 14.0
9	14.0, 15.0	14.0, 14.5
11	14.0, 14.5	13.5, 14.5
16	13.0, 14.0	13.5, 14.0
17	14.5, 15.5	13.0, 14.0
18	15.0, 15.5	14.0, 14.5
19	15.0, 16.0	15.0, 15.0
24	14.0, 14.0	13.0, 13.5
26	22.0, 23.0	13.5, 14.5
27	14.0, 14.5	13.5, 13.5
Mean	16.33	13.93
S.D.	$\pm$2.45	$\pm$0.443
Saline control	13.5, 14.0	14.0, 14.0

Effect of urine (first post-operative day) in increasing the thrombin clotting time of normal plasma. The clotting time on addition of thrombin, of a standard plasma mixed 1 : 1 with urine from each patient is shown. Duplicate assays were made before and after dialysis of the urine samples. Mean values, standard deviations, and clotting times when saline was substituted for urine, are also shown.

Table 76. Transurethral prostatectomy series, EACA treated patients: effect of urine s on the thrombin clotting time of plasma.

Patient Number	Clotting time (seconds) Before dialysis	After dialysis
2	16.5, 17.0	13.5, 13.0
6	14.0, 14.5	14.0, 14.5
10	17.5, 18.5	13.0, 14.0
12	17.5, 18.5	13.0, 13.5
13	21.0, 22.0	14.0, 15.0
14	19.5, 20.0	15.0, 15.0
15	18.5, 19.0	13.5, 13.5
20	13.0, 14.5	14.5, 14.5
21	12.0, 13.0	14.0, 14.5
22	15.5, 16.0	14.0, 15.0
23	17.0, 18.0	14.0, 14.5
25	17.5, 18.0	13.0, 13.5
28	16.0, 17.0	14.5, 15.0
Mean	16.98	14.06
S.D.	±2.482	±0.625
Saline control	13.5, 14.0	13.5, 14.0

Effect of urine (first) post-operative day in increasing the thrombin clotting time of normal plasma. The clotting time, on addition of thrombin, of a standard plasma mixed 1 : 1 with urine from each patient is shown. Duplicate assays were made before and after dialysis of the urine samples. Mean values, standard deviations, and clotting times when saline was substituted for urine, are also shown.

Table 77. Transurethral prostatectomy series, control patients: plasminogen and fibrinogen levels and thrombin clotting times.

Patient Number	Plasminogen (units/ml.)	Fibrinogen (mg./100 ml.)	Thrombin Time (seconds)
1	2.4	245	10.0
3	2.5	297	10.5
4	2.6	310	9.5
5	2.5	262	11.0
7	2.7	316	10.0
8	2.4	293	11.5
9	2.6	275	9.5
11	2.3	258	10.0
16	2.2	220	12.0
17	2.9	318	11.0
18	2.7	302	9.0
19	2.8	424	10.5
24	2.2	215	11.0
26	2.3	362	11.5
27	2.5	398	10.0
Mean	2.5	299.7	10.5
S.D.	±0.2	±59.8	±0.9

Shown are plasminogen and fibrinogen levels, and thrombin clotting times, in plasma samples taken off 4 hours after operation.

Table 78. Transurethral prostatectomy series, EACA treated patients: plasminogen and fibrinogen levels, and thrombin clotting times.

Patient Number	Plasminogen (units/ml.)	Fibrinogen (mg./100 ml.)	Thrombin Time (seconds)
2	2.7	315	9.5
6	2.4	426	10.0
10	2.2	242	10.0
12	2.3	205	10.0
13	2.5	255	10.5
14	2.6	294	9.5
15	2.8	210	11.5
20	2.1	288	11.0
21	2.3	253	11.5
22	2.5	386	9.5
23	2.7	281	10.0
25	2.9	270	12.0
28	2.9	307	9.0
Mean	2.5	287.0	10.3
S.D.	±0.2	±63.0	± 0.9

Shown are plasminogen and fibrinogen levels, and thrombin clotting times, in plasma samples taken off 4 hours after operation.

