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The Studies Concerning the Actions of Ipsilon-Amino-N-Capolic-Acid and Blood Plasmin

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PLASMIN AND ANTIPLASMIN
THEIR PATHOLOGIC PHYSIOLOGY

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Plasmin Research Project

(Received for publication September 15, 1959)

STUDIES ON THE ANTIPLASMIN

It is known that proteolytic enzymes are found in cells and blood. Physiologically, such enzymes have been considered to be related with the protein metabolism of a living organism. Various studies have been made as to whether or not proteolytic enzymes in vivo are identical with digestive enzymes such as trypsin and pepsin[1,2]. And rather recently a specific kind of proteolytic enzyme was found in the human blood[3] and was named "plasmin" or "fibrinolysin."

Various investigations have been made as to the function of plasmin on the living organism, and many grounds have been found out for believing that plasmin in blood and/or in locus becomes active in the case of a series of pathologic states, such as surgical shock, allergy, and also of physical and mental fatigue, that the decomposition of plasma fibrinogen and/or the other proteins in vivo is caused by the above anomalous activity of the mentioned enzyme, and that the products formed by the said decomposition give troubles to the living organism[4,5].

In fact, the activation of plasmin was rapidly observed experimentally by the antigen-antibody reaction, which was brought about in vitro and in vivo (Unger & Mist), and also the toxic substance formed by the action of plasmin on the proteins in blood has the nearly same features as those of histamine, suggesting that the resultant toxic substance may play a part in those disorders in which the activation of plasmin occurred to a certain extent in blood and/or in locus[4,6,7].

An important suggestion was made on proteolytic enzyme, from the viewpoint of experimental medicine, by the fact that the course of inflammation was made to progress favourably in an experimental peritonitis when trypsin was given repeatedly in advance to increase the antiproteolytic power in vivo (Kay)[8].

It has hitherto been known that certain substances such as soyabean trypsin

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inhibitor have an inhibitory effect on plasmin, but such substances are not successfuly used even in animal experiment because of their toxic side action. It is also known that albumin fraction of human serum has an inhibitory power to plasmin (MacFarlane and Biggs)[9], and the said albumin fraction was clinically used by O. Smith and G. Smith[10,11] for the case of pregnancy toxicosis, even-though a mass application of such substance was difficult.

The auther and his colleagues, therefore, started investigations in 1948 searching for synthetic antiplasmin substance, co-operatively working for several years with Nagasawa and his colleagues in the Laboratories of Mitsubishi Chemical Company.* One group of such substances was found out in 1952 from among those synthetic substances as results of the systematic studies made in vitro which showed inhibitory power on the fibrinolytic activity of the plasmin obtained from various serum samples. Among the compounds belonging to the said group, Ipsilon-amino-caproic acid has the inhibitory power to the fibrinolysis with plasmin system at a very low concentration of the order of 10^{-5} mol[12].

The screaning test of anti-plasmin power in the beginning of our investigation was made as follows. A globulin fraction was obtained by diluting the plasmin of man or horse 10 times with aqua dest. and by precipitating and collecting the euglobulin fraction at pH 5.2. The globulin fraction was mixed with fibrinogen solution and a certain quantity of those substances to be measured which had been diluted. Fibrin clot was made by adding thrombin to the above mixture, and the time required for the complete dissolution of fibrin clot at 38°C was measured and compared the said time with that of dissolution of control fibrin clot to which the substance to be measured had not been added.

As to the effect of Ipsilon-amino-caproic acid (hereinafter abbreviated as Ipsilon) to the living organism, the following table shows that anti-anaphylaxis effect of Ipsilon in mice which occurs in protracted course.

	No. of Case examined	Death	Living	Death Rate
Ipsilon was given	37 cases	10	27	27 %
Control	36	23	13	64 %

On the other hand, a remarkable inhibitory effect on the Manteaux reaction was shown by Itoga[13] when local application of Ipsilon was made at the same time. It is noteworthy that such a substance having an inhibitory effect to the Manteaux reaction was scarcely known, and Ipsilon is the most powerful one

* Shosuke Okamoto, Fujio Nagasawa, Eichi Takagi, Yuzo Tsukada, Mikio Yokoi, M. Sato and others were engaged in this cooperative work.

which has been discovered.

Another experiments were made by giving Ipsilon per se to experimental animals, and it was made clear that the repeated administration of a large amount of Ipsilon was practically not toxic. In the case of injecting intravenously 1 g per 1 kg of the weight of a rabbit continuously for ten days once a day, the rabbits were sacrified after the lapse of one week, and the abnormality of organs was examined histologically and proved to be little.

Therefore, clinical examinations on the effect of the intravenous administration of Ipsilon in a appropriate solution were searchingly conducted by Sato and his colleagues and by Itoga and his colleagues. Thus, Sato and others revealed the effectiveness of Ipsilon on some patients where plasmin was active in the venous blood[14]. Itoga and others denoted that the administration of Ipsilon could be effective on some skin diseases[15].

Since then, Ipsilon became to be clinically applicable wider and wider, and the recent achievements made by the members of the committee of our research project are detailed in the several papers of this issue[16,17,18,19].

METHOD AND DISCUSSION

Very marked progress in the chemistry of blood proteins and coagulation factors in recent years has resulted in the rapid development of knowledge and method on the fibrinolysis with plasmin, the more precise investigations being able to be facilitated than those described by us in the form of a patent specification 1953.

In particular, highly purified preparation of bacterial streptokinase has become so easily available to the laboratory work that some studies have been carried out by several workers on the mechanism of Ipsilon on plasma activated with streptokinase (T. Abe, et al.[20], T. Igawa, et al.[21], and S. Okamoto, et al.[22]).

Either clinical or experimental investigations have been variously conducted by numbers of workers and the physiological concept of the fibrinolysis has been developing and changing rapidly. Moreover, the promising development of fibrinolytic therapy in such diseases as a thromboembolic disease and some infection diseases has cast a new light on the physiology of plasmin system in the living organism.

An urgent task, however, was facing us; that is, the most adequate and practical method for revealing some significant aspects of plasmin system in the healthy and patients should be established. This was the reason why it was intended by our committee on Research Project to make a comparison of those

different methods for determining plasmin activity in blood, which have been described and applied by different workers and in different laboratories.

More than 1,200 blood samples furnished from different divisions of the Hospital of Keio University, School of Medicine, were examined in several different methods at the same time in our technical center and the results obtained are briefly summarised in the present paper and will be discussed in the several other papers following.

The improvement of the methods described by MacFarlane et al.[3], Loomis[23], Unger and Mist [6], Astrup and others[24] has been noticed by us and those representative methods were adopted in our technical team, although the excellent fibrin plate method by Astrup is now on the way to be compared with the other methods.

So far as our results obtained until today, the whole clot lysis test by Ratnoff and the euglobulin lysis test by Loomis were both constantly reproduceable, although those different tests seem to represent each different aspect of plasmin system. Results indicated that the whole clot lysis test was the most positive in most cases of the skin diseases suspected to be allergic, and that the euglobulin lysis test was very often positive in some haemorrhagic diseases.

It may be reasonably assumed that results of euglobulin lysis test by Loomis are less influenced by the amount of anti-plasmin than those of whole clot lysis test, and this presents one of the reasons why results from these two methods are different each other. This problem will be later discussed in the paper written by Asahi, Takamura and Okamoto.

Details of the results obtained with streptokinase activation test are now ready to be published by U. Okamoto et al.[25]. These results have been highly reproduceable, but their significance seems to be clearly differnt from those obtained by the methods mentioned.

Results obtained by MacFarlane's test and Kuroyanagi's modification[26] of the said test are delicate, and the results seemed to be largely influenced by the amount and state of anti-plasmin in the sample.

Ultraviolet absorbance test by Unger is sensitive and very ideal when the purified plasmin fractions are used, but it does not seem to be available widely in such complicated sample as blood obtained from patients. The paper by Sasaki and others indicated that the variation of ultraviolet absorbance does not go parallel with the increasing digestion of fibrinogen with plasmin activity. And, the other numerous results obtained in various pathologic states are now under investigation in relating with pathological pattern of the dynamic aspect of plasmin in each disorders.

I should note here that, in most of the cases examined in our technical center such methods as fibrinogen determination by Gramm[27], whole clot lysis test by Ratnoff, euglobulin lysis test by Loomis, streptokinase activation test, Unger's Ultraviolet test, MacFarlane test and its Kuroyanagi's modification were adopted as *the routine methods* for determining the plasmin activity in the blood sample obtained from the healthy person and the patients ailing various diseases.

PLASMIC AND ANTIPLASMIC STATES

Either clinical or experimental investigation has been so variously conducted that only a few achievements will be somewhat tentatively cited in the present paper relating to our investigation.

In the first place, the author should like to note the activation of plasmin was rapidly observed experimentally by the antigen-antibody reaction in vivo and in vitro as well. MacFarlane and Biggs[9], Rocha e Silva[28], Unger and Mist[6], Okamoto and Tsukada[7] respectively noticed and revealed the mechanism of the activation of plasmin with such reactions.

The second point is that the strong actviation of plasmin in blood with some pathologic stages of man proved to cause an increased tendency of bleeding: Ratnoff descrived in 1952 a very typical case of the activation of plasmin in a patient who died from a sever haemorrhage after an operation and in whom no other direct cause of haemorrhage but the activation of plasmin was observed with various kinds of blood examination.

A- or hypo-fibrinogenemia in the course of child delivery has come to be noticed in recent years, and it is now regarded as closely associated with the strong activation of plasmin. A series of such evidences clearly indicated that the strong activation of plasmin can produce, more or less, such a haemorrhage as oozing in a surgical operation.

On the other hand, the successful application of streptokinase to patients ailing thromboembolic diseases or some infection seems to bring about a confusion in the classical concept of the fibrinolysis in vivo. The auther wishes to point out the presence of those two types of pathologic states, where the balance of plasmin system is shifted; the former is a plasmic state, the latter an antiplasmic state. Clinical investigaitons made by Miller and others indicate streptokinase should be available to the latter.

On the contrary, plasmic states in some disorders can be successful combated with the administration of a synthesized antiplasmic substance, i.e. ε-amino-n-caproic acid, as described in those papers written by Okamoto et al., Mikata et al.[17,18], Sato et al.[16] respectively. It may be a somewhat schematic way

to divide the pathologic states into such two types, yet the author regards it as a useful and convenient way for determining whether some new methods such as an antiplasmin treatment, as it were, should be applied or not.

It was clearly demonstrated in our animal experiments that the unfavourable side actions caused by the intravenous administration of streptokinase (of large amount!) were suppressed by the administration of ε. This fact supports the possibility of the effective usage of ε for removing the unfavourable side action caused by the overdosing of streptokinase.

While it is possible to remove the antiplasmic action of ε by giving the streptokinase to patients. The words of Dr. J. Miller (Private communication) may be cited here: That is, "streptokinase in right hand, ε in left hand."

The following papers in this issue will approach to the pathologic physiology of plasmin system in man and animal, suggesting more precise concepts of the fibrinolysis and a rather unique way of treating those disorders belonging to a hyper-plasmic group.

Acknowledgement

The author does wish to express his cordial thanks to Prof. Yoshio Kusama, the chairman of the committee of Research Project on Plasmin and Antiplasmin and also to Prof. Takashi Hayashi, Prof. Komei Miyagi, Prof. Isamu Suda and Prof. Daizo Ushiba, who were the members of the advisory committee of these investigations.

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REACTION MECHANISM BETWEEN ε-AMINOCAPROIC ACID AND PLASMIN, TISSUE ACTIVATOR AND TRYPSIN

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As it has been reported, ε-aminocaproic acid (ε-ACA) has inhibitory activity upon fibrinolysis in vitro as well as in vivo. In this paper experimental studies were discribed on the reaction mechanism between ε-ACA and fibrinolytic enzymes, such as plasmin, tissue activator and trypsin and also inhibitory effect of this substance in the blood of subjects who got its administration.

MATERIALS AND METHODS

1) *ε-aminocaproic acid* was supplied by DAIICHI SEIYAKU Co., Ltd. in the forms of 5% and 20% solutions and white crystallized powder.

2) *Bovine fibrinogen:* by Astrup's method.

3) *Bovine thrombin*[a]: obtained as biothrombin of Seegers.

4) *Human plasmin*[b]: principally by Loomis' method.

5) *Crystalline trypsin*[c]: (MOCHIDA)

6) *Tissue activator* (T.A.): by the method of Astrup and Sterndorff, extracting pig heart muscle homogenate with 2M.KSCN, precipitating at pH 1.0 and dissolving this in phosphate buffer (7.4).

7) *Phosphate buffer* of 1/15 M. at pH 7.4.

8) *Determination of fibrinolytic activity*

A) For the experimental studies the fibrin plate method of Astrup was employed, heated plates for plasmin investigation and standard and heated plates for tissue activator. This method was adopted as it could measure a broad range of fibrinolysis, denaturate and/or eliminate the plasminogen contained in fibrin substrate by heating and detect fibrinolysis more faithfully than in cases of casein- or synthetic substance digestion.

B) For the clinical evaluation euglobulin lysis assay was applied, in which

a), b), c) Kindly supplied by MOCHIDA PHARMACEUTICAL MANUFACTURING Co., Ltd.

euglobulin was prepared by means of simple isoelectric precipitation of plasma at pH 5.2 and this method has advantage of needing easy technique, consuming short time and having pretty high sensitivity to a minimum amount of plasmin at adequate condition.

The lysis time which elapsed from mixing of fibrinogen, euglobulin and thrombin to the complete dissolution of invited fibrin clot, showed good correspondence with plasmin amount, giving a straight line on a double logarithmic graph with axes of lysis time and plasmin concentration, and its average value of 200 minutes at normal human subjects was determined as a standard of plasmin activity and fibrinolytic index (F.I.) of each individual sample could be induced from these two factors.

RESULTS

Inhibitory Activity of ε-ACA upon Plasmin

A serial dilute solutions of ε-ACA prepared with phosphate buffer was mixed with equal volume of plasmin solution and the fibrinolytic activity of them was checked on fibrin plates. The percent inhibition (H) was measured as followed.

$$H = \frac{L_1 - L_2}{L_1}$$

Where L_1 was the control fibrinolytic activity (mm^2) of plasmin which was mixed with equal volume of phosphate buffer and L_2 represented the inhibited fibrinolytic activity of plasmin which was mixed with equal volume of

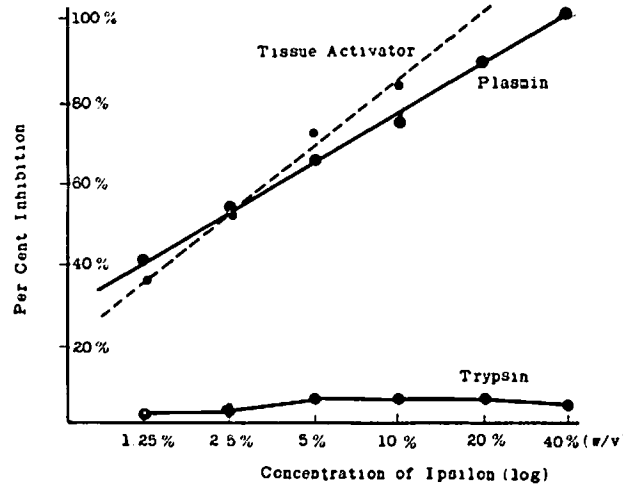

Fig. 1. Inhibition of ε-amino caproic acid upon plasmin, tissue activator and trypsin. (Fibrin plate assays).

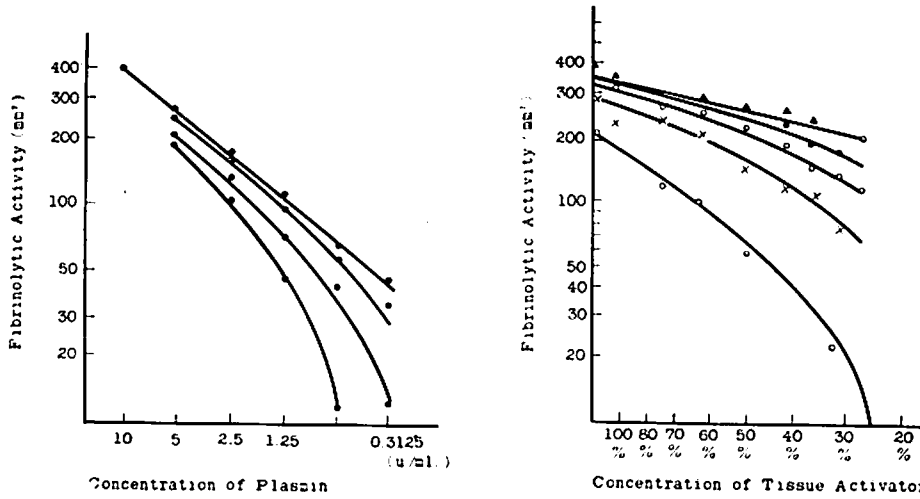

Fig. 2. Inhibition of Ipsilon upon Plasmin and Tissue Activator.

ε-ACA solution.

The logarithmic values of H and ε-ACA concentration showed lineal relationship in Fig. 1. Also in the cases in which plasmin amount was changed and ε-ACA concentration constant, the inhibition grade with a certain amount of ε-ACA was always same, regardless of plasmin concentration, as shown in Fig. 2.

A plasmin-ε-ACA mixture which showed 80% of percent inhibition could get splitted by isoelectric precipitation precedure at pH 5.2 and its precipitate showed nearly 100% fibrinolytic activity as much as the original.

Inhibition of ε-ACA upon Tissue Activator

As it could be seen in Figs. 1 and 2, the relationship between H values and logarithmic concentration of ε-ACA, and between fibrinolytic activity and concentration of T.A. gave quite similar results as in the case of ε-ACA and plasmin. When reaction mixtures of T.A. and ε-ACA which were incubated for different periods, were added to equal aliquots of the same plasminogen solution separately, incubated simultaneously for a certain period, and their isoelectric precipitates at pH 5.2 checked percent inhibition on heated fibrin plates, also very similar results were obtained to those of plasmin and ε-ACA as shown in Fig. 1.

Inhibition of ε-ACA upon Trypsin

The same procedure was employed for trypsin-ε-ACA system as above mentioned and this time no inhibition of ε-ACA upon trypsin was found in fibrinolysis as shown in Figs. 1 and 3.

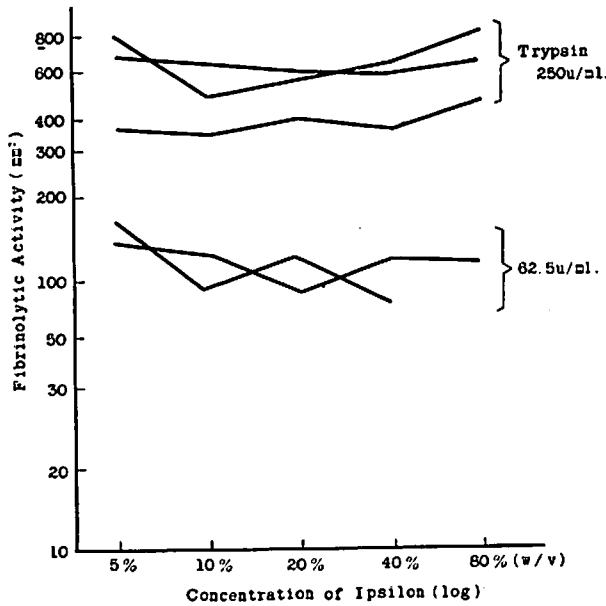

Fig. 3. Inhibition of ε-amino-caproic acid upon Trypsin. (Fibrin plate assays)

In vivo Effects of ε-ACA upon Fibrinolysis of Streaming Blood

F.I. of normal human blood was around 1, but in some cases of blood diseases, nephropathies, circulatory disorders, metropathies, skin diseases including allergic reactions and other diseases as well as in some normal subjects without particular pathological findings to be recognized, it showed higher values; and blood transfusion, electroshock for psychoses and fever therapy for various diseases etc. also invited increased fibrinolysis.

In Fig. 4, F.I. of patients who showed higher fibrinolysis and received intravenous injections of 20 ml of 5% ε-ACA injection, was followed up as time passed before and after injection, and it revealed that the inhibitory effect of ε-ACA became apparent 10–30 minutes after injection, giving low F.I. which was maintained for 2 or 3 hours and then began to return to the original high values. Neither single case was recognized who has F.I. elevated after injection nor decreased inhibition experienced even after long-termed administration of ε-ACA and so far no special side-effect was observed.

DISCUSSION

Numerous substances has been reported to have inhibitory activity upon plasmin, but their reaction mechanism has not been necessarily unveiled. From this point of view it was tried to confirm whether ε-ACA reacted with plasmin

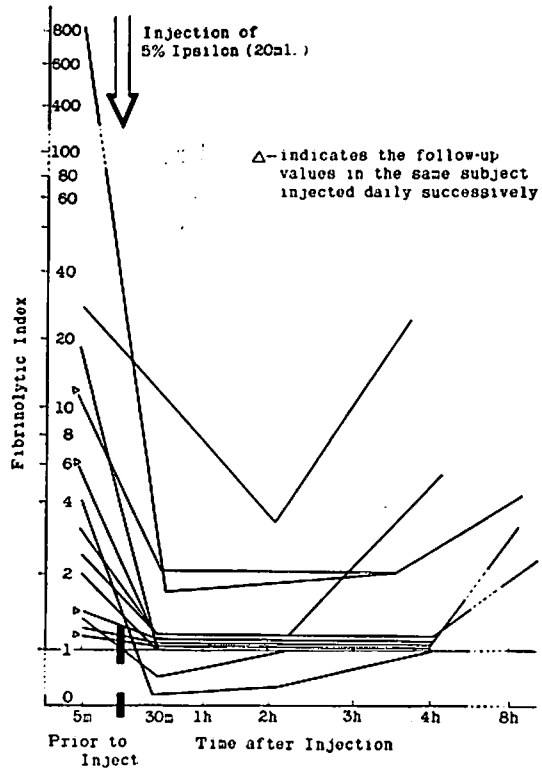

Fig. 4 Inhibition of ε-aminocaproic acid upon fibrinolysis of streaming blood. (Euglobulin lysis assays)

and T.A. stoicheiometrically or catalytically and if the inhibitory effect of ε-ACA was reversible.

As to the interreaction between ε-ACA and plasmin, it was proved to be stoicheiometric from our present experiments, and for that between ε-ACA and T.A. special consideration was paid not to include the plasmin inhibition on fibrin plates by ε-ACA, and it was also of stoicheiometric nature.

Repeated injections of ε-ACA for clinical cases gradually depressed the recovery of F.I. to the initial values and in some haemorrhagic patients they could stop bleeding which appeared at low F.I., e.g. ε-ACA suggested some other effects on bleeding than fibrinolysis inhibition.

As it could be understood in the description of the method, euglobulin lysis assay was applied for clinical evaluation, in which ε-ACA was removed from the euglobulin fraction according to our experiment, and the low F.I. of plasmin samples after ε-ACA injection might confirm the conclusion that this substance exerted inhibition upon tissue activator.

CONCLUSION

1) The reaction mechanism between ε-ACA and plasmin or tissue activator was scrutinized and found to be of stoicheiometric nature, but that substance did not show any inhibitory activity against trypsin on the fibrin plate.

2) The inhibitory effect of that substance was proved in plasm samples early at 30 minutes after injection and maintained for around 3 hours thereafter.

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VISCOSIMETRIC STUDY OF THE ACTION OF ε-AMINO-CAPROIC ACID ON HUMAN PLASMIN

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INTRODUCTION

Recently more attention has been focussed on the action of ε-amino-caproic acid, an antiplasmin substance synthesized by S. Okamoto and others in 1953[1]. U. Okamoto established in 1958 by means of viscosimetric method that ε-amino-caproic acid added to fibrinogen solution inhibits plasmin fibrinogenolysis[2].

Our experiment was conducted using viscosimetric method[2,4,11] with the object of confirming whether plasmin caseinolysis[3-9] would also be inhibited by ε-amino-caproic acid, and if so, to clarify the action in this mechanism[3,9,10].

EXPERIMENTAL

Materials

(1) Casein solution: 7.0% casein solution was prepared, buffered to pH 7.4 with 0.024 M sodium borate buffer, pH 7.4, according to the refining process of casein material described by Philip S. Norman[12], using Hammarsten Casein powder.***** The casein solution was always kept in a refrigerator, at temperature 0°C to 5°C, and the solution was discarded after one week, fresh solution prepared.

(2) Standard human globulin solution (H.G.): The euglobulin fraction was precipitated by diluting the serum taken from five donors by twenty times

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***** Manufactured by Chroma-Gesellschaft Schmitt & Co.

with distilled water and adding 0.5% acetic acid until a pH-value of 5.2 was obtained. The precipitate was resuspended in a volume of 0.024 M sodium borate buffer, pH 7.4 equal to that of the original serum.

We obtained standard human globulin solution after repeating this process twice and used it as *human plasminogen*[13,14]. It was also preserved in a refrigerator, and was handled thereafter in a cold room at 0°C.

(3) Streptokinase* (S.K.): It was prepared as S.K. 10,000 u per ml. in physiological NaCl solution. It was kept at −15°C in a deep freezer, and handled in the cold room.

(4) ε-Amino-caproic acid** (ε-A. C.A.): It was used as 35% solution in distilled water. Only for *procedure 5*, which described later, the powder itself was dissolved in casein solution.

Methods

(1) Viscosimetric Measurements: 3.0 ml. of 7.0% casein solution was put in an Ostwald viscosimeter, which was kept upright in a water bath at a constant temperature 37.0°C (±1/500°C).

After taking control readings, the specific viscosity of casein solution was recorded every 5 min. for two hours after the addition of *Plasmin solution with or without ε-A. C.A.*, 0.33 ml.

The reading at 10 min. after the addition of plasmin solution was taken as the standard and decrements at every 10 min. thereafter were plotted at different concentrations of ε-A. C.A. as shown in Fig. 1a, 2a, 3a, 4a and 5a.

The ratio between the specific viscosity at 10 min. after the addition of plasmin solution and that at 40 min. at different concentrations of ε-A. C.A. is shown on Fig. 1b, 2b, 3b, 4b, and 5b.

(2) The preparation of the plasmin solution was as follows: 0.1 ml (1,000 u) of S.K. was added to 1.0 ml of H.G.

The mixture was left for 20 min. at room temperature and then twice precipitated at pH 5.2 with 0.5% acetic acid. The precipitate was resuspended in 0.55 ml of 0.024 M sodium borate buffer, pH 7.4, and the product was taken as S.K.-activated human globulin with double concentration. At different stages in the foregoing plasmin preparation, ε-A. C.A. of progressive concentrations, i.e. 0.5%, 1%, 2%, 4%, 8% was introduced.

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* Varidase, Lot No. 2200, biophysically and homogeneously refined and offered by Lederle Lab. Div., American Lab. Co., N.Y.

** Powder specially refined and offered by Dai-ichi Seiyaku Co., Tokyo.

Results

Procedure 1: ε-A.C.A. does not affect plasminogen. (Fig. 1)

ε-A.C.A. of different concentrations was added to H.G. and precipitated at pH 5.2 after being kept at 37°C for 10 min.

The precipitate was resuspended in 1.0 ml of borate buffer, pH 7.4, S.K. was added to the suspension and precipitated at pH 5.2 after 20 min. at room temperature. The precipitate was again resuspended in 0.55 ml of borate buffer, pH 7.4. It was used as *plasmin solution with or without ε-A.C.A.*

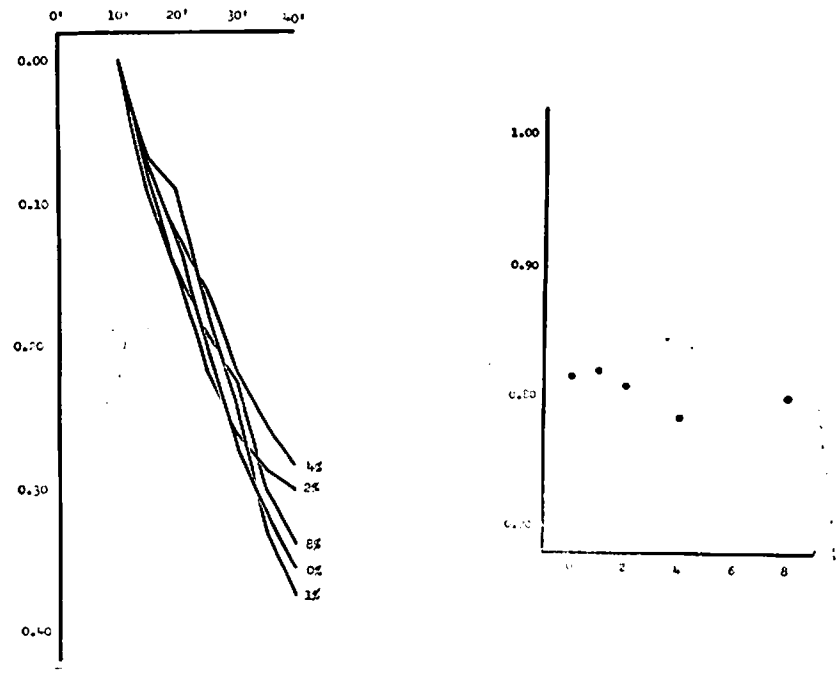

Fig. 1a Fig. 1b

Fig. 1a–5a. Abscissa: Min. after the addition of plasmin.
Ordinate: Specific viscosity reading 10 min. after the addition of plasmin was taken as the standard and decrements every 10 min. thereafter were plotted at different concentrations of ε-A.C.A.

1b–5b. Abscissa: Progressive concentrations (%) of ε-A.C.A.
Ordinate: The ratios between the specific viscosity 10 min. and 40 min. after the addition of plasmin.

As presented in Fig. 1a, the decrements of specific viscosity did not vary with different ε-A.C.A. concentrations.

Fig. 1b shows the ratios of specific viscosity at 10 min. and 40 min., which are not influenced by ε-A.C.A. concentrations.

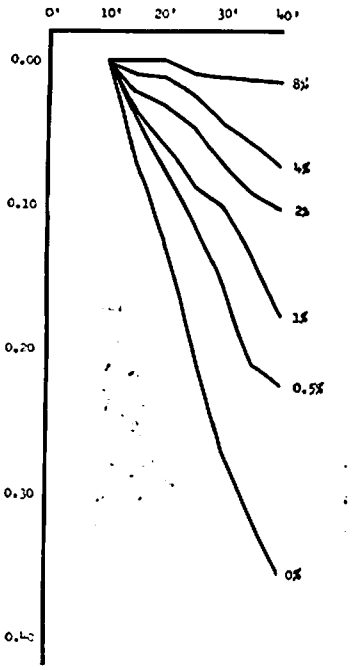

Fig. 2a

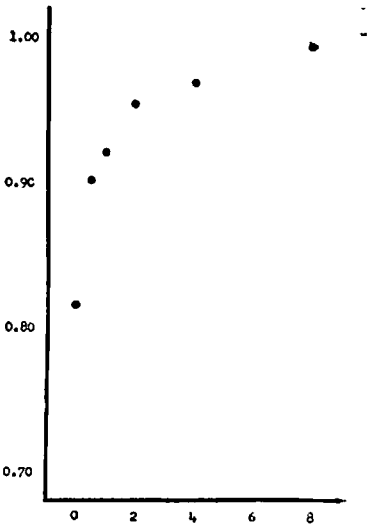

Fig. 2b

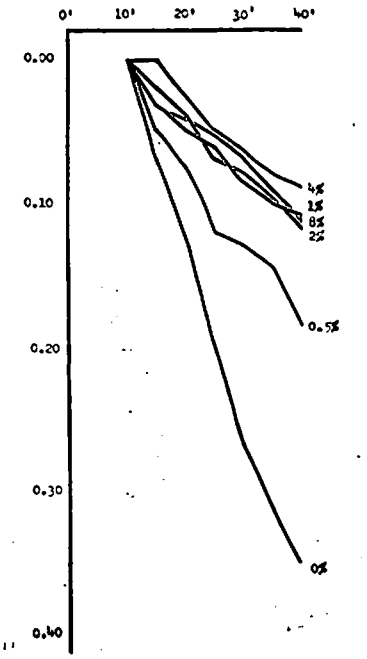

Fig. 3a

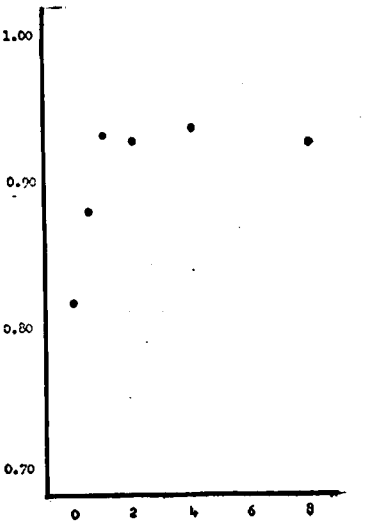

Fig. 3b

Procedure 2: ε-A.C.A. competitively [15] *inhibits S.K.-activation process of human plasminogen.* (Fig. 2)

The mixture of H.G. and ε-A.C.A. was left for 5 min. at room temperature, and then, without precipitation, S.K. was added.

