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AMINO-METHYL-CYCLOHEXANE-CARBOXYLIC ACID: AMCHA

A NEW POTENT INHIBITOR OF THE FIBRINOLYSIS

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Research Projects on Plasmin and Antiplasmin

(Received for publication June 14, 1962)

In 1953, S. Okamoto et al., co-operatively working with the chemist group leaded by Nagasawa, found that epsilon-amino-caproic acid (EACA) had a potent inhibitory effect on the fibrinolysis in vitro and in vivo[1]. S. Okamoto[2] in 1959, and his co-worker, Yokoi[3], in 1960, described in detail the relations of chemical structures to the inhibitory effect on the basis of the results obtained from the examination of more than 300 kinds of chemical compounds. In particular, they mentioned that neither amino-group nor carboxylic group could be replaced without diminishing its original inhibitory effect, and that epsilon compounds had the most potent inhibitory effect of their close homologues[2,3].

Soon after the finding of EACA, a series of animal experiments were carried out by S. Okamoto et al., and the results obtained suggested that EACA would be applied to reverse the "hyperplasminic states" of patients[1,4]. Then, S. Sato[5] and Itoga[6] demonstrated that the clinical signs due to accelerated fibrinolysis were successfully improved by the administration of EACA to patients. Many studies of clinical application of EACA have been conducted along this line by various workers and in various countries[7,8,9,10].

Thus it seems to be nearly accepted that EACA is available to reverse the hyperplasminic states of patients. However, clinical need remained us to search for more potent inhibitor than EACA. Therefore, 1-(aminomethyl)-cyclohexane-4-carboxylic acid (abbreviated as AMCHA) was remarked by S. Okamoto et al. under the close co-operation with the chemist group of Nagasawa[11]. This paper deals with the first step of our studies in evaluating the antifibrinolytic effect of AMCHA in comparison with EACA. The more detailed results will be described by one of the authors and their colleagues in the following papers[12].

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MATERIALS

1-(aminomethyl)-cyclohexane-4-carboxylic acid which was abbreviated as AMCHA in this paper was synthetized in the central laboratories of Mitsubishi Kasei Kogyo Co. AMCHA is a colourless powder having a melting point of 237–238°C (decomposition) and being well soluble in water at room temperature[11]. EACA was synthetized by Daiichi-Seiyaku Co.

Commercial preparations of fibrinogen (Fraction I: Armour Laboratory), casein (Hammersten Co.) and thrombin (Mochida Co.) were used in the following experiments. Streptokinase preparation (Varidase) was offered from American Cynamid Co. A commercial preparation of Tosylarginine-methylester (Minophagen Co.) was used and abbreviated as TAMe. The standard human serum was prepared in our laboratory.

EXPERIMENTALS

The very preliminary examination was first made, and the spontaneously activated fibrinolytic system obtained from human serum was used in order to know whether AMCHA might be promising for clinical application. The measurement of the inhibitory effect of AMCHA was made by admixing a certain amount of the active ingredients with the fibrinolytic system which contained active euglobulin, fibrinogen, thrombin and an adequate amount of 1/20 M of phosphate buffer, and by measuring the time required for the complete dissolution of the formed fibrin clots incubated at 37°C, and comparing the said time with that of control experiments. Even though the inhibitory effect of AMCHA was varied by the difference of the system used, it was evidently observed that AMCHA added to the system caused the retardation of the lysis time and that such a effect was always much stronger than that of EACA. Results obtained from this preliminary experiment indicated that the detailed studies would be urgent.

(A) THE EFFECT OF AMCHA ON THE FIBRINOLYTIC SYSTEM CONTAINING HUMAN SERUM AND STREPTOKINASE

The effect of AMCHA was examined by adopting the plasminogen-streptokinase system with our expectation that AMCHA would most likely affect the activation process of plasminogen. A 0.1 ml of standard human serum, 0.4 ml of 1/20 M phosphate buffer saline solution and 0.1 ml saline solution containing 100 units of streptokinase were mixed together in a test tube at 0°C. Then 0.05 ml of a saline solution containing 5 units of thrombin and 0.3 ml of a 0.33% solution of bovine fibrinogen were added into the test tube. The mixture was incubated at 25°C and the time required for the complete lysis of the formed

clot was measured in seconds. The results obtained presented the control value[18].

The inhibitory effect of AMCHA or EACA was examined by dissolving the agents in the phosphate buffer solution above mentioned; and the relations between the retardation of the lysis time and the concentration of AMCHA were inquired into and compared with EACA. The results were shown in Fig. 1.

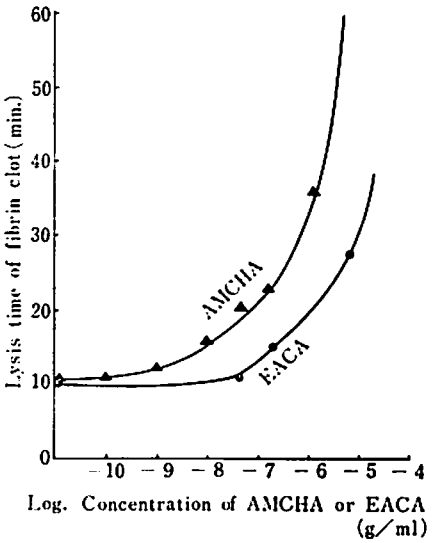

Fig. 1 The inhibitory effect of AMCHA or EACA on the fibrinolytic system containing human serum and streptokinase.

The results in Fig. 1 indicated that the concentration of AMCHA required to double the control lysis time was about 3×10^{-7} g/ml, namely about 2×10^{-6} M, while that of EACA was about 8×10^{-6} g/ml. Therefore, it can be mentioned that the inhibitory action of AMCHA on the streptokinase-activation-process of the plasmin system of human serum in vitro is evidently very potent, that is, more than ten times of the action of EACA.

The inhibitory effect of AMCHA or EACA on the fibrinolysis caused by the partly purified plasmin preparation was, however, rather weak, 10^{-6} g/ml of AMCHA or 10^{-5} g/ml of EACA being required to double the control lysis time. This suggested that AMCHA might more strongly affect the activation process of plasminogen than the activity of plasmin per se. Results obtained from the experiments on the more purified plasmin preparation will be described in the following paper which appears, in this Journal, next to this paper[12].

(B) THE EFFECT OF AMCHA ON THE FIBRINOGENOLYTIC SYSTEM CONTAINING HUMAN SERUM AND STREPTOKINASE

It was reported by U. Okamoto that fibrinogenolysis (fibrinogen splitting by the action of plasmin) was well estimated by the viscosity decreasing of the solution[13]. In the following experiments, 2 ml of 5% fibrinogen saline solution, 0.2 ml of the standard human serum and 0.1 ml of saline solution containing a certain amount of AMCHA or EACA were mixed in a viscosimeter of Ostwald type which was placed in the 38°C (±0.005°C) water bath. After putting 0.1 ml of saline solution containing 1,000 units of streptokinase into the viscosimeter, the measurement of the viscosity was repeatedly made at a certain interval and the results obtained were plotted in Fig. 2. It seemed to indicate that the

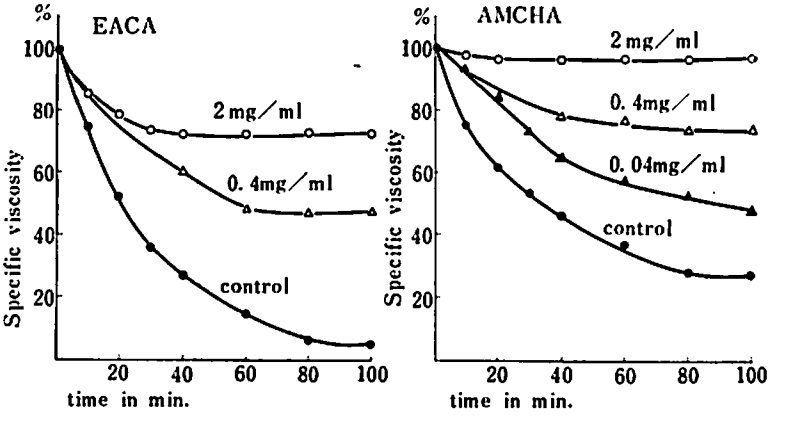

Fig. 2 The inhibitory effect of AMCHA or EACA on the fibrinogenolytic system containing human serum and streptokinase. Ordinates indicate specific viscosity of the reaction mixture; the initial value was expressed as 100%.

inhibitory effect of AMCHA on the mentioned fibrinogenolysis was more potent than that of EACA. It might be noteworthy that the concentrations of AMCHA or EACA requiring for the marked retardation of the viscosity-decreasing were rather high when compared with those concentrations in the fibrinolytic system above mentioned.

The similar results were also obtained by us in the caseinolysis so far as the inhibitory effect of AMCHA or EACA was concerned.

(C) THE EFFECT OF AMCHA ON THE TAMe ESTERASE ACTIVITY

It seems to be broadly accepted that TAMe can be used as a synthetic substrate of plasmin. In fact, it was reported by Sherry et al. that TAMe esterase activity was found in the very plasmin preparation which was purified

by them to the great extent[14]. It is also known that TAMe esterase activity increases markedly in the human serum by adding streptokinase[15]. Therefore, the inhibitory effect of AMCHA on the activation process of TAMe esterase can be expected. Results obtained here, however, seem to contradict to the current opinion mentioned above.

In our experiments, the assay of TAMe esterase activity was performed after the method described by Troll & Sherry in 1954[15]. Human euglobulin solution was prepared by diluting human serum with pure water, precipitating the fraction at pH 5.2 and resolving it in the tris-buffer solution. The reaction mixture was made by admixing 0.1 ml of euglobulin solution with 0.2 ml of 0.1 M TAMe solution, 0.5 ml of tris buffer (0.5 M, pH 9.0) and 0.1 ml of 1% AMCHA or EACA solution. The reaction mixture was incubated at 37°C. Results obtained were plotted in Fig. 3. It was indicated that AMCHA or EACA had no effect on the activity or activation process of TAMe esterase in the reaction mixture which contained serum euglobulin and streptokinase.