Table 79. Suprapubic prostatectomy series: clinical data.

Patient Number	Age	Hb.* (gm/100 ml.)	BUN* (mg./100 ml.)	Blood Pressure* (mm.Hg.) Systolic	Diastolic	Wt. of Tissue removed* (gm.)
1	65	15.9	16	125	85	25
5	74	13.5	16	160	100	39
8	74	9.5	42	110	65	24
9	64	14.3	12	110	80	32
11	57	15.9	13	170	90	71
13	64	16.9	14	170	110	30
14	70	16.5	15	180	95	126
16	66	16.4	14	130	80	60
17	61	14.9	12	135	85	74
18	66	14.6	17	140	80	89
2	82	17.5	17	165	95	110
3	60	15.5	13	115	70	86
4	75	11.6	28	145	95	71
6	75	13.5	14	180	110	24
7	72	12.0	13	140	80	35
10	70	14.3	13	135	85	145
12	78	15.9	18	145	90	56
15	68	16.7	19	160	95	49
19	59	14.4	17	130	80	46

Shown are age, and pre-operative haemoglobin (Hb), blood urea nitrogen (BUN) and blood pressure levels. Weight of prostatic tissue removed is also shown for each patient.

* Measurements made by medical and laboratory staff of Barnes and Jewish Hospitals.

Table 80. Suprapubic prostatectomy series: urine volumes.

Patient Number	Volume (litres) Day after Operation 1	2	3	4	5
1	8.3	6.7	3.9	3.35	3.1
5	11.5	10.0	6.7	5.5	4.4
8	10.0	5.11	3.25	3.0	2.9
9	7.2	4.85	3.2	3.3	2.95
11	4.65	3.55	2.95	2.85	3.05
13	25.5	3.65	4.75	4.3	4.0
14	29.0	5.6	4.95	4.25	4.0
16	6.1	3.85	3.6	3.1	2.9
17	16.0	10.0	5.7	3.75	2.95
18	10.5	4.45	3.1	2.95	2.6
2	135.0	14.5	7.6	3.1	3.3
3	27.0	23.0	8.3	3.15	3.35
4	21.5	4.85	3.45	2.85	3.0
6	31.5	17.0	7.8	4.15	3.05
7	29.5	12.5	5.45	4.2	3.4
10	93.0	4.65	3.8	4.1	4.75
12	73.0	26.0	8.2	6.5	2.8
15	53.0	7.7	4.35	3.85	2.8
19	48.0	17.0	6.5	3.35	3.1

Combined volumes of drainage (per urethram and suprapubic) and eluate from dressings, for each day after operation.

Table 81. Suprapubic prostatectomy series: urinary haemoglobin concentration.

Patient Number	Concentration of haemoglobin (mg./ml.)				
	Day after Operation				
	1	2	3	4	5
1	1.061	1.047	0.799	0.572	0.410
5	1.150	0.863	0.625	0.711	0.458
8	0.970	0.794	1.120	1.071	0.766
9	0.753	0.982	0.629	0.343	0.301
11	0.690	0.591	0.552	0.424	0.280
13	1.040	0.428	0.603	0.290	0.133
14	1.236	1.076	1.179	1.050	0.489
16	0.745	0.800	0.778	0.407	0.290
17	0.888	0.881	0.645	0.504	0.381
18	0.787	0.737	0.625	0.565	0.423
2	0.708	0.637	0.711	0.823	0.758
3	0.782	0.823	0.497	0.940	0.322
4	0.781	0.478	0.557	0.463	0.292
6	0.778	0.541	0.680	0.685	0.220
7	0.633	1.700	0.296	0.209	0.804
10	0.714	0.428	1.315	1.132	0.570
12	0.552	0.178	0.152	0.146	0.245
15	0.891	0.676	0.718	0.758	0.774
19	0.753	0.742	0.689	0.588	0.342

Shown for each patient is daily concentration of haemoglobin in pooled drainage (per urethram and suprapubic) and eluate from dressings.