It was twice precipitated at pH 5.2 after 20 min. at room temperature.

Procedure 3: ε-A.C.A. noncompetitively[15] *inhibits the remaining plasminogen activation process after plasminogen has been partially converted to plasmin.* (Fig. 3)

That mixture of H.G. and S.K. was left for 20 min. at room temperature, to which without precipitation, ε-A.C.A. was added. This was twice precipitated at pH 5.2 after 5 min. at room temperature.

Fig. 4a

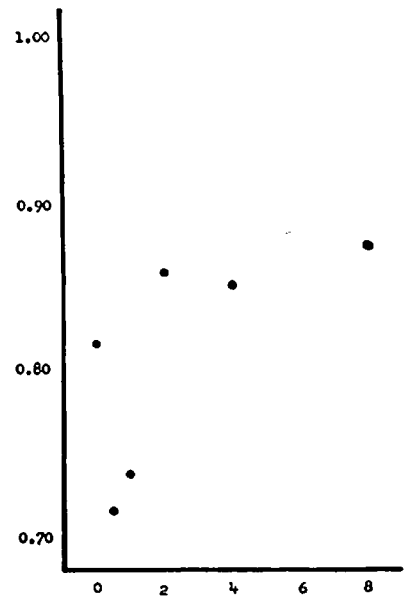

Fig. 4b

ε-A.C.A. was found to inhibit the remaining plasminogen activation non-competitively at the concentrations higher than 1%.

Procedure 4: ε-A.C.A. inhibits plasmin activity noncompetitively[15] *at higher concentrations (more than 2%), but enhances plasmin activity at lower concentrations (less than 1%).* (Fig. 4)

The mixture of H.G. and S.K. was left for 20 min. at room temperature, and then precipitated at pH 5.2. The precipitate was resuspended in borate buffer pH 7.4, and ε-A.C.A. was added. It was precipitated again at pH 5.2 after 10 min. at 37°C.

Procedure 5: ε-A.C.A. added to casein solution inhibits plasmin activity non-competitively[15] *at high concentrations, but does not enhance plasmin activity at lower concentrations.* (Fig. 5)

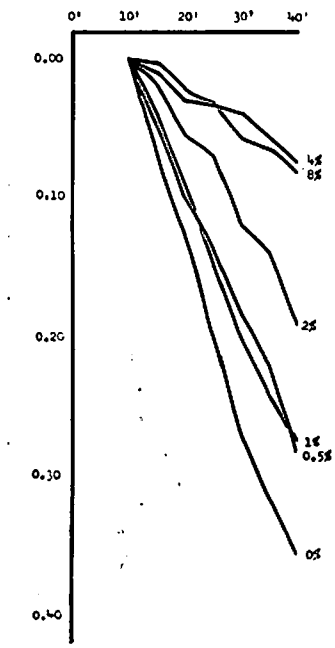

Fig. 5a

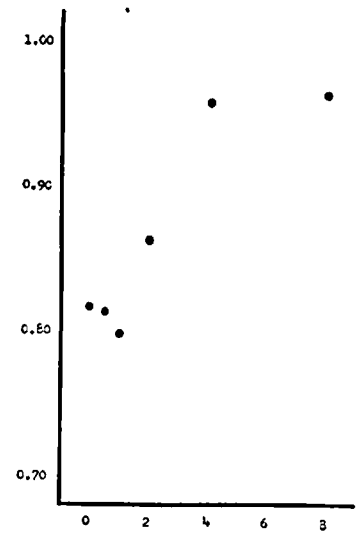

Fig. 5b

Solutions of casein with progressive concentrations of ε-A.C.A. powder were prepared: 16.65 mg, 33.30 mg, 66.60 mg, 133.20 mg and 266.40 mg. S.K.-activated human globulin was added to these solutions to yield mixtures containing each 0.5%, 1%, 2%, 4% and 8% of ε-A.C.A.

DISCUSSION

It was reported by S. Okamoto et al[1] in 1953 that ε-A.C.A. inhibits the spontaneous lysis of fibrin clot with plasmin prepared from various serum samples. Recently Abe, T. et al[16] demonstrated that, by fibrin plate method, ε-A.C.A. had an inhibitory effect against plasmin prepared from serum according to Loomis' method.

Ablondi, F.B. et al[10], however, revealed that ε-A.C.A. proved to be a weak competitive inhibitor of the caseinolytic activity of plasmin and to be a potent inhibitor of the S.K.-activated fibrinolytic system.

It was also reported by Alkjaersig, N. et al[3] that ε-A.C.A. competitively inhibited the activation of human plasminogen by S.K. and ε-A.C.A. in concentrations higher than 0.06 M was a noncompetitive inhibitor of the proteolytic activity by plasmin, but in low concentrations it enhanced the proteolytic action of plasmin.

In our experiment, data similar to those of Alkjaersig et al were presented by viscosimetric study on caseinolysis with plasmin.

However, the difference from the results of Alkjaersig et al lies in our data that the above-mentioned enhancing effect was absent when ε-A.C.A. in low concentrations was added to casein solution before the latter was affected by plasmin.

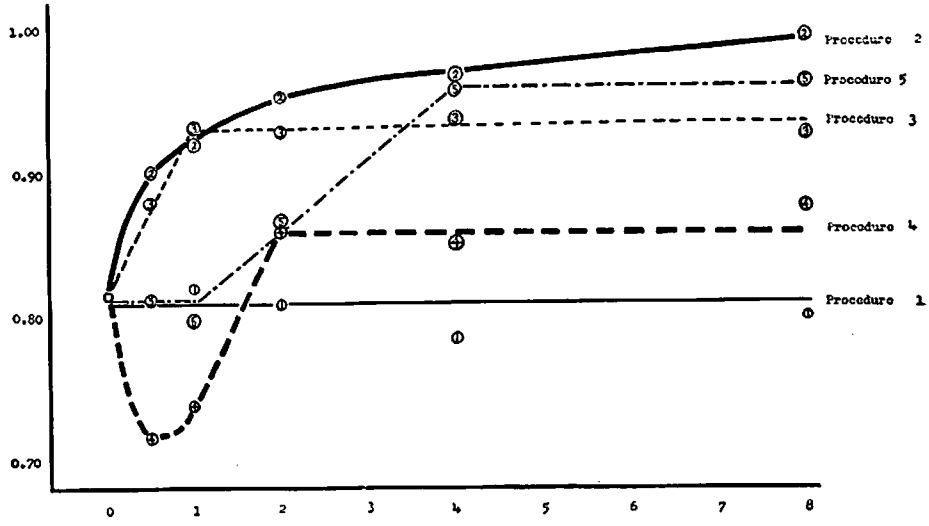

Fig. 6 Abscissa: Progressive concentrations of ε-A.C.A. (%).
Ordinate: The ratios between the specific viscosity at 10 min. after the addition of plasmin and that at 40 min.

Our results, therefore, seem to emphasize the possibility that the interaction among ε-A.C.A., substrate and plasmin would be more complicated than ever expected.

At the sametime we should like to note that a casein solution of higher concentration than those of the previous workers was used in our investigation and the rate of plasmin action was indirectly measured solely by means of the viscosimetry on such a casein solution.

SUMMARY

By means of the viscosimetric method, the inhibitory effect of ε-A.C.A. on plasmin caseinolysis was studied.

(1) ε-A.C.A. inhibits *competitively* S.K.-activation process of human plasminogen.

(2) a) At high concentrations, over 2%, ε-A.C.A. inhibits *noncompetitively* caseinolytic activity of plasmin produced from human globulin with S.K.

b) At low concentrations, below 1%, it enhances caseinolytic activity of plasmin.

This enhancing effect was not seen when ε-A.C.A. was added to casein before the latter was affected by plasmin. (Fig. 6)

I should like to thank the members of the Technical Center of the Research Project on Plasmin and Antiplasmin and Prof. S. Okamoto.

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SOME OBSERVATIONS ON A REMARKABLE FIBRINOLYTIC ACTIVITY IN THE EXTRACT OF NASAL TISSUES AND THE RELATED TISSUES

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INTRODUCTION

Chemical aspects of lung have been studied minutely by a great number of workers, very few of whom, nevertheless, have noticed the significant relation of chemical activity of lung to that of nasal tissues. Jobling & Petersen[1], Weiss[2], Rocha e Silba[3], Ungar[4], Collumbine & Rydon[5] and others investigated proteolytic activity of lung tissues, relating with the pathogenesis of lung diseases, and obtained the results that proteolytic activity in lung might play an important role in the process of inflammation and allergic change of lung.

In nasal tissues, however, any investigations have not been undertaken yet of the proteolytic system, although nasal tissues might be regarded as a part of the respiratory system. We are facing problems characteristic inflammations or allergic disorders of nasal tissues seem to present, and trying to solve them with favour of advanced study of proteolysis of lung.

MATERIALS AND METHODS

Tissue materials used: Fresh tissues used in the preparation were taken out, on the occasion of operations, of patients ailing such diseases as sinusitis, tonsillitis, gastritis, stomach ulcer and stomach cancer.

Mucous membrane of maxillary sinus and of inferior turbinate, faucial tonsil, pharyngeal tonsil and stomach mucous membrane taken from the patients, were washed in isotonic saline solution, dried on filter paper, and kept in a deep

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freezer at −20°C.

Crude preparation: The tissues were suspended in ten times its wet weight of ice-cold isotonic saline solution and homogenized by glass-homogenizer of a Potter type. After centrifugation of the homogenate, the supernatant fluids obtained were used as crude enzyme solution. These treatments were carried out in a cold room at 0°C.

Tissue fractions[6]: The tissues were suspended in ten times its wet weight of ice-cold 0.25 M sucrose solution, and homogenized. The homogenate was then submitted to differential centrifugation. The homogenate was first centrifuged for 10 minutes at 600 g. The sediment was suspended in 0.25 M sucrose solution and also centrifuged for 10 minutes at 600 g. The supernates summed up were centrifuged for 10 minutes at 8,500 g. That sediment was again suspended in 0.25 M sucrose solution, and then centrifuged for 10 minutes at 8,500 g, which was known as mitochondria. The remained supernate, separated from mitochondria, was centrifuged for one hour at 18,000 g. The sediment was suspended in 0.25 M sucrose and also centrifuged for one hour at 18,000 g, at last microsome obtained.

Fibrinogen: The preparation of Armour Laboratories was dissolved in isotonic saline solution, making 0.2% of solution.

Thrombin: The preparation of Parke Davis & Co. was dissolved in isotonic saline solution, making 10 u/cc of solution.

Plasminogen: As a source of plasminogen, the globulin fraction of human plasma was used. The human plasma was diluted with distilled water, adjusted to pH 5.2 with 0.5 per cent acetic acid to precipitate the euglobulin fraction. After centrifugation, the precipitate was redissolved in saline buffer at pH 7.4 (1/15 M phosphate buffer) to the original volume.

Determination of the fibrinolytic activity: Slightly modified Ungar's method[7] was adopted. Each crude tissue extract was divided into three samples, usually of 1.8 cc each. The samples of crude extracts were mixed with 1.8 cc of 1/15 M phosphate buffer (pH 7.4). Sample A and B were added to 2.0 cc of 0.2 per cent fibrinogen solution. Sample C was added to 2.4 cc of isotonic saline solution. Sample A was mixed with 0.4 cc of 10 u/cc thrombin solution. As a control was used the mixture of 3.6 cc of 1/15 M phosphate buffer, 2.0 cc of 0.2 per cent fibrinogen solution and 0.4 cc of 10 u/cc thrombin solution. After these treatments, Sample A and the control formed fibrin clots. Therefore, these four samples were placed for 30 minutes in a hot water bath at 37°C. After incubation, 0.4 cc of thrombin solution was added to clot sample B remaining fibrinogen. All these samples were filtered and the filtrate read on the Beckmann

spectrophotometer. The optical density reading of sample A against C, corrected by subtracting the absorption of a fibrinogen-thrombin blank, gave the fibrinolytic activity. The reading of sample B against C, corrected by subtracting the absorption of a fibrinogen thrombin blank, gave the fibrinogenolytic activity.

RESULTS

1. Effect of the crude extract of maxillary mucous membrane on fibrin or fibrinogen.

Twenty-five minutes was dissolution time by the extract in ten times its wet weight of isotonic saline solution on fibrin clot. It took solution of two times its crude original extract of isotonic solution 28 minutes; the solution of four times its crude original extract of isotonic solution 30 minutes (Table 1). Sample B formed fibrin clot after addition of thrombin.

Table 1

Action of the Extract from the Mucous Membrane Affected by Maxillary Sinusitis for Fibrin at 37°C

Extract of maxillary mucous membrane	Extract from isotonic saline solution with 10 times weight of the wet original mucous membrane	Isotonic saline solution with 2 times weight of the original extract	Isotonic saline solution with 4 times weight of the original extract
Dissolution time of fibrin clot	25 min	28 min	30 min

1/15 M phosphate saline solution (pH 7.4) 1.8 cc + extract 1.8 cc + 2.0 cc of 0.2 % fibrinogen + 0.4 cc of thrombin (10 units per cc).

The optical density reading of fibrin clot lysis was as seen in Figure 1 and Figure 2. The fibrinogenolysis was found not to occur, judging from the optical density readings. The optical density reading of fibrinolysis were either high concentration of extract with large value of absorbance or high concentration of extract with smaller value of absorbance than the low one. It was found that fibrinolysis times did not always keep pace with the absorbances. This relation should be investigated furthermore.

2. Effect of the crude extract of inferior turbinate on fibrin or fibrinogen.

The dissolution time by the extract in ten times its wet weight of isotonic saline solution on fibrin clot was 15 minutes; the solution in two times its crude original extract of isotonic solution 18 minutes, in four times extract 20 minutes, in eight times extract 25 minutes. Sample B formed fibrin clot after addition of thrombin (Table 2).

The optical density reading of fibrinolysis product is presented in Figure 3.

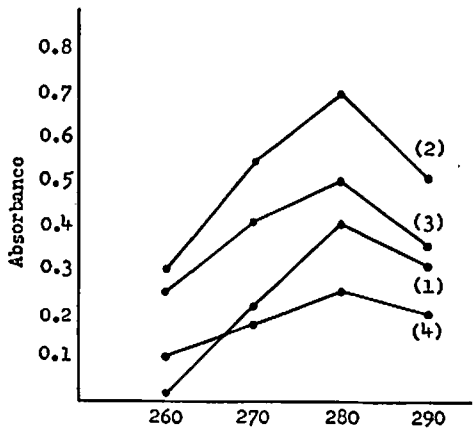

Fig. 1

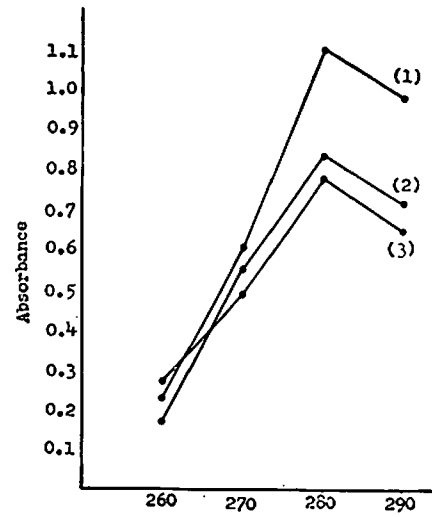

Fig. 2

Fig. 1 & 2. Action of the extract from the mucous membrane affected by maxillary sinusitis for fibrin for 30 min at 37°C. Wave length (mμ)

1/15 M phosphate saline solution (pH 7.4) 1.8 cc+extract (from isotonic saline solution with 10 times weight of the wet original mucous membrane) 1.8 cc+2.0 cc of 0.2 % fibrinogen+0.4 cc of thrombin (10 units per cc).

(1): Original extract.
(2): Isotonic saline solution with 2 times weight of the original extract.
(3): Isotonic saline solution with 4 times weight.
(4): Isotonic saline solution with 8 times weight.

The fibrinogenolysis did not occur in the optical density readings. The high concentration of the extract showed the short dissolution time of fibrin clot but low value of absorbance. The relation of the optical density readings of the maxillary mucous membrane extract to the inferior turbinate one was as follows Figure 4.

Table 2

Action of the Extract from the Mucous Membrane of Inferior Turbinate for fibrin at 37°C

Extract of inferior turbinate mucous membrane	Extract from isotonic saline solution with 10 times weight of the wet original mucous membrane	Isotonic saline solution with 2 times weight of the original extract	Isotonic saline solution with 4 times weight of the original extract	Isotonic saline solution with 8 times weight of the original extract
Dissolution time of fibrin clot	15 min	18 min	20 min	25 min

1/15 M phosphate saline solution (pH 7.4) 1.8 cc+extract 1.8 cc+2.0 cc of 0.2 % fibrinogen +0.4 cc of thrombin (10 units per cc).

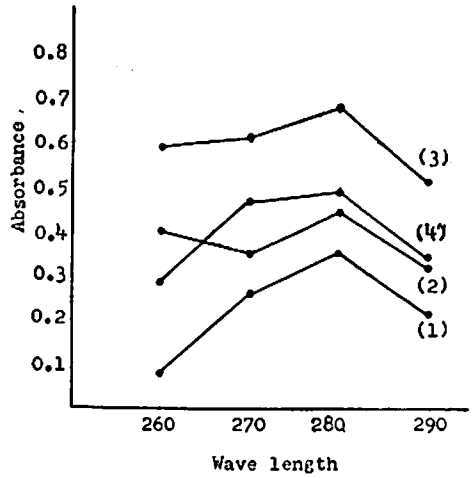

Fig. 3

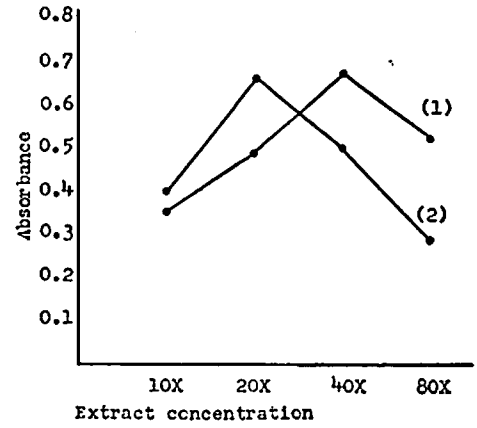

Fig. 4

Fig. 3. Action of the extract from the mucous membrane of inferior turbinate for fibrin for 30 min at 37°C. Wave length (mμ)

1/15 M phosphate saline solution (pH 7.4) 1.8 cc+extract (from isotonic saline solution with 10 times weight of the wet original mucous membrane) 1.8 cc+2.0 cc of 0.2 % fibrinogen+0.4 cc of thrombin (10 units per cc).

(1): Original extract.
(2): Isotonic saline solution with 2 times weight of the original extract.
(3): Isotonic saline solution with 4 times weight.
(4): Isotonic saline solution with 8 times weight.

Fig. 4. Comparison of fibrinolysis activity in each concentration of extracts from mucous membrane of maxillary sinus and inferior turbinate at 37°C for 30 min. wave length 280 (mμ).

(1): Inferior turbinate.
(2): Maxillary sinus.

3. Effect of the crude extract of faucial tonsil, pharyngeal tonsil and stomach mucous membrane on fibrin or fibrinogen.

In these experiments fibrinolysis and fibrinogenolysis were not observed after incubation for 30 minutes at 37°C, and the optical density readings were not measured.

4. Effect of addition of cystein to the extract of mucous membrane of maxillary sinus on fibrinolysis.

As such enzyme as cathepsin is activated by cystein which inhibits plasmin it was by addition of cystein that the fibrinolytic activity was analyzed in the isotonic saline extract of the mucous membrane affected by maxillary sinusitis. According to the stated modification of Ungar's method, the effect of cystein, the final concentration of which was adjusted to 1%, was observed. The dissolution time of fibrin clot by addition of cystein was markedly delayed long and the

optical density reading decreased, that is, the fibrinolytic activity was decreased to a certain extent (Figure 5).

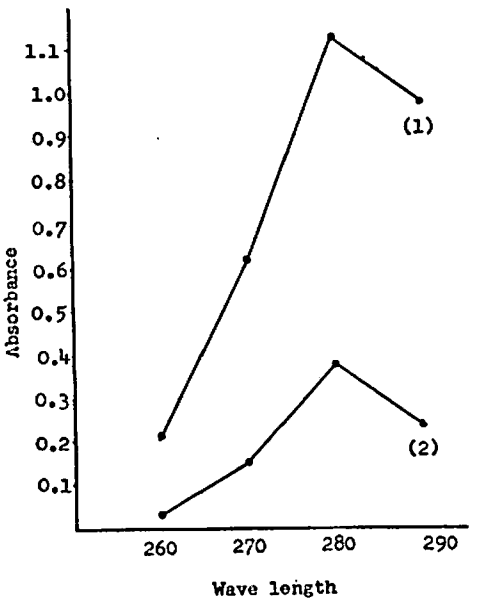

Fig. 5. Effect of addition of cystein to the extract from maxillary mucous membrane on fibrinolysis. Wave length (mμ)

(1): 1/15 M. phosphate saline solution (pH 7.4) 1.8 cc + extract (from isotonic saline solution with 10 times weight of the wet original mucous membrane).

(2): The solution to which cystein was added to (1) (final concentration 1%).

This fact showed that the fibrinolytic activity in the mucous membrane affected by maxillary sinusitis differed from cathepsin activity.

5. Effect of addition of ε-aminocaproic acid to the extract of mucous membrane affected by maxillary sinusitis.

According to the stated Ungar's method, the effect of ε-aminocaproic acid was observed, the final concentration of which was adjusted to 1%. It has been known that ε-aminocaproic acid is a plasmin inhibitor.

Results obtained indicated that ε-aminocaproic acid inhibited completely the fibrinolytic activity in the extract of mucous membrane affected by maxillary sinusitis.

Comparison of the fibrinolytic activities found in the mucous membrane of maxillary sinus, inferior turbinate and nasal polyp

Preceding experiments showed that the nasal tissues had fibrinolytic activity, each of them a little different activity. We adopted the Astrup's plate[8] method

to make distinct the differences of the fibrinolytic activity of them: the mucous membrane affected by maxillary sinusitis, the mucous membrane of inferior turbinate and nasal polyp.

The fibrinogen was contaminated with bovine plasminogen, thereby rendering the fibrin plate (0.1 per cent solution of bovine fibrinogen) susceptible to the action of plasminogen activator. The product of two perpendicular diameters of the lysed zone was used as a measure of proteolytic or activator activity (Figure 6).

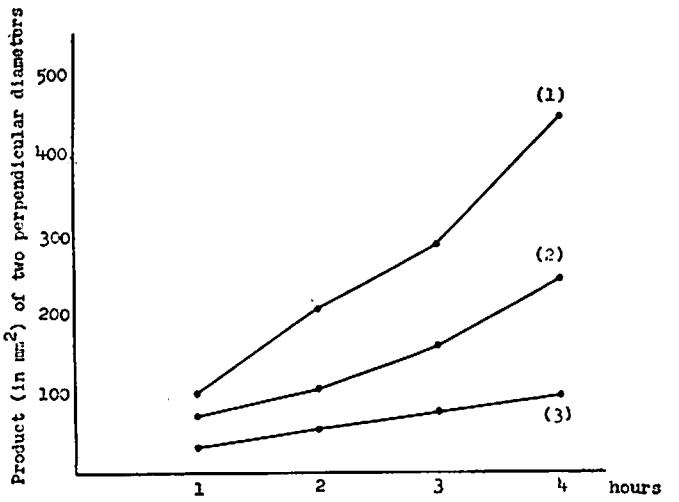

Fig. 6. Action of the extract from the mucous membrane of inferior turminate (1), maxillary sinus(2) and nasal polyp (3) (extract from isotonic saline solution with 10 times weight of the wet original material).

Ordinate: Product (in mm²) of two perpendicular diameters of the lysed zones (average of 3 single determinations).

Abscissa: Reaction time.

The fibrinolytic activity in the mucous membrane of inferior turbinate was more potent than that in the mucous membrane affected by maxillary sinusitis which was more potent than that of nasal polyp which was active.

In the experiment adopting the heated (for 45 minutes at 85°C) fibrin plate method, the lyzed zone by the extract in ten times its wet weight of isotonic saline solution was not observed.

But by the extract in three or four times its wet weight of isotonic saline solution, the lyzed zone was observed to some degree. It will be made certain by advanced experiments whether this fibrinolytic activity is plasmin proper or plasminogen activator.

Effect of fractions from maxillary mucous membrane extract on plasminogen

According to the Tagnon's method[9] a clot was formed, when the fibrinogen solution was mixed with plasminogen solution, tissue fractions (mitochondria, microsome and last supernate), and thrombin added. The time of dissolution of fibrin clot was measured, which told that the activity of the extract was found not in the fraction of microsome, supernate, but in the fraction of mitochondria.

Table 3
'Action of Maxillary Sinus Mucous Membrane Fractions on Plasminogen

Tube No.	Reagents in CC				Dissolution time of Clot (min)
	Total extract	Mitochondria	Microsome	Supernate	
1	0.3				25
2		0.3			20
3			0.3		120+
4				0.3	120+

All fraction were suspended in a volume equal to that of the original extract.
Fibrinogen 0.2 cc+globulin solution 0.1 cc+1/15 M phosphate buffer to make a total of 1 cc. Thrombin (on glass rod) added to each tube pH 7.4 temperature 37°C.

But also an experiment, where fibrinogen solution was mixed with mitochondria from extract, thrombin added, but plasminogen was not added, showed fairly fibrinolytic activity (Table 3).

Three similar fractionation experiments of faucial tonsil, were carried out, in all of which it was proved that fibrinolytic activity was absent in two particulated fractions and supernate.

DISCUSSION

It would be thought that there are differences in the physico-chemical process of morphological tissue. In the oto-rhino-laryngological field, there are also the differences in that of mucous membrane of maxillary sinus, faucial tonsil, pharyngeal tonsil (and stomach mucous membrane). When these tissues become morbid in inflammation, the differences are possibly more accelerated. The pathologic-physiological study of the inflammatory tissues would be of considerable help to that of physico-chemical mechanisms of the normal ones.

To investigate the proteolytic activity of each inflammatory tissue, a test was made for substrate of fibrin and fibrinogen. Fibrinolytic activity was observed in isotonic saline extracts of such tissues as mucous membrane of inferior turbinate and of maxillary sinus, but in those of faucial tonsil, pharyngeal tonsil and stomach mucous membrane it was not observed. It was found

that all tissues did not contain fibrinogenolysis.

By the plate method the fibrinolytic activity in nasal tissues was analyzed. Nasal polyp was one of the proper nasal tissues severely destroyed by inflammation or allergy. The mucous membrane affected by maxillary sinusitis had still the morphological structure of a nasal tissue, though it was considerably influenced by inflammation. The mucous membrane of inferior turbinate possessed its normal nasal tissue. It was examined in Astrup's plate method how the morphologically modified tissues changed the proteolytic activity. The result indicated that the mucous membrane of inferior turbinate is more potent than that affected by maxillary sinusitis which is more active than nasal polyp.

As for proteolytic enzymes there are plasmin, trypsin, pepsin, cathepsin and papain, etc., which have each its own varied characters. The peculiarities are as follows. (1) KCN, H_2S and others which activate cathepsin inhibit plasmin activity; (2) such inhibitory substances of cathepsin as H_2O_2 clearly activate plasmin activity. Accordingly plasmin is an enzyme with different nature from those of cathepsin, papain and others. We tested, to make the property clear, how the fibrinolytic activity in nasal tissues was changed by the addition of cystein, and found the fibrinolytic activity goes down at that time. It was also found that ε-aminocaproic acid inhibits the activity completely.

According to Astrup, Müllertz[8] and Sherry[10], the bovine fibrin used as a substrate in the method contains bovin plasminogen as a contamination, because no procedure is available which may produce bovine fibrinogen free from plasminogen.

Therefore the method of fibrin clot dissolution responds to fibrinolytic enzymes as well as to activators which are able to transform bovine plasminogen into plasmin. But it is possible to destroy the plasminogen by means of a heat denaturation, still with most of the sensitivity of the fibrin towards the proteolytic enzymes and to make it insensitive to fibrinolytic activator. Using the heat fibrin plate method by Astrup et al, we observed whether the tissue extracts dissolved the fibrin or not, and found the extract also dissolved the fibrin to some degree. Characteristics of the fibrinolytic activity are under investigation.

By Tagnon[9], and by Lewis and Ferguson[11] it was confirmed that animal tissues contained a fibrinolytic activator existing particularly in the microsome fraction. But Astrup[12] could not find the fibrinolytic activity in microsome by the method of Tagnon. And using potassium thiocyanate he succeeded in isolation of a soluble fibrinolytic activator from animal tissues. In order to acertain the view, we designed an experiment of function in tissue fraction. Results obtained showed, contrary to Tagnon's test, that fibrinolytic activity

existed in mitochondria, not in microsome, and moreover mitochondria presented the fibrinolytic activity without plasminogen. This experimental fact might signify that the contaminant plasminogen in bovine fibrinogen was activated by mitochondria or any enzyme in mitochondria which dissolved the fibrin. This nature is to be researched into. The active agent in tonsil was not detected in mitochondria, microsome and supernatant respectively.

Spector[13], Duthie and Chain[14] reported that polypeptide by the action of proteolytic enzyme offered a reasonable mechanism for the locally increased capillary permeability, and leucocyte infiltration was observed whenever tissue was injured. Also Collumbine and Rydon[5] stated that a substance or substances, similar to Menkin's leucotaxine, was found in blister fluids produced thermally or by the action of the vesicants mustard gas, and in phosgene lung oedema fluids.

According to Ungar et al[4], the addition of the specific antigen to slices of lung taken from a sensitized guinea pig, or the addition of anaphylactoid agents (tween 20, Octadecylamine, morphine, and 48/80) to tissue slices from normal animals, or the perfusion of lung with these agents, caused protein breakdown and liberation of histamine and heparin.

Suppression of histamine and heparin released by inhibition of proteolysis suggested that the latter was the more fundamental recation.

Buhrmester, Wenner[15], Baxter and Rose[16] fixed quantity of histamin in nasal tissue, and pointed out that nasal polyp and normal nasal mucous membrane contain histamine. They also investigated the variation of quantity of histamine in case of the tissue injured.