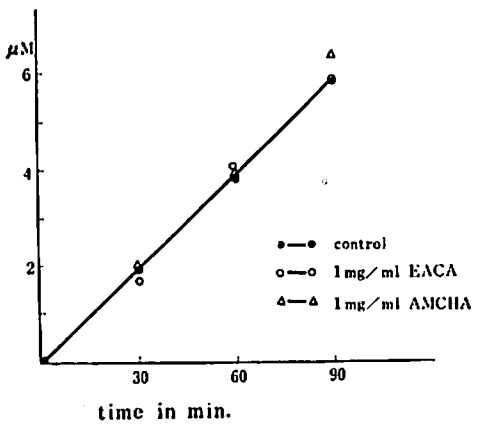

Fig. 3 Effect of AMCHA or EACA on the TAMe esterase activity. Ordinate indicates the amount of carboxylic acid freed from TAMe after incubation of various time at 37°C; abscissa indicates the time of incubation in min.

(D) THE EFFECT OF THE INTRAVENOUS ADMINISTRATION OF AMCHA TO RABBITS

The studies on the effect of AMCHA on the fibrinolytic system in vitro suggested that the administration of AMCHA to animals might be more effective that of EACA for suppressing the streptokinase activation test of fibrinolysis of the circulatory blood.

In the experiments shown in Table 1, 250 mg, 100 mg and 25 mg of AMCHA

or EACA were respectively given intravenously to rabbits weighing ca. 2.5 kg. Blood samples were drawn by venopuncture at the times mentioned in Table 1, and the actions of AMCHA or EACA were measured by the streptokinase activation test of the fibrinolysis. The results obtained are shown in Table 1 and summarized as follows.

1) When 250 mg of AMCHA or EACA solved in saline solution was given intravenously to rabbits, a very marked retardation of the lysis time of the streptokinase activation test of fibrinolysis was observed one hour to six hours after the injection of these two agents.

2) The action of AMCHA, however, was different from that of EACA when 100 mg or 25 mg of these agents were given to rabbits. The intravenous injection of 100 mg or 25 mg of AMCHA produced a marked retardation of lysis time, while that of EACA was obviously weaker than of AMCHA.

3) Then in giving rabbits, such a small amount of the active agents as 5 mg, the action of AMCHA was compared with that of EACA. Results obtained are shown in Table 2.

Table 1.

The effect of the intravenous administration of AMCHA or EACA to rabbits

Chemical substance	Dosage (mg)	Lysis time (sec.)			
		Before experiment	After experiment		
			1~2 hrs.	3~4 hrs.	5~6 hrs.
AMCHA	250	1,320	> 2,400	> 2,400	> 2,400
EACA	250	1,200	> 2,400	> 2,400	> 2,400
AMCHA	100	1,080	> 3,600		1,926
EACA	100	900	1,950		1,884
AMCHA	25	1,986	> 3,600	> 3,600	2,592
EACA	25	1,992	2,586	2,424	2,166

Table 2.

The effect of the intravenous administration of a smaller amount of AMCHA or EACA to rabbits.

Chemical substance	Dosage (mg)	Lysis time (sec.)					
		Before experiment	After experiment				Inhibitory effect
			1/2~1 hrs.	1.5~2 hrs.	4 hrs.	6 hrs.	
AMCHA	5	465	750	588	510	510	exists
AMCHA	5	690	1,230	1,080	990	840	exists
AMCHA	5	1,050	1,968	1,350	1,320	1,170	exists
EACA	5	420	450	450	450	438	none
EACA	5	480	480	510	510	450	none

In three rabbits to which 5 mg of AMCHA was given intravenously, the retardation of lysis time was obviously confirmed in each blood sample which was taken 30 minutes to six hours after the injection. In two rabbits to which 5 mg of EACA was given intravenously any retardation of lysis time was hardly confirmed.

The mentioned results indicated that some relatively small amount of AMCHA could reveal its potent antifibrinolytic effect to the circulatory blood though the same amount of EACA was little effective.

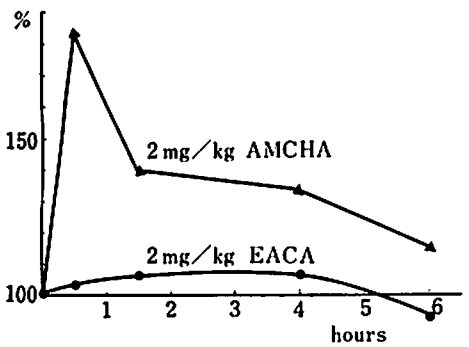

Fig. 4 The antifibrinolytic effect of the intravenous administration of 2 mg per kg of AMCHA or EACA to rabbits. Ordinate indicates per centage of the retardation of the lysis time estimated by the streptokinase activation test.

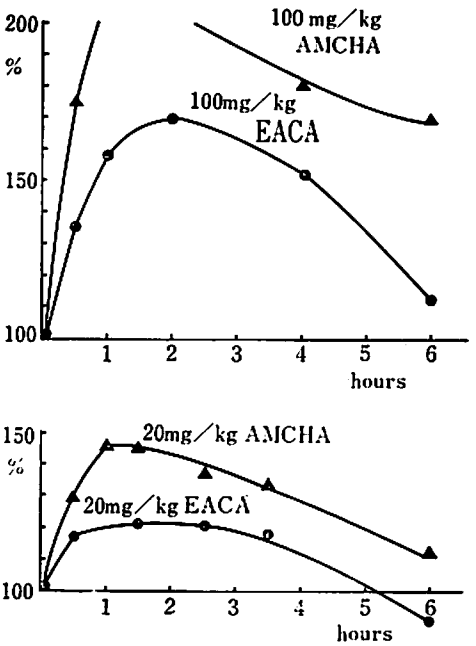

Fig. 5 The effect of the oral administration of AMCHA or EACA to rabbits.

(E) THE EFFECT OF AMCHA GIVEN PER OS TO RABBITS

In order to present the basis of the clinical application of AMCHA and compare the effect with that of EACA, oral administration was performed in rabbits. Results obtained indicated that the antifibrinolytic effect of AMCHA was well demonstrated when a small amount of AMCHA was given per os to rabbits.

It could be said that a certain amount of the oral administration of AMCHA showed the more potent antifibrinolytic action in its rate and duration. Clinical application of AMCHA per os to patients were thus suggested to be promising.

(F) REVERSAL BY AMCHA OF LYTIC SYSTEM IN BLOOD STREAM PRODUCED IN RABBITS

It has been reported by the authors et al.[16] or Miller et al.[17] that the intravenous administration of EACA was effective to reverse the activated fibrinolysis of the circulatory blood in animal experiments. The same effect was examined in the following experiments using AMCHA instead of EACA.

In order to activate the lytic system in the circulatory blood of rabbits, 5 ml of human serum and 30,000 units of streptokinase were given intravenously to rabbits. The activation of the lytic system in blood was easily demonstrated by estimating the time required for the complete lysis of the formed clots of fresh blood samples (natural clot lysis). Results shown in Fig. 6a indicated that the very activated state of lytic system in blood lasted for 70 minutes or more after the injection of streptokinase.

Results shown in Fig. 6b, however, indicated that, even though the natural clot lysis time was shortened to 30 min. by the activation procedure above

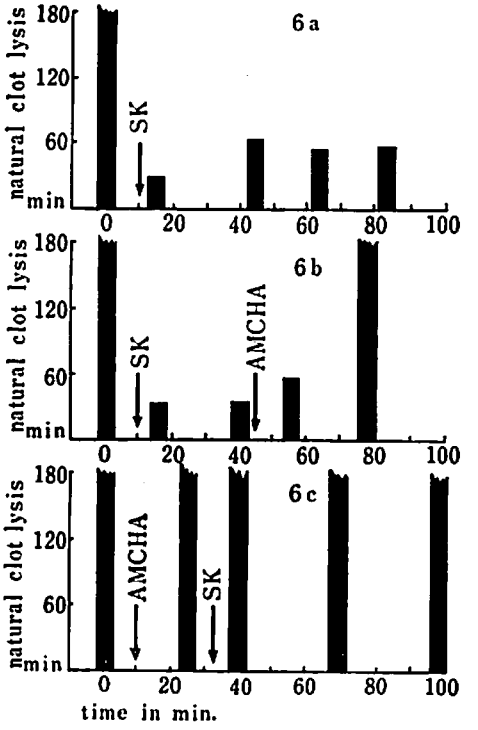

Fig. 6 Reversal or inhibition by AMCHA of the activation of lytic system in blood of rabbits.

mentioned, it was turned normal by the injection of 5 ml of 5% AMCHA solution

to the rabbit. It was also indicated that the administration of AMCHA prior to the activation procedure prevented the appearance of the rapid clot lysis in circulatory blood which would otherwise occur (Fig. 6c).

DISCUSSION

Since the discovery of a fetal hemorrhage with accelerated fibrinolysis by Soulier et al. in 1952[19], attention was paid by several workers to the evidence that the usual treatments for haemostasis were not effective. Some attempts, however, had been started to search for antifibrinolytic agent by Jobling et al. in 1915[20], and by Rosenmann in 1938[21]. The systematic studies by S. Okamoto et al. had been also started in 1948, and the potent inhibitory effect of EACA on fibrinolysis was reported by them in 1953[1]. Kaulla et al. in 1953 examined the antifibrinolytic effect of antibiotics[22]. In 1958 Mounter reported the inhibition of plasmin by phosphorus compounds, but they were toxic[24]. Sarker examined the antifibrinolytic effect of the close homologues of EACA in 1958, and confirmed that EACA was most effective among those compounds[23].

In recent years, EACA has been rather broadly applied to ameriolate the fibrinolytic hemorrhage or the allied syndromes. A very low toxity of EACA and its highly specific action to the fibrinolytic system have been generally accepted. However, it was also noticed by the authors that some workers administered to patients such a large amount of EACA as 30 g per day in order to suppress the very extensively accelerated fibrinolysis of blood. This was the reason why a more potent inhibitor than EACA was inquired into, even though EACA is yet regarded by the authors as one of the most ideal inhibitors for the clinical purpose.

1-(aminomethyl)-cyclohexane-4-carboxylic acid which was abbreviated as AMCHA in this paper was synthetized by Nagasawa, Takagi, Yokoi and Mangyo in the central laboratories of Mitsubishi Kasei Kogyo Co.[2].

Studies of the mode of action of AMCHA will involve many a problems, so results described in this first paper of AMCHA will be limited to the approach of evaluating the inhibitory effect of AMCHA and comparing it with that of EACA.