Table 82. Suprapubic prostatectomy series: post-operative urinary haemoglobin loss.

Patient Number	Blood loss (gm. haemoglobin)					
	Day					Total, Days 1-5
	1	2	3	4	5	
1	8.806	7.014	3.116	1.916	1.271	22.123
5	13.225	8.630	5.438	3.911	2.015	33.219
8	9.700	4.057	3.640	3.231	2.221	22.831
9	5.422	4.763	2.013	1.132	0.888	14.218
11	3.209	2.098	1.628	1.208	0.854	8.997
13	26.52	1.562	2.864	1.247	0.532	32.725
14	35.844	6.026	5.836	4.463	1.956	54.125
16	4.545	3.080	2.801	1.262	0.841	12.529
17	14.208	8.810	3.677	1.890	1.124	29.709
18	8.264	3.280	1.938	1.667	1.100	16.249
2	95.580	9.237	5.404	2.551	2.501	115.273
3	21.114	18.929	4.125	2.961	1.079	48.208
4	16.792	2.318	1.922	1.320	0.876	23.228
6	24.507	9.197	5.304	2.843	0.671	42.522
7	18.674	2.125	1.613	0.878	0.273	23.563
10	66.402	19.902	4.997	4.641	2.708	98.668
12	40.296	4.628	1.246	0.949	0.674	47.793
15	47.223	5.205	3.123	2.918	2.167	60.636
19	36.114	12.610	4.479	1.970	1.060	56.263

Shown for each patient is daily post-operative blood loss and total blood loss for the 5 day period of observation (Data calculated from tables 80 and 81). The results are also shown in figure 26.

Table 83. Control of urinary blood loss with EACA in a patient with blood loss after prostatectomy.

Day	Urine Volume (ml.)	Urinary Hb.Conc. (mg./ml.)	Rate of Hb. loss in urine (gm./24 hrs.)
1	9800	5.680 ✱	55.66
2	1920	0.156	0.299
3	1670	0.097	0.162
4	1320	0	0
5	1460	0	0
6	1800	0.077	0.138
7	1770	0.374	0.662
8	1680	0.071	0.119
9	6900	2.540 ✱	17.53
10	1730	0.948	1.640
11	1940	0.747	1.449
12	2110	0.502	1.059
13	1900	0.283	0.538
14	1360	0.382	0.520

Details of urinary blood loss in patient 1, chapter 12. The data are graphically displayed in figure 28.

✱ Diluted 1 : 9 before assay.

Table 84. Urinary haemoglobin loss in a patient with prolonged bleeding after prostatectomy, treated with EACA.

Day	Time Interval (hours	Urine Volume (ml.)	Urinary Hb.Conc. (mg./ml.)		Urinary Hb.Loss (gm.)	Rate of Urinary Hb.loss (gm./24hrs.)
3	24	13,300	7.74	(a)	102.9	102.9
4	17	1,460	1.397		2.04	2.88
4-5	23	6,400	6.16	(a)	39.42	41.04
5-6	20	4,700	2.84	(b)	13.35	16.03
6-7	24	3,670	0.955		3.504	3.50
7	12	910	0.604		0.550	1.10
8	24	1,580	0.758		1.200	1.20
9	24	1,600	0.657		1.051	1.05
10	24	1,650	0.697		1.150	1.15
11	24	2,420	2.440	(b)	5.905	5.91
12	12	2,930	4.780	(b)	14.01	28.02
	12	1,920	1.484	(c)	2.849	5.70
13	24	1,920	1.146		2.200	2.20
14	24	1,760	0.744		1.309	1.31
15	24	1,880	0.702		1.320	1.32
16	24	1,510	0.848		1.280	1.28
17	24	1,880	0.686		1.290	1.29
18	24	2,710	2.260	(c)	6.12	6.12
19	12	4,900	3.368	(b)	16.50	33.00
19-20	24	5,200	3.460	(b)	18.0	18.0
20-21	24	2,930	1.706	(c)	5.157	5.16
21-22	24	1,810	1.658	(c)	3.000	3.00
22-23	24	1,790	1.006		1.800	1.80
23	12	870	0.092		0.080	0.16
24	12	650	0.097		0.063	0.13
	12	680	0.266		0.181	0.36
25	12	520	0.823		0.428	0.86
	12	670	0.198		0.133	0.27
26	12	730	0.063		0.046	0.092
	12	610	0.071		0.043	0.086
27	24	1,660	0.055		0.091	0.182
28-45	-	-	0.030		-	-