Whenever plasmin system in nasal mucous membrane was activated by antigen-antibody or kinase function derived from bacterial infection, such product as polypeptide increased in quantity and might accelerate to make locally capillary permeability, leucocyte infiltration and oedem, and would cause a tissue disturbance. The inhibition of plasmin activity to a certain low concentration would obstruct the cleaning function by plasmin activity and bring deposition of fibrin to tissue, fibrinous hypertrophie.

Astrup[17] stated that the tissue containing fibrinokinase is an organ with tendency of bleeding. And it is characteristic that nose has frequent opportunities of bleeding.

Astrup[18] confirmed that there was fibrinokinase in lung of human. It is of great interest that nose has a plasmin-like substance as in lung, when we recall that nasal tissue might be regarded as a part of the respiratory system organ. On the other hand, there is not fibrinolytic activity in stomach mucous

membrane of the digestive organ.

We hope the facts and the considerations which we detailed would make an useful offer for future investigations.

CONCLUSION

1. Fibrinolytic activity was measured in isotonic saline extract of such fresh tissue as (1) the mucous membrane of inferior turbinate and maxillary sinus (2) faucial tonsil and pharyngeal tonsil, and (3) stomach mucous membrane, which were all taken from patients on the occasion of their operations.

2. Results obtained indicated that: (1) the strongest activity was observed in the mucous membrane of inferior turbinate, (2) stronger activity in the mucous membrane affected by maxillary sinusitis, (3) fibrinolytic activity was weaker in nasal polyp, and (4) fibrinolytic activity was not observed at all in faucial tonsil, pharyngeal tonsil and stomach mucous membrane.

3. Fibrinolytic activity of nasal tissues was decreased to a certain extent by the addition of cystein; and completely inhibited by ε-aminocaproic acid, which has been known as a specific inhibtor of plasmin.

4. Fibrinolytic activity was found in the mitochondria fraction obtained from nasal tissues, but not in that from tonsil.

5. The nature of this fibrinolytic activity in detail are under further investigation.

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A SUPPRESSING EFFECT OF ε-AMINO-N-CAPROIC ACID ON THE BLEEDING OF DOGS, PRODUCED WITH THE ACTIVATION OF PLASMIN IN THE CIRCULATORY BLOOD

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INTRODUCTION

The more precise concept of fibrinolysis has long been inquired by the members of workers in different fields and from different angles; yet the evidences obtained are so contraversial that some fundamental investigations are, and will be, required to formulate the advanced concept of the phenomena of fibrinolysis which have been observed in various conditions of patients and experimental animals.

In fact, current interest on fibrinolysis has been raised by the promising development of fibrinolytic therapy[1] on such disorders as thromboembolic disease, which led some workers to regard the fibrinolysis as one of the repairing mechanism of a body[2].

However, another concept has been made from the very intense, pathological fibrinolysis, which was observed in some fatal conditions as so called "endless bleeding" of patients. One of the simplest examples of such pathological fibrinolysis, reported by Ratnoff[3] in 1952, was that his patient died after an operation for endless bleeding, the blood examination indicating very intense plasmin

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activity but no other significant primary causes of the bleeding. By many other investigators it has been also reported that a- or hypo-fibrinogenemia caused by pathological fibrinolysis resulted in the enhanced bleeding tendencies of surgical and obstetrical patients.

Thus the contemporary achievement poses two different tasks. One is investigation on the favourable effect of fibrinolysis just as seen in fibrinolytic therapy. The other is on the conceptive effect of pathologically accelerated fibrinolysis as seen in hemorrhage of dramatic intensity of certain patients.

Here a special attention was paid by us to the evidence that such an intense hemorrhage was not responsible to the usual treatments. This is the reason why it was undertaken by S. Okamoto and his colleagues to search for a synthetized antiplasmic substance in 1947. After having examined more than 300 kinds of the synthetized chemical compounds, ε-amino-n-caproic acid* was discovered as one of the most effective substances to suppress specifically the activation of plasmin and/or the plasmin activity in vitro and in vivo[4]. Side actions of ε were confirmed to be negligible even when a considerably large amount of ε was repeatedly administrated to experimental animals.

Taking advantage of the discovery of ε, the following animal experiments were designed by us to study (1) the relation of the intense fibrinolysis to hemorrhage and (2) the effect of administration of ε to the fibrinolysis and to the accompanied bleeding.

METHODS

a) Medium sized dogs, which had a healthy appearance and weighed 8 kg to 12 kg, were used. Narcotizing the dogs by the intravenous injection of sodium pentobarbiturate (sodium 5-ethyl-5-(1-methylbutyl)-barbiturate) solution, the blood pressure was recorded by the ordinary manometer, which was connected with the catheter inserted into the carotid artery. The trachea tube was inserted in the trachea to make ventilation easier. Through a small tube inserted in the trachea tube, oxygen was supplied to prevent anoxia of dogs. Injections of adequate cardiotonic agents to dogs were made when the condition of the dog became weakened.

b) It has generally been accepted that: (1) The blood of dogs shows a weak or no response to streptokinase**. (2) Human serum can be strongly activated with SK because of the presence of the proactivator-activator-system which is found in human serum and is very responsive to SK. (3) Plasminogen

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* abbreviated as ε in this text.
** hereinafter abbreviated as SK.

in dog's blood can be easily activated with SK in the presence of a small amount of human serum. Thus, based upon the results obtained from the fundamental experiments as described later, the following method was adopted to activate plasmin system in the circulatory blood of dogs. A kind of the Castellani's absorption method was adopted to remove natural aggulutinins of human serum to dog's red cells. About 50 cc of human serum was kept for 30 minutes at 0°C with an equal amount of washed red cells of dogs. By centrifuging the mixture at the refrigerated centrifuge, 50 cc of human serum was obtained. The sample was kept in a cold room. Before the administration of the serum to dogs, it was warmed for 15 minutes at room temperature and injected intravenously to dogs. It is demonstrated in the present paper that the administration of this human serum to the dogs caused no unfavourable side effects. According to the blood examination made on the blood sample taken after the administration of the human serum, there was only a small change of plasmin activity in the blood. Then, a given amount of SK was injected intravenously to dogs in order to cause the strong activation of plasmin in the circulatory blood.

c) Blood samples were taken time to time from dog veins during the experiment. Plasmin activity and the content of plasma fibrinogen in the sample were determined by the routine methods[5, 6, 7]. Fibrinogen content in plasma was gravimetrically measured according to the Gramm's method.

The method to examine variations of the bleeding tendency of dogs under given conditions was as follows:

(1) The skin of dog was incised 5–6 cm long at several parts on the trunk, and each incision was opened across about 3 cm wide.

(2) A certain size of filter paper, which had been weighed previously, was closely placed on the wound surface for 30 seconds, so that the blood oozed from the wound surface was absorbed into the filter paper as much as possible.

(3) The amount of blood absorbed was calculated by measuring again the weight of the filter paper used. The weight of blood absorbed was understood to indicate the degree of "oozing."

d) The effect of the intravenous administration of ε on plasmin activity as well as on the bleeding tendency was examined, and appropriate and necessary controls were taken.

Details of the experimental method were given in the case report of animal experiments.

RESULTS

a) *Influence of the addition of a small amount of human serum on the activation of plasminogen with SK in the blood of dogs.*

These experiments, carried out in vitro, were undertaken to determine quantitative details of the experimental method to activate the plasmin system in the circulatory blood of dogs by the intravenous administration of human serum and SK. Fifteen test tubes were divided into three groups, A, B and C, respectively. 0.5 cc of the oxalated plasma of dogs, various amounts of SK dissolved in 0.1 cc of a physiological saline solution, 0.05 cc of human serum and a physiological saline solution of ε were mixed in different combinations as described in the following table. Then, fibrin clot was made by adding 0.05 cc of 2% $CaCl_2$ and some amount of physiological saline solution to make total volume 1.0 cc. Test tubes were then incubated at 37°C for 30 minutes. After that, the total dry weight of fibrin in each reaction mixture was gravimentrically measured according to the method of Gramm.

The following table shows percentages of the breakdown of fibrin in each tube: (Taking standard fibrin as 100)

Test tube No.	Reaction mixture				Percentage of the breakdown of fibrin
	Dog plasma (cc)	Human serum (cc)	SK (unit)	2.5 % ε solution (cc)	
A-1	0.5	—	0	—	0
A-2	0.5	—	10	—	3
A-3	0.5	—	100	—	9
A-4	0.5	—	1,000	—	70
A-5	0,5	—	10,000	—	100
B-1	0.5	0.05	0	—	0
B-2	0.5	0.05	10	—	100
B-3	0.5	0.05	100	—	100
B-4	0.5	0.05	1,000	—	100
B-5	0.5	0.05	10,000	—	100
C-1	0.5	0.05	0	0.1	0
C-2	0.5	0.05	10	0.1	3
C-3	0.5	0.05	100	0.1	5
C-4	0.5	0.05	1,000	0.1	2
C-5	0.5	0.05	10,000	0.1	100

From the above mentioned results, we confirmed that the addition of 0.05 cc of human serum to 0.5 cc of dog plasma in the presence of 10 units of SK resulted in sufficient activation of plasmin and the complete breakdown of fibrin, but, when ε was added, the breakdown of fibrin by plasmin was strongly arrested. These results presented enough evidence for determining the experimental conditions of sufficient activation of plasmin in the circulatory blood of dogs. On the basis of the results, 50 cc of human serum and 10,000 units of SK were administrated intravenously to dogs weighing about 10 kg, we expecting sufficient activation of plasmin in the circulatory blood.

b) *Influence of the sole administration of SK on plasmin activity and oozing.*

Case No. 1: Experimental Record No. 3. Date: Oct. 25, 1958.

Dog's name: Elizabeth Adult, Sex: Female, Body weight: Ca. 10 kg.

(1) Control blood sample was taken at 3:00 p.m. and the following result was obtained: i) Natural clot lysis test: The coagulation time was 4 minutes 30 seconds and clot lysis did not occur within 3 hours. ii) The content of plasma fibrinogen was 630 mg/dl of plasma.

(2) At 3:45 p.m., 20,000 units of SK, which was dissolved in 2 cc of physiological saline solution, was injected intravenously into the dog. At 3:55 p.m., a second blood sample was taken. Plasmin activity of this sample was examined by the method of natural clot lysis test. The coagulation time was 3 minutes 30 seconds and clot lysis occurred in 50 minutes. The content of plasma fibrinogen was 625 mg/dl of plasma.

(3) Results described in item (1) and (2) indicated that the sole administration of a sufficient amount of SK to the dog was accompanied only by slight activation of the plasmin system in the circulatory blood and that, by the sole administration of SK, any noticiable oozing from the incised wound was not observed at all.

(4) It was indicated also (i) that, by the sole administration of SK, there was only a very weak response of the plasmin system in the circulatory blood of the dog, without accompanying any sign of oozing, and (ii) that these results coincided with the results obtained in vitro under similar experimental conditions described in A group in the above table.

Case No. 2: Experimental Record No. 24. Date: May 30, 1959.

Dog's name: James Adult, Sex: Male, Body weight: Ca. 9 kg.

(1) At 4:09 p.m., the blood pressure was 120–130 mm Hg and oozing was 11.5 mg/sec. Control blood sample was taken at the same time and the following result was obtained. i) Natural clot lysis test: The coagulation time was 4 minutes and 10 seconds, and lysis did not occur within 3 hours. ii) Lewis' euglobulin lysis test: 2.38 units/0.5 cc of plasma. iii) Δ of absorbance at 280 mμ U.V.: 0.008. iv) Whole clot lysis test: 8 days. v) The content of plasma fibrinogen: 710 mg/dl of plasma.

(2) At 4:23 p.m., 50,000 units of SK was injected intravenously into the dog. At 4:26 p.m., a second blood sample was taken and the following result was obtained. i) Natural clot lysis test: The coagulation time was 3 minutes 10 seconds and lysis of the clot did not occur within 3 hours. ii) Lewis' euglobulin lysis test: 2.38 units/0.5 cc of plasma. iii) Δ of absorbance of 280 mμ U.V.: 0.023. iv) Whole clot lysis test: 5 days. v) The content of plasma fibrinogen: 600 mg/dl of plasma.

Throughout the experiment, blood pressure did not show any change and no increased oozing was observed.

(3) It was indicated (i) that, by the intravenous administration of SK, plasmin activity in the circulatory blood of the dog was only very slightly activated without accompanying any sign of increased bleeding, and (ii) that these results coincided with those obtained in vitro tests described in A group in the above table.

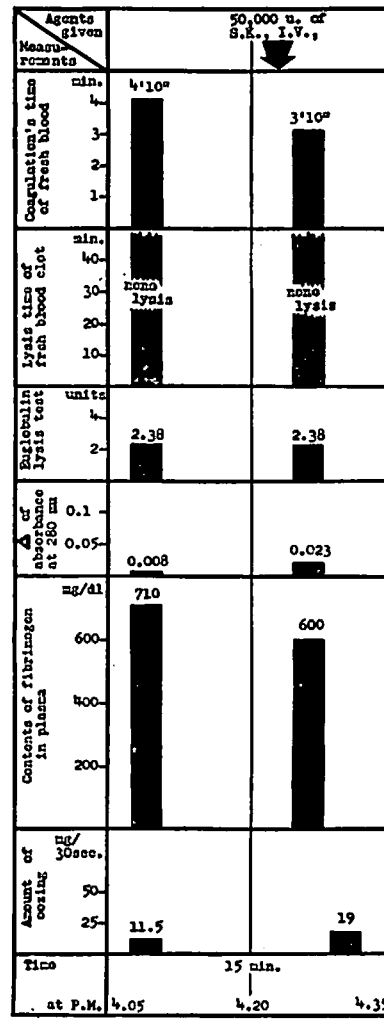

Fig. 1. Influence of the sole administration of S.K. on plasmin activity and oozing.

c) *Influence of the administration of human serum and SK on plasmin activity and oozing.*

Case No. 3: Experimental Record No. 4. Date: Oct. 31, 1958.

Dog's name: Shiro Adult, Sex: Male, Body weight: Ca. 10 kg.

(1) At 3:36 p.m., control blood sample was taken. Plasmin activity of the sample was examined and the following result was obtained. i) Natural clot lysis test: The coagulation time was 7 minutes and the natural clot lysis time was 28 minutes. ii) The content of plasma fibrinogen: 642 mg/dl of plasma. At 4:06 p.m., the amount of oozing was 9 mg/30 sec.

(2) At 4:10 p.m., 50 cc of human serum which underwent Castellani's absorption, was injected intravenously. A second blood sample was taken at 4:12 p.m. and the following result was obtained. Natural clot lysis test: The coagulation time was 4 minutes 30 seconds and the natural colt lysis time 25 minutes. Increased oozing was not observed. The blood pressure was 155–165 mm Hg.

These results showed that natural clot lysis in the blood and oozing was not enhanced by the sole administration of human serum.

(3) At 4:38 p.m., 100,000 units of SK was administered intravenously to the dog. Plasmin activity of the sample, which was taken at 4:40 p.m. was examined. i) The coagulation of fresh blood did not occur within 3 hours, indicating a strong activity of plasmin in the blood sample. The amount of oozing was 10 mg/30 sec and the blood pressure was 110 mm Hg. ii) The blood pressure was maintained

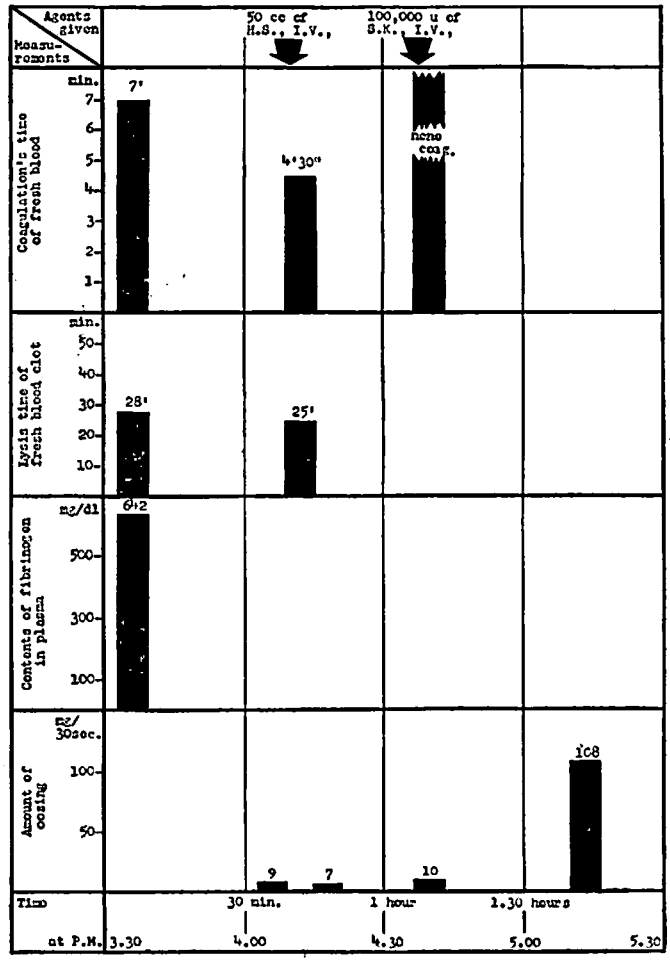

Fig. 2. Influence of the administration of human serum and S.K. on plasmin activity of oozing.

at 175–165 mm Hg, by the administration of Noradrenalin, upon which oozing started to occur very strongly. The amount of oozing measured increased to 108 mg/30 sec.

(4) This result showed (i) that plasmin activity in the circulatory blood and oozing from the incised wound remained actually unchanged by the preliminary administration of human serum which underwent Castellani's absorption and (ii) that by the successive administration of SK in addition to human serum, plasmin activity became high and oozing was distinctively enhanced, just as expected from the results obtained in vitro tests of B group in the above table.

d) *Inflence of the administration of ε on the high activity of plasmin and oozing caused by the administration of human serum plus SK.*

Case No. 4: Experimental Record No. 5. Date: Nov. 5, 1958.

Dog's name: Robert Adult, Sex: Male, Body weight: Ca. 10 kg.

(1) Control blood sample was taken at 3:15 p.m. and the following results were obtained. i) Natural clot lysis test: The coagulation time was 8 minutes 30 seconds and clot lysis did not occure within 3 hours. ii) Lewis' euglobulin lysis test: 2.5 units/0.5 cc of plasma. iii) Δ of absorbance at 280 mμ U.V. was not detectable. iv) The content of plasma fibrinogen: 852 mg/dl of plasma. The blood pressure was 125–135 mm Hg and oozing did not occur from the incised wound.

(2) At 3:36 p.m., 50 cc of human serum which underwent Castellani's absorption was injected intravenously and at 4:23 p.m., 70,000 units of SK was administered intravenously to the dog. Plasmin activity of the sample, which was taken at 4:26 p.m. was examined and the result was as follows. i) Natural clot lysis test: The coagulation time was 5 minutes 30 seconds and lysis time of the clot was 45 minutes. ii) Lewis' euglobulin lysis test: More than 5.0 units/0.5 cc of plasma. iii) Δ of absorbance at 280 mμ U.V.: 0.24. iv) The content of plasma fibrinogen: 682 mg/dl of plasma. At the same time, the blood pressure, which underwent a transient fall during the intravenous administration of SK, recovered nearly to normal by the intravenous administration of Noradrenalin. The amount of oozing was 10 mg/30 sec.

This result showed that plasmin in the circulatory blood of the dog was activated very strongly by the intravenous administration of human serum plus SK.

(3) At 4:50 p.m., when the blood pressure had recovered to normalcy by the intravenous administration of 100 cc of Dextran, oozing was observed, and the amount of oozing was 70 mg/30 sec. Oozing occurred strongly after the recovery of the blood pressure. At 5:05 p.m., about 150 cc of Dextran was administered intravenously.

(4) At 5:15 p.m., 100 cc of 5% ε solution was administrated intravenously. The blood pressure recovered to normalcy, and the amount of oozing was 40 mg/30 sec. At the same time, a third blood sample was taken and plasmin activity examined. The result was as follows. i) Lewis' eugloblin lysis test: 2.5 units/0.5 cc of plasma. ii) Δ of absorbance at 280 mμ U.V.: 1.08. iii) The content of plasma fibrinogen: 308 mg/dl of plasma.

(5) At 6:04 p.m., 20 cc of 5% ε solution was administrated supplementally further to decrease plasmin activity. The last blood sample was taken at 6:35 p.m., and plasmin activity was examined. The result was as follows. i) Lewis' euglobulin lysis test: 0.8 units/0.5 cc of plasma. ii) Δ of absorbance at 280 mμ U.V.: was not detactable. iii) The content of plasma fibrinogen was 472 mg/dl of plasma. At the same time, the amount of oozing was 25 mg/30 sec and the blood pressure remained normal.

This result showed that plasmin activity and oozing caused by the administration of human serum plus SK had decreased by the intravenous administration of ε.

Case No. 5: Experimental Record No. 17. .Date: April 13, 1959.

Dog's name: Jupiter II Adult, Sex: Male, Body weight: Ca. 10 kg.

(1) At 4:00 p.m., control blood sample was taken. Plasmin activity was examined and the result was as follows. i) Natural clot lysis test: The coagulation time of the fresh blood was 2 minutes and the lysis time of natural clot was 21 minutes. ii) Lewis' euglobulin lysis test: 0.81 units/0.5 cc of plasma. iii) Δ of absorbance at 280 mμ U.V. was not detectable. iv) The contents of plasma fibrinogen: 720 mg/dl of plasma.

The blood pressure was 130–135 mm Hg at 4:23 p.m. The amount of oozing was 11 mg/30 sec.

(2) At 4:29 p.m., 50 cc of human serum was injected intravenously into the dog. The second blood sample was taken at 4:30 p.m. The plasmin activity of the sample was as follows. i) Natural clot lysis test: The coagulation time of the natural clot was 3 minutes and the lysis time was 16 minutes. ii) Lewis' euglobulin lysis test: 1.47 units/0.5 cc of plasma. iii) Δ of absorbance at 280 mμ U.V. was negative. iv) The content of plasma fibrinogen: 640 mg/dl of plasma.

The blood pressure was 125–130 mm Hg. The amount of oozing was 5 mg/30 sec.

These results showed that, by the administration of human serum, plasmin activity was little changed and the amount of oozing was not increased.

(3) At 4:43 p.m., 70,000 units of SK was administered intravenously to the dog and the third blood sample was taken at 4:55 p.m. The plasmin activity of the sample was examined. i) Natural clot lysis test: The coagulation time was 3 minutes 30 seconds and the lysis time 16 minutes 30 seconds. ii) Lewis' euglobulin lysis test: 6.0 units/0.5 cc of plasma. iii) Δ of absorbance at 280 mμ U.V. was not detectable. iv) The content of plasma fibrinogen: 260 mg/dl of plasma. At 4:55 p.m., the blood pressure was 130–135 mm Hg and oozing was increased to 35 mg/30 sec.

(4) At 5:06 p.m., the intravenous administration of 30,000 units of SK was carried out supplementally with intent to enhance the activation of plasmin as much as possible.

(5) At 5:10 p.m., blood examination was carried out and the result was as follows. i) Natural clot lysis test: The coagulation time was 3 minutes and the lysis

time 6 minutes. ii) Lewis' euglobulin lysis test: 5.0 units/0.5 cc of plasma. iii) Δ of absorbance at 280 mμ U.V. was negative. iv) The content of plasma fibrinogen: 220 mg/dl of plasma.

At 5:11 p.m., the blood pressure was 120-130 mm Hg. Oozing was observed to have increased to 38 mg/30 sec.

(6) Twenty cubic centimentres of 5% ε solution was injected intravenously to the dog at 5:46 p.m., we expecting to suppress plasmin activity. A blood sample was taken at 5:56 p.m. and the result obtained was as follows: i) Natural clot lysis test: The coagulation time was 2 minutes 30 seconds and lysis of the clot did not occur within 3 hours. ii) Lewis' euglobulin lysis test: 1.56 units/0.5 cc of plasma. iii) Δ of absorbance at 280 mμ U.V. was negative. iv) The content of plasma fibrinogen: 460 mg/dl of plasma.

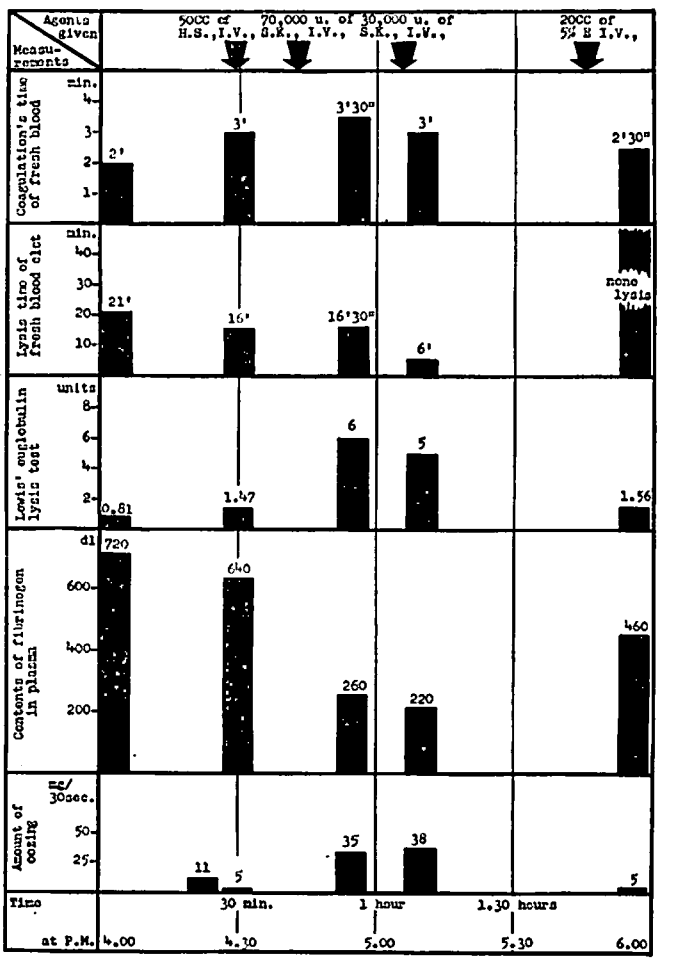

Fig. 3. Influence of ε on high activity of plasmin and oozing caused by administration of human serum plus streptokinase.

At 5:55 p.m., the blood pressure was 160–165 mm Hg and oozing decreased distinctly to 5 mg/30 sec.

(7) This result showed that high plasmin activity in the blood and oozing caused by the administration of human serum plus SK, were distinctly suppressed by the intravenous administration of ε.

Case No. 6: Experimental Record No. 20. Date: April 24, 1959.

Dog's name: Orient II Adult, Sex: Male, Body weight: Ca. 12 kg.

(1) Control blood sample was taken at 5:00 p.m. and plasmin activity was examined. i) Natural clot lysis test: The coagulation time was 5 minutes and lysis of the clot did not occur within 3 hours. ii) Lewis' euglobulin lysis test: Lower than 0.56 units/0.5 cc of plasma. iii) Whole clot lysis test: 3 days. iv) The content of plasma fibrinogen: 680 mg/dl of plasma.

At 5:00 p.m., the blood pressure was 165–166 mm Hg and the amount of oozing was 9 mg/30 sec.

(2) At 5:50 p.m., 50 cc of human serum was administered intravenously to the dog and at 5:53 p.m. a second blood sample was taken. Plasmin activity was examined and the result was as follows: i) Natural clot lysis test: The coagulation time was 4 minutes an lysis of the clot did not occur within 3 hours. ii) Lewis' euglobulin lysis test: Less than 0.56 units/0.5 cc of plasma. iii) Whole clot lysis test: 3 days. iv) The content of plasma fibrinogen: 700 mg/dl of plasma.

At 5:00 p.m., the blood pressure was 160–162 mm Hg and the amount of ooizng was 15 mg/30 sec.

This result showed that, by the sole administration of human serum, neither plasmin activity nor oozing tendency underwent any noticeable change.

(3) At 6:03 p.m., 50,000 units of SK was injected intravenously, whereby a transient fall of the blood pressure to 75 mm Hg occurred at 6:08 p.m., which recovered to normalcy after about 10 minutes.

(4) At 6:25 p.m., 30,000 units of SK was administered supplementally. The third blood sample was taken at 6:30 p.m. The plasmin activity of the sample was examined and the result obtained was as follows: i) Natural clot lysis test: The coagulation time was 1 minute and lysis time of the clot was 6 minutes. ii) Lewis' euglobulin lysis test: More than 10 units/0.5 cc of plasma. iii) Whole clot lysis test: 2 hours and a half. iv) The content of plasma fibrinogen was 160 mg/dl of plasma.

The amount of oozing increased to 115 mg/30 sec. at 7:35 p.m., and the blood pressure was 148–152 mm Hg.

This result indicated that a strong activation of plasmin and an increased oozing were produced by the administration of human serum plus SK.

(5) Twenty cubic centimetres of 5% ε solution was injected intravenously at 7:48 p.m. and supplemented by the intravenous administration of 20 cc of 5% ε solution at 8:43 p.m.

The last blood sample of the experiment was taken at 8:46 p.m. The plasmin activity of the sample was examined and the result was as follows: i) Natural

clot lysis test: The coagulation time was 4 minutes and lysis time of the clot was 11 minutes. ii) Lewis' euglobylin lysis test: 0.714 units/0.5 cc of plasma. iii) Whole clot lysis test: 4 days. iv) The content of plasma fibrinogen: 400 mg/dl of plasma.

At 7:48 p.m., the amount of oozing was 55 mg/30 sec and the blood pressure was 180–190 mm Hg.

(6) This result showed that, by the administration of ε, high plasmin activity and oozing caused by the administration of human serum plus SK were distinctly suppressed.

Case No. 7: Experimental Record No. 22. Date: May 13, 1959.

Dog's name: Keiko Adult, Sex: Female, Body weight: Ca. 9 kg.

(1) Control blood sample was taken at 5:08 p.m. The plasmin activity of the sample was examined. i) Natural clot lysis test: The coagulation time was 2 minutes and lysis of the clot did not occur within 3 hours. ii) Lewis' euglobulin lysis test: Less than 0.56 units/0.5 cc of plasma. iii) Δ of the absorbance at 280 mμ U.V.: 0.056. iv) Whole clot lysis test: 4 days. v) The content of plasma fibrinogen: 570 mg/dl of plasma.

The oozing was 5 mg/30 sec at 5:30 p.m. and the blood pressure was 130–140 mm Hg.

(2) At 5:45 p.m., 50 cc of human serum was injected intravenously. At 5:51 p.m., the second blood sample was taken. Plasmin activity was examined and the result obtained was as follows: i) Natural clot lysis test: The coagulation time was 1 minute 15 seconds and lysis of clot did not occur within 3 hours. ii) Lewis' euglobulin lysis test: Less than 0.56 units/0.5 cc of plasma. iii) Δ of the absorbance at 280 mμ U.V.: 0.017. iv) Whole clot lysis test: 4 days. v) The content of plasma fibrinogen: 590 mg/dl of plasma.

The blood pressure was 130–140 mm Hg.

(3) At 6:04 p.m., 50,000 units of SK, dissolved in 5 cc of physiological saline solution, was injected intravenously into the dog. At 6:13 p.m., the third blood sample was taken and the amount of oozing increased to 25 mg/30 sec. The plasmin activity of the sample was examined and the result obtained was as follows: i) Natural clot lysis test: The coagulation time was 2 minutes 10 seconds and the lysis time of the clot was 3 minutes 15 seconds. ii) Lewis' euglobulin lysis test: More than 50 units/0.5 cc of plasma. iii) Δ of the absorbance at 280 mμ U.V.: 0.160. iv) Whole clot lysis test: 30 minutes. v) The content of plasma fibrinogen: 180 mg/dl of plasma.