As to the in vitro test of the inhibitory effect of these agents, fibrin, fibrinogen, casein and TAMe were used as substrates, and the clot lysis time for fibrin, viscosity decreasing for fibrinogen, ultraviolet absorbance for casein and acid liberation for TAMe were respectively estimated. The reaction mixture was made by admixing the one of the mentioned substrates with the human standard serum and streptokinase.

Results obtained from the fibrinolysis test (Fig. 1) indicated that the inhibitory effect of AMCHA was far more potent than that of EACA. Calculation made from the lowest concentrations of AMCHA or EACA for doubling the lysis time of the control clots indicated that the action of AMCHA was ca. 27 times of that of EACA.

Action of AMCHA given to rabbits intravenously or orally was examined by administering various amounts of AMCHA or EACA and estimating the streptokinase activation test of blood samples taken from the rabbits at various intervals. Results obtained (Tables 1. and 2., Figs. 4, 5 and 6) indicated that the more potent effect of AMCHA than EACA was clearly demonstrated when some small amounts of the agents were given to the animals. Results shown in Table 2, for instance, indicated that the inhibition of the streptokinase activation test was markedly observed in the blood samples taken from rabbits 6 hours after the intravenous administration of 5 mg of AMCHA; while the inhibition was not observed at all even in the blood samples taken 30 min. to one hour after the intravenous administration of the same amount of EACA. Results obtained from the oral administration (Fig. 5) indicated that the inhibition by AMCHA was also more potent than EACA in its grade and duration. Results shown in Fig. 6 demonstrated reversal or inhibition by AMCHA of lytic system in blood stream produced in rabbits by streptokinase.

Thus, basing upon the mentioned results, the authors should like to suggest that it is urgent to extend the investigation of AMCHA towards the clinical application. It is also known that toxity of AMCHA is very weak when examined by acute and chronic tests in animals (unpublished). The authors expect that AMCHA will be effectively applied, either intravenously or orally, to suppress the extensively accelerated fibrinolysis in blood of patients which otherwise may require the administration of a very large amount of EACA.

The remained problem is to discuss the results obtained from examination on TAMe esterase activity. However, one of the authors, U. Okamoto is now studying this subject. The other aspect of the mode of action of AMCHA will be described in the following papers.

SUMMARY

1) A new potent inhibitor of the fibrinolytic system was found. Its chemical name was 1-(aminomethyl)-cyclohexane-4-carboxylic acid and abbreviated as AMCHA in our laboratories.

2) The inhibitory effect of AMCHA in vitro was far more potent than that of EACA when examined by the fibrinolytic system.

3) The inhibitory effect of AMCHA given to rabbits intravenously or orally was also more potent than that of EACA when the effect was examined by the streptokinase activation test of blood samples taken at various intervals.

4) Reversal by AMCHA of the accelerated fibrinolysis in circulatory blood produced in rabbits by streptokinase was demonstrated.

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INFLUENCE OF AMCHA ON THE ACTIVITY OF FIBRINOLYSIN (PLASMIN)

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(Received for publication June 14, 1962)

INTRODUCTION

Since Epsilon-Amino Caproic Acid (EACA) was found to be a potent inhibitor of fibrinolysis by Okamoto et al. in 1953[1], a number of studies were carried out either experimentally or clinically. The exertion of discovering a far more potent inhibitor has been continued by the same discoverers, and in consequence, at present time, a new synthetic substance called AMCHA has been found[2,3]. Mechanism of EACA to plasmin system in vitro has been studied by Alkjaersig[4], Ablondi[5], Fukutake[6], etc.; the knowledge as to inhibitory action of AMCHA is however yet insufficient, though it has been outlined in the previous paper by one of the authors and U. Okamoto[3].

The further studies upon the mode of action of AMCHA to plasmin system, thus, have been performed by the authors in attempt to confirm the previous report[3] and to extend the knowledge of this new potent antiplasminic substance.

EXPERIMENTAL

Materials

1. AMCHA was synthetized by Nagasawa et al. in the Central Laboratories of Mitsubishi Kasei Kogyo Co., Tokyo.

2. Epsilon-Amino Caproic Acid (EACA) was furnished by Daiichi Seiyaku Co., Tokyo.

3. Streptokinase (SK) used was a commercial preparation, Varidase (lot #2200–76) of Lederle Laboratories Division, American Cyanamid Co., New York.

4. Thrombin: This was a commercial preparation, Japan Blood Bank, Osaka.

5. Purified plasminogen: It was prepared from crude human plasminogen by modified Klein's method[7,8]. The crude human plasminogen was obtained from 20 fold diluted human serum by isoelectric precipitation at pH 5.2 with 1% acetic acid.

* Professor of Physiology.

6. SK-activated-plasmin: Purified plasminogen and necessary amount of SK were mixed together and incubated at 25°C for 30 minutes. This solution was used as SK-activated-plasmin.

7. Fibrinolysin-Merck: It was offered by Merck Sharp & Dohme Research Laboratories.

8. Trypsin: Crystaline Trypsin of Novo Laboratories, Copenhagen was used. The stock solution containing 0.25 mg/ml in 0.025 N-HCl was diluted 2 to 16 times in Buffer solution.

9. Fibrinogen: Bovine fibrinogen, Cohn's Fraction I, Armour, was purified by Brombäck's technique[9].

10. Casein: The Hammarsten casein of E. Merck, Germany was purified by the method of Norman[10].

11. Tosyl-arginine-methylester (TAMe): It was supplied by Minophagen Co., Ltd. Tokyo.

12. Buffer: Borate saline buffer, pH 7.4, as described by Palitzsch[11] was used for caseinolytic assay and fibrinolytic assay of plasmin. Veronal buffer, 0.1 M, pH 7.8 was used for fibrin plate test, and Tris buffer, 0.2 M, pH 9.0 for TAMe esterolytic assay.

Methods

1. Fibrinolytic Assay: The method of Lewis et al.[12] was partially modified. The total volume of the standard clot employed was 1.0 ml which contained 0.1 ml of saline solution of SK-activated-plasmin or Fibrinolysin-Merck, 0.2 ml of purified fibrinogen saline solution (clottable protein 0.22%), and 0.1 ml of 20 u/ml thrombin solution. Borate saline buffer was used as the solvent of fibrinogen and for the adjustment of total volume. AMCHA or EACA was added in the assay system prior to the addition of enzyme.

2. Fibrin plate test: Heated fibrin plate test and unheated fibrin plate test were both used[13]. AMCHA or EACA was added in fibrinogen solution before the formation of fibrin clot plate.

3. Caseinolytic Assay: The procedure was followed as described by Norman[10]. AMCHA or EACA was added in casein solution before the addition of Fibrinolysin-Merck.

4. TAMe esterolytic Assay: The assay was performed according to the procedure described by Troll & Sherry[14].

Results

A. PRELIMINARY OBSERVATIONS OF THE EFFECT OF AMCHA UPON THE FIBRINOLYTIC SYSTEM

It has been known that effect of inhibitors on SK-activated-plasmin varies

with changing the amount of SK employed. In attempt to determine the adequate amounts of SK under the condition of the considerably purified system used here, the relations between the amounts of SK and the lytic activity of the system were examined; and from the results obtained, which were plotted in Fig. 1, some adequate amounts of SK for the following experiments were decided, and in the experiments shown in Fig. 2, four and two units of SK were respectively used in each test system.

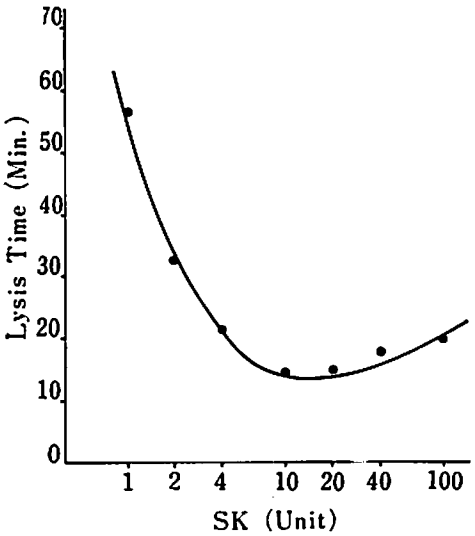

Fig. 1 Relation between the fibrinolysis with SK-activated-plasmin and the amount of SK used for activation of plasminogen.

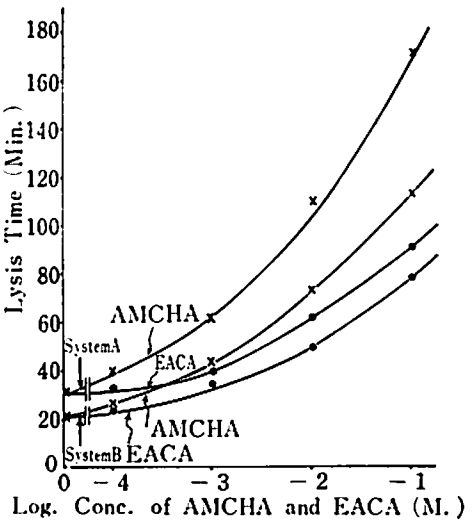

Fig. 2 Inhibitory effect of AMCHA and EACA upon the fibrinolysis with SK-activated-plasmin in fibrin clot system.
×——× AMCHA •——• EACA
System A contains 2 units of SK: System B 4 units.

As is demonstrated in Fig. 2, clot lysis time was prolonged when AMCHA was added in the assay system, and the degree of this prolongation was greater than when EACA was added.

It was roughly estimated that the inhibitory effect of AMCHA upon fibrinolysis in this assay system was approximately 5 times to 10 times greater than that of EACA by comparing the concentrations at which the lysis time was twice of the control values.

It should be noticed, however, whether or not the inhibitory action of AMCHA was localized only upon the activity of plasmin itself. Because of the presence of SK which contained in the assay system and of the contaminated plasminogen which could not be removed perfectly from fibrinogen even by Brombäck & Brombäck's purification technique, activation process of plasminogen

may have been occurring during the assay period, so that the inhibition by AMCHA on the activation process may have been involved.

B. EFFECT OF AMCHA UPON FIBRINOLYSIS WITH THE PURIFIED PLASMIN

To distinguish the suppressing effect of AMCHA upon the fibrinolytic activity of plasmin from that upon the activation process of plasminogen was the purpose of the following experiments. Purified plasmin, Fibrinolysin-Merck was used throughout in the serial experiments.