Shown are urine volumes, and urine haemoglobin concentration and content for patient 2 (chapter 12) with prolonged haematuria after prostatectomy. The data are graphically displayed in figure 30. See also table 85.

(a) Diluted 1:9 before assay.
(b) Diluted 1:3 before assay.
(c) Diluted 1:1 before assay.

Table 85. EACA dosage in a patient with prolonged bleeding after prostatectomy.

Day	Time Interval (hours)	EACA Dosage (gm)	EACA Dosage rate (gm/24 hrs.)
3	24	Nil	Nil
4	16	12	17
4-5	24	30	30
5-6	24	15	15
6-7	24	12	12
7-8	24	6	6
8-9	24	6	6
9-10	24	3	3
10	8	1	3
11	24	Nil	Nil
12	12	Nil	Nil
12-13	20	16.5	20
13-14	28	7	6
14-15	36	4.5	3
16-17	36	3	2
17	12	Nil	Nil
18	24	Nil	Nil
19	12	Nil	Nil
19-20	24	16	16
20-21	24	12	12
21-22	24	6	6
22-23	24	4	4
23-24	24	3	3
24-25	24	2	2
25-26	36	4.5	3
27-34	-	-	3
35	12	1.5	3
35-38	72	6	2
38-39	36	1.5	1.0
39-44	-	-	0.5

Details of EACA dosage in patient 2 (chapter 12) with prolonged haematuria after prostatectomy. The data are displayed graphically in figure 30. See also table 84.

Table 86. Urinary haemoglobin loss and EACA dosage in a patient with prolonged bleeding after prostatectomy.

Day	Time Interval (hours)	Urine Volume (ml.)	Urinary Hb. conc. (mg./ml.)	Rate of Hb.loss in urine (gm./24hrs.)	EACA dosage rate (gm/24hrs.)
8	12	1530	0.462 *	4.474	-
8-9	24	1770	0.295	0.522	20
9-10	24	1920	0.089	0.171	-
10	12	660	0.058	0.076	-
11	24	2810	0.926	2.602	-
12	12	3090	0.858	5.302	-
	12	890	0.225	0.400	22
13	12	1850	0.087	0.161	6
	12				
14	12	670	0.150	0.200	6
	12	960	0.625	1.200	
15	12	720	0.229	0.330	15
	12	780	0.084	0.132	
16	24	1440	0.087	0.125	6
17	24	1700	0	0	6
18	9	1650	0	0	6
18-19	24	2710	0.720	1.951	17
19	15	1380	0.109	0.240	6
20	24	1390	0.130	0.181	6
21	24	1450	0.069	0.101	6
22	24	1720	0.058	0.100	6
23	24	1610	0.063	0.101	6
24	24	1800	0.056	0.101	6
25	24	1710	0	0	-

Details of urinary blood loss and EACA dosage in patient 3 (chapter 12), with prolonged bleeding after prostatectomy. The data are also shown in figure 31.

* Diluted 1:1 before assay.