The amount of oozing increased to 50 mg/30 sec at 6:18 p.m. and the blood pressure was 125–130 mm Hg.

(4) This result showed that plasmin activity in the blood was extremely accelerated and oozing was increased several times by the administration of human serum and SK.

(5) At 7:07 p.m., 20 cc of 5% ε solution was administered intravenously. Then twice at 7:40 p.m. and 8:14 p.m., injections of 20 cc of 5% ε solution were carried out.

The last blood sample was taken at 8:21 p.m. The plasmin activity of the sample was examined and the result was as follows: i) Natural clot lysis test: The coagulation time was 5 minutes 50 seconds and lysis of the clot did not occur within 3 hours. ii) Lewis' euglobulin lysis test: 0.83 units/0.5 cc of plasma. iii) Δ of the absorbance of 280 mμ U.V. turned negative. iv) Whole clot lysis test: 5 days. v) The content of plasma fibrinogen: 240 mg/dl of plasma.

At 8:21 p.m., the amount of oozing was 24 mg/30 sec and the blood pressure was 130–140 mm Hg.

(6) This result showed that intense plasmin activity and oozing, which had been caused by the administration of human serum and SK, were decreased by the repeated administration of ε.

e) *Influence of the preliminary administration of ε on the activation of plasmin and oozing caused by the administration of human serum and SK.*

Case No. 8: Experimental Record No. 7. Date: Nov. 13, 1958.

Dog's name: Marie Adult, Sex: Female, Body weight: Ca. 9 kg.

(1) Control blood sample was taken at 3:45 p.m. The plasmin activity of the sample examined was as follows: i) Natural clot lysis test: The coagulation time was 4 minutes 45 seconds and lysis of the clot did not occur within 3 hours. ii) Lewis' euglobulin lysis test: 0.5 units/0.5 cc of plasma. iii) Δ of the absorbance at 280 mμ U.V. was not detectable. iv) The content of plasma fibrinogen: 950 mg/dl. At 4:25 p.m., the amount of oozing was 1 mg/30 sec and the blood pressure was 100 mm Hg.

(2) At 4:33 p.m., 20 cc of 5% ε solution was injected intramuscularly and at 4:35 p.m. 40 cc of 5% ε solution was injected intravenously. At 4:40 p.m., 50 cc of human plasma, which had received Castellani's absorption with the dog's erythrocytes, was administered intravenously and, at 4:54 p.m., 50,000 units of SK was also injected intravenously into the dog. The plasmin activity of the second blood sample, which was taken at 4:56 p.m., was as follows: i) Natural clot lysis test: The coagulation time was 5 minutes 30 seconds and clot lysis occured 30 minutes later. ii) The content of plasma fibrinogen: 230 mg/dl of plasma. At 5:00 p.m., the blood pressure fell transiently to 80 mm Hg, but it recovered within 5 minutes. The amounts of oozing measured at 5:00 p.m. and at 5:10 p.m. were both 0 mg/30 sec.

(3) This result showed that the preliminary administration of ε was effective in suppressing the activation of plasmin in plasma and oozing caused by the administration of human serum plus SK.

Case No. 9: Experimental Record No. 23. Date: May 22, 1959.

Dog's name: Hercules Adult, Sex: Male, Body weight: Ca. 11 kg.

(1) Control blood sample was taken at 4:25 p.m. The plasmin activity of the sample was as follows: i) Natural clot lysis test: The coagulation time was 3 minutes 30 seconds and lysis of the clot did not occur within 3 hours. ii) Whole clot lysis test: 4 days. iii) The content of plasma fibrinogen: 1,000 mg/dl of plasma. The

blood pressure was 140–150 mg Hg.

no noticeable oozing could be observed.

(2) At 4:39 p.m., 40 cc of 5% ε solution was administered intravenously and 20 cc of 5% ε solution was given intramuscularly. The second blood sample was taken at 4:46 p.m. The plasmin activity of the sample examined was as follows: i) Natural clot lysis test: The coagulation time was 2 minutes 40 seconds and lysis of the clot did not occur within 3 hours. ii) Whole clot lysis test: 4 days. iii) The content of plasma fibrinogen: 1,010 mg/dl of plasma.

The amount of oozing was 5 mg/30 sec and the blood pressure 150–160 mm Hg at 4:43 p.m.

(3) At 4:52 p.m., 50 cc of human serum was administered intravenously and at 5:04 p.m., 50,000 units of SK was injected intravenously. The third blood sample was taken at 5:06 p.m. The plasmin activity of the sample was as follows: Natural clot lysis test: The coagulation time was 1 minute and lysis of the clot did not occur within 3 hours.

The blood pressure fell slightly from 165–175 mm Hg to 140–145 mm Hg, but immediately it recovered to the former level after 10 minutes. The amount of oozing was 5 mg/30 sec at 5:08 p.m.

This result showed that, by the preliminary administration of ε, plasmin activity and oozing were not increased in spite of the administration of 50 cc of human serum and 50,000 units of SK.

(4) The supplementary administration of 50,000 units of SK was provided intravenous-

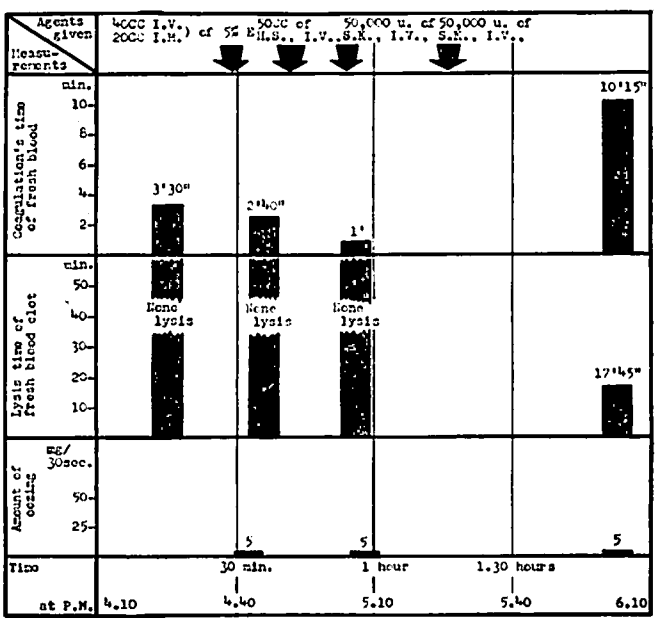

Fig. 4. Influence of the preliminary administration of ε on the activation of plasmin and oozing caused by the administration of human serum and S.K.

ly at 5:26 p.m.

(5) The blood sample which was taken at 6:03 p.m. at the end of this experiment was as follows: i) The coagulation time of fresh blood was 10 minutes 15 seconds and the lysis time of this clot was 17 minutes 45 seconds later. ii) Whole clot lysis test: Coagulation did not occur. iii) The content of plasma fibrinogen: 150 mg/dl of plasma. The blood pressure was 145–150 mm Hg. The amount of oozing was 5 mg/30 sec.

This result showed that the preliminary administration of ε was effective in suppressing the activation of plasmin of plasma and oozing caused by the administration of 50 cc of human serum and of the total of 100,000 units of SK which previously proved to be sufficient to produce the activation of plasmin and an obvious oozing.

f) *Influence of the administration of ε on the oozing tendency caused by the administration of Heparin.*

Case No. 10: Experimental Record No. 18. Date: April 20, 1959.

Dog's name: Pegasus Adult, Sex: Male, Body weight: Ca. 12 kg.

This experiment was carried out in order to ascertain whether the administration of ε was effective against the oozng tendency which was caused without significant change of plasmin by another anti-coagulant Heparin. During the experiment, oxygen gas was provided to the dog through tubes inserted intratracheally.

(1) The amount of oozing measured at 6:15 p.m. was 11 mg/30 sec.

(2) At 6:25 p.m., 50 mg of Heparin was injected intravenously. At 6:30 p.m., oozing occurred from the incised wound, and the amount of oozing increased to 75 mg/30 sec.

(3) At 6:36 p.m., the supply of oxygen gas was stopped, and the trachea tube was narrowed to produce the anoxic state in the dog. Oozing was intensely increased with the disturbance of ventilation. At 6:45 p.m., the amount of oozing became as remarkable as 205 mg/30 sec.

(4) At 6:55 p.m., the narrowed trachea tube was reopened and the supply of oxygen gas was again provided; oozing decreased thereafter. At 7:10 p.m., the amount of oozing was 46 mg/30 sec.

(5) At 7:15 p.m., the trachea tube was narrowed again to a quarter of the original area and the supply of oxygen gas was stopped. Oozing resumed and increased. At 7:25 p.m., the amount of oozing was 165 mg/30 sec.

(6) At 7:42 p.m., 20 cc of 5% ε solution was administered intravenously to the dog during the state of anoxia, but the increased oozing was not suppressed. The amount of oozing, which was measured 5 minutes after the injection of ε, was 183 mg/30 sec.

(7) Resuming the supply of oxygen gas at 8:08 p.m., the oozing clearly decreased to 40 mg/30 sec at 8:13 p.m.

(8) Plasmin activity examined during the course of the experiment showed no significant change.

(9) This result showed (i) that oozing occurred more or less after the intravenous administration of Heparin, (ii) that, during the anoxic states, oozing increased 5 tiems and (iii) that the administration of ε was not effective in lessening oozing caused by Heparin. Attention was paid to these results, in which activation of plasmin was not observed in bleeding caused by Heparin.

From this evidence, it is clear that oozing can be produced not only by the activation of plasmin, but also by the administration of Heparin plus anoxia. Emphasis is placed on the evidence that ε was not effective against oozing caused by Heparin plus anoxia.

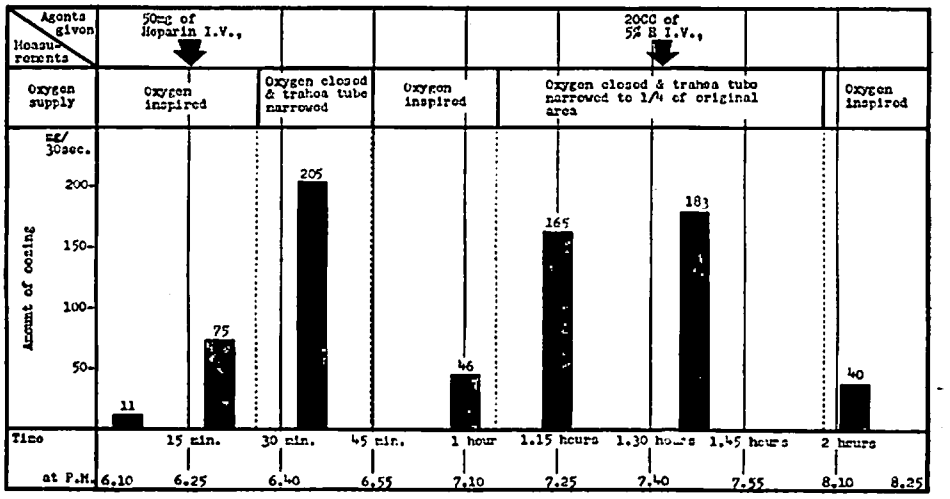

Fig. 5. Influence of ε on the oozing tendency caused by the administration of heparin.

DISCUSSION

It is known that the intravenous, sole administration of SK to dogs resulted in slight or no activation of plasmin in the circulatory blood. In fact, the results obtained from cases Nos. 1 and 2 showed that there was only a weak response of the plasmin system of dogs to the intravenous, sole administration of a sufficient amount of SK.

Results obtained from cases Nos. 3, 4, 5, 6 and 7 showed that no change of the plasmin system in the blood of dogs occurred by the intravenous, sole administration of human serum which underwent the mentioned Castellani's absorption.

The effect of the intravenous administration of human serum plus SK to dogs was examined in cases Nos. 3, 4, 5, 6, and 7.

(1) Plasmin activity was observed to be very strongly activated in the circulatory blood of dogs, when examined by the method of natural clot lysis tests of

fresh blood carried out at 37°C, euglobulin lysis test[5], the measurements of extinction of 280 mμ Ultraviolet test[6] and whole clot (plasma clot) lysis test[7].

(2) When plasmin in the circulatory blood was strongly activated by the intravenous administration of human serum plus SK, a distinct increase of bleeding i.e. an increased oozing from the incised wound was recorded.

By the intravenous administration of ε which was carried out after the occurrence of an obvious oozing with the activation of plasmin system in dogs, the inhibition of the activity of plasmin in the circulatory blood of dogs and the decrease of the oozing tendency from the incised wound were both observed. These results were demonstrated in cases Nos. 4, 5, 6 and 7.

Effects of ε administrated intravenously to dogs prior to the administration of human serum plus SK were demonstrated in results obtained from cases Nos. 8 and 9. These results showed that the preliminary administration of ε diminished the degree of the activation of plasmin to be caused by the intravenous administration of human serum plus SK and prevented the occurance of any noticeable oozing, which could appear after the intravenous administration of human serum plus SK without the previous administration of ε.

We summarize (1) that, when plasmin activity in the circulatory blood of dogs was fully activated by the intravenous administration of human serum plus SK, an increased oozing from the incised wound occurred, and (2) that, by the administration of ε, the activation of the plasmin system in the blood and the oozing from the incised wound were clearly suppressed.

It was previously mentioned by us that the activation of the plasmin system in the circulatory blood or possibly in the locus can be a cause of some kinds of disorders, and that the administration of ε to patients suffering from such disorders was effective in combating the activation of plasmin and in treating the disorder associated with the activation of plasmin system[4].

Though we have not neglected the possibility that the activation of plasmin in some pathologic states can be produced by a more primary or more profound "cause" such as the release of cytoactivator of plasmin from the injured tissue or the occurrence of antigen antibody reaction in case of diseases suspected to be allergic, emphasis should be placed on another aspect of the activation of plasmin, that is, the activation of plasmin and the resultant high activity of plasmin can be "a cause" of some pathologic states such as bleeding with hypofibrinogenemia or disturbed mechanism of clot formation in more or less injured regions. If, by some example the activation of plasmin can be regarded to be the result of another injury, such illustration does not deny the possibility that

the activated plasmin can be a direct cause of disorders such as bleeding.

We say, after reasonable consideration, (1) that the model experiments in dogs to produce pathologic states with high activation of plasmin in the blood and to improve the state with the administration of ε, presented sufficiently scientific evidence, confirming the relation of cause and effect of antiplasmin therapy, (2) that the evidence demonstrated here coincided with the clinical evidence, that is, the administration of ε was effective in combating the activation of plasmin and the related pathologic states, and (3) that the method to combat the activation of plasmin system or high activity of plasmin in the circulatory blood with the administration of ε can be merit and of utility in treating the pathologic states associated with and caused by the activation of the plasmin system or high activity of plasmin.

The following are naturally deduced from the evidence demonstrated above:

(1) A strong activation of plasmin in the blood experimentally produced can cause an increased bleeding tendency of dogs.

(2) The activation of plasmin and high plasmin activity in the blood as well as an increased tendency of oozing was clearly suppressed by the intravenous administration of ε which was carried out either prior or posterior to the experimental process of activating plasmin in the blood.

(3) Such an increased tendency of oozing as made by giving Heparin and producing an experimental anoxia in dogs was not accompanied by any signs of the activation of plasmin in the blood, nor improved at all by the intravenous administration of ε.

(4) The experimental activation of plasmin in the blood in these experiments was not a result but a cause of the increased tendency of oozing.

(5) The activation of plasmin found in the blood of some kinds of patients is not a simple, final result, but it can be a cause of bleeding or other associated disorders, as previously stated by us.

The essential evidence of the antiplasmic therapy, that is, the successful application of ε in suppressing the activation of plasmin or high plasmin activity of human blood and in treating those kinds of diseases which were accompanied and regarded to be caused by the activation of plasmin in the blood, was reproduced and experimentally confirmed by the obvious evidence obtained from the mentioned animal experiments. The typical coincidence is found between the clinical and the experimental evidence: In such disorders as (i) bleeding in metropathia hemorrhagica[8], bleeding tendency of aplastic anemia or leucemia[9] which were revealed by the other members of the project at School of Medicine, Keio University, and as (ii) oozing experimentally produced in dogs, high activity

of plasmin of the blood and the increased bleeding tendency were both at the same time observed, and the suppression of the high plasmin activity by the administration of ε resulted in the distinct improvement of the above-mentioned bleeding.

It was reasonably deduced from the evidences described in the present paper, that the method to combat the activation of plasmin system or high activity of plasmin in the circulatory blood by the administration of ε can be available in treating the pathologic states associated with and caused by the activation of plasmin system or high activity of plasmin.

SUMMARY AND CONCLUSION

a) It was demonstrated in these experiments that the intravenous administration of a specially prepared human serum and a certain amount of SK resulted in a strong activation of plasmin and a decrease of plasma fibrinogen in the circulatory blood of dogs.

b) It was also recognized that high activity of plasmin in the circulatory blood of dogs was distinctly accompanied by the marked increase of bleeding, i.e. the oozing tendency from incised wounds of the skin of dogs.

c) It was proved that the intravenous administration of a certain amount of ε was effective in suppressing the bleeding tendency as well as high plasmin activity in the blood, both of which had been produced by the mentioned procedure.

d) The intravenous administration of ε, which had been undertaken prior to the intravenous administration of human serum and SK, was very effective in arresting the occurrence of the oozing tendency and the activation of plasmin in those dogs which received the intravenous administration of human serum and SK.

e) However, the intravenous administration of ε was not effective in arresting the oozing tendency of dogs which had the preliminary intravenous administration of a physiological saline solution containing 50 mg of Heparin sulfate and experimental anoxia. It was in particular noticed that the activity of plasmin was unchanged in this case.

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CLINICAL USE OF ε-AMINO-N-CAPROIC ACID ON METROPATHIA HEMORRHAGICA

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INTRODUCTION

Since a synthetized antiplasmic substance, i.e., ε-amino-n-caproic acid*** was, after long endeavour, discovered by Okamoto, Nagasawa, Tsukada, Takagi and Yokoi[1], it has been presented to be solved whether the clinical administration of ε is effective in treating those patients suffering from the pathologically high activity of plasmin in the circulatory blood and/or in the illed locus.

The pathological fibrinolysis has variously been investigated in the obstetrical field, yet nothing had been reported about the bleeding of so called metropathia hemorrhagica until it was preliminary reported by our group in 1956[2], that the administration of ε is effective in treating the functional bleeding of uterus where high plasmin activity is observed in the circulatory blood of the patients. A difficult task, facing us, was to present adequate evidences of controls improving the clinical effectiveness of ε in treating the bleeding of metropathia hemorrhagica. Therefore, it was particularly intended (1) to examine the effectiveness, if any, of ε by comparing it with the results of the use of placebo injection of glucose solution, and (2) to examine statistically the results of the administration of ε in treating said metropathia hemorrhagica by comparison with the results of the administration of Bothermone (a mixture of male and female hormones) which was applied in treating metropathia hemorrhagica in our clinic.

METHOD

1) The case history and clinical status of patients were carefully inquired, examined and recorded in routine. Diagnosis was made after adequate and

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*** abbreviated as ε in this text.

sufficient examination including cervical smear test for detection of cancer and biopsy of endometrial tissue for confirming the hyperplasia.

2) Twenty cubic centimetre of 5% sterilized distilled aquenous solution of ε was injected intravenously to a group of patients every day, and the effect of the administration of ε on hemorrhage from uterus in patients was examined at each consultation.

3) Twenty cubic centimetre of 5% glucose solution in a placebo injection was given intravenously to another group of patients under careful inspection. When uterus bleeding remained nearly constant or increased by the administration of placebo injection, treatment with ε was began and these results were examined.

4) Bothermon was given subcutaneously to a third group of patients under the careful inspection. The results obtained were compared with those obtained from the first or the third group.

5) Concerning to clinical examination, plasmin activity in the blood was also examined by routine methods under the guidance of Okamoto of Keio Physiology Laboratory. The Ratnoff's method was used in our laboratory in plasmin experiment, but in order to make more thorough research, the methods of Ratnoff, MacFarlane, Kuroyanagi, Lewis & Furguson, and Ungar were respectively adopted. Besides those methods fibrinogen quantitative determination was used.

RESULTS

a) *Clinical cases of the sole administration of ε after placebo injection.*

Case No. 1. Keio Card No. 8527. Patient Name: S.H., female, Age: 37.

1) Diagnosis: Metropathia Hemorrhagica.

2) Anamnesis: Married 17 years ago. No pregnancy and delivery in the past. Menstruation cycles 30 days. Average duration of each menstruation 7 days. Amount of bleeding at each menstruation normal. Last menstruation continued from June 20 to 26, 1958. On August 10, pain started to be felt on the lower abdomen. On August 27, uterine bleeding started. The patient came to the hospital on September 6, 1958.

3) Status at the first examination on September 6: The uterine body was enlarged but its consistence is normal. By the cervical smear test, cancer was negative, and hysteric secretion was bloody. No other significant sign was found. Thus, the above-mentioned diagnosis was given. The examination of venous blood indicated that euglobulin lysis was accelerated.

4) Course and effect of the administration of ε:

On September 6, 20 cc of 5% glucose solution was injected intravenously, and on September 7, the patient complained that bleeding from the uterus increased.

On September 7, 20 cc of 5% ε solution was injected intravenously. The uterine bleeding which had lasted for eleven days was stilled on the following day.

5) Side effect: None.

While the placebo injection was ineffective, one injection of ε stopped the uterine bleeding.

Case No. 2. Keio Card No. 8615. Patient Name: T.N., female, Age: 23.

1) Diagnosis: Metropathia Hemorrhagica.
2) Anamnesis: Married in 1958. No pregnancy and delivery in the past. Menstruation cycle 28 days. Average duration of each menstruation 4 days. Amount of bleeding in each menstruation is normal. Last menstruation continued from August 13 to 19, 1958. On September 3, uterine bleeding started. The patient came to the hospital on September 9, 1958.
3) Status at the first examination on September 9: The uterine body was normal, and its consistence was normal. Bloody secretion was observed. No other significant sign was found. The above-mentioned diagnosis was given after the first examination.
4) Course and effect of the administration of ε: On September 9 and 10, 20 cc of 5% glucose solution was injected intravenously. The uterine bleeding, however, increased by a amount day by day. On September 11 and 12, 20 cc of 5% ε solution was injected intravenously. The uterine bleeding fairly decreased after the first injection and completely ceased aftr the second injection. No unfavorable side effect was found.

The administration of ε was effective in treating the patient suffering from metropathia hemorrhagica, but the preliminary administration of placebo, i.e. the two intravenous injections of glucose solution were not effective at all.

Case No. 3. Keio Card No. 8680. Patient Name: Y.S., female, Age: 46.

1) Diagnosis: Metropathia Hemorrhagica.
2) Anamnesis: Married 26 years ago. No pregnancy and delivery in the past. Menstruation cycle 25 days. Average duration of each menstruation 2 days. Amount of bleeding in each menstruation normal. Last menstruation continued from August 21 to 23, 1958. On September 2, uterine bleeding started. The patient came to the hospital on September 10, 1958.
3) Status at the first examination on September 10: The uterine body was found to be smaller than normal and its consistence is soft palpable. Dark reddish bloody secretion was positive. No other significant sign was found. By the smear test, cancer was negative, and hyperplasia endmetrii was found by the endometrial biopsy. The above-mentioned diagnosis was given. The examination of venous blood showed that plasmin activity was normal.
4) Course and effect of the administration of ε: On September 10, 20 cc of 5% glucose solution was injected intravenously. On the following day, bleeding still continued. Twenty cubic centimetre of 5% ε solution was injected intravenously,

and the administration of ε was repeated on September 12 and 13. The bleeding gradually decreased after the first administration of ε and ceased with the second injection. No unfavorable side effect was found.

While the placebo injection was ineffective, the administration of ε was effective in treating this case.

Case No. 4. Keio Card No. 8693. Patient Name: S.T., female, Age: 37.

1) Diagnosis: Metropathia Hemorrhagica.
2) Anamnesis: Married 10 years ago. Two normal pregnancies and deliveries in the past. Menstruation cycle was 28 days. Average duration of each menstruation 5 days. Amount of bleeding in each menstruation normal. Last menstruation continued from August 20 to 26, 1958. On August 10, uterine bleeding occurred and thereafter the dizziness started. On September 2, 6 and 8, uterine bleeding occurred again. The patient came to the hospital on September 10, 1958.
3) Status at the first examination on September 10: The uterine body was larger than normal but its consistence was normal. No bleeding complained, though secretion was bloody. Cancer was negative by the smear test. No other significant sign was found. The above-mentioned diagnosis was given. The result of the examination of venous blood indicated that normal plasmin activity, as the blood sample was taken in the period when bleeding was negative.
4) Course and effect of the administration of ε: From September 10, 20 cc of 5% glucose solution was injected intravenously every day for three days. The bleeding gradually increased, and by 3 injections it obviously increased. Since September 13, 20 cc of 5% ε solution was injected intravenously every day for 3 days. The bleeding gradually decreased from the following day of the first injection of ε After the second injection, the bleeding almost stopped, but the final result of the administration of ε was not confirmed, because the patient had menstruation from September 16 to 21. After menstruation, however, uterine bleeding did not occur. No unfavorable side effect was found.

Three injections of placebo were ineffective, but the administration of ε was effective in treating this case.

Case No. 5. Keio Card No. 8905. Patient Name: M.T., female, Age: 35.

1) Diagnosis: Metropathia Hemorrhagica.
2) Anamnesis: Married 14 years ago. Three normal pregnancies and deliveries, and one curettage of uterus in the past. Menstruation cycle 30 days. Average duration of each menstruation 5 days. Amount of bleeding in each menstruation normal. Last menstruation continued from August 21 to 24, 1958. On September 15, uterine bleeding started. The patient came to the hospital on September 18, 1958.
3) Status at the first examination on September 18: The uterine body was somewhat larger and softer than normal. Erosio portionis was found and blood clot was found thereon. Secreta was bloody. By the smear test, cancer was negative and hyperplasia endometril was proved by the endometrial biopsy. The above-mentioned diagnosis was given based on this examination. The result of the examination of venous blood showed that euglobulin lysis was accelerated, indicating higher

plasmin activity in plasmin system.

4) Course and effect of the administration of ε: On September 18, 20 cc of 5% glucose solution was injected intravenously but bleeding did not decrease. On September 19 and 20, 20 cc of 5% ε solution was injected intravenously. By the first injection, the bleeding gradually decreased and completely ceased after the second. No unfavorable side effect was found.

Bleeding which was not improved by the administration of a glucose solution, ceased by only two injections of ε. This result indicates that the administration of ε was effective in treating the uterine bleeding in this case.

b) *Clinical case of sole administration of ε after Bothermon administration.*

Case No. 6. Keio Card No. 7680. Patient Name: K.T., female, Age: 27.

1) Diagnosis: Metropathia Hemorrhagica.
2) Anamnesis: Married four years ago. Two curettages of uterus in the past. Menstruation cycle 30 days. Average duration of each menstruation 4 days. Amount of bleeding in each menstruation little. Last menstruation continued from July 25 to 29. On August 2, uterine bleeding started. The patient came to the hospital on August 9, 1958.
3) Status at the first examination on August 9: The uterine body was dug-egg-sized large, but softer. Bloody secretion was positive. No other significant sign was found. The case was diagnosed as Metropathia Hemorrhagica.
4) Course and effect of the administration of ε: a) From August 9, 2 cc of Bothermon was injected subcutaneously every day for 4 days. But the bleeding did not cease and no improvement was observed. b) On August 18, treatment with ε was started. Twenty cubic centimetre of 5% ε solution was injected intravenously on August 18, 19, 20, 21 and 25. After the second injection, the bleeding started to decrease, and after the third injection completely ceased. The fourth and the fifth injections were carried out to prevent the occurrence of bleeding and obtained a successful result. No unfavorable side effect was found.

The uterine bleeding, which had not improved by Bothermon, completely ceased by the administration of ε.

c) *Clinical case of the intermittent use of ε.*

Case No. 7. Keio Card No. 7514. Patient Name: M.S., female, Age: 41.

1) Diagnosis: Metropathia Hemorrhagica.
2) Anamnesis: Married 13 years ago. No pregnancy and delivery in the past. Menstruation cycle 30 days. Average duration of each menstruation 4 days. Amount of bleeding in each menstruation little. Last menstruation continued from July 21 to 24, 1958. On July 30, uterine bleeding started. The patient came to the hospital on August 2, 1958.
3) Status at the first examination on August 2: The uterine body was slightly enlarged. Dark-reddish bloody secretion was observed. By the smear test cancer was negative. No other significant sign was observed. The case was diagnosed

as metropathia hemorrhagica.

4) Course and effect of the administration of ε: Twenty cubic centimetre of 5% ε solution was injected intravenously on August 2. Bleeding almost disappeared by one injection. On August 5, the 2nd injection was carried out. On August 14, the bleeding which ceased by two injections, occurred again and the patient came again to the hospital on the following day. The treatment with ε started again. Twenty cubic centimetre of 5% ε solution was injected intravenously in eight times from August 15 to 23. The bleeding gradually decreased by the administration of ε, and completely ceased. No side effect was found.

The results demonstrated a typical case of the intermittent use of ε. The administration of ε was effective in all the cases in suppressing uterine bleeding, and the intermittent abolishment of ε treatment made bleeding occur again. Thus, the evidence was presented to deduce the effectiveness of ε on uterine bleeding.

d) *Other clinical cases of sole administration of ε.*

Case No. 8. Keio Card No. 7690. Patient Name: I.O., female, Age: 26.

1) Diagnosis: Metropathia Hemorrhagica.
2) Anamnesis: Married 24 years old. Two normal pregnancies and deliveries in the past. Menstruation cycle 28 days. Average duration of each menstruation 5 days. Amount of bleeding in each menstruation normal. Last menstruation continued from July 31 to August 4, 1958. On August 6, bloody secretion started. The patient came to the hospital on August 9, 1952.
3) Status at the first examination on August 9: The uterine body was slightly smaller. A small amount of bloody secretion was observed. The disease was diagnosed as metropathia hemorrhagica. Blood examinations were carried out, and the result showed that euglobulin lysis was strongly accelerated, indicating high activity of plasmin in the blood.
4) Course and effect of the administration of ε : Twenty cubic centimetre of 5% solution was injected intravenously on August 9 and 11. By the first injection, bleeding almost disappeared; by the second, the bleeding completely ceased. On August 12, the blood examination was carried out when the bleeding completely disappeared. The result showed that euglobulin lysis in the blood turned to normal. No side effect was observed.

The administration of ε was effective in suppressing the uterus bleeding and high activity of plasmin in the blood.

Case No. 9. Keio Card No. 2886. Patient Name: E.I., female, Age: 23.

1) Diagnosis: Metropathia Hemorrhagica.
2) Anamnesis: Married one year ago. No pregnancy and delivery, but one curettage in the past. Menstruation cycle 25 days. Average duration of each menstruation 3 days. Amount of bleeding at each menstruation normal. Last menstruation started from August 23, and continued more than 3 weeks, indicating that normal menstruation turned to pathologic bleeding. The patient came to the hospital on Sepember 17, 1958.

3) Status at the first examination on September 17: The uterine body was found slightly enlarged and softer. Dark reddish bloody secretion positive. Hyperplasia endometrial biopsy. No other significant sign was observed. The result of the examination of venous blood was that euglobulin lysis was accelerated, indicating the activation of plasmin system.
4) Course and effect of the administration of ε: On September 17, 18, and 19, 20 cc of 5% ε solution was injected intravenously. After the first injection the uterine bleeding almost ceased. After three injection, it completely stopped. No unfavorable side effect was observed.

The administration of ε was effective in treating this case of metropathia hemorrhagica.