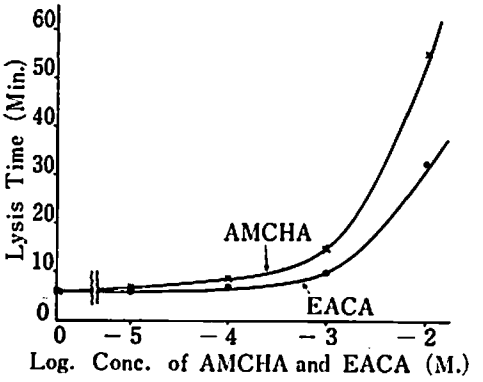

Fig. 3 Inhibitory effect of AMCHA and EACA upon the fibrinolysis with Fibrinolysin-Merck in fibrin clot system.
×——× AMCHA •——• EACA

The result was shown in Fig. 3. In this instance where Fibrinolysin-Merck was substituted for the SK-activated-plasmin in the plasmin-fibrin clot system, the concentration of AMCHA and EACA at which the lysis time was brought to twice of control value were $10^{-3.5}$ M and 10^{-3} M respectively. These values were observed in the experiments to be variable depending upon the activity of plasmin given, but the rate of inhibitory action of AMCHA was always over that of EACA.

According to fibrin plate test, similar findings were observed. The data were presented in Table 1, A. The lysis area appeared on fibrin plate, which represents the activity of plasmin, was inversely proportional to the logarithm of the given concentration of AMCHA or EACA.

The degree of the inhibition was expressed by percentage and shown in Fig. 4. The question was still present, however, whether activation process may have been involved or not in the assay system used here. This will be discussed later.

Table 1 (A)

Effect of AMCHA & EACA upon fibrinolysis with plasmin and trypsin measured by unheatedplate test.

(B)

Effect of AMCHA & EACA upon fibrinolysis with plasmin measured by heated plate test.
Plasmin: Fibrinolysin-Merck 50 Fibrinolytic units/ml.
Trypsin: Novo trypsin 400 u/ml.

		A							B			
Test		Unheated							Heated			
Enzyme		Plasmin				Trypsin			Plasmin			
		Lysis Area (mm^2)			Mean (mm^2)	Lysis Area (mm^2)		Mean (mm^2)	Lysis Area (mm^2)			Mean (mm^2)
Control		690	681	672	681	364	384	374	352	326	333	337
AMCHA	(M)											
	−5	525	490	602	539	352	360	356	366	414	378	386
	−4	473	440	476	463	360	326	343	410	429	415	417
	−3	360	352	344	352	360	320	340	342	340	344	342
	−2	122	150	156	144	324	216	320	250	252	260	254
	−1	35	25	30	30	320	320	320	161	173	161	165
EACA	−5	594	615	636	615	356	340	350	458	426	400	428
	−4	525	525	546	532	340	320	330	466	496	442	468
	−3	425	415	430	420	302	298	300	408	411	421	410
	−2	238	224	216	226	291	299	295	330	315	315	320
	−1.5	120	120	120	120	300	288	294	200	212	194	202
	−1	0	0	0	0	286	284	285	110	99	90	93

The observations by heated plate test were quite different from the above (Table 1, B, Fig. 4). The inhibitory effect was seen in higher concentration; over 10^{-2} M of AMCHA, and $10^{-1.5}$ M of EACA. On the other hand, enhancing effect appeared in lower concentration of both compounds. That is, the activity of AMCHA upon the fibrinolysis by plasmin was stronger at the site of inhibition, and weeker at the site of enhancement as compared with EACA.

No effect of AMCHA & EACA upon the fibrinolysis with trypsin just indicates the specificity of their inhibitory action (Table 1).

C. EFFECT OF AMCHA UPON CASEINOLYTIC AND TAMe ESTEROLYTIC ACTIVITY WITH PLASMIN

Various amounts of AMCHA were added in casein solution prior to the addition of plasmin, and caseinolytic assay was carried out. For comparison, EACA was also employed. Fig. 5 indicates that AMCHA as well as EACA did not show any effect upon the digestion of casein with plasmin except the slight

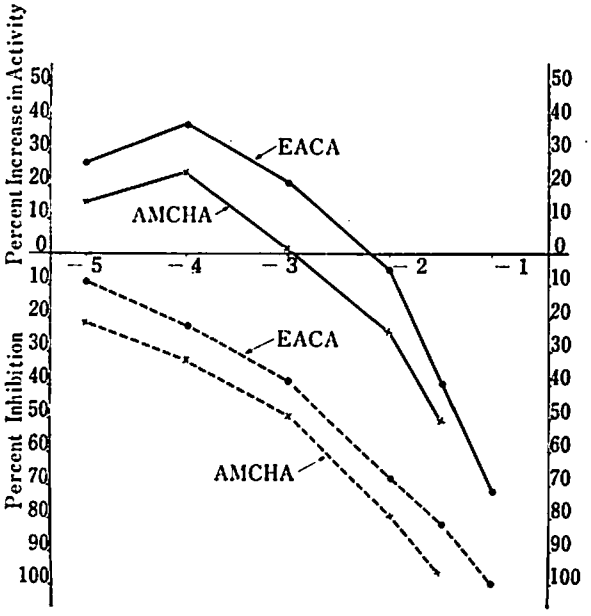

Fig. 4 Effect of AMCHA and EACA upon fibrinolysis with plasmin.
Abscissa: Log. Conc. of AMCHA and EACA
Oridinate: Enhancing and inhibitory effect expressed by percentage. Calculated from Table 1, A and B.
×——× AMCHA } heated plate test
•——• EACA }
×——× AMCHA } unheated plate test
•——• EACA }

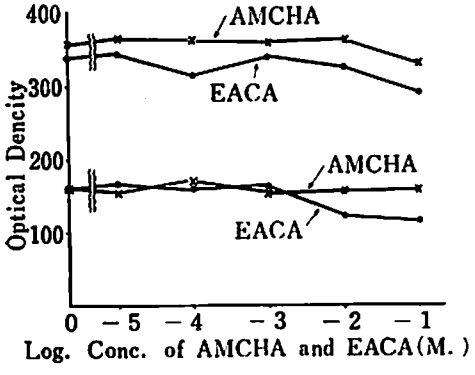

Fig. 5 Effect of AMCHA and EACA upon caseinolysis with plasmin.
×——× AMCHA
•——• EACA

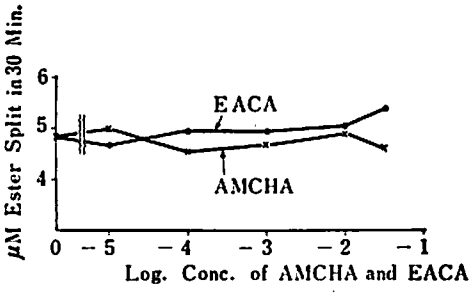

Fig. 6 Effect of AMCHA and EACA upon TAMe-Esterolytic activity with plasmin.
×——× AMCHA
•——• EACA

inhibitory effect in more than 10^{-1} M of EACA.

No effect of AMCHA was observed upon the TAMe hydrolytic activity with plasmin. The observations on the effect of EACA were nearly same as shown in Fig. 6.

DISCUSSION

Results obtained indicated that the inhibitory effect of AMCHA upon fibrinolysis with plasmin was remarkable either in plasmin-fibrin clot system (Figs. 2 and 3) or in plasmin-fibrin plate system (Table 1, A, Fig. 4) unless the fibrin was heated. Thus it was strongly suggested that activation process of plasminogen may be affected by AMCHA and EACA. However it was not sure that Fibrinolysin-Merck employed was free of SK. Even if SK was completely absent, plasminogen contaminated may be activated by the plasmin in auto-catalytic manner. In order to obtain the more precise information concerning the problems as mentioned above, the studies using a more direct technique must be planned. It has been proceeding, and the report will be given in subsequent paper[15].

So far as fibrinolytic assay test is concerned, therefore, only heated plate test most likely indicates the activity of plasmin itself[16]. It is difficult, however, to interpret the phenomenon, observed on heated plate, of enhancing effect by AMCHA and EACA. There was an explanation by Alkjaersig[4] for the similar phenomenon observed with EACA by illustrating the analogous action of quaternary amines to cholin esterase and plasmin[17,18]. But, the evidence was lack. It is considered that AMCHA and EACA might possess the protective action of plasmin against its auto-inactivation, likewise some of substrates i.e. fibrinogen and fibrin[19]. If so, when a certain concentration of AMCHA or EACA are contained in assay system, plasmin activity would probably become greater rather than when these compounds are not included. It would be one of the possibilities to account for the phenomenon.

The other point which interests us, beside that, is the fact that such enhancing effect was throughly extinguished, when a trace amount of plasminogen was present. This evidence will give a base to reasonable considerations for the clinical application of AMCHA as well as EACA.

The results obtained that no effect was observed on caseinolysis (Fig. 5) and TAMe esterolysis (Fig. 6) with plasmin in wide range of concentration of AMCHA and EACA are of interest and supposed to be a matter to discuss. It has been reported by Alkjaersig[4] that EACA inhibited caseinolytic action of plasmin noncompetitively in concentration exceeding 6×10^{-2} M, while by

Ablondi[5] that EACA did so but competitively in concentration 4×10^{-2} M. But in our experiment, it is unlikely that inhibition occured by EACA in concentration of this degree. This discrepancy would be responsible for the difference of the experimental condition.

Why was plasmin activity inhibited by AMCHA and EACA of about 10^{-2} M or $10^{-1.5}$ M when substrate was fibrin, and was not when substrate was casein or TAMe? If plasmin were formed from different type of precausor, that is, there were two or more components in plasmin, as currently proposed 'fast' and 'slow' factors in antiplasmin of human serum or guinea pig serum[20], they would act differently on their substrate, and they would undergo the inhibitory action of AMCHA and EACA differently. The problem propose could be understood by the above assumption.

SUMMARY

1) Effect of AMCHA upon fibrinolysis, caseinolysis and TAMe esterolysis with plasmin was examined and compared with that of EACA. It is evident that AMCHA has inhibitory activity upon fibrinolysis with plasmin but not caseinolysis and TAMe esterolysis, and the inhibitory activity of AMCHA is approximately 5 times greater than EACA.