Table 87. Urinary EACA content and urokinase activity in a patient with renal carcinoma and haematuria, treated with EACA.

Day of observation	EACA conc. (mg./ml.)	EACA conc. (Molarity)	EACA 24 hr. output (gm.)	Urokinase Zone of lysis (mm^2)	Urokinase conc. (Units/ml.)	Urokinase Activity per 24 hrs. (Units x 10^2)
1	-	-	-	272 (130*)	18.9	35.15
2	-	-	-	170 (131*)	18.3	38.98
3	-	-	-	156 (132*)	15.8	30.18
4	2.91	2.2×10^{-2}	4.802	0 (133*)	0	0
5	1.03	7.9×10^{-3}	1.936	0 (134*)	0	0
6	0.14	1.07×10^{-3}	0.281	176 (135*)	8.6	17.28
7	0.028	2.14×10^{-4}	0.055	130 (136*)	12.2	23.91
8	0	0	0	165 (137*)	21.5	32.04
9	-	-	-	132 (138*)	15.5	28.06
10	-	-	-	156 (139*)	20.3	35.93

Shown are EACA content and urokinase activity in patient 4 (chapter 12), with haematuria due to renal carcinoma. The data are also shown in figure 32.

* Standard urokinase dilution curve number (table 46).

Table 88. Urinary haemoglobin loss in a patient with renal carcinoma and haematuria, treated with EACA.

Day of observation	Urine Volume (ml.)	Haemoglobin conc. (mg./ml.)	Haemoglobin loss/ 24 hrs. (gm.)
1	1860	0.834	1.55
2	2130	0.620	1.32
3	1910	0.974	1.86
4	1650	0.164	0.27
5	1880	0.097	0.18
6	2010	0.269	0.54
7	1960	0.633	1.24
8	1490	1.174	1.75
9	1810	1.077	1.95
10	1770	0.932	1.65

Shown are urine volumes and urinary haemoglobin concentration and content in patient 4, (chapter 12), with haematuria due to renal carcinoma. The data are also shown in figure 32.

APPENDIX 4

STANDARD METHODS

APPENDIX 4

STANDARD METHODS

Thrombin clotting time

The method described is that of Fletcher et al. (1959).

To 0.1 ml. plasma is added 0.3 ml. of the "thrombin titration mixture" of Seegers and Smith (1942). This is made up with 6 ml. saline, 1 ml. tris buffer, 0.1 Molar, pH 7.5, 2 ml. 0.7 per cent calcium chloride and 2 ml. 15 per cent acacia solution. The clotting time of the plasma with "thrombin titration mixture" is estimated at 37°C after addition of 0.1 ml. thrombin, 10 N.I.H. units/ml.

Fletcher, A.P., Alkjaersig, N., Sherry, S. (1959), J.clin.Invest., 38, 1096.

Seegers, W.H., Smith, H.P. (1942), Amer.J.Physiol., 137, 348.

Fibrinogen assay

The method described is that of Ratnoff and Menzie (1951) as modified by Alkjaersig (1960). In this assay fibrinogen is clotted with thrombin; the fibrin so formed is/

is hydrolysed with sodium hydroxide and the tyrosine released is estimated colourimetrically. A constant proportion of tyrosine in the fibrinogen molecule is assumed (1:11.7).

In a 15 ml. test tube are placed "0.2 ml." glass beads (diameter 0.15 mm.), 6.0 ml. saline, 0.1 ml. thrombin solution, 100 N.I.H. units/ml., 0.2 ml. 2.5% calcium chloride and 0.2 ml. plasma. The tube is shaken but not inverted and the fibrin is caught up on the glass beads. After standing for 1 hour at 4°C the tube is shaken again and centrifuged for 10 mins. at 2,000r.p.m. The glass beads and adherent fibrin are washed 3 times with saline. After final centrifugation and decantation of the washing fluid, 0.4 ml. 10 per cent sodium hydroxide is added and the tube boiled in a water bath for 20 minutes. After cooling to room temperature, 0.6 ml. 5 per cent trichloracetic acid, 2 ml. 0.5 N sodium hydroxide and 0.6 ml. dilute (1:2) Folin Ciocalteu reagent are added. After standing for 15 minutes for colour development, optical density at 650 mμ is read against a reagent blank. Readings are converted to fibrinogen concentration, mg./100 ml., from a standarded tyrosine curve.