Case No. 10. Keio Card No. 3225. Patient Name: K.T., female, Age: 36.
1) Diagnosis: Metropathia Hemorrhagica.
2) Anamnesis: Married 26 years old. Two normal pregnancies and deliveries, and one curettage in the past. Menstruation cycle 30 days. The amount of bleeding in each menstruation was normal. Average duration of each menstruation was 3 days. Last menstruation continued from February 14 to 16. Uterine bleeding started from March 4, 1958. The patient came to the hospital on March 12, 1958. Cancer was negative by the smear test. The disease was diagnosed as metropathia hemorrhagica. Ergot was administered to suppress the bleeding on March 12. But dark reddish bloody secretion did not decrease. From March 13, 20 cc of 5% ε solution were injected intravenously every day for three days. The bleeding completely ceased by the injections of ε.
3) Status of the first examination made on September 14: On September 14, 1958, uterine bleeding appeared again. The patient came to the hospital on September 20. Hyperplasia endometrii was observed by the endometrial biopsy. Blood examination was carried out on September 20 and the result was that plasmin activity was slightly activated.
4) Course and effect of the administration of ε: The administration of ε was carried out from September 20 every day for three days. After 2 injections, the bleeding almost disappeared and, after the third injection, completely disappeared. No unfavorable side effect was observed.

The treatment with ε was effective against the two cases of bleeding, which started on March 4 and September 14 respectively.

Case No. 11. Keio Card No. 7624. Patient Name: T.K., female, Age: 34.
1) Diagnosis: Metropathia Hemorrhagica.
2) Anamnesis: Married 12 years ago. Three normal pregnancies and deliveries in the past, and one curettage one year ago. Menstruation cycle 28 days. Average duration of each menstruation 3 days. Last menstruation continued from June 23 to 25, 1958. On July 17, bloody secretion started. The patient came to the hospital on August 6, 1958.
3) Status at the first examination made on August 6: The uterine body was enlarged and softer. Bloody secretion was positive. Cancer was negative by the smear

test. Decidua basalis and villus with a part of degeneration was observed by the endometrial biopsy. The disease was diagnosed as metropathia hemorrhagica. The result of blood examination was that euglobulin lysis was accelerated, showing higher plasmin activity in the blood.

4) Course and effect of the administration of ε: On August 6, curettage was carried out. On August 7, 8 and 9, 20 cc of 5% ε solution was injected intravenously. Bleeding decreased after 2 injections of ε. By three injections of ε, it almost disappeared. No unfavorable side effect was found.

Though curettage was carried out prior to the administration of ε. The treatment with ε was effective and improved the bleeding rapidly.

Case No. 12. Keio Card No. 8885. Patient Name: T.K., female, Age: 41.

1) Diagnosis: Metropathia Hemorrhagica.
2) Anamnesis: Married 26 years old. Two pregnancies and deliveries, and one abortus in the past. Menstruation cycle 28 days. Average duration of each menstruation 2 days. Last menstruation continued from August 15 to 16. From the beginning of September, a small amount of bleeding was observed. The patient came to the hospital on September 17, 1958.
3) Status of the first examination made on September 17: The uterine body was enlarged and softer. Bloody secretion was positive. Hyperplasia endometrii was proved by the endometrial biopsy. Thus, the disease was diagnosed as metropathia hemorrhagica. Blood examination was carried out on September 17, and the result was that whole clot lysis was accelerated, indicating high plasmin activity.
4) Course and effect of the administration of ε: Twenty cubic centimetre of 5% ε solution were injected intravenously in one injection each of September 17 and 19. After the first injection, bleeding almost disappeared, and by the second injection, completely ceased. No unfavorable side effect was found.

The administration of ε was effective in suppressing the uterine bleeding which had been started prior to menstruation.

Case No. 13. Keio Card No. 8965. Patient Name: S.K., female, Age: 26.

1) Diagnosis: Metropathia Hemorrhagica.
2) Anamnesis: Married on 1958. Two pregnancies and two curettages. Menstruation cycle 30 days. Average duration of each menstruation 5 days. Last menstruation continued from September 1 to 5, 1958. A small amount of bloody secretion was found sometime for 2 years. The patient came to the hospital on September 20, 1958.
3) Status at the first examination made on September 20: The uterine body was normal. Dark reddish bloody secretion was positive. The disease was diagnosed as metropathia hemorrhagica. Blood examination was carried out on September 20, and the result was that whole clot lysis was strongly accelerated, indicating high plasmin activity in the blood.
4) Course and effect of the administration of ε: On September 20, 20 cc of 5% ε solution was injected intravenously. At the examination on September 21, it was observed that the bleeding disappeared and ceased completely by one injection.

No unfavorable side effect was found.

The administration of ε was effective in suppressing the uterine bleeding accompanied by high plasmin activity.

Case No. 14. Keio Card No. 8409. Patient Name: K.S., female, Age: 30.

1) Diagnosis: Metropathia Hemorrhagica.
2) Anamnesis: Married in the age of 22. Five pregnancies and four deliveries and one abortus. At the beginning of June, the patient underwent curettage for extrauterino pregnancy, and thereafter had a nearly ordinary menstruation. Menstruation cycle irregular, but the amount of bleeding normal. Average duration of each menstruation was 3 days. The patient came to the hospital on September 3, 1958.
3) Status at the first examination of September 3: The uterine body was normal. A small amount of bloody secretion was observed. Cancer was negative by the smear test. Hyperplasia endometrii was proved by the endometrial biopsy. The disease was diagnosed as metropathia hemorrhagica. Blood examination was carried out and the result was that whole clot lysis was strongly accelerated, indicating high plasmin activity in the blood.
4) Course and effect of the administration of ε: On September 9, 10 and 11, 20 cc of 5% ε solution was injected intravenously. By two injections bleeding almost disappeared and by three injections, it completely ceased. No unfavorable side effect was found.

The treatment with ε was effective in treating this case of metropathia hemorrhagica.

Case No. 15. Keio Card No. 9402. Patient Name: F.W., female, Age: 52.

1) Diagnosis: Metropathia Hemorrhagica.
2) Anamnesis: Married 30 years ago. Five normal pregnancies and deliveries. No menstruation. On October 7, 1958, uterine bleeding and pain on lower abdomen occurred. The patient came to the hospital on October 8, 1958.
3) Status at the first examination made on October 8: The uterine body was normal and softer. Erosio portions were observed. Dark reddish bloody secretion was positive. Cancer was negative by the smear test. No other significant sign was found. The disease was diagnosed as metropathia hemorrhagica. The result of blood examination carried out on October 8, indicated the normal plasmin in the blood.
4) Course and effect of the administration of ε: Twenty cubic centimetre of 5% ε solution was injected intravenously on October 9, 10 and 11. By one injection, bleeding gradually decreased, by two injections almost disappeared and by three injections completely cease. No unfavorable side effect was found.

The administration of ε was effective in treating this case of metropathia hemorrhagica.

Case No. 16. Keio Card No. 7700. Patient Name: S.N., female, Age: 25.

1) Diagnosis: Metropathia Hemorrhagica.

2) Anamnesis: Married one year ago. No pregnancy and delivery. Menstruation cycle 29 days. The amount of bleeding in each menstruation was normal. Average duration of each menstruation was 4 to 7 days. Last menstruation continued June 27 to July 1, 1958. On July 27, a big amount of uterine bleeding occurred. The patient came to the hospital on August 9, 1958.
3) Status at the first examination made on August 9: The uterine body was smaller and its hardness normal. Bloody secretion was positive. Cancer negative by the smear test. Hyperplasia endometrii was found by the endometrial biopsy. No other significant sign was observed. The disease was diagnosed as metropathia hemorrhagica and hyperplasia uterine.
4) Course and effect of the administration of ε: On August 9, 20 cc of 5% ε solution was injected intravenously. Bleeding was confirmed by the clinical examination made on August 11. The result of blood examination carried out on August 11, was that whole clot lysis and euglobulin lysis were both accelerated, indicating high plasmin activity in the blood. On August 11 and 12, the treatment with ε was carried out. By three injections, uterus bleeding completely ceased. No unfavorable side effect was found.

The administration of ε was effective in treating this case of metropathia hemorrhagica.

DISCUSSION

It has been broadly accepted that Metropathia Hemorrhagica can be caused by the unbalance of sex hormones and can be treated by the administration of those hormones, though what actually causes Metropathia Hemorrhagica has not yet been confirmed. Thus, therapy used for such disorder has not led from any fundamental theory substantially.

Smith and Smith[3] in 1952, however, reported that plasmin is active in venous blood at the time of menstruation; it led Otsuka[2] and Sato[4] to fined the evidence that plasmin in blood is extremely active in Metropathia Hemorrhagica.

This discovery led Takayama and Sato[5] to presume that an antiplasmic substance, i.e., ε might have a favourable effect on metropathia hemorrhagica. In fact, it was reported from our group that the administration of ε to patients suffering from metropathia hemorrhagica had a considerable effect clinically in treating the disease.

This investigation was undertaken to present some sufficient evidences enough to prove the clinical effectiveness of ε on metropathia hemorrhagica.

The patients under investigation were diagnosed as suffering from metropathia hemorrhagica, in which menstruation was not regarded as a cause of the bleeding and other kinds of hemorrhagic cause such as uterus cancer were denied by the clinical and laboratory routine examination. In particular, em-

phasis was placed in endometrial biopsy and smear test to deduce and confirm the diagnosis.

The high plasmin activity in the blood was found in 9 out of 12 cases of metropathia hemorrhagica. The results indicated that, in metropathia hemorrhagica, plasmin system in the blood are high activated in general.

These investigations were designed.

i) To examine the effect of the sole administration of ε in a sufficient number of patients suffering from metropathia hemorrhagica and to present a detailed record of each case.

ii) To demonstrate a adequate and sufficient evidence of control by using placebo injection or by using Bothermon injection; the latter is known to be effective in treating metropathia hemorrhagica.

iii) To deduce statistically a conclusion on the utility of the administration of ε by comparison of the course of metropathia hemorrhagica with the sole administration of ε with the course of the same disease under the administration of placebo or Bothermon.

To 30 cases of metropathia hemorrhagica, one venous injection of 20 cc of 5% ε solution was given every day as a rule, and the course was observed and recorded. The results obtained from 30 cases showed that in 50% of which hemorrhage ceased by one or two injections of ε, and in 79.9% of which hemorrhage ceased with the third injection of ε at most.

In other 70 cases, the course and results of the application of Bothermon were observed. In 41.3% of the 70 patients, bleeding stopped after one or two injections of Bothermon, and in 62.4% after three injections at most. The herementioned results were compared with those obtained by the sole administration of ε. Thus, it was deduced and concluded that the use of the administration of ε was significantly better than that of Bothermon.

The another adequate control cases were presented to discuss and examine the effectiveness of ε from various standpoints.

i) The course and results of the application of placebo are shown in case Nos. 1, 2, 3, 4, 5 and 6, as per attached sheets. It was first proved that the performance with placebo injections were ineffective. A remarkable improvement was demonstrated by a few injections of ε to those patients in whom placebo injection had been under taken and proved to be ineffective. These results clearly indicated that the effectiveness of ε was not due to auto-suggestion.

ii) In case No. 7, Bothermon was four time administrated to the patient resulting any improving at the disorders. Then, the treatment with ε was started

and succeeded in ceasing the bleeding of metropathia hemorrhagica. The course of this case seems to present a type of control for revealing the effectiveness of ε.

iii) In case No. 8, a more adequate control case was presented by adopting the intermittent use of ε. That is, the administration of ε suppressed the uterine bleeding. The abolishment of ε treatment, however, reversed the disorders, and it was arrested completely by resuming the treatment with ε.

CONCLUSION

a) In 10 out of 12 cases of metropathia hemorrhagica caused by the disfunction of the ovarium, plasmin activity in the venous blood was higher than normal and it was suppressed by the administration of ε.

b) In 29 out of 30 cases of metropathia hemorrhagica, the uterine bleeding was successfully treated, at most, with five injections of ε carried out once a day.

c) From the results of the sole administration of ε or the administration of ε with previous placebo injection of a glucose solution and also from the results of the administration of Bothermon, it was statistically deduced that the effectiveness of ε in arresting and treating uterus bleeding of these patients was more obvious than that of Bothermon and it was due solely to the administration of ε.

d) From the results obtained in this investigation, it was confirmed that no unfavorable side effects was produced by the intravenous administration of ε.

e) The treatment with ε of these 30 patients of metropathia hemorrhagica, i.e. disfunctional uterine bleeding, was evidently effective and useful in the clinical performance.

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VARIATIONS OF PLASMIN IN THE HEMORRHAGIC BLOOD DISEASES

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On fibrinolysis, a number of studies have been reported by many investigators. In 1936 MacFarlane reported that the blood of a patient whose gall bladder was resected remained liquid without adding any anticoagulant, and Yudin (1936) also observed the same phenomenon in the blood of one who died of trauma. In Russia, the blood of a man who died suddenly could be transfused without adding any anticoagulant, and such was described clinically by Mole (1943), Wexler and Ellis (1944) and many others. Following these observations, Reiman[1] reported the hemorrhagic diseases accompanying the accelerated fibrinolysis in blood, and such fibrinolysis were reported by Tagnon[2] at delivery, by Weiner[3] at premature separation of placenta, by Mathey[4] at operation of the lung and also by Tagnon[5], Crane[6] and Aboulker[7] at prostatic cancer, etc.[8-11] However, only a few studies have been reported on hemorrhagic blood diseases accompanied by fibrinolysis, and only leukemia[12,13], thrombocytopenic purpura[14] or polycythemia vera[15] have been studied.

Although the occurrence of hemorrhage in the blood diseases is naturally related to the disturbance of blood clotting factors, in this study plasmin activities in cases of aplastic anemia and acute myeloic leukemia were estimated in order to elucidate the relation of plasmin activity to hemorrhagic tendency. Furthermore, significance of plasmin in these hemorrhagic diseases was studied by administrating the synthetized antiplasmin ε (ε-amino-n-caproic acid)**. Then, the relations between steroid hormone and plasmin activity were investigated in order to confirm the more detailed hemostatic mechanism of this hormone, because in the studies so far published, for example the one reported by

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** Abbreviated as ε in the following.

Stefanini[16], only the increase of capillary resistance or of thrombocyte production have been implicated as the factor related to hemostatic effect of steroid hormone.

METHODS

The observations were made in 7 cases of aplastic anemia and 2 cases of acute myeloic leukemia. Blood and urine of these patients were taken periodically, and plasmin activities were estimated. In the case of blood transfusions, the materials were taken before the transfusion to avoid influence of this procedure to the plasmin activity.

The following methods were employed for estimating the activities.

1) Ratnoff's method (whole plasma test)[17].
2) Lewis and Furguson's method[18] in which euglobulin fraction was used as an indicator. (Euglobulin test)
3) Whole plasmin test[19] that estimates whole plasmin in the serum by adding 100 units of streptokinase.
4) Colgan's test[20] that estimates plasmin in urine.

Then, fibrinogen was measured by Gram's method. Of these only the Euglobulin and the whole plasmin test were shown in the figure.

Various methods of hematological examinations also were performed on the patients. Of these, erythrocyte- and leucocyte-count, hemoglobin-concentration and capillary fragility were shown in the figure. The dosage of ε was 40 cc of 5% solution intravenously injected daily.

RESULTS

The plasmin activities determined by the Euglobulin test showed an increase corresponding with the degree of hemorrhage, and the results from the whole plasmin test confirmed a increase in activity from the Euglobulin test. The activities determined by Ratnoff test, however, did not shown a corresponding relationship and few positive cases were found using Colgan's urine test. In our cases, the rise and fall of plasmin activity was determined from the results of the Euglobulin test and the whole plasmin test.

Table 1

	Whole plasma test (Ratnoff)	Euglobulin test (Lewis & Furguson)	Whole plasmin test in serum (S.K. activation)	Colgan's urine test	Fibrinogen
	days	Lew u/cc	Lew u/cc	u/cc	mg/100 cc
19/12	5	1.12	663	<1	250
26/1	3	1.66	463	1	250
6/2	3	1.12	520		240
20/2		1.82	520		240

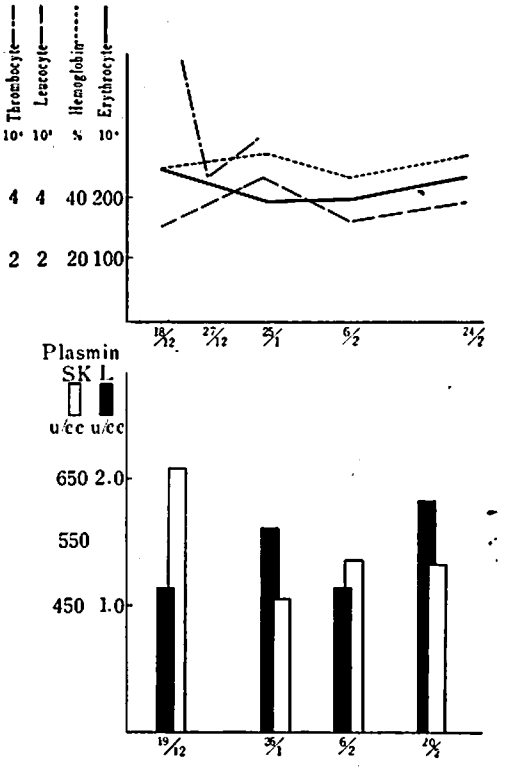
Thrombocyte
Leucocyte
Hemoglobin
Erythrocyte
10⁴ 10³ % 10⁴
4 4 40 200
2 2 20 100
18/12 27/12 25/1 6/2 21/2
Plasmin
SK L
u/cc u/cc
650 2.0
550
450 1.0
19/12 25/1 6/2 20/2

Fig. 1

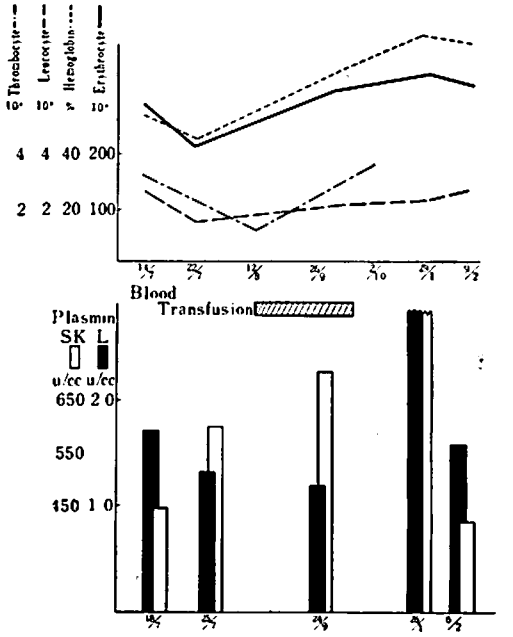
4 4 40 200
2 2 20 100
Blood
Transfusion
Plasmin
SK L
u/cc u/cc
650 2 0
550
450 1 0

Fig. 2

Case No. 1. (Fig. 1, Table 1) Aplastic anemia. H.S., Female, Age: 49.

In this case, neither gum bleeding nor nasal hemorrhage was observed, and the plasmin activity was always normal as indicated in Fig. 1 and Table 1.

Case No. 2. (Fig. 2, Table 2) Aplastic anemia. F.M., Female, Age: 39.

No gum bleeding and nasal hemorrhage was observed, but the plasmin activities were found to be increased only at menstrual period.

Table 2

	Whole plasma test (Ratnoff) days	Euglobulin test (Lewis & Furgson) Lew u/cc	Whole plasmin test in serum (S.K. activation) Lew u/cc	Colgan's urine test u/cc	Fibrinogen mg/100 cc
18/18	6	1.66	438	<1	320
25/7	8	1.26	595	<1	200
24/9	3	1.12	695	<1	121
26/1	3	4.44	833	<1	260
6/2	3	1.60	420		240

Case No. 3. (Fig. 3, Table 3) Acute myeloic leukemia. M.A., Male, Age: 36.

The plasmin activity determined by the Euglobulin test was so highly increased during the episode of subcutaneous hemorrhage and melena while blood transfusion and 20 mg of prednisolone was being administered. Increasing the dosage of prednisolone even to 30 mg did not reduce the bleeding and the patient died from melena at last. It was also observed that the capillary resistance was remarkably reduced and thrombocyte-count was rather increased.

Table 3

	Whole plasma test (Ratnoff) days	Euglobulin test (Lewis & Furguson) Lew u/cc	Whole plasmin test in serum (S.K. activation) Lew u/cc	Colgan's urine test u/cc	Fibrinogen mg/100 cc
19/12	5	1.12	520	<1	200
23/12	4	1.12	420	<1	290
9/1	5	2.0	400	<1	230
20/1	5	2.0	168	<1	200
26/1	4	4.44	420	<1	220
6/2	4	5.72	208		500

Case No. 4. (Fig. 4, Table 4) Aplastic anemia. W.T., Male, Age: 38.

Table 4

	Whole plasma test (Ratnoff) days	Euglobulin test (Lewis & Furguson) Lew u/cc	Whole plasmin test in serum (S.K. activation) Lew u/cc	Colgan's urine test u/cc	Fibrinogen mg/100 cc
18/7	2	3.6	318	1	25
25/7	8	1.26	705	<1	330
1/8	9	1.32	618	<1	170
12/8	3	1.32	495	1	520
21/8	4	1.42	500	1	170

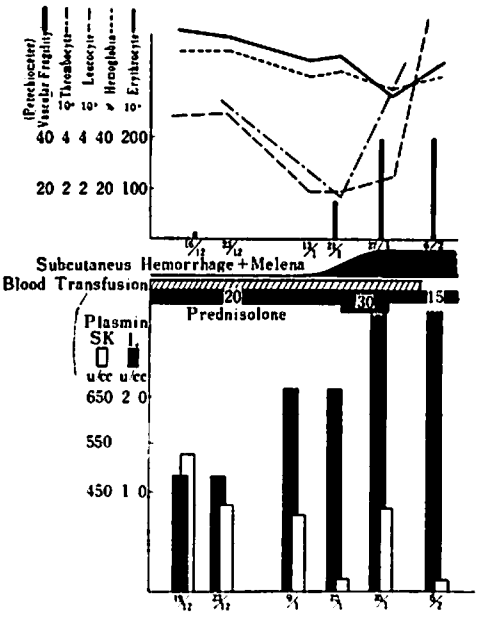
(Petechiae)
Vascular Fragility
Thrombocyte
Leucocyte
Hemoglobin
Erythrocyte
40 4 4 40 200
20 2 2 20 100
Subcutaneus Hemorrhage + Melena
Blood Transfusion
20
30
15
Prednisolone
Plasmin
SK L
u/cc u/cc
650 2 0
550
450 1 0

Fig. 3

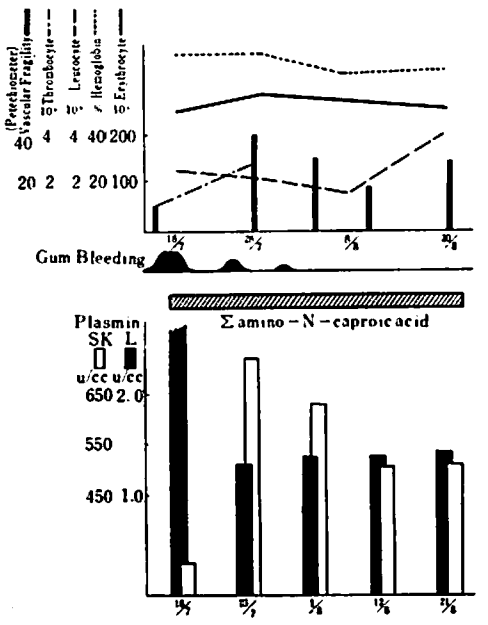
(Petechiae)
Vascular Fragility
Thrombocyte
Leucocyte
Hemoglobin
Erythrocyte
40 4 4 40 200
20 2 2 20 100
Gum Bleeding
Σ amino-N-caproic acid
Plasmin
SK L
u/cc u/cc
650 2.0
550
450 1.0

Fig. 4

Although the plasmin activity was greatly increased and fibrinogen decreased during the episode of gum bleeding, these symptoms gradually improved by the administration of ε, and the plasmin activity was decreased to normal with recovery of gum bleeding and fibrinogen was also increased.

Case No. 5. (Fig. 5, Table 5) Aplastic anemia. H.I., Male, Age: 19.

Nasal hemorrhage was persisting and it completely disappeared during the administration of ε; the plasmin activity also turned to normal.

Table 5

	Whole plasma test (Ratnoff) days	Euglobulin test (Lewis & Furguson) Lew u/cc	Whole plasmin test in serum (S.K. activation) Lew u/cc	Colgan's urine test u/cc	Fibrinogen mg/100 cc
18/7	6	1.66	318	1	175
25/7	12	1.12	595	<1	170
1/8	5	1.12	625	<1	250
12/8	3	1.12	505	1	250
21/8	4	1.12	595	<1	100
26/1	3	2.36	695	<1	160

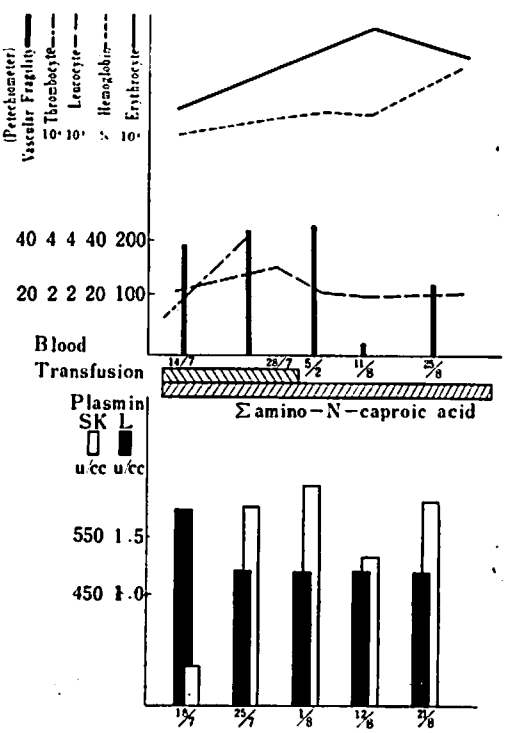

Fig. 5

Case No. 6. (Fig. 6, Table 6) Aplastic anemia. M.K., Male, Age: 57.

The plasmin activity and subcutaneous bleeding increased when the dosage of prednisolone was decreased from 30 mg to 20 mg, but these were improved by blood transfusion and increase of the dosage to 30 mg. These phenomena always reappeared when the dosage of prednisolone was decreased to 20 mg.

Table 6

	Whole plasma test (Ratnoff) days	Euglobulin test (Lewis & Furguson) Lew u/cc	Whole plasmin test in serum (S.K. activation) Lew u/cc	Colgan's urine test u/cc	Fibrinogen mg/100 cc
18/12	5	1.26	208		180
23/12	4	1.12	520	<1	210
9/1	3	2.50	320	<1	250
20/1	5	2.00	400	<1	230
26/1	4	3.34	380	<1	260
6/2	2	1.12	520		260
20/2		3.64	420	<1	240

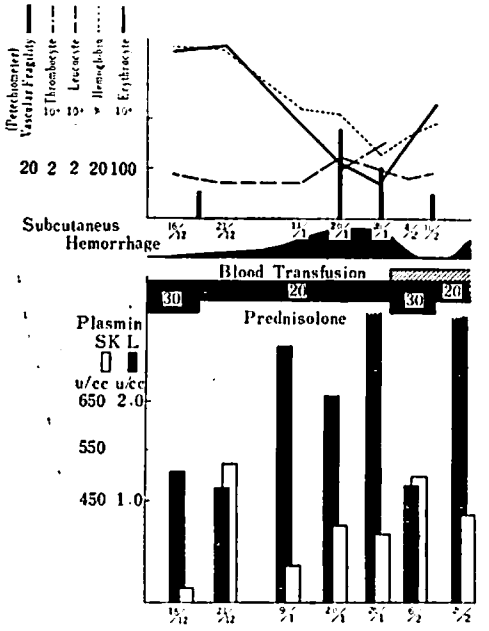

Fig. 6

Case No. 7. (Fig. 7, Table 7) Aplastic Anemia. K.K., Female, Age: 17.

The patient had a marked gum bleeding, which could not be ameliorated by the administration of 10 mg of prednisolone. But the hemorrhage was decreased during the administration of ε with prednisolone. The activity showed reincrease, thereafter, with gum bleeding during the administration of 20 mg of prednisolone and blood transfusion. These symptoms recovered by increasing the dosage of prednisolone to 40 mg, while reappeared by decreasing it to 30 mg. The capillary resistance was

reduced with the bleeding, but thrombocyte-count did not changed.

Table 7

	Whole plasma test (Ratnoff) days	Euglobulin test (Lewis & Furguson) Lew u/cc	Whole plasmin test in serum (S. K. activation) Lew u/cc	Colgan's urine test u/cc	Fibrinogen mg/100 cc
11/12	5	1.54	595	<1	260
18/12	5	1.42	463		440
23/12	4	1.26	595	<1	260
9/1	4	1.12	416	<1	350
20/1	5	2.5	420		330
26/1	3	4.0	520		390
6/2	3	1.74	555		270
20/2		2.66	345	1	180

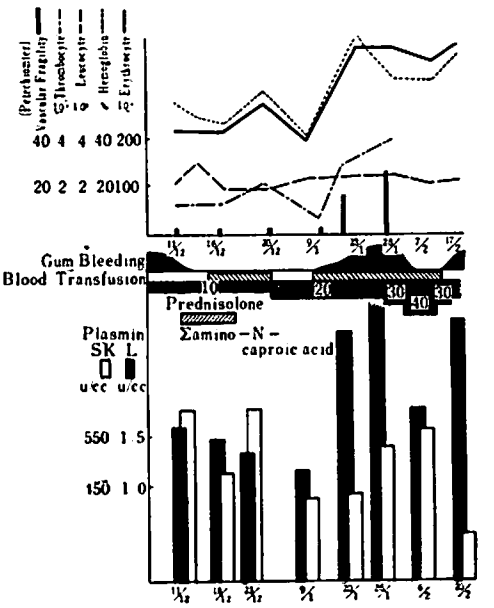

Fig. 7

Case No. 8. (Fig. 8, Table 8) Aplastic anemia. H.S., Male, Age: 21.

Table 8

	Whole plasma test (Ratnoff) days	Euglobulin test (Lewis & Furguson) Lew u/cc	Whole plasmin test in serum (S. K. activation) Lew u/cc	Colgan's urine test u/cc	Fibrinogen mg/100 cc
11/12	5	1.34	325	<1	240
18/12	5	1.12	345		140
23/12	4	1.26	375	2	150
9/1	4	1.12	325	<1	220
20/1	5	5.0	208	<1	150
26/1	4	2.5	278	<1	220
6/2	3	2.10	345		200
20/2		1.42	420	<1	130

Gum bleeding appeared during blood transfusion, but diminished by the administration of 30 mg of prednisolone with ε. This bleeding reappeared when the administration of ε was stopped, and the plasmin activity in urine was increased, then recovered by the administration of 50 mg of prednisolone with ε. But the plasmin activity was reincreased by decreasing the dosage of prednisolone without any bleeding and thereafter turned to normal.