2) Inhibitory activity of AMCHA upon activation process of plasminogen was suggested.

3) Enhancing effect of AMCHA and EACA upon fibrinolysis with plasmin and the difference of the action of AMCHA and EACA between the effect upon fibrinolysis with plasmin and caseinolysis or TAMe esterolysis were discussed.

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CLINICAL USE OF AMCHA ON CERTAIN DERMATOSES

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Our experience in clinical use of antiplasmin drug ε-amino-n-caproic acid on several kinds of skin diseases has been reported already[1-5]. Recently a new antifibrinolytic substance was synthesized, and named as AMCHA[6-8]. We tried to prove the effectiveness of the new antiplasmin drug AMCHA and intended to reveal the relation between plasmin activity in the blood and the effectiveness of administration of AMCHA.

GENERAL METHOD OF CLINICAL EXAMINATION

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

2) Aqueous solution of AMCHA was injected to out-patients every day as a rule. To infant patients, 2 to 5 ml of 5% AMCHA solution was injected subcutaneously or intravenously, and to the other patients 5 ml of the solution intravenously.

3) 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 AMCHA.

RESULTS

Three cases of acute dermatitis, and two cases of autosensitization dermatitis were selected for the investigation. In reproducing the degree of subjective or objective signs, the following symboles were used.

+++.....Very strong
++ .....Moderately strong

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\+ .....Clearly present but weak

± .....Very slight

Case 1. Keio Card No. 8433, Yasuko Hori (23) Female

1) Diagnosis: Acute dermatitis (on left arm).

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

3) Case history: About one week prior to examination the lesion appeared on left arm. No previous treatment was done. Patient was seen at O.P.D. on October 23, 1961.

4) Status at the first examination: Causalgia (++), red swelling (+++). Acute exudative inflammation was apparently observed. Blood examination was carried out on October 23. The result showed the acceleration of euglobulin lysis, indicating high activity of plasmin.

5) Course and effect of treatment with AMCHA: Five ml of 5% AMCHA solution was injected intravenously for three days from October 23. By the first injection, exudation began to disappear. After the second injection, red swelling disappeared and causalgia was much improved; and after the third injection, it almost healed up. Blood examination was carried out 24 hours after the second injection. The result showed still accelerated euglobulin lysis.

The administration of AMCHA was interrupted for one week. On November 2, blood examination was again carried out, and the result revealed normal activity of plasmin.

6) Side effect: None.

7) External application: Boric acid and zinc ointment.

8) Result: It is recognized that the administration of AMCHA was conspicuously effective and plasmin activity in blood became lower than before the administration of AMCHA.

Case 2. Keio Card No. 9093, Reiko Yoneda (5) Female

1) Diagnosis: Autosensitization dermatitis (on the whole body).

2) Anamnesis: No significant anamnesis related to diagnosis.

3) Case history: Since about one month patient developed several nummular eczematous lesion of legs, and three days prior to examination generalized *eruption* appeared all over the body. Patient was seen at O.P.D. on November 24, 1961.

4) Status at the first examination: Itching (++), red spots (+), papules (+), vesicles (++), crusts (+), exudation (+). Blood examination was carried out on November 24. The result showed the acceleration of whole clot lysis, indicating high plasmin activity.

5) Course and effect of treatment with AMCHA: Since November 25, 5 ml of 5% AMCHA solution was injected intravenously for eight days. After the second injection, exudation began to decrease; after the eighth injection the lesion almost healed up. Blood examination was carried out at this time, and normal activity of plasmin was noticed.

6) Side effect: None.

7) External application: Boric acid and zinc ointment.

8) Result: It is recognized that the administration of AMCHA was effective in treating autosensitization dermatitis and plasmin activity became lower than before AMCHA treatment.

Case 3. Keio Card No. 9302, Michiko Fujimaki (20) Female

1) Diagnosis: Acute dermatitis (on face, neck and hands).

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

3) Case history: About three days prior to examination the lesion appeared on above mentioned area. Patient was seen at O.P.D. on December 5, 1961.

4) Status at the first examination: Causalgia (++), red swelling (++). Acute exudative inflammation was apparently observed. Blood examination was carried out on December 7, prior to AMCHA treatment. The result revealed hypofibrinogenemia, indicating high plasmin activity in the blood.

5) Course and effect of treatment with AMCHA: Five ml of 5% AMCHA solution was injected intravenously from December 7. After the first injection, red swelling and causalgia were much improved; after the fourth injection, the lesion almost healed up. Blood examination at this time showed normal content of plasma fibrinogen.

On December 19, blood examination was again carried out, and it revealed normal activity of plasmin.

6) Side effect: None.

7) External application: Boric acid and zinc ointment.

8) Result: It is recognized that the administration of AMCHA was effective, and that, by the administration of AMCHA, the course of improvement was satisfactory, and plasmin activity in blood became lower than before the treatment.

Others: One case of autosensitization dermatitis with high plasmin activity and one case of acute dermatitis with normal activity of plasmin were also treated with the same method, and the administration of AMCHA was conspicuously effective in treating these cases and suppressing plasmin activity.

CONCLUSION

Three cases of acute dermatitis, and two cases of autosensitization dermatitis were treated with 5% AMCHA solution. The blood examination, performed before AMCHA treatment, showed high plasmin activity in 4 out of these cases, and normal plasmin activity was observed in one case of acute dermatitis.

The results of our investigations revealed that the administration of 5% AMCHA solution was effective for treating all these cases, and that plasmin activity in blood became lower than before the administration of AMCHA in 4 cases with high activity of plasmin, and no unfavorable side effect was

observed.

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THE EFFECT ON THE PLASMINIC ACTIVITY OF AMCHA IN CASES WITH CEREBRAL VASCULAR LESION

PRELIMINARY REPORT

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(Received for publication May 12, 1962)

As mentioned previously[1], we confirmed the fact that plasminic activity was enhanced in the cases with intracranial hemorrhage.

Accordingly, we have been using Ipsilon aminocaproic acid injection for the treatment of this condition, and more recently, we had an opportunity to use AMCHA[2-4], a more powerful antiplasmin preparation, to some cases.

The results are presented here.

Case 1: Male, Age 50; a boilerman, Clinical diagnosis; cerebral hemorrhage.

1) C.C.: Coma.
2) P.I.: This known hypertensive (200 mm Hg. for 3–4 years) had been fairly well without medication until Feb. 7, 1962, when he was found in coma at about 11 P.M. on the roof of building where he was working. There was no evidence of head injury or alcohol ingestion. On Feb. 8, he was admitted to our ward.
3) Physical examination: Large, well nourished. Face flushed. Pulse 90 regular with good tonus. Respiration labored. Temp. 39°C. Conscious comatous. B.P. 210–123. Anemia, jaundice (−). Pupils regular round myotic without light reflex. Corneal reflex (−). Fundi unable to examine. Lips dried, some blood coagula attached. No odor on breath. No deviation of tongue and uvula. Nuchal rigidity (−). Heart enlarged to left Ca. 1 f.b. sounds pure. Lungs breath sounds clear. Abdomen bulged, forborygmi (−). Liver and spleen not palpable. Extremities flaccidly paralyzed, biceps and triceps reflex right (+), left (−) patellar and ankle jerk bilaterally present, but not accentuated. Abdominal reflex (−). Pathologic reflexes (−).
4) Course in the Ward: Shortly after the admission, he vomited several times with fever elevation up to 41°C. A spinal tap revealed bloody fluid indicating the possibility of intraventricular hemorrhage. The patient underwent a hypothermic treatment with his body temperature kept at 32°C. Tracheotomy was performed to secure his air way. Oxygen tent, retention catheter and intravenous infusion

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were applied. Via the infusion, cortisone, AMCHA, Cytochrom C, GABA etc. were administered. Five cc of 5% AMCHA solution was administered twice a day. After one week of the hypothermic treatment, the patient still remained in coma, but pathologic reflexes became positive in all extremities. At this point, hypothermia was discontinued.

On Feb. 15, a sudden fall of blood pressure was noted with quick recovery by vasoconstrictive agent. Thereafter the patient was digitalized but coma and remitting fever up to 42°C had continued till March 20.

On March 21, his pulse rate decreased suddenly with drop of temperature to 35°C. This condition had persisted until his death on April 9.

5) Laboratory examination: At the early stage of the disease, leucocytosis up to 15,000 with 89% neutrophile was noted. In blood serum, a hypokalemic tendency (K. 3.5 mEq/l) was noted.

Urinalysis; Protein (卄)—(+)
Sugar (−), Urobilinogen (+)—(±)
R.B.C. 10—20/H.P.F.

Cerebral fluid;

(Feb. 8) Opening pressure 130, bloody
Nonne-Apelt (+), Pandy (+)
Protein 35 mg/dl

(Mar. 12) Opening pressure 190
Xanthochromic
R.B.C. 246/3
Protein 40 mg/dl

Table 1
Plasminic activity on Case 1

	Feb. 8 10:00 A. M.	Feb. 8 11:00 A. M. 30 min. after AMCHA injec.	Feb. 9
Fibrinogen (mg/dl)	3.8	2.7	1.62
Whole clot lysis (days)	8	14	14
Whole plasmin of serum (units)	10.75	7.08	6.17
Euglobulin lysis (units)	0.71	less than 0.56	less than 0.56

6) Pathological findings:

From the upper border of medulla to the level of the 5th cranial nerve, there was a massive hemorrhage of the brainstem on the right side and small one on the left side with hemosiderin deposition. Dilatation of ventricle and incisular herniation were noted. Atherosclerotic changes in basilar artey were moderate. Several hemorrhagic infarcts were also noted in the bilateral claustrum.

7) Comment: Though palsminic activity in this case was not significantly elevated in the early stages of the disease, its shifting toward antiplasmic direction after AMCHA injection was exhibited in those laboratory data. It seems noteworthy that a case with medullary hemorrhage could have a relatively long period of

survival by daily injections of AMCHA.

Case 2: Male, Age 50; Clinical diagnosis; Cerebral hemorrhage.

1) C.C.: Disturbance of speech and left hemiplegia.
2) Course: On Jan. 4, 1961, this man noticed that his body inclined to left with vertigo while he was walking. Shortly thereafter, disturbance of speech and left hemiplegia developed. Some recovery had been attained by massage and physical treatment before his admission on 14th of October. On admission physical examination revealed a moderate degree of speech disturbance and left hemiparesis with elevated deep tendon reflexes. Chaddock and Rossalimo's reflex were noticed on this side. Urine protein was negative, fundi Keith-Wagner IIa and B.P. 147/88. Plasminic activity was as shown in Table 2.