Alkjaersig, N., (1960), in N I.H. Conference on Thrombolytic Agents, eds. Roberts, H.R., Geraty, J.D.; University of North Carolina Press, Chapel Hill, p. 316.

Ratnoff/

Ratnoff, O.D., Menzie, C. (1951), J.Lab.clin.Med., 37, 316.

Plasminogen assay

The method used was the caseinolytic assay of Remmert and Cohen (1949) as modified by Alkjaersig et al. (1959b). Antiplasmin is first destroyed by incubating the plasma with acid. The acid is neutralised with alkali and buffer, and streptokinase is then added to convert the plasminogen to plasmin. The plasmin so produced is assayed by a caseinolytic technique, the amount of tyrosine released from the casein being a measure of the amount of plasmin present.

The casein solution used is prepared by boiling for 20 minutes 25 gm. casein ("Hammarsten" quality, Nutritional Biochemicals Corporation, Cleveland, Ohio) in 500 ml. phosphate buffer, 0.1 Molar, pH 7.6. The solution is filtered while hot and after cooling, the pH is readjusted to 7.6.

To 0.5 ml. plasma is added 0.5 ml. 1/6 N hydrochloric acid. After standing for 15 minutes at room temperature to destroy antiplasmin, 0.5 ml. 1/6 N sodium hydroxide is added, followed by 1.0 ml. phosphate buffer, 0.1 Molar, pH 7.6, 0.5 ml. streptokinase solution 2,000 units/ml. (Varidase - Lederle), and 2.0 ml. 5 per cent casein solution. After addition of casein the assay mixture is incubated at 37°C for 62 minutes/

minutes. At 2 minutes and 62 minutes 2 ml. aliquots are taken and to each is added 2 ml. 10 per cent trichloracetic acid. After centrifugation (2000 r.p.m. for 10 minutes) 1 ml. of the supernatant is added to 5 ml. 0.5 N sodium hydroxide and 1.5 ml. 5 per cent trichloracetic acid, followed by 1.5 ml. dilute (1:2) Folin Ciocalteu reagent. After standing for 15 minutes for colour development, the optical density of the 62 minute sample is read at 650 mμ with the 2 minute sample as blank. Tyrosine release is read off a standard curve; one casein unit equals 180 mg. tyrosine released in 1 hour.

Alkjaersig, N., Fletcher, A.P., Sherry, S., (1959b), J.clin.Invest., 38, 1086.

Remmert, L.F., Cohen, P., (1949), J.biol.Chem., 181, 431.

Assay of EACA in urine by the high voltage electrophoresis technique of Sjoerdsma and Hanson

The method consists of isolation of EACA by high voltage paper electrophoresis, development of colour by a ninhydrin and copper spray, elution of the EACA bands from the paper and measurement of optical density in a spectrophotometer. The method produces separation of EACA from the normally occurring urinary amino acids with the exception of alanine (Sjoerdsma and/

and Hanson 1959), who also found that over an EACA range of 0 to 30 μg, EACA concentrations are proportional to the optical density of the eluted band in the spectrophotometer. Because of limitation as to sample size (maximum about 40 μl.) EACA concentration of less than about 2.5 mg./100 ml. cannot be assayed.

Reagents

(1) Buffer for electrophoresis. Formic acid, acetic acid, acetone and water in proportions 15:10:15:65 v/v, with a final pH of 1.2.