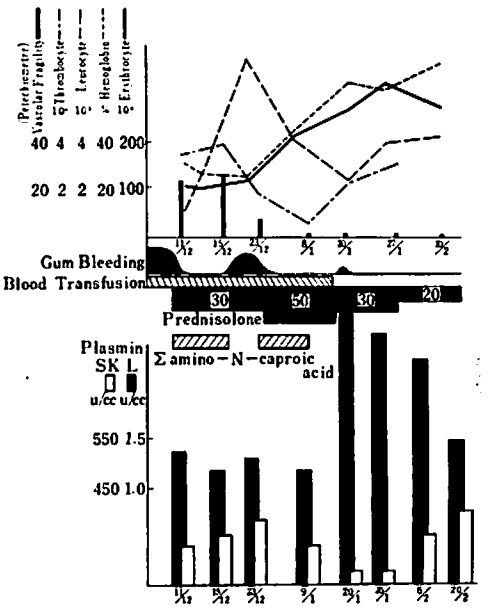

Fig. 8

Case No. 9. (Fig. 9, Table 9) Acute myeloic leukemia. B.H., Male, Age: 60.

Marked gum bleeding was improved by the administration of ε and blood-transfusion. Thereafter no bleeding was observed also during the administration of prednisolone, but the plasmin activity was increased by decreasing the dosage of prednisolone without bleeding.

Table 9

	Whole plasma test (Ratnoff) days	Euglobulin test (Lewis & Furguson) Lew u/cc	Whole plasmin test in serum (S. K. activation) Lew u/cc	Colgan's urine test u/cc	Fibrinogen mg/100 cc
11/12	5	1.54	555	<1	600
18/12	2	1.12	595		180
19/12	5	1.12	375	<1	300
23/12	4	1.26	595	<1	380
9/1	2	2.00	420	<1	310
20/1	2	2.00	400	<1	220
26/1	4	2.50	625	<1	220

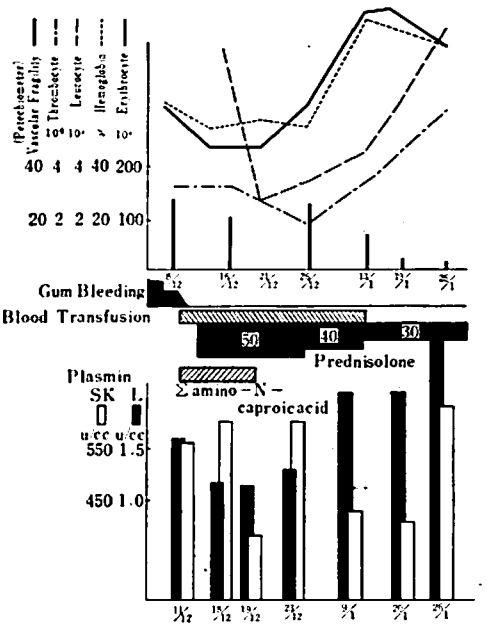

Fig. 9

DISCUSSION AND SUMMARY

The reason why only a few studies have been reported on the fibrinolysis accompanying blood diseases may be due to difficulty in measuring plasmin activity and also to neglect of the importance of plasmin in the blood disease accompanied by disturbance of thrombocyte and other clotting factors. Most cases of leukemia [12,13] and thrombocypotenic purpura[14] reported so far showed marked decreases of thrombocyte-count, except for only one case of leukemia. All cases reported here also showed decrease of thrombocyte-count, and doubtlessly had disturbance in these clotting factors.

It was observed that the plasmin activities were not increased even in aplastic anemia as seen in cases 1 and 2 when so-called oozing bleeding such as gum bleeding and nasal hemorrhage did not accompany. Marked increases were induced however, when the oozing-bleeding occured as seen in cases 3, 4, 6 and 7, and case 3 of acute myeloic leukemia died with marked increase of the plasmin activity and melena, in spite of increasing of thrombocyte-count.

In any cases, on the other words, hemorrhage and plasmin activity were found to be parallel to each other, while no marked changes were seen in their thrombocyte-count. The hemorrhage caused by fibrinolysis was said to be oozing bleeding from injured tissue, mucous membrane and serous membrane, and also

petechial bleeding. All cases observed in our study also had the oozing bleeding such as gum and nasal hemorrhage. Therefore, the plasmin activity might have an important role in various kinds of hemorrhage in our cases.

This is further indicated by the fact that some cases showed a decrease of plasmin activity and hemorrhagic tendency when ε was administered.

The cause of increase of the plasmin activity is not fully understood yet, and those of aplastic anemia and acute myeloic leukemia is quite unknown. But the increase is naturally induced when the plasminogen is activated by liberation of various activators such as tissue activator, activator in plasma, urinary activator, trypsin, bacterial streptokinase or staphylokinase and then the pasmin activity covers the antiplasmin activity. Thus it seemed likely that the destruction of many leucocytes in leukemia would bring the activation of plasmin together with bacterial infection. On the other hand, Kwaan et al.[21] observed that the plasminogen activator was liberated by localized ischemia of venous wall, also observed increase of the fibrinolytic activity by adrenalin, histamin or serotonin, and concluded that adrenergic vasoconstriction of the vessels brought the localized ischemia and liberation of the plasminogen activator. Virchow[22] and Mole[23] found that the fibrinolytic activty in man suddenly died of anoxia was found to be the stron gest in the peripheral capillary and this was confirmed by Tagnon's[24] finding that the fibrinolytic activity would be accelerated by anoxia. The diseases reported in this paper also were accompanied by severe anemia, and it is easy to assume that the plasminogen activator would be liberated by anoxia.

On the cause of hemorrhage due to plasmin activation, the lysis of fibrinogen and other clotting factors are problems much discussed. According to Soulier and his associators[25], factor V, factor VII, prothrombin and AHF were lysed in which the factor V was the most, and Niewiarouski and Latallo[26] reported that AHF, factor V and plasma thromboplastin were lysed but no effect was observed on Christmas factor, factor VII and thromboplastic activity of the thrombocyte. Although these lytic factors may also have to be studied in detail, there were no certain changes in our clotting tests.

As to fibrinogen levels, on the other hand, it was said that hemorrhage could be induced by 100 mg percent of fibrinogen and Sawitsky described that 174 mg percent of fibrinogen induced hemorrhage. But fibrinogen levels in our cases was not decreased in despite of increasing of plasmin activity, except in some cases.

Since there was no obvious lytic activity due to activation of plasmin in this study, the problem of thrombolysis is also to be regarded as a cause of the hemorrhage. The thrombolysis is said to be[26,27] induced not only by the

markedly increased proteolytic activity, but also only by the increase of plasminogen activator, even though the increase of proteolytic activity is slight. On this phenomenon, it is believed that plasminogen activator is absorved on the surface of fibrin, thence induce the activation of plasminogen in fibrin and thrombolysis will occur rapidly. In our cases, even though the cause of hemorrhage may not depend only on the increase of thrombolytic activity, this is an interesting problem as a cause of hemorrhage, if the localized liberation of plasminogen activator by anoxia, previously described, is taken into consideration.

From these it was concluded that many undesolved problems were seen in the relations between plasmin activation and hemorrhage, and further investigations is necessary.

Increases in thrombocyte-count or capillary resistance have been mentioned as being a hemostatic mechanism of steroid hormone as stated by Stefanini[16]. But in our cases, peripheral thrombocyte-or bone marrow megakaryocyte-count were not always increased by the administration of prednisolone. Even though a slight increase of capillary resistance was observed in some cases, but these increases alone would be unable to explain the hemostatic function of this hormone.

As in case 6 and 7, 30–40 mg of prednisolone induced the decrease of plasmin activity and also recovery of hemorrhage, while decrease of dosage to 20 mg produced the reappearance of increased plasmin activity and hemorrhage. In case 8 and 9, increased plasmin activity reappeared after decrease of the dosage of prednisolone without hemorrhage, but only case 8 showed a increase of thrombocyte-count.

From these facts it was observed that the plasmin activity and hemorrhage was inhibited by steroid hormone, but hemorrhage could not be always increased by the plasmin activation occurred after decreasing of the dosage of prednisolone.

According to Ungar[29], plasmin activity was decreased with the administration of ACTH in his animal experiment, and he concluded that ACTH accelerated the binding action of plasmin and antiplasmin by way of the adrenal cortex and the spleen, however, MacFarlane[30] observed the fibrinolysis by injection of adrenalin into a severe addison's patient whose adrenal was destroyed completely, and concluded that the adrenal cortex had not direct effect on the plasmin activity. At any rate, further study will be required to clarify the relations between steroid hormone and plasmin.

The hemorrhagic tendency, which was the most important symptom of aplastic anemia and acute myeloic leukemia and was the direct cause of death, was due not only to disturbance of thrombocyte and other clotting factors but also to the activation of plasmin, however, the cause of plasmin activation could be only

speculated. Steroid hormone would have an important role in hemostatic mechanism by inhibiting the plasmin activity.

CONCLUSIONS

The plasmin activity was estimated by various methods in 7 cases of aplastic anemia and 2 cases of acute myeloic leukemia, and the relations between hemorrhage and plasmin activity were observed.

1. Gum bleeding, nasal hemorrhage and subcutaneous bleeding were observed with increasing of plasmin activity.

2. In some cases, ε-amino-n-caproic acid inhibited the bleeding and decreased the plasmin activity also.

3. Administration of some dosage of steroid hormone decreased the plasmin activity and inhibited the bleeding.

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THE STUDY ON PLASMIN IN CASES WITH CEREBRAL VASCULAR LESION

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The studies on plasmin system in the field of internal medicine have been made exclusively in hemorrhagic hematologic diseases up to date.

Recently, we are investigating on this enzyme system on the patient ailing cerebral vascular lesions, and will present some of our results in this paper.

METHOD AND MATERIAL

Materials: The number of cases studied up to date is 45, involving both sexes aged from 45 to 71 years old.

The cases consist of 9 intracranial hemorrhages, 13 cerebral infarctions, 20 arteriosclerosis (including those with hypertension) and 3 retinal bleedings. All of the arteriosclerotic patients chosen for this survey reveal ophthalmoscopically moderate to severe retinal arteriolosclerosis. (K.W. Gr. II-III-IV)

Method: For the determination of plasmin activity, venous blood of patient was used. After withdrawal, the blood sample was immediately mixed with 1/10 volume of 3.8% sodium citrate solution and kept at 4°C in a refrigerator. The routine method to determine the activity are as follows.

1) Whole clotolysis test (by Ratnoff's method) [1]
2) Euglobulin lysis test (by Lewis's method) [2]
3) Streptokinase activated plasmin activity (whole plasmin unit) (Colgan's method) [3]
4) Quantitative determination of fibrinogen [4]

These methods were partially modified for the use at our technical center. The criterion for the plasmin activity is as follows.

As to whole clotolysis test, occurrence of this phenomen within 3 days was

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interpreted as "accelerated" (positive), between the 4th and 6th day as "normal" and after the 7th day as "prolonged."

In streptokinase activated plasmin activity, below 20 u. was interpreted as negative (−), from 21 u. to 26 u. as (±) and over 27 u. as positive (+).

For euglobulin lysis test, below 0.7 u. was decided as negative above 1.0 u. as positive.

As to fibrinogen, below 2 mg/ml was considered to be decreased and above 4 mg/ml to be increased.

RESULTS

Results in 9 cases with intracranial hemorrhage (cerebral hemorrhage 8 and subarachnoid hemorrhage 1) are as follows. (Interval between the attack and blood sampling varied from 4 hours to 31 months).

With whole clotolysis test, plasmin activity normal 3, increase (+) 3, decrease 2, non examined 1, streptokinase activated plasmin activity (+) 4, (±) 5, (−) 0. Euglobulin lysis test was negative in all examined cases. Fibrinogen normal 5, decrease 2 and increase 2.

The case where plasmin activity was most remarkable was 59 years old male died one day after the attack of cerebral hemorrhage. In this case results were as follows; whole clotolysis test—3rd day (+), streptokinase activated plasmin −27.8 u. (+), fibrinogen −1.1 mg/dl. (decreased). Euglobulin lysis test was not performed.

Of 3 cases with retinal bleeding, whole clotolysis test was normal in 1 case, prolonged in 2 cases. Streptokinase activated plasmin activity was (+) in 1 case, (±) in 2 cases. Fibrinogen was normal in all cases.

Of 13 cases wih cerebral infarction, whole clotolysis test was normal in 5 patients and prolonged in 7 cases. Streptokinase activated plasmin activity was positive in only one case, (±) in 11 cases and negative in 1 case. Euglobulin

Table 1

Activity of Blood Plasmin in Cases with Cerebral Vascular Lesion

Case No.	Name	Sex	Age	Diagnosis	Interval from the attack to sampling	Plasmin—Activity			
						Fibrinogen (mg/ml)	Whole clotolysis (days)	Whole plasmin (unit)	Euglobulin-lysis test (unit)
15	K. K.	M	48	Cerebral hemorrhage	8 months	2.8	10	25.64	less than 0.56
16	M. O.	M	46	〃	2 〃	3.0	9	30.30	〃 0.56
20	T. K.	M	60	〃	10 〃	1.9	4	27.77	〃 0.56
21	H. O.	M	64	〃	18 〃	3.1	4	25.64	〃 0.56
32	E. K.	M	62	〃	12 〃	4.4	3	27.80	〃 0.56

Case No.	Name	Sex	Age	Diagnosis	Interval from the attack to sampling	Plasmin—Activity			
						Fibrinogen (mg/ml)	Whole clotolysis (days)	Whole plasmin (unit)	Euglobulinlysis test (unit)
36	K. K.	M	45	〃	1 day	3.8	6	25.64	〃 0.56
37	M. N.	M	59	〃	4 hours	1.1	3	27.80	—
106	M. Y.	F	52	〃	5 months	2.4	—	22.22	—
29	A. N.	M	44	Subarachnoid hemorrhage	4 days	8.3	3	20.83	less than 0.56
13	M. K.	F	58	Retinal bleeding		3.4	5	30.30	〃 0.56
14	K. T.	F	57	〃		2.6	10	23.81	〃 0.56
23	M. O.	F	68	〃		3.2	8	24.69	〃 0.56
2	T. M.	M	55	Cerebral thrombosis	19 months	3.1	6	25.64	〃 0.56
3	S. T.	M	71	〃	6 〃	2.7	6	25.64	〃 0.56
4	T. H.	M	67	〃	19 〃	3.4	8	25.64	〃 0.56
5	S. N.	M	63	〃	7 〃	2.8	8	28.98	〃 0.56
6	K. I.	M	64	〃	4 〃	4.5	7	19.61	〃 0.56
9	B. S.	M	60	〃	7 〃	6.3	6	23.81	〃 0.56
17	T. S.	M	60	Cerebral embolism	5 〃	7.5	11	23.81	〃 0.56
18	Y. K.	M	66	Cerebral thrombosis	5 〃	4.3	4	21.50	〃 0.56
19	S. M.	M	61	〃	31 〃	4.3	5	23.81	〃 0.56
22	K. S.	M	58	〃	2 days	3.5	9	23.81	〃 0.56
25	N. S.	M	61	〃	6 months	4.2	9	20.83	〃 0.56
105	N. M.	F	63	〃	5 〃	2.8	—	25.04	0.69
39	S. S.	M	69	〃	1 day	2.7	8	22.20	less than 0.56
1	N. T.	M	67	Arteriosklerosis		3.1	6	23.81	〃 0.56
10	S. T.	M	63	〃		3.1	6	30.30	〃 0.56
11	T. G.	F	55	〃		3.7	3	25.64	〃 0.56
101	M. O.	F	60	〃		3.2	2	16.66	〃 0.56
102	M. U.	M	69	〃		3.2	2	16.66	〃 0.56
107	H. K.	F	54	〃		3.1	—	19.61	〃 0.56
109	I. Y.	F	62	〃		4.0	—	25.64	0.57
112	N. I.	F	65	〃		2.7	—	22.22	less than 0.56
115	I. I.	F	58	〃		2.5	9	23.81	〃 0.56
116	Y. T.	F	53	〃		2.2	7	20.83	〃 0.56
117	T. K.	M	65	〃		3.8	4	10.41	〃 0.56
118	Y. A.	M	49	〃		2.4	3	25.64	〃 0.56
119	Y. S.	F	52	〃		2.9	4	17.54	0.58
121	T. S.	M	74	〃		2.8	3	25.64	less than 0.56
122	D. T.	M	56	〃		2.9	4	25.64	〃 0.56
123	K. M.	F	60	〃		3.0	3	23.81	〃 0.56
124	K. S.	M	48	〃		3.1	2	30.30	〃 0.56
125	T. S.	F	59	〃		2.7	6	27.77	〃 0.56
126	K. N.	F	47	〃		2.4	7	22.22	〃 0.56
27	R. N.	F	56	〃		4.1	4	23.81	〃 0.56

Table 2
Activity of Blood Plasmin in Cerebral Vascular Disease

Diagnosis	Number of cases	Fibrinogen			Whole clotolysis			Whole plasmin			Euglobulin-lysis test	
		Decr-ease	Nor-mal	Incr-ease	Accel-erated	Nor-mal	Prolo-nged	+	±	−	+	−
Intracranial hemorrhage	9	2	5	2	3	3	2	4	5	0	0	9
Retinal bleeding	3	0	3	0	0	1	2	1	2	0	0	3
Cerebral infarction	13	0	7	6	0	5	7	1	11	1	0	13
Arterio-sclerosis	20	0	19	1	7	7	3	3	12	5	0	20

lysis test was negative in all cases. Fibrinogen normal 7, increase 6.

Of 20 cases with arteriosclerosis, whole clotolysis was accelerated in 7 cases, normal in 7 cases, prolonged in 3 cases. Streptokinase activated plasmin activity was (±) in 12 cases, (+) in 3 cases and (−) in 5 cases. Fibrinogen was normal in 19 cases, increased in 1 case.

DISCUSSION AND SUMMARY

Comparing the result in cases with cerebral hemorrhage to those in cases with cerebral infarction, in the former, whole clotolysis test is positive (accelerated) in one third of the cases, streptokinase activated plasmin activity is positive in about half of the cases and none of them is negative, while in the latter, whole clotolysis test is not accelerated in any cases, but is prolonged in 7 cases, streptokinase activated plasmin activity is (±) in the majority of the cases and (+) in only 1 case.

It is a very interesting fact that, in the cases of so called cerebral apoplexy, the value of whole clotolysis test and whole plasmin show a special tendence for hemorrhage and infarction respectively.

Plasmin was positive not in all cases with hemorrhage. This is probably due to the interval between the attack and blood sampling or to some other factors.

In cases with arteriosclerosis (including those hypertension) as the control for the cases with apoplexy, it was remarkable that streptokinase activated plasmin was negative in one fourth of the cases. On the other hand, whole clotolysis test was accelerated in several cases. These cases are considered very interesting as to the prognosis for the cerebral apoplexy, and beeing followed up carefully at our clinic.

In conclusion, a definate relationship between cerebral vascular lesion, particularly intracranial hemorrhage and plasmin activity was suggested.

We are planning to make further investigation in more number of case as to the change of plasmin level in relation with time from attack and so on.

We wish to express our thanks to S. Okamoto, M.D., Professor of Physiology, Kobe Medical College.

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THE INHIBITORY EFFECT OF IPSILON-AMINO-CAPROIC ACID ON THE TUBERCULIN REACTION

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Some observations were attempted to examine the inhibitory action of Ipsilon-amino-caproic Acid** on the Tuberculin reaction. On this study Ipsilon showed a powerful inhibitory action on the Tuberculin reaction, when it was injected intracutaneously as mixed solution of Tuberculin and Ipsilon. Those who were injected the above solution, reacted positively to the ordinary Tuberculin test. It was considered however, that in vitro Ipsilon might destroy the Tuberculin effective substance in the mixed solution of Tuberculin and Ipsilon. Next, when Ipsilon was injected at the same region where Tuberculin reaction*** had already been done in a general way a certain period before, Ipsilon showed an inhibitory action on this occasion, too.

METHOD

The first experiment: On the right fore-arms of six Tuberculin-positive children the T-R (1:2000 Tuberculin solution) was given. On the left fore-arms of the same subjects Tuberculin-Ipsilon mixture(one volume of 1:1000 Tuberculin plus the same volume of Ipsilon solution) was injected. Both right and left regions of the arms were examined about redness and induration after 24 and 48 hours of the injection.

The second experiment: T-R (1:2000 Tuberculin solution) was given on both fore-arms of four Tuberculin-positive children. On the same regions of the left fore-arms of these subjects 2.5% of Ipsilon solution was injected intracutaneously after 12 and 24 hours of the Tuberculin injection. The effect of Ipsilon was examined after 24 and 48 hours of the first Tuberculin injection.

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** hereinafter abbreviated as Ipsilon.
*** hereinafter abbreviated as T-R.

RESULTS

In the table, group 1 is the subjects of the first experiment, and group 2 is of the second one.

Effect of Ipsilon on Tuberculin Reaction.

Group	Case No.	Case Name	Age (years)	Sex	Side of arms	Reading of diameter of redness and Induration: After 24 hours	Reading of diameter of redness and Induration: After 48 hours	Inhibitory effect
Group 1	1	NY	4	Male	Left Right	3×3/10×14 5×5/20×25	3×3/ 0 5×5/25×25	+
	2	YD	10	Male	L R	0 / 0 3×3/10×9	0 / 4×4 3×3/10×5	+
	3	HN	10	Male	L R	0 / 4×4 4×4/25×27	0 / 0 4×4/20×18	+
	4	KY	9	Female	L R	0 / 4×4 10×8/13×15	0 / 5×5 10×5 /13×10	+
	5	OT	14	Female	L R	0 / 6×5 0 /10×8	0 / 5×5 2×3/ 7×8	−
	6	TO	10	Female	L R	0 /17×15 0 /16×13	0 /14×12 0 /11×10	−
Group 2	7	HI	7	Male	L R	0 / 3×25 0 /13×12	0 / 0 0 /10×11	+
	8	AM	8	Male	L R	0 / 6×4 0 /14×14	0 / 2×2 0 / 5×5	±
	9	NZ	12	Male	L R	2×3/ 4×4 2×3/14×15	0 / 0 0 /10×9	+
	10	TH	13	Male	L R	0 / 8×6 0 / 8×8	0 / 0 0 / 8×9	+

Note: In the reading, Denominator is the diameter of outer redness and numerator is the inner redness.

In group 1 the inhibtory action of Ipsilon on T-R was seen in four cases among six. In case 1 injection was carried out immediately after mixing of Tuberculin and Ipsilon solutions. In case 2 injection was carried out after 8 hours of mixing them. In cases 5 and 6 the injection was given after a long period of mixing of the two kinds of the solution, and T-R was not inhibited by Ipsilon. This result means that Ipsilon does not destroy the Tuberculin effective substance in vitro.

In group 2, inhibitory action of Ipsilon on T-R was noted on three cases among four. This was another demonstration that inhibitory action of Ipsilon on T-R was not due to the destruction of Tuberculin effective substance by Ipsilon, but due to the direct action of Ipsilon against body tissue. Ipsilon concentration of 2.5% is enough to inhibit T-R. To examine the side effect of Ipsilon, only Ipsilon was injected intracutaneously and observed its reaction after 24 and 48 hours of injection, but neither redness nor induration was noted.

CONCLUSION

Ipsilon seemed to inhibit T-R. It is thought that the inhibitory action of Ipsilon on T-R is not due to the destruction of Tuberculin effective substance by Ipsilon, but due to the direct action of Ipsilon against body tissue.

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CLINICAL USE OF ε-AMINO-N-CAPROIC ACID ON ECZEMA OR OTHER KINDS OF SKIN DISEASES SUSPECTED TO BE ALLERGIC

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INTRODUCTION

It has been reported by several authors that eczema or other kinds of skin diseases suspected to be allergic are treated effectively by antiplasmic therapy in most cases.

But these reports were only clinical data, and plasmin activity in the blood was examined only in a few cases.

We have tried to prove the effectiveness of antiplasmin drug ε-amino-n-caproic acid*** and intended to reveal the relation between plasmin activity in the blood and the effectiveness of the administration of ε.

This investigation was done by evaluating the plasmin activity together with antiplasmic therapy and the changes of plasmin activity as followed on patients who were suffering from eczema or other kinds of skin diseases suspected to be allergic, were carried out at the Dermatology Department of the School of Medicine, Keio University, and some results obtained were as follows.

GENERAL METHOD OF CLINICAL EXAMINATION

i) The clinical status of patients was carefully observed and recorded according to clinical routine in every consultation.

ii) A sterilized distilled aqueous solution of ε was as a rule, injected to out-patients every other day and to in-patients every day. To infant patients, 2 to 5 cc of 5% ε solution was injected subcutaneously or intravenously, and to other patients 20 cc of 5% ε solution intravenously.

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*** Abbreviated as ε in this text.

iii) Relating to the clinical examination, plasmin activity in the blood was also examined by routine methods. The acceleration of whole clot lysis and euglobulin lysis and the decrease of the content of plasma fibrinogen were noticed. Appropriate and necessary controls were taken to examine the effect of ε.

RESULTS

The clinical diagnosis of 46 patients under investigation and the results of the blood examination performed before treatment with ε are summarised in the following table.

Relation Between Plasmin Activity and Diagnosis

Diagnosis / Plasmin activity	Eczema	Dermatitis	Urticaria	Others
Higher	12	14	4	1
Normal	3	4	1	1
Lower	0	2	1	3
Total	15	20	6	5

As shown in the above table, higher plasmin activity in the blood was found in 12 out of 15 cases of eczema, 14 out of 20 cases of dermatitis, 4 out of 6 cases of urticaria, and one out of 5 cases of other skin diseases. On the other hand, lower plasmin activity was only found in one case of chronic urticaria, 2 out of 2 cases of sub-acute dermatitis and 3 out of 5 cases of other skin diseases.

In 35 out of 46 cases in which plasmin activity was examined, the administration of ε was carried out at our clinics. In these cases, we particularly conducted research on the relation between plasmin activity in the blood, clinical signs and effect of ε. The results were as follows:

Diagnosis / Plasmin activity	Eczema	Dermatitis	Urticaria	Others
Higher	11 (10)	12 (11)	4	0
Normal	3 (2)	3 (1)	0	0
Lower	0 (0)	0 (0)	1	1
Total	14 (12)	15 (12)	5	1

Remark: Figures in parentheses indicate numbers of cases accompanied by exudative inflammation and are included in figures out of parentheses.

Plasmin activity in the blood was higher in 11 out of 14 cases of eczema, in 12 out of 15 cases of dermatitis and in 4 out of 5 cases of urticaria. In case

of higher plasmin activity, acute exudative inflammation was observed in 10 out of 11 cases of eczema, and in 11 out of 12 cases of dermatitis. Acute exudative inflammation was observed in 12 out of 14 cases of eczema and in 12 out of 15 cases of dermatitis. Such inflammation attacked the patient suddenly accompanied by such objective symptoms as intensive flush, swelling and exudation. The treatment with ε was effective in 13 out of 14 cases of eczema, especially in all the cases which showed higher plasmin activity and were accompanied by acute exudative inflammation, and was also effective in 13 out of 15 cases of dermatitis, especially in 11 out of 12 cases of acute exudative inflammation, and in 10 of 11 cases which showed higher plasmin activity. The treatment with ε was effective in 4 cases which showed higher plasmin activity, out of 5 cases of urticaria.

Thus, the administration of ε was effective in 30 out of 34 cases of eczema, dermatitis and urticaria, especially in 24 out of 27 cases which showed higher plasmin activity.

Plasmin activity in the blood was lower than normal in cases No. 13 and No. 21 in which sign of exudative inflammation was little. The result obtained from these cases indicated that the administration of ε was ineffective in these cases.

Normal plasmin activity in the blood was observed in some cases of eczema and acute dermatitis. In these cases, the administration of ε was generally effective but not so prominent as in the cases of the same diagnosis in which higher plasmin activity was observed.

Investigations on the effect of the sole administration of ε to 12 patients suffering from such skin diseases with acute exudative inflammation were carried out in spite of the difficulty in clinical practice. In fact, such patients tend to ask for additionl treatments with ointment. The results obtained from those patients, of which details were shown in the description of the following clinical cases (Nos. 5, 10, 14, 31, 33, 34, 35, 37, 38, 39, and 40), indicated that the sole administration of ε was effective in all 11 cases.

In case No. 37, any other treatment than the intermittent use of ε was adopted. First, eczema apparently improved after the administration of ε, remaining still higher plasmin activity in the blood. The administration of ε was then interrupted for a week and the eczema got obviously exacerbated accompanying high activity of plasmin in the blood. By resuming the sole administration of ε, eczema got rapidly and completely cured. In case No. 31, the intermittent, sole administration of ε was also carried out. By the application of ε in the first stage, the disease improved. But after the interruption of the

administration of ε the exacerbation of signs was observed. The restarting of the administration of ε resulted in a successful improvement of the acute signs.

In case No. 6, the effectiveness of the intermittent administration of ε was examined with the continuous use of ointments. The application of first proved to be effective; the interruption of the administration of ε in an early stage reversed skin diseases despite the continuous use of ointments. When the administration of ε was resumed, the skin disease was cured. The results obtained from this case correspond to the results obtained from the sole, intermittent use of ε to such a disease.

As a result of the careful inspection of the results of hundreds of injections of ε given to these patients, no unfavorable side effect was observed.

CLINICAL CASES

In reproducing the degree of subjective or objective signs, the following symboles were used.

+++	..... Very strong
++	..... Moderately strong
+	..... Clearly present but weak
±	..... Very slight

(1) *Clinical cases of the sole, intermittent administration of ε.*

a) *Case 37. Keio Card No. 7176, Eiko Endo (27) Female*

1) Diagnosis: Acute eczema (on face and neck).

2) Anamnesis: No significant anamnesis related to the diagnosis.

3) Case history: About a month before the first examination, eczema appeared on face and neck. Medical treatment with calcium compound and vitamins was given at another hospital, but in vain. The patient came to the hospital on October 1, 1958.

4) Status at the first examination: Itching (++), causalgia (++), red swelling (+++), erosion (±). Acute exudative inflammation was observed.
Blood examination was carried out on October 1. The result showed that whole clot lysis was strongly accelerated, indicating high activity of plasmin.

5) Course and effect of treatment with ε: No other treatment than ε was applied in this case 20 cc of 5% ε solution was injected intravenously for six days from October 1. By the first injection, exudation began to disappear. After the second injection, secretion and redness decreased; after the fourth injection, the eczema seemed to have almost healed; and after the sixth injection, it healed completely. Blood examination was carried out 24 hours after the sixth injection.
The result showed that whole clot lysis remained accelerated. The administration of ε was interrupted for four days. On October 14, eczema recurred and blood examination was carried out. The result indicated the accerelation of

whole clot lysis of the blood. The administration of ε was restarted; after first injection all signs of eczema began to improve and after the fourth injection the eczema was cured completely.

On July 3, 1959, when no significant sign was observed, blood examination was carried out to determine plasmin activity in the healthy state. The result obtained was that plasmin activity in the blood was normal. This fact indicated that higher plasmin activity in the blood had appeared in the course of eczema and not in the healthy state.

6) Side effect: None.

7) Result: High plasmin activity of blood was found in this case. Conspicuous effectiveness of the sole administration of ε, carried out intermittently, constitutes the sufficient control for demonstrating that the curing of the disease was solely due to the sole administration of ε.

b) *Case 31. Keio Card No. 6872, Yoshiko Ikeda (22) Female*

1) Diagnosis: Acute eczema (on face and limbs).

2) Anamnesis: No significant anamnesis related to diagnosis.

3) Case history: Eruption appeared four years before at the upper and lower limbs. Since then improvement and exacerbation interchanged. Two months before the first examination, similar eruptions appeared again on the same parts. Treatments with Sodium thiosulfate, Vitamin B_2, Methionine at another hospital showed no effect. The patient came to the hospital on September 17, 1958.

4) Status at the first examination: Itching (++), red spots (++), papules (++), little blisters (+) and on the whole body scales (+), red coloured and swelling face (+). Acute exudative inflammentory tendency was apparently observed.

Blood examination was carried out on September 17. Whole clot lysis and euglobulin lysis were accelerated, indicating higher plasmin activity.