Table 2
Plasminic activity on Case 2

	Nov. 13 1961	Dec. 25 1961	Dec. 25 after AMCHA injec.	Jan. 13 1960	Jan. 13 after AMCHA injec.
Fibrinogen	2.60	3.35	3.67	2.86	2.30
Ratnoff's test	2 hours	4 days	>14 d.	>14 d.	>14 d.
Whole plasmin of Euglobulin (units)	22.98	23.80	11.90	27.77	13.33
Whole plasmin of Serum (units)		20.83	12.82	20.83	11.11
Euglobulin lysis (units)	0.56	0.56	0.59	0.83	0.63

In this case 5% AMCHA was injected 5 cc once a day intravenously and blood sample was drawn 30 minutes after the injection.

3) Comment: In this patient, plasminic activity by whole clot lysis was definitely enhanced as a 2 hours or 4 days. Whole plasmin revealed also high value. However, by AMCHA injections, whole clot lysis has prolonged and whole plasmin value reduced, indicating the suppresion of plasminic activity.

Case 3: Female, Age 41, Clinical diagnosis; Retinal bleeding.

1) C.C.: Visual disturbance.
2) Course: On Jan. 5, 1962, this woman developed suddenly visual disturbance of the right eye, which brought her to the ophthalmology clinic of this hospital. A

Table 3
Plasminic activity on Case 3

	Jan. 22 1962		Jan. 29 1962		Feb. 5	
	Before injec.	After injec.	Before injec.	After injec.	Before injec.	After injec.
Fibrinogen (mg/dl)	1.30	1.50	2.70	2.62	1.70	1.60
Whole clot lysis (days)	1	1	11	14<	1	1
Whole plasmin of Euglobulin (units)	19.60	11.49	23.80	16.60	20.20	19.60
Whole plasmin of Serum (units)			20.20	13.88	18.51	12.82
Euglobulin lysis (units)			<0.56		<0.56	<0.56

diagnosis of retinal bleeding was made. She had no past history of tuberculosis or other abnormalities were disclosed internally. Her plasmic activity was as shown in Table 3.

The method of AMCHA injection and blood sampling were just same as in *Case 2*.

3) Comment: In this case, again those laboratory data of fibrinogen content and whole clot lysis indicate a marked elevation of plasminic activity in the early stage of the disease. Following the injection of AMCHA, the activity, particularly whole plasmin was reduced.

In conclusion, it was demonstrated that the AMCHA injection to the cases with cerebral hemorrhage and retinal bleeding could a beneficial effect on the course of the disease by suppressing their plasminic activity. No particular side effects are noted in all cases.

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THE RELATIONSHIP OF PLASMIN TO GLAUCOMA

CLINICAL USE OF ε-AMINOCAPROIC ACID AND AMCHA ON GLAUCOMA

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INTRODUCTION

The pathogenesis of glaucoma has been studied by many investigators, and various etiologies have been proposed, but none of them has been definitely proved.

A consideration of the chemical and physical factors involved in the development of glaucoma will of necessity be elaborated and developed from a discussion of the mechanisms that maintain the normal function of the eye ball.

In previous studies, the authors reported the relationship between the plasmin activity and various eye disorders.

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 glaucoma.

METHODS AND MATERIAL

Material: The observations were made in 16 cases. The cases consist of 5 simple glaucoma, 5 inflammatory glaucoma and 6 secondary glaucoma.

Methods: For the determination of plasmin activity, venous blood of patients was used.

The routine method to determine the activity is as follows, 1) Whole clot lysis test (by Ratnoff's method), 2) Euglobulin lysis test (by Lewis's method), 3) Whole plasmin test (by Colgan's modification), 4) Quantitative determination of fibrinogen.

RESULTS

(2) Simple glaucoma (Table 1):—As shown in Table 1, of 5 cases with simple glaucoma, streptokinase activated plasmin activity was positive in only one case (case 5). In other 4 cases, plasmin activity was normal.

NO.	Sex	Age	Fibrinogen (mg/ml)	Whole clotolysis (days)	Whole Plasmin (unit)	Euglobulin lysis test (unit)	Intraocular pressure
1	♀	35	2.22	14	17.54	Less than 0.56	R=23.0 mmHg L=19.0
2	♀	16	2.20	14	11.90	" 0.56	R=17.5 L=17.5
3	♀	78	2.64	8	17.54	" 0.56	R=24.0 " L=23.0 "
4	♀	54	3.22	8	18.51	" 0.56	R=19.0 " L=19.0 "
5	♂	27	2.52	9	33.33	" 0.56	R=36.0 " L=28.0 "

Table 1 The plasmin activity in the cases with Simple Glaucoma

(2) Inflammatory glaucoma (Table 2):—As shown in Table 2, of 5 cases with inflammatory glaucoma, streptokinase activated plasmin activity was positive except case 2.

NO.	Sex	Age	Fibrinogen (mg/ml)	Whole clotolysis (days)	Whole plasmin (unit)	Euglobulin lysis Test (unit)	The intraocular pressure (mmHg)
1	♂	62	2.52	5	22.22	Less than 0.56	R.=17.5 L.=82.0
2	♂	65	2.68	14	19.04	" 0.56	R =63.0 L.=17.0
3	♀	72	2.88	7	30.30	" 0.56	R =42.5 L.=62.0
4	♀	62	4.14	7	22.22	" 0.56	R =57.0 L.=17.0
5	♂	56	3.22	5	27.77	" 0.56	R.=57.5 L.=24.5

Table 2 The Plasmin activity in the Cases with inflammatory glaucoma

(3) Secondary glaucoma (Table 3):—As shown in Table 3, higher plasmin activity in the blood was found in 4 out of 6 cases of secondary glaucoma. These results indicate that significant relation was found between the results of fibrinolysis and pathologic state such a inflammatory or secondary glaucoma.

(4) The effect of ε-aminocaproic acid (Ipsilon) on intraocular pressure:—The dosage of ε was 10–20 cc of 20% solution intravenously injected. The observations were made in 16 cases. The cases consist of 2 simple glaucoma, 5 acute inflammatory glaucoma, 1 absolute glaucoma and 8 secondary glaucoma. Ocular tension was measured with Schiøtz tonometer before and after the ad-

NO.	Sex	Age	Fibrinogen (mg/ml)	Whole clotolysis (days)	Whole plasmin (unit)	Euglobulin lysis test (unit)	The intraocular pressure	
1	♂	48	0.34	14	15.15	Less than 0.56	R=17.5 mmHg L=82.0 "	Ocular injury
2	♂	26	4.12	14	26.63	" 0.56	R=60.0 " L=prothese	Sympathic ophthalmia
3	♂	71	2.30	4	27.80	" 0.56	R= 9 mmHg. L=15 "	Uveitis
4	♂	27	2.14	14	19.59	" 0.56	L=35 "	Bechet's disease
5	♀	47	3.20		18.51	" 0.56	R=60 " L=13 "	Hemorrhagic glaucoma
6	♂	27	2.00	6	18.51	" 0.56	R=15 " L=62 "	Retinal vein thrombosis

Table 3 The plasmin activity in the cases with secondary glaucoma

ministation of ε, and expressed as millimeters Hg. In most cases, venous blood samples were drawn before and after administration of ε for determination of plasmin activity.

As shown in Table 4, intraocular pressure was reduced in 12 out of 16 cases after the intravenous administration of ε, and 4 cases were not effective. The degree of fall on intraocular pressure was ranged from 5 mmHg to 27 mmHg. Generally, the maximum observed hypotensive effect was noted from a half-hour to 2 hours after the administration of ε but was variable case by case (Fig. 1).

NO.	Sex.	Age	Types	Intraocular pressure (mmHg)			C.p.p. plasmin
				before	after	Maximam decreasing	
1	♂	42	secondary , glaucoma	66.0	41.0	− 25.0	
2	♂	62	"	41.0	25.0	−16.0	C.P.P. (+)
3	♂	22	"	50.0	36.0	−14.0	C.P.P. (+)
4	♂	34	"	39.0	39.0	0	
5	♀	57	"	54.0	37.0	−17.0	
6	♂	57	"	28.0	22.0	−16.0	
7	♀	64	"	22.0	16.0	−6.0	
8	♂	4	"	50.0	38.0	−12.0	
9	♀	47	absolute glaucoma	60.0	54.0	−6.0	
10	♀	47	acute glaucoma	R.71.0 L.58.0	42.0 36.0	−29.0	C.P.P. (++)
11	♀	46	"	45.0	31.0	−22.0 −14.0	C.P.P. (+++)
12	♂	56	"	46.0	46.0	0	C.P.P. (−)
13	♂	32	"	62.0	62.0	0	
14	♂	59	simple glaucoma	R.18.0 L.55.0	13.0 55.0	− 5.0 0	
15	♂	32	"	24.0	17.0	− 7.0	
16	♀	61	absolute glaucoma	R.50.0 L.45.0	50.0 45.0	0	

Table 4 The change of intraocular pressure by ε-A.cA injection

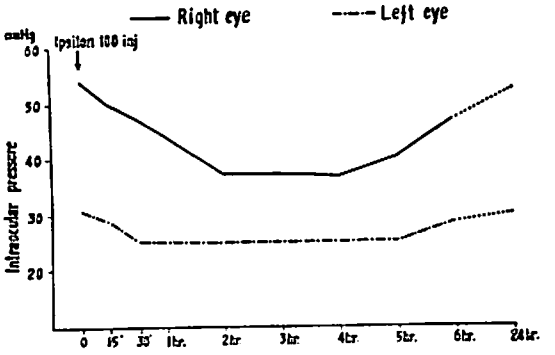


Fig. 1 Case 5. Secondary glaucoma
Changes of intraocular pressure following intravenous administration of Ipsilon.

Thereafter, the intraocular pressure began to rise unless surgical or additional medical means were employed. It was difficult to determine when the hypotensive effect of ε was ended, because the duration of hypotensive effect was very different by each cases. The most dramatic responsc to ε has been observed in cases of acute angle-closure glaucoma. It was observed in our experiments that the effect of ε on the intraocular pressure decreased gradually by repeated administration of ε as shown in following case reports.