(2) Spray reagents (a) Ninhydrin, 0.5 per cent, in butanol previously saturated with a mixture of pyridine, acetic acid and water in proportion 10:1:95 v/v, with a pH of 6.

(b) Copper reagent, prepared by mixing a saturated solution of cupric nitrate, 10 per cent nitric acid, water and methanol in proportions 1:0.2:4:95 v/v.

Method

The method to be described differs from that of Sjoerdsma and Hansen in the paper used (Schleicher and Schuell No. 598 instead of Whatman No. 3) and the voltage (1,000 V instead of 2,000 V).

An E-C horizontal high voltage apparatus was used (EC Apparatus Co., Swarthmore, Pa.). Sample size was/

was from 5 - 40 µl and EACA standards of 5, 10, 20 and 30 µg. were run with each assay. Samples were run for 1 hour at 1,000 V.

On removal from the apparatus, the paper was dried at 80°C for 1 hour. It was then sprayed with the ninhydrin reagent and the colour developed by heating at 80°C for 30 minutes. The purple colour obtained was then converted to a more stable light red colour by spraying with the copper reagent. The paper was allowed to dry in a current of cool air. The EACA band, identified by reference to the standard, was then cut out and eluted for 30 minutes with 4 ml. of methanol in a stoppered test tube. A clear portion of the paper of corresponding size was eluted also and used as a blank. Optical density of the unknown was read against the blank at 508 mµ. in a Beckman DU spectrophotometer. From the optical densities obtained with the EACA standards, a graph was constructed and from this the EACA concentration in the urine sample was read. The mean molar extinction coefficient of EACA treated in this way was 0.35.

Sjoerdsma, A., Hanson, A , (1959), Acta chem. Scand., 13, 2150.

Euglobulin lysis

The fraction of plasma which precipitates in dilute solutions/

solutions at pH 5.3 (the euglobulin fraction) contains plasminogen activator, plasminogen and fibrinogen, but antiplasmin is largely in the supernatant (Macfarlane and Pilling, 1946). If the euglobulin precipitate is resuspended and clotted with thrombin, much shorter lysis times are found than with the whole plasma from which the euglobulin precipitate was prepared (for a normal plasma, hours instead of days). The technique is therefore more convenient than whole plasma clot lysis time determination.

Method

The method used was that of Sherry et al. (1959c). The test is set up within 30 minutes of withdrawal of the sample, which is stored at 4°C . To 7.4 ml. acetic acid, pH 5.3, is added 0.6 ml. plasma. After standing 10 minutes at 4°C, the precipitate is centrifuged down at 2,000 r.p.m. for 10 minutes in a refrigerated centrifuge. The supernatant is poured off, the precipitate is dissolved in 0.7 ml. barbitone buffer in 0.9 per cent saline, pH 7.4, and clotted with 0.1 ml. thrombin, 10 N.I.H. units/ml. Time for complete clot lysis at 37°C is noted.

Calculation of results

Results are expressed in arbitary units. It has been shown by Sherry and Alkjaersig (1957) that in fibrinolytic assays, activity is a direct function of the reciprocal of the lysis time, and accordingly a logarithmic plot/

plot of lysis times against units of activity shows a linear relationship. Euglobulin lysis times are expressed in terms of arbitary units of activity derived from such a plot. A lysis time of 30 minutes is assigned an activity of one unit, and the values for other lysis times are obtained by dividing the lysis times observed into 30. Normal euglobulin lysis times are over 300 minutes (i.e. less than 0.1 unit) and if lysis has not taken place the test is discontinued at 300 minutes, the result being presented as "less than 0.1 unit".

Macfarlane, R.G., Pilling, J., (1946), Lancet, 2, 562.

Sherry, S., Alkjaersig, N., (1957), Thrombos.Diathes. haemorrh. (Stuttg.), 1, 264.

Sherry, S., Lindemeyer, R.I., Fletcher, A.P., Alkjaersig, N., (1959c), J.clin.Invest., 38, 810.