5) Course and effect of treatment with ε: Five cubic centimetre of 5% ε solution was injected intravenously every other day five times, and then every day for the following three days, without using external ointments. After the fourth injection, an inprovement of symptoms was perceived, and after the fifth injection, red swelling in the face disappeared and itching was almost unfelt.

On September 27, the administration of ε was stopped because almost all signs had healed. At this time, blood examination was carried out. The result showed that plasmin activity turned to normal in whole clot lysis and euglobulin lysis. On October 11, the patient had a relapse. The patient came again to the hospital on October 16. Red swelling and itching were perceived at the face, especially both eyelids, and the eruption on both lower limbs was also exacerbated. The result of blood examination carried out on October 16 showed that plasmin activity was normal in whole clot lysis and euglobulin lysis.

Treatment with the sole administration of ε started again. Namely 20 cc of 5% ε solution was injected intravenously every day for six days. After the

first injection, red swelling and itching subsided rapidly. After the third injection most of signs disappeared.

6) Side effect: None.

7) Result: It is recognized that the sole, intermittent use of ε was successful in the course of the treatment.

(2) *Clinical cases of the sole administration of ε.*

a) *Clinical cases of patients suffering from urticaria against which the administration of Antihistamin Drugs was ineffective.*

It is generally known that such kinds of urticaria, against which the administration of Antihistamin Drugs is ineffective, will improve with difficulty by the other ordinary treatments.

Case 5. Keio Card No. 4805, Hikaru Motoakiba (24) Male

1) Diagnosis: Chronic urticaria.

2) Anamnesis: No important, significant anamnesis related to the diagnosis.

3) Case history: Suffered from urticaria for a month before hospitalization. Only transient improvement for ten hours or less was perceived each time by an injection of Antihistamin drug. The patient came to the hospital on July 2, 1958.

4) Status at the first examination: Red spots on the skin were observed, and itching complained. Artificial urticaria was positive.
Blood examination was carried out on July 2 prior to the administration of ε. The result thereof was that whole clot lysis was strongly accelerated, indicating the accelerated fibrinolysis by plasmin in vivo.

5) Course and effect of the sole administration of ε: Starting from July 2, 5 cc of 5% ε solution was injected intravenously three times a week, and six injections in all. No other treatment than ε was given. After the second injection, eruption disappeared in the daytime, appearing only during the night; after the fifth injection, urticaria did not appear at all.

6) Side effect: None.

7) Result: The administration of ε was recognized distinctly effective, because the chronic urticaria which had lasted for ten months healed completely within a couple of weeks by the sole administration of ε.

Case 10. Keio Card No. 5059, Kiyoshi Shibata (28) Male

1) Diagnosis: Chronic Urticaria.

2) Anamnesis: No particular, significant anamnesis related to the diagnosis.

3) Case history: Urticaria began to appear three months previously, at least once a day in the evening. Only transient improvement for ten hours or less was perceived each time, by an injection of antihistamin drugs. The patient came to the hospital on July 11, 1958.

4) Status at the first examination: Artificial urticaria (+), red spots (+), itching (+). The result of a blood examination carried out on July 11, prior

to the administration of ε, showed that plasmin fibrinogen had decreased, indicating the accelerated breakdown of plasma fibrinogen.

5) Course and effect of the sole administration of ε: Starting from July 11, 5 cc of 5% solution was injected three times a week, totalling twelve injections. After the fourth injection, the frequency of appearance of urticaria diminished; and after the ninth injection it seldom appeared. After the twelveth, it healed completely.
6) Side effect: None.
7) Result: The sole administration of ε was recognized as conspicuously effective, because such chronic urticaria which had lasted three months, healed completely with twelve injections of ε.

Case 14. Keio Card No. 5196, Kitaro Soma (63) Male

1) Diagnosis: Acute urticaria.
2) Anamnesis: No particular anamnesis related to the diagnosis.
3) Case history: Urticaria started appearing when the patient took Ephedrine for treating asthma ten days before the first examination. By the administration of Antihistamin drugs, it disappeared for a while each time, but it appeared again about ten hours after.
4) Status at the first examination: The patient came to the hospital several hours after he took Ephedrine on July 16, 1958. Blood examination was carried out on that day prior to the administration of ε. The result thereof was that whole clot lysis was slightly accelerated and the content of plasma fibrinogen had decreased to 130 mg/dl indicating higher plasmin activity of blood.
5) Course and effect of the sole administration of ε: Five cubic centimetre of 5% ε solution was injected intravenously every day for six days. After the fourth injection, Urticaria did not appear at all.
6) Side effect: None.
7) Result: It is recognized that the course of improvement was very satisfactory, and that treatment with ε was conspicuously effective.

b) *Clinical cases of patients suffering from acute eczema or dermatitis.*

Case 33. Keio Card No. 7011, Kazuko Nagai (23) Female

1) Diagnosis: Acute dermatitis (on the face).
2) Anamnesis: No particular anamnesis related to the diagnosis.
3) Case history: In the morning of the day before the first examination, appeared eruption, the cause of which was unknown. The patient came to the hospital on September 24, 1958.
4) Status at the first examination: Causaligia (++), red swelling (+++). Acute exudative inflammation apparently observed.
Blood examination was carried out on September 24, prior to the administration of ε. The result thereof was that whole clot lysis was accelerated indicating higher plasmin activity.

5) Course and effect of the sole administration of ε: No other treatment than ε was carried out in this case. At first 5 cc of 5% ε solution was injected intravenously, and thereafter 20 cc of 5% ε solution was given every day for four days. After the first injection red swelling and causalgia subsided conspicuously, and after the fourth injection the dermatitis healed completely. Blood examination was carried out on September 29 when the disease had healed. The result showed that whole clot lysis had turned to normal.
6) Side effect: None.
7) Result: It is recognized that, by the sole administration of ε, higher plasmin activity became normal and acute dermatitis was healed completely. This fact shows that the effectiveness of ε is based on the antiplasmic action of ε.

Case 34. Keio Card No. 7018, Fumiko Sakaeda (24) Female

1) Diagnosis: Dermatitis due to shellfish poisoning (on the whole body).
2) Anamnesis: The patient often suffered from urticaria.
3) Case history: On September 23, 1958, half an hour after the patient ate soya bean soup of shellfish, itching and redness appeared on the whole body. Next morning the patient came to the hospital.
4) Status at the first examination: Itching (+++), causalgia (+++), redness (++). Acute exudative inflammation apparently observed. Blood examination was carried out on September 24, prior to the administration of ε. The result thereof was that whole clot lysis was accelerated and hypofibrinogenemia was proved, indicating high plasmin activity in the blood.
5) Course and effect of the treatment with ε: From September 24, 10 cc to 20 cc of 5% ε solution was injected intravenously two times a day for two days, and then 20 cc of 5% ε solution was injected once a day for the following four days. After the second injection symptoms became worse for a time; and at the blood examination carried out on September 26, whole clot lysis and euglobulin lysis were found to be accelerated.
But afterwards it showed a gradual improvement. After the fifth injection the dermatitis began to desquamate and healed completely.
6) Side effect: None.
7) Result: It is recognized that the satisfactory course of improvement was shaped by the sole administration of ε to the patient in whom high plasmin activity was found in the blood.

Case 35. Private Card of Dr. Hatano, No. 365 Fujiro Miyake (52) Male

1) Diagnosis: Microbid (on almost the whole body, especially both armpits, buttocks and the limbs).
2) Anamnesis: No particular anamnesis related to the diagnosis.
3) Case history: About three weeks before the first examination eruption appeared on the above-mentioned parts. None of Ca-Methioninate, Vitamin B_2, Chloromycetin, Terracortril ointments and Baramycin ointment were effective at another hospital. The patient came to the hospital on April 4, 1958.
4) Status of the first examination: Itching (+), exudation (+), red spots (+),

papules (+), crusts (+).

Blood examination was carried out on April 7. The result thereof was that whole clot lysis was accelerated, indicating high plasmin activity.

5) Course and effect of the treatment with ε: No other treatment than ε was carried out in this case. Twenty cubic centimetre of 5% ε solution was injected intravenously every day for forty-two days, and improvement in all signs went on but original eruption on the buttock remained.
6) Side effect: None.
7) Result: It is recognized that the administration of ε was effective in treating the microbid.

Case 38. Keio Card No. 7195, Kyoko Tanaka (10) Female

1) Diagnosis: Acute eczema (on both cheeks, the back of both ears, the back, buttocks and limbs).
2) Anamnesis: No significant anamnesis related to the diagnosis.
3) Case history: About a week before the first examination, eczema appeared on both cheeks, the back of both ears, the back, buttocks and limbs. The patient came to the hospital on October 2, 1958.
4) Status at the first examination: Itching (++), exudation (+++), red spots (++), papules (++), little blisters (+). Acute exudative inflammation apparently observed.

Blood examination was carried out on October 2 prior to the administration of ε. The result was that whole clot lysis was normal.

5) Course and effect of the administration of ε: No other treatment than ε was applied in this case. Since October 2, 20 cc of 5% ε solution was injected intravenously every day for nine days; but no ointments were used. After the second injection, the eczema became a little better; after the sixth injection it dried up to a great extent. After the seventh injection it desquamated generally and only itching was sometime felt, and after the ninth injection the eczema almost healed. Blood examination was carried out at this time. The result showed that the content of plasma fibrinogen has increased, suggesting the lowered activity of plasmin during the treatment with ε.
6) Side effect: None.
7) Result: It is recognized that the administration of ε was conspicuously effective, and that, by the administration of ε, the course of improvement was satisfactory, and plasmin activity in blood became lower than before the administration of ε.

Case 39. Keio Card No. 7213, Roku Fuchigami (40) Female

1) Diagnosis: Acute dermatitis (on neck and breast).
2) Anamnesis: No significant anamnesis related to the diagnosis.
3) Case history: When the patient put on a nylon blouse, dermatitis appeared on the neck and breast. The patient came to the hospital on October 31, 1958.
4) Status at the first examination: Red spots with distinct boundaries (++),

papules (++). Acute exudative inflammation was observed. Blood examination was carried out prior to the administration of ε. The result thereof was that whole clot lysis was slightly activated.

5) Course and effect of the administration with ε: No other treatment than ε was applied in this case. Five percent ε solution was injected intravenously 20 cc each on October 3 and 6. But no ointment was used. The day after the first injection, papules disappeared, red spots became faint, and itching improved. By the two injections the dermatitis was completely cured. The result of blood examination carried out on October 13 was that whole clot lysis had turned to normal.
6) Side effect: None.
7) Result: It is recognized that the course of improvement was very satisfactory and that the administration of ε was conspicuously effective in treating the dermatitis and suppressing plasmin activity.

Case 40. Keio Card No. 7416, Sachiko Samukawa (32) Female

1) Diagnosis: Acute dermatitis (on the face).
2) Anamnesis: No significant anamnesis related to the diagnosis.
3) Case history: Itching on the face started to occur ten days before the first examination. Shaving with a razor four days before, exacerbated itching and caused red swelling. It was treated at another hospital, but no improvement was obtained. The patient came to the hospital on October 13, 1958.
4) Status at the first examination: Swelling (+), redness (++), millet seed size pustules (+), itching (++). Acute exudative inflammation was observed. The result of the blood examination carried out on October 13 prior to the administration of ε was that plasmin activity was normal.
5) Course and effect of treatment with ε: Twenty cubic centimetre of 5% ε solution was injected intravenously in one or two injections a day for thirteen days. Any other pharmaceuticals and ointments were not used in this case. After the first injection, swelling subsided, and exudation and itching decreased. After the fifth injection pain and itching were not felt, but only the faint red swelling remained. After the ninth injection every signs greatly improved. But the use of a cosmetic cream, several hours after the ninth injection, exacerbated itching, pain and redness. ε was given one or two times a day for seven days, and the dermatitis was completely cured.
Blood examination was made on October 30 when the dermatitis was cured. The result was that plasmin activity was normal.
6) Side effect: None.
7) Result: It is recognized that the course of improvement was satisfactory and that the treatment with ε was conspicuously effective and reduced higher plasmin activity to normal.

(3) *Clinical cases of intermittent administration of ε together with external ointments or other pharmaceuticals.*

Case 6. Keio Card No. 4492, Sawa Furukawa (47) Female

1) Diagnosis: Acute eczema (on the back head, neck and both elbows).
2) Anamnesis: One year before the first examination eczema-like eruption appeared at the neck and face.
3) Case history: On the middle of April 1958, eczema started to appear at the back of the head, the neck and the face, and it continued for more than two months.
4) Status at the first examination: The first examination was made on June 21, 1958. Itching (++) pain (±), causalgia (±), red spots (+), infiltration (+), exudation (+), swelling of the lympatic grand at the neck (+). Acute exudative inflammation was observed.
   In spite of the external use of Boric acid ointment, 2% Phenol calamine lotion, in addition to ten injections of Ca-Methionate and three injections of Vitamin B_2, the status got worse.
   On July 7, besides the above-mentioned signs, itching papules were newly found on both elbows. Blood examination was carried out on July 7. The result was that whole clot lysis was accelerated and the content of plasma fibrinogen decreased, indicating higher plasmin activity.
5) Course and effect of treatment with ε: Starting from July 7, 5 cc of 5% ε solution was injected every day and Glyteer carbowax was used externally. After the first injection exudation greatly improved. After the third injection, it disappeared, only faint red spots and itching remained. After the sixth injection carried out on July 12, all signs improved, but only slight itching and slight redness remained. On July 13, the injection of ε was not undertaken. On July 14, the symptoms were found to have changed for the worse.
   Therefore three injections of Vitamin B_2 and one of Ca-Methionate were given in addition to ointments, but ε treatments was not given. Even with this treatment, the symptoms hardly improved.
   From August 6, the treatment with ε was restarted; 5 cc of 5% ε solution was injected every other day for ten days, and zinc oil was used additionally. On August 8, redness, exudation and itching were weakened. Still on August 13, the redness and papules remained slightly and itching was sometimes felt. On August 15, the disease was considered to have improved.
6) Side effect: None.
7) Result: The course of treatment by the intermittent use of ε present, adequate evidence for proving the effectiveness of ε on the acute eczema.

Case 8. Keio Card No. 4954, Yoshio Kunigane (39) Male

1) Diagnosis: Microbid (on the whole body).
2) Anamnesis: No particular anamnesis related to the diagnosis.
3) Case history: Five months before similar efflorescense appeared on the whole body, and two months after healed by the administration of Antibiotics at another hospital. A months after it recurred, and symptoms remained unimproved. The patient came to the hospital on July 8, 1958.

4) Status at the first examination: Red spots (++), papules (+), pustules (+), blood crusts (+), itching (++), and exudation (+++) on whole body. Acute exudative inflammation apparently was observed. The focus of microbid was considered to be at the right elbow. Blood examination was carried out on July 8, prior to the administration of ε. Whole clot lysis was normal, but euglobulin lysis and hypofibrinogenemia indicated accelerated fibrinolysis by plasmin in vivo.
5) Course and effects of treatment with ε: Some other pharmaceuticals were used in the first stage in addition to ε; but in the final stage ε was solely administered. Improvement was finally obtained by the sole administration of ε. Starting from July 8, 5 cc of 5% ε solution was injected intravenously every other day and eleven injections were given in all. Three injections of Ca-Methionate were also given. The day after the first injection of ε, efflorescense showed indication of drying, and itching was reduced. After the third injection, pigmentary deposit was perceived. But a faint itching was still felt. On September 20, microbid appeared again on both lower thighs. Twenty cubic centimetre of 5% ε solution was injected, as a rule, every other day and ten injections in all were given. In addition to ε, Ca-Methionate was injected intravenously in three injections. Symptoms almost disappeared by this treatment, but eruption was found again at the right elbow and on both lower thighs on September 30. The result of blood examination was that whole clot lysis and euglobulin lysis were accelerated. The sole administration of ε started on September 30. After 20 cc of 5% ε solution was injected intravenously in two injections, efflorescense dried and redness with microbid completely disappeared though the focus still remained.
6) Side effect: None.
7) Result: It was recognized that the administration of ε was effective and useful in treating main signs of microbid.

(4) *Others:*

The below table shows the results of other clinical cases.

From these results, it is clearly understood that:

1) ε was effective in treating all 12 cases of eczema and dermatitis accompanied by remarkable signs of acute exudative inflammation. In 11 out of these 12 cases plasmin activity of the blood was higher than normal and in the remaining one it was normal. This type of skin disease is called "acute type" in the table.
2) ε was, however, ineffective in treating 2 cases of skin desease in which plasmin activity of the blood was lower than normal.
3) The expression of "subacute type" was used for 6 cases of chronic inflammation accompanied by slightly acute inflammation, in half of which cases plasmin activity remained normal, while the remaining cases showed somewhat higher plasmin activity. The administration of ε in 3 out of 6 cases were effective, but remaining cases ineffective.

CONCLUSION

Most of those cases of eczema and other kinds of skin disease suspected to be allergic, in which such objective symptoms as flush, swelling and exudation were intense and such subjective symptoms as itching and causalgia were conspicuous, showed higher plasmin activity in the blood examination.

A sufficient number of clinical investigations indicated that the administration of ε was effective for treating those kinds of skin diseases. The results obtained from the intermittent use of ε presented the appropriate control.

Results obtained from the sole administration of ε or the intermittent, sole administration of ε to such patients indicated that the effectiveness of the administration of ε was solely due to the action of ε.

As a result of the careful inspection of such patients, no unfavorable side effect was observed.

The administration of ε is, therefore, effective and useful for treating patients suffering from eczema and other kinds of skin diseases, with signs of acute exudative inflammation, in which plasmin activity in blood is higher than normal.

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No.	Keio Card No.	Diagnosis	Status of the first examination		Plasmin activity	Administration of 5% ε solution	Other drugs	Results
2	4760 Age 33 Male	Dermatitis lacquer	Acute type	itching (++) pain (++) red spots (++) erosion in part (++)	High±ə	5 cc×6	Zinc oil. Polyethyleneglycol ointments	Effective. No side effect.
26	6288 Age 28 Female	Acute dermatitis	Acute type	causalgia (++) itching (++) redness (++) blisters (++)	Higher	20 cc×6 5 cc×6	Salycilic acid ointment	Effective No side effect.
27	6118 Age 57 Female	Acute dermatitis	Acute type	redness (+) exudation (++) itching (++)	Higher	5 cc×7	Glucuronic acid Zinc oil Ca-Methionate	Effective. Signs gradually improved. No side effect
15	5372 Age 74 Male	Acute eczema	Acute type	exudation (++) red spots (++) blisters (++) itching (+)	Higher	5 cc×16	Phenol zinc lotion	Extremely effective. All signs completely improved by 6 injections. No side effect
30	6691 Age 60 Female	Acute eczema	Acute type	itching (++) red spots (++) papules (+)	Normal	5 cc×4	Prednisolone	Effective. No side effect.
12	5160 Age 5 Female	Chronic eczema with acute exacerbation	Acute type	itching (++) red spots (++) papules (++) exudation (++)	Higher	2.5 cc×1 5 cc×1	Boric acid and zinc ointment	Conspicuously effective. By 1 injection exudation disappeared. No side effect
18	5538 Age 6 Female	Chronic eczema with acute exacerbation	Acute type	itching (++) papules (++) exudation (++) lichenification (++)	Higher	5 cc×11	Boric acid ointment. Hydrocortisone ointment	Effective. All signs improved by 3 injections. No side effect
23	5912 Age 4 Female	Chronic eczema with acute exacerbation	Acute type	red spots (++) itching (++) papules (+)	Higher	5 cc×3	T. S. ointment	Effective. By 2 injections all signs improved. No side effect.
24	5937 Age 12 Female	Chronic eczema with acute exacerbation	Acute type	itching (++) red spots (++) papules (++)	Higher	5 cc×9	T. S. ointment	Effective. By 6 injections all signs improved. No side effect.

7	4957 Age 21 Female	Acute dermatitis and microbid	Acute type	exudation (++) papules (++) swelling (++) red spots (+) pustules (+) crusts (+) itching (+)	Higher	5 cc×4 20 cc×27	Boric acid and zinc ointment. Vitamin A and D. Phenol zinc ointment	Effective. By 2 injections main signs improved. No side effect
43	7570 Age 48 Male	Acute dermatitis and autosesitized dermatitis	Acute type	itching (++) redness (++) blisters (++) exudation (++)	Higher	20 cc×8	Boric acid and zinc ointment Baramycin ointment	Conspicuously effective. By 2 injections exudation disappeared, by 4 injections all signs improved. No side effect.
42	7445 Age 26 Male	Acute eczema and microbid	Acute type	itching (++) papules (+++) pustules (+++)	Higher	20 cc×12	Boric acid and zinc ointment. Baramycin ointment Glyteer ointment	Effective. By 5 injections all signs improved. No side effect.
44	7641 Age 31 Male	Acute urticaria	Acute type	itching (++) wheal (+++) red spots (++) swelling (++)	Higher	20 cc×3	Phenol zinc lotion	Conspicuously effective. All signs completely improved by 3 injections. No side effect.
32	6944 Age 58 Female	Acute and chronic eczema	Acute type	itching (++) exudation (++) infiltration (+)	Higher	5 cc×1 20 cc×2	Boric acid and zinc lotion. T.S. ointment. Prednisolone	Effective. All signs improved gradually. No side effect
19	5611 Age 54 Male	Acute dermatitis	Sub-acute type	exudation (++) redness (++) fissure (++) crusts (++) pain (+)	Normal	5 cc×9	Zinc oil Phenol zinc lotion Vitamin A and D ointment	Effective. All signs improved gradually by 5 injections. No side effect.
45	7699 Age 25 Female	Acute dermatitis	Sub-acute type	red spots (+) itching (+)	Normal	20 cc×6	Phenol zinc lotion	Effective. All signs improved by 4 injections. No side effect.
46	8044 Age 23 Female	Acute eczema	Sub-acute type	itching (+) red spots (+) papules (+)	Normal	20 cc×2	Boric acid zinc ointment	Effective. All signs improved by 2 injections. No side effect.

No.	Keio Card No.	Diagnosis	Status of the first examination		Plasmin activity	Administration of 5 % ε solution	Other drugs	Results
28	6462 Age 20 Female	Subacute eczema	Subacute type	itching (+) papules (+) blisters (+) erosion (+)	Higher	5 cc×2 20 cc×6	Zinc oil T.S. ointment	Ineffective No side effect.
11	2659 Age 41 Female	Autosensitized dermatitis	Subacute type	itching (+) papules (+)	Slightly higher	5 cc×3	Phenol zinc lotion	Ineffective. No side effect.
36	7145 Age 41 Female	Acute dermatitis	Subacute type	red spots (++) papules (++) scales (+) itching (+++)	Higher	20 cc×7	Vitamin A and D ointment (at only seventh injection)	Ineffective. After 5 sole administration of ε, all signs slightly improved. By seventh injections, additionally using Vitamin A and D ointment, all signs exacerbated, and it did not improve in spite of the repeated administration of ε. No side effect.
21	4291 Age 19 Female	Herpetiform dermatitis (Duhring)		itching (++) red spots (+) blisters (+)	Lower	20 cc×8	—	Ineffective. No side effect.
13	3895 Age 21 Female	Chronic urticaria		—	Lower	5 cc×11	—	Ineffective. No side effect.

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CLINICAL USE OF IPSILON FOR THE PREVENTION OF THE ALLERGIC REACTIONS FROM BLOOD TRANSFUSION

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It has been gradually admitted that plasmin has a significant part in the appearance of anaphylaxis. Rocha e Silva[1] previously reported that the appearance of anaphylaxis was caused by liberation of Hystamin from the liver due to fibrinolysis. It has been noticed that cases of urticaria or chillness often appeared after blood transfusion. Therefore we are reporting here the results of study worked on the relationship between plasmin and urticaria or chillness appeared after blood transfusion, and plasmin activities were determined by various methods. As blood was transfused ε-amino-n-caproic acid[2]** was used and its effect was also identified.

MATERIALS AND METHODS

One to two hundred ml. of fresh blood was transfused. As the subject of the following investigation, only these patients who were known to develop urticaria or chillness always after transfusion were chosen. Plasmin activity was determined by the routine methods[3,4,5,6]. Fibrinogen was gravimetrically determined according to the method of Gramm. Changes in plasmin activity by simple transfusion were compared with that which were determined after blood transfusion preceded by the intravenous injection of 40 ml of 5% ε solution.

RESULTS OF EXPERIMENTS

The results obtained in each cases were as follows.

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** Abbreviated as ε in this text.

Case 1: Aplastic anemia. K.K. Female, 18 years-old.

Every time A-type blood was transfused, urticaria occurred conspicuously, and the plasmin activity was found to be increased in Ratnoff test and remarkable increase was found in whole plasmin. After the use of ε, however, urticaria had never appeared at all and the plasmin activity also turned to normal.

Case 2: Aplastic anemia. F.M., Female 48 years-old.

Whenever O-type blood was transfused, urticaria occurred, and the plasmin activity was proved to be markedly high in Euglobulin test. But after the use of ε only slight urticaria was seldom noticed.

Case 3: Lung abscess. H.K., Female, 64 years-old.

Every transfusion of AB-type blood was followed by urticaria and in Ratnoff test the exasperation of plasmin activity was remarkable. Also in this case urticaria had never appeared after the use of ε.

Case 4: Aplastic anemia. H.S., Male, 25 years-old.

At the time of A-type blood transfusion urticaria and chillness never failed to appear, but remarkable exasperation was not noticed in the activity, and only fibrinogen decreased. After the use of ε the side actions never occurred, and fibrinogen showed normality.

Case 5: Thrombocytopenic purpura. N.S., Female, 43 years-old.

Every time A-type blood was transfused, urticaria and chillness were so conspicuous that blood transfusion had sometimes to be suspended. In Ratnoff

Case No.		Ratnoff test days	Euglobulin test Lew u/ml	Whole plasmin test in serum Lew u/ml	Colgan's urine test u/ml	Fibrinogen mg/dl
1	Before transfusion	6	1.66	437	<1	320
	Urticaria	3	1.12	852	<1	344
	Transfusion+ε	8	1.26	595	<1	200
2	Before transfusion	5	1.42	462	<1	440
	Urticaria	5	3.34	487	<1	210
	Transfusion+ε	5	1.12	487	<1	260
3	Before transfusion	7	1.12	462	<1	716
	Urticaria	3	1.12	520	<1	360
4	Before transfusion	7	1.12	208	<1	190
	Urticaria	6	1.12	260	<1	140
	Transfusion+ε	7	1.12	208	<1	230
5	Before transfusion	9	1.12	397	<1	210
	Urticaria	5	1.12	320	<1	200
	Transfusion+ε	8	1.12	208	<1	230
6	Before transfusion	6	1.12	439	<1	260
	Urticaria	2	1.12	417	<1	250
	Transfusion+ε	6	1.12	198	<1	280

test the plasmin activity showed much exasperation, comparing with that before transfusion. However, after the use of ε the side-reactions never occurred, but blood transfusion was not interrupted at all.

Case 6: Erythroleucemia. K.Y., Male, 40 years-old.

Every transfusion of A-type blood was followed by conspicuous chillness and marked exasperation was shown in Ratnoff test. But they had never appeared after the use of ε, and the activity showed normality.

CONCLUSION

For urticaria and chillness occurring after blood transfusion, it must be firstly considered carefully whether appropriate blood type may be used or a provider may be allergic. However, as far as we experienced there were good many cases in which plasmin activities showed exasperation, which could be controlled by the use of ε.

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STUDIES ON THE URINARY FIBRINOLYTIC ACTIVITY IN NORMAL AND PATHOROGIC STATES

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INTRODUCTION

The plasmin system in the circulatory blood has been regarded as so complicated that various studies from different angles would be necessarily undertaken. The urinary fibrinolytic activity, however, has been studied only by a few workers.

In 1952 Colgan et al.[1] reported that the urinary fibrinolytic activity was strongly increased in dogs which had received a fatal dose of X-ray irradiation and afterwards died of severe lung-haemorrhage. It was observed by us that the urinary fibrinolytic activity increased in about half of those fishermen of the 5th Fukuryu-Maru who had been exposed to a large amount of irradiation from the radioactive falling out.

This investigation was undertaken to examine the urinary fibrinolytic activity in healthy persons and in patients ailing various disorders which were suspected to be related with the activation of plasmin system either in blood or in urine.

METHODS AND MATERIALS

Materials

Urine: Urine samples obtained were frozen with dry-ice aceton and kept in a deep freezer at −20°C. Just before the measurement, the stock samples were thawn at room temperature.

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ε-amino-n-caproic acid*: Synthetized ε-amino-n-caproic acid was kindly furnished by Daiichi Seiyaku Co., Ltd.

Fibrinogen: 0.3% bovine fibrinogen of Armour Laboratories in phosphate-saline buffer solution was used.

Thrombin: Thrombin topical of Park Davis was used. Stock solution containing 100μ. of thrombin per 1 ml of phosphate saline buffer solution at pH 7.4 was kept at −20°C.

Streptokinase**: Varidase, a mixture of streptokinase and streptodornase, kindly furnished by the Lederle Laboratories Division, American Cynamid Co., was used. Stock solution, containing 1000 u. of streptokinase per ml of phosphate-saline buffer solution was kept at −20°C.

Buffer: Phosphate-saline buffer, pH 7.4, was used throughout.

Measurembent of urine fibrinolytic activity.

A modification of Colgan's[1] method was used. pH of human urine was adjusted to 7.4. 0.5 ml of urine was diluted with phosphate buffer.

To a series of tubes containing 0.5 ml of urine dilutions, 0.2 ml of buffered saline and 0.3 ml of fibrinogen solution were added. After the mixture was stirred, the tubes were incubated at 37°C for 30 minutes in a water bath. Then, to each tube, 1 drop of 100 u/ml thrombin solution was added, and the tubes were stirred rapidly. They were incubated again for 30 minutes at 37°C in a water bath. At the end of the incubation period, the tubes were examined for clot lysis. The end point was considered to be the highest urine dilution.

Thus, 1 unit of urine fibrinolytic activity is the amount of the presence in the non diluted urine dilution.

If 0.5 ml urine diluted 4 times causes clot lysis under the said condition, 0.5 ml of undiluted urine was called to contain 4 units of fibrinolytic activity.

The measurements of blood plasmin

The blood plasmin was measured in the Ratnoff's test [2], MacFarlane's test[3], Ungar's ultraviolet absorption test[4] and Lewis' & Ferguson's test[5].

Gravimetrical determination of plasma fibrinogen was made by the Gramm's method[6].

RESULTS

1. *Urinary fibrinolytic activity in healthy persons*

The activity of urinary fibrinolysis of 13 healthy persons was measured

* hereinafter abbreviated as ε.
** hereinafter abbreviated as SK in this paper.

by the said method. The result was shown in Table 1. In healthy persons, one unit, or less than, of the urinary fibrinolytic activity was observed in all cases.

Table 1
Urinary Fibrinolytic Activity in Healthy Persons

Case No.	Age	Sex	Fibryn. activity (units)
1	33	M	1
2	28	M	1
3	18	M	<1
4	29	M	<1
5	38	M	<1
6	26	F	1
7	21	F	<1
8	22	F	<1
9	27	F	<1
10	22	F	<1
11	41	F	<1
12	41	F	<1
13	45	F	<1

2. *Urinary fibrinolytic activity of patients ailing various diseases*

Fibrinolytic activity in urine of 125 patients suffering from various kinds of diseases was measured.

Higher activity of the urine fibrinolysis than 1 unit was observed in 14 cases out of 125 (in 11%).

In 8 cases out of 27 of diseases belonging to malignant tumors (in 30%), the fibrinolytic activity in the urine was found higher than 1 unit.