Case 1.—68 years-old, male, secondary glaucoma: He was admitted to our hospital with a chief complaint of blurred vision and eye pain for one week after contusion of eye. We observed that traumatic cataract and subluxation of the lens. On admission, intraocular pressure involved eye was 60 mm Hg, the pupillary diameter was 5 mm and gonioscopy revealed a closed chamber angle. Acetazolamide (Diamox) 500 mg was given and pilocarpine 3% were started every 2 hours. After 5 days,

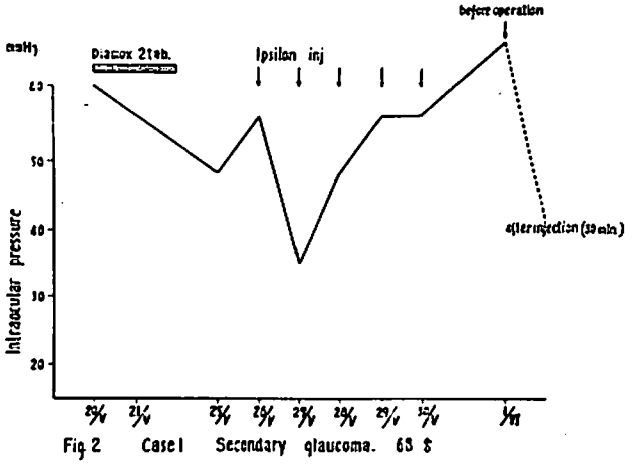


Fig. 2 Case 1 Secondary glaucoma. 68 ♀

intraocular tension was 48 mmHg in the right eye. Then, Diamox and pilocarpine was stopped, the intraocular tension elevated to 56 mmHg on the following day. The ε 20%, 10 cc was given intravenously. Fig. 2 shows the response to ε of this case. Marked reduction in intraocular pressure was noted on the following day. But inspite of repeated administration of ε the decrease in intraocular pressure produced by ε could not be maintained. ε was given about 30 min, prior to operation. Measurement of tension was performed immediately prior to operation. The intraocular pressure quickly fell to 41 mmHg and iridectomy was performed on the eye without difficulty. ε has proven equally effective in acute primary glaucoma and in angle closure glaucoma due to swelling of a cataractous lens.

Case 2.—62 year old, male, secondary glaucoma: Case history: First attack of glaucoma occured in 4 Oct. 1960. In this time, the intraocular pressure reduced to normal by administration of Diamox and pilocarpine. A second attack occured in 20 March 1961. Table 5 shows the plasmin activity in this case at a attack of glaucoma

Date	Fibrinogen (mg/ml)	Whole clotolysis (days)	Whole plasmin (unit)	Euglobulin lysistest (unit)	Intraocular pressure		CPP
20/III	2.52	5	22.22	less than 0.56	82.0 mmHg	before operation	(+)
19/V	3.38	14	14.18	" 0.56	19.0 "	after operation	(−)
1/VI	2.60	9	9.80	" 0.56	22.0 "	after Ipsilon Injection	(−)

Table 5 Case 2 Secondary glaucoma 62 ♀

and normal stage of the intraocular pressure. A third attack occured in 22 May 1961. Then, the administration of ε 20%, 10 cc, was started. Fig. 3 shows the response to ε of this case. At first day May 22, marked reduction in intraocular pressure was noted

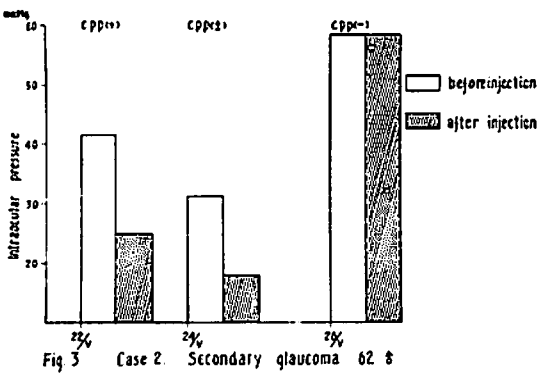
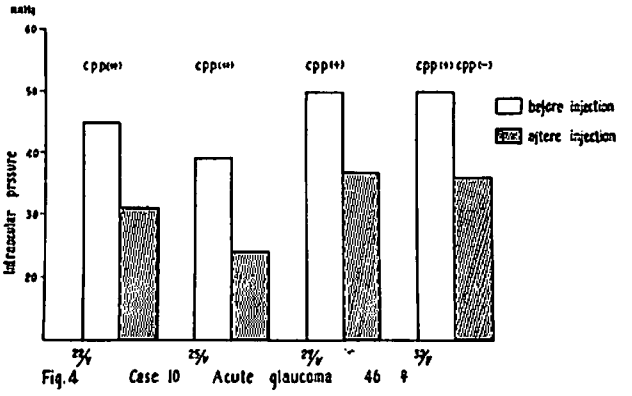


Fig 3 Case 2. Secondary glaucoma 62 ♀

30 minutes later, and pressure was in the normal range. In May 24, response was essentially the same as that of the preceeding day, but pressure rapidly rose again

to 59 mmHg in May 26 and despite of the use of ε the intraocular pressure remained unchanged. As shown in Table 5 and Fig. 3, the effect of ε on the intraocular pressure was approximately proportional to the capillary permeability promoting action in the blood.

Case 3.—46 year-old, female, acute angle-closure glaucoma (Fig. 4): She had



symptoms of acute angle-closure glaucoma for 18 hours prior to her admission to the hospital. On admission, intraocular pressure in the involved eye was 45 mmHg, and gonioscopy revealed an entirely closed chamber angle. Following the intravenous administration of AMCHA 5 cc, intraocular pressure fell 30 mmHg, in the following day, tension had been reduced to 24 mmHg. But, after 2 days, the intraocular tension began to rise, and the effect of the agent has not been so marked as that of the preceeding day.

COMMENTS

Glaucoma has been the most important and interesting problems in recent ophthalmology but the etiology is still unknown.

A great deal of interest and study has been directed toward elucidating the mechanisms responsible for controlling the intraocular pressure. Sallmann, Löwenstein, Ohashi and Nagai have provided evidences of centrally elicited changes in the eye. Recently, Akagi described that an automatic center of the intraocular pressure exist in the diencephlon. Linner, Prijot and others suggested the mechanisms controlling intraocular pressure is mediated via the autonomic nervous system. Another workers have studied the relationship between hormonal system and intraocular pressure. In present report, the authors suggested that the existence of some relationship between the elevation of intraocular tension and the plasmin activity.

Fig. 5 is a schematic figure of the mechanisms responsible for elevation of

intraocular pressure and relationship between the elevation of tension and autonomic nerve system, fibrinolytic enzyme system and hormonal system. The relationship between plasmin and eye diseases with special reference to stress is

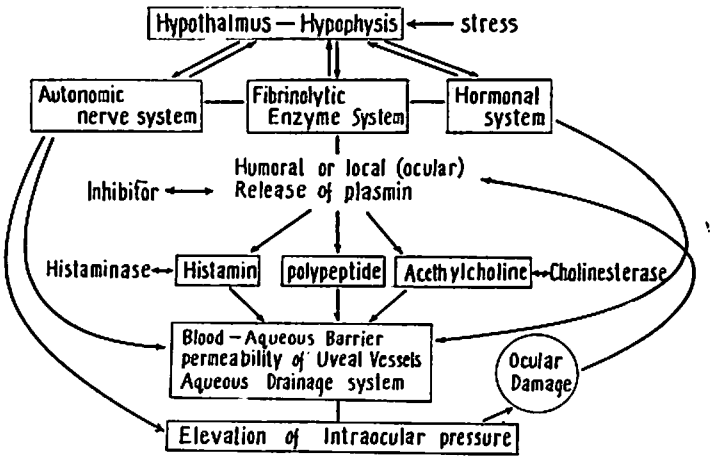


Fig. 5

important problem to be investigated further. Ikeda suggested that the stress plays and important roll in the pathogenesis of uveitis and also glaucoma as disposing or provoking agents.

It is quite clear that emotional upsets from a great variety of causes play a role in closed angle glaucoma. Croll, in 1960 reported that the death of a loved one, some type of anxiety states may trigger an attack of acute closed angle glaucoma. It is very interesting problem for us to perform a study of the relationship between increased ocular tension and the plasmin activity during various emtoional stimuli. MacFarlane concluded from his patient studies that spontaneous fibrinolytic activity occurs in the blood of human subjects following a variety of disturbing stimuli such as surgical operations, shock, trauma, anxiety, violent exercise and in some pathological states. It can also be reproduced by the injection of adrenalin, and this observation, coupled with the nature of the other stimuli mentioned, suggests that activation of the proteolytic system of the blood is part of the alarm reaction.

Intravenous ε is effective in lowering intraocular pressure. The most useful application of ε has been in acute angle closure glaucoma and secondary glaucoma.

In eyes with high tensions, surgery is facilitated by the reduction in pressure which can be produced by intravenous ε given immediately preoperatively. In few cases, we observed the lesser hypotensive effect or no effect by the adminis-

tration of ε. Its reason is unknown but, the dosage of administration of ε or other factors are responsible for the lesser hypotensive effect in such cases. The ocular hypotensive action of ε would appear to result from ability to reduce the plasmin activity in the blood and aqueous fluid. Ungar described that the damage which plasmin can cause either directly by attacking tissues, especially the capillary wall, or indirectly releasing histamine or other toxic protein break down products, is a function of the interval during which it circulates in an active form. In the light of certain observations histamine seems to be released as a result of a proteolytic process. The amount of histamine liberated is therefore probably related to the interval during which plasmin is free.

We supposed that the plasmin and such toxic substances as histamine or polypeptide formed by the action of plasmin on the proteins in blood and aqueous humor may play a part in the glaucoma. Recently, we have been studing experimentally, the relations between the intraocular tension and fibrinolytic enzyme system under simple conditions.

An experiment as follows using rabbits was made. Employing normal rabbits, the change of the tonographic values produced by the retrobulbar injection of sufficient amount of streptokinase (SK) were measured with the Keio electric tonometer. The intraocular tension increased slightly and the rate of inflow markedly increased after SK injection.

Then, we measured the fibrinolytic activity in the aqueous humor of rabbits or human using the modified Sasaki's method. a) The strongest activity was observed in the aqueous humor of glaucoma. b) The fibrinolytic activity increased by the retrobulbar injection of SK. c) The strongest antifibrinolytic activity was observed in secondary aqueous humor after paracentesis.