Table 2
Urinary Fibrinolytic Activity in Hematologic Diseases

Name of diseases	No. of cases examined	No. of cases with higher activity
Aplastic anemia	51	1
Leucemia	27	3
Agranulocytosis	3	0
Purpura	4	1
Hemophilia	1	0
Benzol poisoning	1	1
	87	6

In the hematologic diseases, however, urinary fibrinolysis was accelerated only in a few cases, though higher activity of blood plasmin was very often observed in these cases. In other diseases examined no acceleration of fibrinolysis was observed in the urine. Therefore, the appearance of the higher fibrinolytic activity than 1 u. in urine was, by us, regarded as an abnormal sign

which might be resulted from some pathologic states.

3. *Urinary fibrinolytic activity with pregnancy and exposure to the slight radium irradiation.*

Twenty-seven urine samples from healthy pregnant women and 19 from nurses working in a radium irradiation room were measured.

Results obtained were as follows.

In 19 pregnant women out of 27 and in 12 nurses out of 19 observed was higher activity of fibrinolysis in urine.*

4. *Nature of fibrinolytic activity that appeared in urine.*

To determine the nature of urinary fibrinolysis, the activators and inhibitors on blood plasmin were applied to the urine samples. S. Okamoto et al. had examined the action of hydrogen peroxide, α- and β- SH- compounds, ε and others on blood plasmin, reporting that blood plasmin could be identified with the action of the said compounds.

In this report, action of SK, hydrogen peroxide, cystein and ε on the urinary fibrinolytic activity was examined in vitro; results were shown in Table 3.

Table 3

The Action of SK, H_2O_2 and Cystein on the Urinary Fibrinolytic Activity in vitro

Reagents added / Case No.	None	With SK (150 u)	With H_2O_2 (0.1 %)	With cystein (1/1000 M)
1	4 u	8 u	16 u	16 u
2	4	8	16	16
3	2	8	—	—
4	2	4	—	32
5	2	8	—	—

Table 4

Inhibitory Action of ε on the Urinary Fibrinolytic Action in vitro

Subjects	Case No.	Without ε	With ε (1 %)	Period of pregnancy
Pregnant women	1	2 (units)	<1 (unit)	38 weeks
	2	4	<1	32 〃
	3	2	<1	33 〃
	4	2	<1	34 〃
	5	2	<1	30 〃
Nurses working in radiation room	6	8	<1	
	7	4	<1	
	8	8	<1	
	9	4	<1	
	10	2	<1	

* It was noticed that no other pathologic signs were observed in the urine tests with those pregnant women.

Inhibitory action of ε on the urinary fibrinolysis of pregnant women and nurses servicing in a radium irradiation room was determined. One per cent of ε completely inhibited the urinary fibrinolysis of those samples in vitro.

DISCUSSION

No systematic studies on the urinary fibrinolysis had been undertaken, in the relation of blood fibrinolysin, until the rapid development of investigation on blood fibrinolysin has been largely achieved by numerous workers, even though so called proteolytic agent in urine has long been noticed, either clinicaly or experimentally.

In recent years, however, more precisely advanced knowledge of blood fibrinolysin has favoured the study of the enzymatic mechanism of urinary fibrinolysis. In fact, MacFarlane[7,8] and Bjerrehuns[9] described the nature of the proteolytic agent in urine and noted that it had similar properties to those of blood fibrinolysin. Astrup[10] and others investigated more precisely the nature of the urinary fibrinolytic agent by means of their fibrin plate method, and claimed that proteolytic agent in normal urine was not an enzyme, but activator of plasmin. Their conclusion was such that the urinary proteolysis for fibrin clot should be caused by plasmin which was, artificially as it were, produced by an interaction between plasminogen and the urine activator during the measurement.

In the present paper, it was intended not to develop the further analytical study of the urinary fibrinolytic activity but to examine the rate of the urinary fibrinolysis in healthy persons, nurses servicing in radium room, pregnant mothers, patients ailing various diseases and, in particular, patients suffering from those diseases in which plasmin activity in blood was most observed higher than normal. It was an advantage in conducting our investigation that recent works cooperatively made by the members of the committee of Research Project of Plasmin and Antiplasmin had presented the detailed data; those were available to know in which state plasmin activity in blood was generally higher than normal.

Urine samples were obtained from healthy persons and from those patients whose plasmin was suspected to be activated in blood. Thus, in 184 urine samples, the fibrinolytic activity of the urine was semiquantitatively measured according to the method which was first reported by Ungar and Mist[4] and applied to urine by Colgan et al.[1]

Results obtained showed that the urinary fibrinolytic activity was one unit, or less than, in the urine of healthy 13 persons. Higher activity of urinary fibrinolysis than one unit was found only in 14 out of 114 patients who were

suffering from malignant tumor or haematologic diseases.

In all patients out of 11 suffering from the other diseases than those above mentioned, the urinary fibrinolytic activity was also one unit or less. These results indicated that the criterion of judgement of results on urinary fibrinolysis by Colgen et al. can be available even to clinical results of the urinary fibrinolytic activity.

Here the authors would like to notice two evidences: One is that the fibrinolytic activity in the urine was found higher than 1 unit in 8 cases out of 27 of diseases belonging to malignant tumor (in 30%). The other is that, in the haematologic diseases, urinary fibrinolysis was accelerated only in 6 cases out of 87, though higher activity of blood plasmin was very often observed in such haematologic diseases.

Higher fibrinolytic activity in urine, however, was observed very often in pregnant mother and nurses who received a small amount of irradiation; that is, in 19 out of 27 cases of pregnancy (in 70%) and in 12 out of 19 cases of nurses servicing in a radium room (in 63%). The detailed data of such nurses have been described by Yamashita and Suzuki[12].

A preliminary study on the nature of the urinary fibrinolysis from authors' view-point was undertaken and the results obtained were summarized in Tables 3 and 4. It is noteworthy (1) that the urinary fibrinolysis was accelerated by the addition of SK, H_2O_2 or cystein, and (2) that the activity was strongly inhibited by ε. The effect of cystein seems to present a problem to be further investigated. That is because the fibrinolysis in urine can resulte from the more complicated enzyme mechanism than the action of so called plasmin and activator system.

However, the fact that ε, i.e. a specific inhibitor of plasmin activation, completely inhibited the urinary fibrinolysis led us to consider that fibrinolytic action in the urine might be largely depending on the fibrinolytic enzyme and its activator system similar to the blood fibrinolytic system.

Finally the authors like to emphasize the difficulties in establishing more practical method for revealing the dynamic aspect of plasmin system in blood and/or in tissue. In fact, the urinary fibrinolytic agent behaves in a different way than that in blood. So far as the clinical investigation at the present step is concerned, it seems useful to measure the plasmin activity in blood and urine respectively and by several different procedures. Further investigation of the problem is now being conducted in our laboratories.

SUMMARY

1) In 184 cases of healthy persons, patients ailing some diseases and nurses, fibrinolytic activity of the urine was seminquantitatively measured according to the method which was first reported by Unger & Mist and applied to urine by Colgan et al. The criterion of judgement of the results was also based upon that of Colgan et al.
2) Fibrinolytic activity of the urine was found higher than normal in 19 cases of pregnancy out of 27 (70%), and in 12 out of 19 cases of nurses who received a small amount of irradiation (63%). Fibrinolytic activity of blood, however, remained normal in the most of those cases.
3) Fibrinolytic activity of urine was found normal in 50 cases of aplastic anemia out of 51 and in 24 out of 27 cases of leucemia and erythroleucemia. Fibrinolytic activity of blood in most of these patients was found to be higher than normal.
4) In 8 cases of malignant tumor out of 27, fibrinolytic activity of the urine was higher than normal. In about half of such patients, fibrinolytic activity of blood was found also to be higher than normal.
5) These accelerated fibrinolytic activity of the urine samples turned normal when ε-amino-n-caproic acid was added to the reaction mixture in vitro.

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STUDIES ON THE URINARY FIBRINOLYTIC ACCELERATION WITH SMALL DOSE IRRADIATION OF IONIZING RADIATION

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INTRODUCTION

The matter of radiation injury has lately come to be regarded as more important than ever, with newly born opinions of its pathological physiology, its prevention and the treatment. Needless to say that it is best not to be irradiated, but the doctors, the engineers and the nurses who are treating patients with ionizing radiation, receive more or less irradiation of the rays, however careful they may be, and unexceptionally suffer from the so-called chronic radiation injury.

In order to prevent chronic radiation injury, a film badge, or a pocket-dosimeter is used to measure the exposure-dose; if, judging from the decided maximum permissible dose, one should be found to be over exposed, he will be necessarily put in another position. But regrettably there are a few in advertent cases of over-exposure, because the over-exposure is not immediately accompanied by either subjective or objective symptoms. An examination of blood given every month, which is itself not so reliable, is inadequate for the detection of the changing condition of the body.

Recently Loomis[1] and Colgan[2] reported that the irradiation of ionizing radiation causes the activation of Plasmin in blood and urine, and moreover Ungar[3] conffirmed that it is much easier to measure the activity of Plasmin in urine than in blood.

The aim of this experiment is to know whether Plasmin in urine is activated

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by such a small amount of irradiation as nurses may be exposed during giving treatments.

METHODS OF THE EXPERIMENT

The measurement of the activity of Plasmin in urine of the nurses before and after Radium or Radio-isotope handling, was done. Total amount in use are ^{60}Co tubes 885 mc, and ^{60}Co Needles 42 mc, ^{60}Co Cells 55.4 mc, Radium tubes 100 mg and the others Radium-Needles 10 mg; daily treating amount of Radium and ^{60}Co are about 450 mc.

The duty of nurses in the radium room is three days a week, and they handle radium or radioisotopes for six days a month. The exposed dose measured by a film badge, was 150–450 mr/2W.

THE RESULTS OF THE EXPERIMENT

Case 1. Name: K.M., Age: 21. Four months' service in the radiology department. Exposured dose was about 50 mr.

Table 1

	Colgan's units		No. of leucocytes
	Non treated	Adding ε invitro	
Before the duty	4	<1	4,300
After the duty	1	<1	4,200
10 days after the duty	4	<1	4,400

In this case no subjective or objective symptoms was preceived, nor was the fluctuation of leucocytes.

Case 2. Name: T.H., Age: 25. Eight months' service in the radiology department. The irradiation dose about 60 mr.

Table 2

	Colgan's units		No. of leucocytes
	Non treated	Adding ε in vitro	
Before the duty	4	<1	3,900
After the duty	8	<1	4,100

She subjectively had a tired feeling all over the body, and perceived slight pigmentation at the tips of fingers. As she had been worked long time in the radiology department, and the activity of Plasmin was also remarkably high, she was forced to given up her work and took enough rest. The following table

shows the condition of Plasmin in blood, measured 12 days after her service. Plasma-Fiblinogen is normal, but Plasmin is seen to be activated.

Table 3

Fibrinogen (Plasma)						2.1 mg/dl			
Ratnoff's test						3 days			
MacFarlane's test (24 hours)									
2×	4×	8×	16×	32×	64×	128×	256×	512×	∞
	±	±	±	±	±	+	+	+	
Kuroyanagi's modification (24 hours)									
2×	4×	8×	16×	32×	64×	128×	256×	512×	∞
			−	±	+				
S.K. activation (S.K. 100 unit) (Lewi's & Furguson's test)						Time 6′ 10″ Lewi's unit 27			
Ungar's U.V. test									
6×dilution	260 mμ		270 mμ		280 mμ		290 mμ		
	−0.060		−0.073		−0.080		−0.057		
Lewi's & Furguson's test						3 hours Lewi's unit 0.56			

Case 3. Name: A.Y., Age: 22. Two months' service in the radiology department. The irradiation dose about 110 mr.

Table 4

	Colgan's units		No. of leucocytes
	Non treated	Adding ε in vitro	
Before the duty		<1	5,700
After the duty	1	<1	5,600
10 days after the duty	2	<1	5,500

The amount of irradiation is extremely large, but the activity of Plasmin is low and no subjective symtoms was felt.

Case 4. Name: J.I., Age: 26. Seven months' service in the radiology department. The irradiation dose about 90 mr.

The activation of Plasmin after duty was exceedingly obvious. The result of Plasmin determination in blood was as follows.

In this case, just as in case No. 2, Plasmin in blood was also found activated.

Table 5

	Colgan's units		No. of leucocytes
	Non treated	Adding ε in vitro	
Befor the duty	2	<1	5,100
After the duty	8	<1	4,900

It seems that the longer one serves in the radiology department, the more Plasmin may be accumulated. These results forces us to think that long continuous duty in a radium room is harmfull. She felt no subjective symptoms.

Table 6

Fibrinogen (Plasma)						3.1 mg/dl			
Ratnoff's test						3 days			
MacFarlane's test (24 hours)									
2×	4×	8×	16×	32×	64×	128×	256×	512×	∞
−	−	−	±	±	±	±	+	+	
Kuroyanagi's modification (24 hours)									
2×	4×	8×	16×	32×	64×	128×	256×	512×	∞
			−	±	±				
S.K. activation (S.K. 100 unit) (Lewi's & Furguson's test)						Time 6 min. Lewi's unit 27.8			
Ungar's U.V. test									
6×dilution	260 mμ		270 mμ			280 mμ		290 mμ	
	+0.011		+0.019			+0.024		+0.017	
Lewi's & Furguson's test						Time 160 min. Lewi's unit 0.63			

Case 5. Name: H.I., Age: 22. Six months' service in the radiology department. The irradiation dose about 70 mr.

Table 7

	Colgan's units		No. of leucocytes
	Non treated	Adding ε in vitro	
Before the duty	2	<1	4,800
After the duty	4	<1	4,900
10 days after the duty	1	<1	4,800

In this case anti-plasmin (Ipsilon 20 cc) was intravenously injected once

before the second duty, and Plasmin in urine was measured. Plasmin after duty was (±) as follows.

Unchanged Plasmin showed the effect of Ipsilon for the prevention of the injury.

Table 8

	Colgan's units		No. of leucocytes
	Non treated	Adding ε in vitro	
Before the duty	2	<1	5,000
After the duty	2	<1	4,900

Case 6. Name: K.K., Age: 19. Three months' service in the radiology department. The irradiation dose about 70 mr.

Table 9

	Colgan's units		No. of leucocytes
	Non treated	Adding ε in vitro	
Before the duty		<1	5,700
After the duty		<1	5,600

Ipsilon 20 cc was intravenously injected once before duty of radium service. Urine Plasmin was (−) both before and after duty.

Case 7. Name: Y.H., Age: 20. A month service in the radiology department. The irradiation dose about 90 mr.

Table 10

	Colgan's units		No. of leucocytes
	Non treated	Adding ε in vitro	
Before the duty		<1	5,300
After the duty	4	<1	5,100

No subjective symptoms were felt.

CONSIDERATION

It was reported that in the cases of irradiation of small dose of ionizing rays for a short duration any physiological or blood change was not noticed, but our experiments showed that there was some physiological changes seen by blood or urine examination. It is regrettable that the present method of clinical examination is not complete enough to reveal such slight changes caused. That

is, as seen in tables, the number of leucocytes is almost unchanged, not substantially, but owing to the error of the measurement. In these cases plasmin value of urine was highly activated, so it seems to be quite significant. In our experiments it is certain that even with the small amount of irradiation urine plasmin tends to be activated, but this phenomenon is not enough to conclude the occurrence of radiation injury.

Plasmin in urine (++) and in blood (+) are in a parallel relation. MacFarlane[4] stated that Plasmin in blood was completely different from that in urine in their physical and chemical natures. The measurement of Plasmin in blood is too complicated to be used practically in an individual clinical examination. The measurement of Plasmin in urine may be enough to show the activation.

The duration of urine plasmin activation seems to continue for a fairly long time, as it was determined slightly even ten days after giving up the work in the radium room.

CONCLUSIONS

(1) The measurement of urine plasmin of nurses working in the radium room succeeded in establishing that plasmin is activated even with a slight irradiation of ionizing rays.

(2) The activity of plasmin seems to last for a fairly long time.

(3) In the case of a slight irradiation no outstanding morphological blood changes are generally noticed. The measurement of plasmin may be a more effective method for clinical detection of slight irradiation effects.

(4) Administration of antiplasmin (Ipsilon) before irradiation shows some preventive action on the radiation injury.

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WHOLE CLOT LYSIS TEST AND EUGLOBULIN LYSIS TEST IN RENAL DISEASES; THEIR RELATIONS TO THE CLINICAL SIGNS

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INTRODUCTION

It is naturally a difficult task to reveal some relation of clinical signs to the results of plasmin tests in patients ailing renal diseases of various kinds and of various grade. A thoughtful precaution should be paid before such investigation would be undertaken. Yet some suggestion for such trials has previously been given for the broad, screening works which were made by our committee on the project in scanning plasmin activity in blood of healthy persons and patients ailing diseases of various kinds. In fact, Itoga et al have pointed out that higher plasmin activity in blood was rather often observed in patients ailing nephritis. This is reason why a investigation was conducted by us to examine plasmin activity in the blood of patients suffering from several kinds of renal diseases and to search for some relation of clinical signs to plasmin activity of blood in those patients.

MATERIALS AND METHODS

Determination of the plasmin activity in blood and the other kinds of clinical and laboratory tests were carried out simultaneously in such cases of renal diseases as acute nephritis (6 cases), chronic nephritis (17 cases), nephrosis syndrome (2 cases), nephrosclerosis (1 case), diabetic nephropathy (1 case), idiopathic renal bleeding (4 cases) and renal tuberculosis (1 case).

The blood samples for determining the plasmin activity were taken from the cubital vein of patients in the morning.

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The determinations of plasmin activity in blood were performed according to the whole clot lysis test by Ratnoff and the euglobulin lysis test by Loomis.

RESULTS

Plasmin activity in the blood of patients ailing various renal diseases was examined and the results obtained were summarized in Table 1.

Table 1

Fibrinolytic Activity in the Blood of Patients Suffering from Renal Disease

Diseases	Cases			
	Total No. of patients	Total No. of speciemen	No. of speciemen with positive* result	
Acute nephritis	6	6	3	1 solely in whole clot lysis test 2 solely in euglobulin lysis test
Chronic nephritis	17	26	17	6 solely in whole clot lysis test 7 solely in euglobulin lysis test 4 in both
Nephrose syndrome	2	2	0	
Nephrosclerose	1	1	1	in both
Diabetic nephropathy	1	1	1	in both
Idiopatic renal bleeding	4	5	2	solely in euglobulin lysis test
Renal tuberculosis	1	1	0	
Total	32	42	24	

* Positive result means higher activity of plasmin then normal in the blood speciemen.

In 20 out of 32 examples obtained from patients of acute or chronic nephritis, plasmin activity was found to be higher than normal in whole clot lysis test and/or in euglobulin lysis test.

The various kinds of clinical and laboratory examinations were made in those patients. Critical values of results in each test were shown in Table 2 for evaluating the grade of pathologic findings. The authors intended to knows, if present, the relation between the results of plasmin test and those of the other clinical and laboratory signs.

In acute nephritis, no significant relation was found between the results of fibrinolysis and those of clinical and laboratory tests. In chronic nephritis, however, some relation between them were remarked. Similar relation was also noticed in other kind of chronic renal diseases.

As shown in Table 3, patients ailing the chronic renal diseases can be divided into four groups in such way as,

a) Group I, where whole clot lysis test and euglobulin lysis test were both negative,

Table 2

Critical Values of Results in Clinical and Laboratory Test

I	Whole clot lysis test	3 days
II	Euglobulin lysis test	1 unit (Lewis)
III	Urine findings	
	quantity	500 cc
	spetific gravity	1010
	protein	10 mg/dl
	erythrocyte	1/1 VF (200×)
	cast	
	epithel (renal)	appearance
IV	Blood pressure and retinal findings	
	max.	150 mmHg
	min.	90 mmHg
	mean	100 mmHg
	blood pressure of Art. centralis Retinae max.	100 mmHg
	retinal findings	Keith Wagener IIa
V	Serum protein	
	total	5.9 g/dl
	plasmafibrinogen	<2.0 mg/ml or> 5.0 mg/ml
	albumin	3.9 g/dl
	albumin globulin ration	1.29
VI	Non-protein Nitrogen	40.0 mg/dl
VII	Serum cholesterol	260 mg/dl
VIII	Urine function test	
	phnolsulfo-phethalein test	50 %
	urine dilution test (Fisherberg and Mosenthal)	1010
	urine concentration test (〃)	1019
IX	Blood sedimentation rate medium	20 mm
X	Blood cell count	
	erythrocyte	299×10^4
	leucocyte	>7900 or< 4900
	hemoglobin	10.0 g/dl

b) Group II, where whole clot lysis test was positive but euglobulin lysis test was negative,

c) Group III, where euglobulin lysis test was positive but whole clot lysis test was negative, and

d) Group IV, where euglobulin lysis test and whole globulin lysis test were both positive.

The other clinical and laboratory tests than those of plasmin were also divided into several groups in Table 3. Grouping of those tests was made basing upon the pathologic significance of each test.

Table 3

Relation between the Results from Fibrinolysis Tests and the Clinal, Laboratory Signs in Chronic Renal Diseases

	I Group	II Group	III Group	IV Group
Whole clot lysis test	negative	positive	negative	positive
Euglobulin lysis test	negative	negative	positive	positive
Serum protein	−	−	++	−
Sclerotic signs	−	−	+	++
Renal function	−	+	++	+
Vascular change	−	+	+	+
Sedimentation rate	−	+	+	+
Urine findings	+	+	++	+

The following signs were used in such way as

++ Pathologic findings are generally severe.

\+ Pathologic findings are generally moderate.

− Pathologic findings are generally slight.

Thus, the relations between the results of plasmin tests and those of each test group were obviously demonstrated in Table 3, and can be summarized in the following way.

a) Urinary findings were more or less pathologic, but any other significant involvements were not observed in most of the examples belonging to group I where two kinds of plasmin tests were both negative.

b) Besides the pathologic findings in urine, remarkable involvements in renal function test, blood sedimentation rate and urine analysis were noticed in most of the examples belonging to group II where whole clot lysis test was solely positive.

c) In addition to the pathologic signs mentioned in above items, (a) and (b), low plasma protein and vascular involvements were observed in most of the examples belonging to group III where euglobulin lysis test was solely positive.

Table 4

A: Results of the clinical and laboratory test in the cases where whole clot lysis test was solely positive. (II Group)

Tests	No. of case examined	No. of cases with abnormality
Phenolsulfo-phthalein test	5	4
Urine concentration test	6	5
Blood sedimentation rate	6	4
Urine findings	6	6

B: Results of the clinical and laboratory test in the cases where euglobulin lysis test was solely positive. (III Group)

Tests	No. of cases examined	No. of cases with abnormality
Serum protein (total)	7	4
Serum albumin	7	6
A/G	7	5
Blood pressure min.	7	5
Blood pressure mean	7	6
E.C.G.	6	4
Retinal findings.	7	5
Blood pressure of A. cent. retinae	5	3
Phenolsulfophthalein test	7	5
Sedimentation rate	7	5
Urine findings	7	7

C: Results of the clinical and laboratory tests in the cases where both tests were positive. (IV Group)

Tests	No. of cases examined	No. of cases with abnormality
Serum cholesterol	5	5
Blood pressure max.	6	3
Blood pressure mean	6	3
Blood pressure of Art. cent. retinae	4	3
Retinal findings	5	3
Urine concentration test	4	3
Sedimentation rate	5	5
Urine findings	6	6

d) In most of the examples belonging to group IV where whole clot lysis test and euglobulin lysis test were both positive, broad involvements were found. Among those signs, cholesthemia was found to be remarked and sclerotic signs were observed.

The detailed results of each example were shown in Table 4, and the course of two clinical cases in Tables 5 and 6.

DISCUSSION AND SUMMARY

A difficult problem in conducting such investigation was to decide the method of measuring plasmin activity in blood. It has generally accepted that results obtained by euglobulin lysis test is less disturbed by the amount of antiplasmin in serum than those obtained by whole clot lysis test. On the other hand, whole clot lysis test can be more sensitive than the former to the balance between

Table 5

Results of Clinical and Laboratory Tests in each clinical cases

	Diseases	Acute nephritis		Chronic nephritis				Nephrose Syndrome	Nephro-Sclerose	Diabelic Nephro-pathy
Patients	Datum	28.Jan. '59	28.Jan. '59	28.Jan. '59	6, Mar. '59	6, Mar. '59	23.Jan. '59	3, Feb. '59	3, Feb. '59	3, Feb. '59
	Name	S.A	F.S	M.S	T.Y	M.K	N.O	Y.T	S.T	S.K
	Age and sex	23 m	25 f	24 m	44 m	31 m	59 f	48 m	68 f	58 f
Fibrinolytic teste	Whole clot lysis test	4	4	4	4	3	3	4	2	3
	Euglobulin lysis test	100′ 1.00	150′ 0.67	125′ 0.87	50′ 2.00	118′ 0.85	80′ 1.25	125′ 0.80	35′ 2.86	95′ 1.05
	Streptokinase activated plasmins test	7′ 30″ 22.2	7′ 30″ 22.2	9′ 30″ 17.5	8′ 30″ 20.2	9′ 18.5	5′ 30″ 29.8	8′ 30″ 19.5	7′ 30″ 22.2	6′ 10″ 27.0
Blood examines	Total protein g/dl	6.4	7.0	7.8	5.6	6.0	5.8	4.8	7.0	7.0
	Albumine g/dl	4.0	4.4	4.0	3.2	2.7	3.2	1.4	4.0	4.4
	A/G	1.68	1.71	1.04	1.33	0.82	1.19	0.41	1.36	1.64
	Fibrinogen g/dl	2.1	3.3	3.3	4.5	5.2	1.0	5.7	2.6	3.1
	None protein N. mg/dl	23.7	18.2	25.0	29.7	24.4	64.0	36.5	30.4	
	Serum cholesterol mg/dl		176	165	240	222	321	500↑	264	280
	Sedimentation rate	4	11	7	15	59	65	103	52	24
Urine function	Phenolsulfophthalein test %	80	55		20	50	20	30	50	
	Urine dilution test	1007	1006	1007	1010	1002	1006	1005	1010	1015
	Urine concentration test	1020	1015	1015	1016	1010	1010	1010	1016	1030
Cardio-vascular change	Retinal findings (Keith Wagener)	0	0	0	3a		3a	0~1	2b	4
	Blood pressure of Art. cent retinae		65/34		102/70		120↑/58	90/48	122↑/46	120/45
	Blood pressure max./min.	132/90	115/65	174/130	154/90	126/64	190/100	98/68	156/110	194/86
	Blood pressure mean	104	82	145	111	85	130	78	125	122
	Electro cardio gram		(−)		Hyper K	(−)	(−)	(−)	(−)	(−)
Urine findings	Quantity	1800	600	2300	2000	1300	700	3000	750	1800
	Spetific gravity	1015	1016	1012	1010	1010	1015	1010	1010	1015
	Rote cell	2~3/1	5~6/1	7~8/1	1~0/1	5~6/1	0~1/1	1/10	1/5~6	1~2/1
	Casts	(−)	(−)	(−)	(−)	(+)	(−)	(+)	(−)	(−)
	Protein mg/dl	22	18	13	350	57	232	380	10	11
Blood cell count	Erythrocyte 100×100	470	358	430	333	382	202	269	418	399
	Hemoglobin	100	80	96	63	78	49	70	97	100
	Leucocyte	7000	5200	5200	6100	5600	6500	7100	5800	5700
Note							6, Feb. died	using of Prednisolone		

Table 6

The Course of Clinical Cases

		No. 1			No. 2		
Patients	Datum	2, Dec. '58	19, Jan. '59	6, Mar. '59	2, Dec. '58	19, Jan. '59	6, Mar. '59
	Name	T.O	〃	〃	M.I	〃	〃
	Age and sex	28 m	〃	〃	28 f	〃	〃
Fibrinolytic tests	Whole clot lysis test	7	3	5	7	5	2
	Euglobulin lysis test	100′ 1.00	180′ 0.56	180′ 0.56	60′ 1.67	180′ 0.56	105′ 0.95
	Streptokinase activated plasmin test	16′ 10.4	10′ 16.8	13′30″ 12.3	9′30″ 17.5	8′30″ 19.5	9′ 18.5
Blood examines	Total protein g/dl	6.0	7.2	7.4	6.0		6.6
	Albumin g/dl	3.2	4.5	4.9	3.3		4.4
	A/G	1.12	1.7	2.0	1.26		1.76
	Fibrinogen g/dl	0.25	0.24	0.27	0.37	0.40	0.42
	None-protein N mg/dl	42.3	20.0		21.6	30.0	26.0
	Serum cholesterol mg/dl	159		226	258		382
	Sedimentation rate (medium)	15	8	4	41	11	14
Urine function	Phenolsulfo-phthalein test		25			80	
	Urine dilution test	1008	1006	1002	1009		
	Urine concentration test	1016	1024	1028	1019		
Cardio vascular changes	Retinal findings (Keith Wagener)	II a			II a		
	Blood pressure of Art. cent. retinae	127/41			85/59		
	Blood pressure max./min.	148/80	164/98	128/60	140/90	126/80	110/80
	Blood pressure mean	103	120	83	107	95	90
	Electro cardio gram	(−)			damage (+)		
Urine findings	Urine quantity cc	900	1.200	700	400	900	900
	Specific gravity	1015	1013	1012	1012	1012	1010
	Rote cell	30～40/1	15～16/1	7～10/1	3～4/1	7～8/1	3～4/1
	Casts	1/10 granula	(−)	(−)	1/5～6 hyaline	(−)	(−)
	Protein mg/dl	16.8	12	11.2	416	196	328
Blood cell count	Erythrocyte	406	448	532	277	298	377
	Hemoglobin sahli	90	87	110	55	78	70
	Leucocyte	5500	5200	5500	5100	5100	4400
Note		Treatment with ε-amino-caproic acid was started after 1rst examination.					

plasmin and antiplasmin. Furthermore, such a understanding is a simplest scheme of the dynamic aspect of the plasmin system in the circulatory blood.

Therefore, it seems at least, to be necessary to note the results of whole clot lysis test and those of euglobulin lysis test as well.

In the present investigation, whole clot lysis test, euglobulin lysis test and the other kinds of clinical signs were examined in 32 patients ailing several kinds of renal diseases. Results obtained were compared each other and summarized in Tables 1 and 3.

It was first noticed that high plasmin acctivity in blood was most often observed in the cases of nephritis: The plasmin activity was higher than normal in 17 out of 23 cases of acute and chronic nephritis.

It was then recognized that patients ailing the chronic renal diseases can be devided into the following four groups, basing upon the results of whole clot lysis test and euglobulin lysis test.

a) In 6 examples of chronic nephritis (Group I) where whole clot lysis test and euglobulin lysis test were both negative, urinary findings carried out simultaneously were more or less pathologic; but any other pathologic tendencies were not present in either clinical or laboratory tests.

b) In those 6 examples of chronic nephritis (Group II) where whole clot lysis test was solely positive, remarkable involvements in renal function test, blood sedimentation rate and urine analysis were observed in general.

c) In those 7 examples (Group III) where euglobulin lysis test was solely positive, low plasma protein, vascular changes were noticed in addition to the pathologic sign already mentioned in item (b) and (c).

d) In those 6 examples of chronic renal diseases (Group IV) where whole clot lysis test and euglobulin lysis test were both positive, cholesthemia was remarked in general.

Attention should be paied on the evidence that increased rate of blood sedimentation was very generally observed in the cases where one kind of plasmin test is at least positive. (Groups II, III and IV).

The authors should like to point out that clinical signs of patients in group III are more severe than those in group II. The evidence, that significance of whole clot lysis test and euglobulin lysis test are different each, recalls our memory that euglobulin lysis test was positive in hematologic disease and whole clot lysis test was positive in skin diseases suspected to be allergic. It seems that a significant problem is facing us to be investigated further.

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