CONCLUSION

In the studies already reported, an attempt was made to clarify the pathologic physiology of fibrinolytic enzyme system in the patients suffering from glaucoma,

1) High fibrinolytic activity in the blood was most often observed in such a case as inflammatory and secondary glaucoma. On the other hand, fibrinolytic activity remained normal in the most cases of simple glaucoma.

2) After administration of ε-aminocaproic acid to patients with all types of glaucoma marked reduction of intraocular pressure resulted, along with significant decrease of blood fibrinolytic activity. (ε-aminocaproic acid is an antiplasmin substance synthesized by S. Okamoto and others in 1953).

We do wish to express the thanks to Prof. Kuwabara, Prof. Okamoto and members of the technical center of Research Project on Plasmin.

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STUDIES ON VARIATION IN PLASMIN (BLOOD FIBRINOLYSIN) ACTIVITY IN CASES WITH CEREBRAL APOPLECTIC DISEASES

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Relation between cerebral apoplexy, which stands at the head of Japanese death rate, and blood fibrinolysin (plasmin) activity is not yet sufficiently elucidated. According to Ooneda[5,7], plasmin may play a role in the development of angionecrosis (fibrinoid degeneration) of the cerebral arteries, which might be the direct cause of hypertensive intracerebral hemorrhage. Concerning relation between cerebral vascular lesions and plasmin, there is a study by Goto[2]. It has been difficult to perform a thorough study on variation in plasmin activity, since patients immediately after apoplectic stroke have rarely been observed, on account of the special nature of this disease, in hospitals provided with the facilities. Fortunately, as first-line practitioners, we have had many chances to observe apoplectic patients immediately after the stroke and to measure plasmin activity time to time in the course of the disease. With dead cases, we carried out autopsies in order to study relation of plasmin activity with cerebral hemorrhage and cerebral infarction.

MATERIAL AND METHODS

Investigations were performed on blood samples from 285 patients with cerebral vascular lesions observed in Mihara Hospital in Isesaki City from April 1961 to February 1962 (Table 1).

Blood samples for the determination of plasmin activity were taken from veins of the forearm, and after addition of 1/10 volume of 3.8% sodium citrate solution, they were kept at 4°C in a refrigerator for 12 to 24 hours to separate blood plasma. The used reagents were as follows: Bovine fibrinogen (Armour

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Table 1.
Materials; Cerebral vascular diseases 285 cases (Fatal cases 46 and autopsy cases 25).

Cerebral hemorrhage	84
Cerebral infarction	102
Undetermined cases (Cerebral hemorrhage? infarction?)	53
Subarachnoid hemorrhage	7
Angiospastic encephalopathy	9
Cerebral arteriosclerosis or hypertension	30

Laboratories); dissolved in physiological saline to 3.3 mg per ml. Thrombin (Park Davis); dissolved in physiological saline to 100 u/ml. Streptokinase; Varidase from Lederle Laboratories (streptokinase 100,000 u and streptodornase 25,000 u/vial); dissolved in 10 ml of physiological saline. Determination of plasmin activity was performed by the following three methods:

1) Okamoto's[4] method (streptokinase-activated plasmin activity method). To the above mentioned citrated plasma was added 1/10 volume of 3.8% $CaCl_2$, and after mixing, the mixture was allowed to stand at 37°C for 30 minutes to induce clotting. The produced fibrin clot was removed with a glass rod, and the remainder was further subjected to centrifugation to obtain serum. In a small tube kept in ice water, 0.45 ml of phosphate buffer saline (pH 7.4), 0.1 ml of serum from the patient and 0.1 ml of streptokinase were mixed. After 5 minutes, was further added 0.05 ml of 100 u/ml thrombin, and then rapidly 0.3 ml of 0.33% fibrinogen. The mixture was stirred, and immediately it was transferred to a water bath at 25°C. During this procedure, fibrin clot was formed and then dissolved, and time from the addition of fibrinogen solution to complete dissolution of fibrin clot was measured with a second watch. As the control was used normal human serum, for which the above mentioned time was about 8 minutes. This was taken as the standard.

2) Ratnoff's[8] method (whole clot lysis test).

3) Macfarlane-Kuroyanagi's[3] method.

RESULTS

Comparison of plasmin activity between cases of cerebral hemorrhage within 24 hours and those over 2 weeks after the attack revealed that the activity was evidently higher among the former than the latter. The plasmin activity tended to fall from 2 weeks after the attack (Figs. 1 and 2, Table 2).

a) Plasmin activity measured by the Okamoto's method, that is, fibrin clot lysis time, was within 8 minutes for all the 15 cases of cerebral hemorrhage

within 24 hours after apoplectic stroke (Fig. 1, a), and within 6 minutes for 8 of them. Some showed remarkably high activity such as 2–3 minutes. In contrast, high plasmin activity was infrequently showed by 21 cases at 2 weeks or more after the stroke, and high value of less than 6 minutes was shown by none (Fig. 1, b).

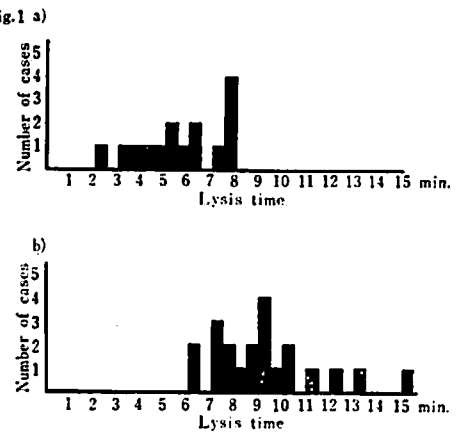


Fig. 1 a) Plasmin activity measured by Okamoto's method in 15 cases with cerebral hemorrhage within 24 hours after apoplectic attack.
b) Plasmin activity measured by Okamoto's method in 21 cases with cerebral hemorrhage 2 weeks or more after the attack.

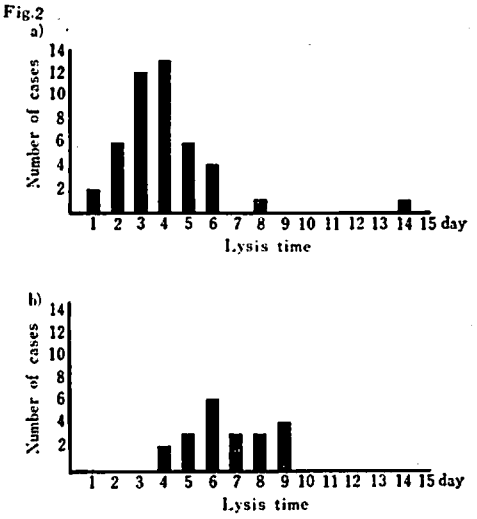


Fig. 2 a) Plasmin activity measured by Ratnoff's method in 45 cases with cerebral hemorrhage within 24 hours after apoplectic attack.
b) The activity measured by Ratnoff's method in 21 cases with cerebral hemorrhage 2 weeks or more after the attack.

b) Determination of plasmin activity by the Ratnoff's method for 45 cases within 24 hours after apoplectic stroke showed the marked activation of the enzyme in all except 2, fibrin clot being dissolved within 6 days (Fig. 2, a). It was of interest that a patient who was attacked by apoplexy during diagnostic examination showed immediately after it not so high plasmin activity, the lysis time being 8 days, but that after 3 and a half hours he showed remarkably accelerated activity with lysis time of 4 days. Later he died from intraventricular hemorrhage. A case who died from massive intracerebral hemorrhage gave the lysis time of 1 day (Figs. 2, a and 6, c). In general, the lysis time was evidently longer after 2 weeks and over than within 24 hours following the stroke, indicating the tendency of fall of plasmin activity with the lapse of time (Fig. 2).

c) Plasmin activity as determined by the Macfarlane-Kuroyanagi's method

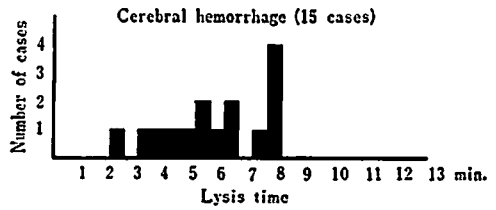
was also found activated in cases of cerebral hemorrhage within 24 hours after the stroke (in 15 out of 35 cases examined, especially remarkably in 6). None at 8 days or more the attack showed activity of 2 plus or 3 plus (Table 2).

Table 2.

Relationship between plasmin activity measured by Macfarlane-Kuroyanagi's method and the elapsed time after the attack in cases of cerebral hemorrhage.

Plasmin activity	Cases within 24 hours	Cases from 2 to 7 days	Cases over 8 days
—	20	42	8
1+	7	3	2
2+	2	2	0
3+	6	2	0

Plasmin activity was compared between cerebral hemorrhage and infarction within 24 hours after apoplectic stroke, and it was more frequently found elevated cases in the former than in the latter (Fig. 3). As afore-mentioned, the results by the Okamoto's method showed that for all cases of cerebral hemorrhage, the clot lysis time was within 8 minutes, while none of cerebral infarction cases showed such high plasmin activity as to dissolve fibrin clot within 7 minutes (Fig. 3). Further, as seen in Fig. 4, which presents results by the Ratnoff's method, plasmin activity was evidently accelerated in 43 out of 45 cases of



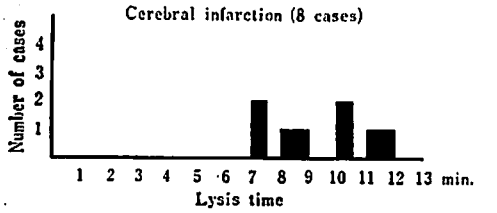
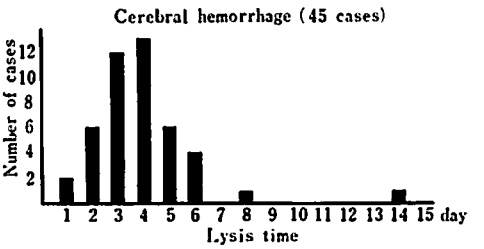


Fig. 3 Comparison of plasmin activity measured by Okamoto's method between cerebral hemorrhage and infarction within 24 hours after the attack.



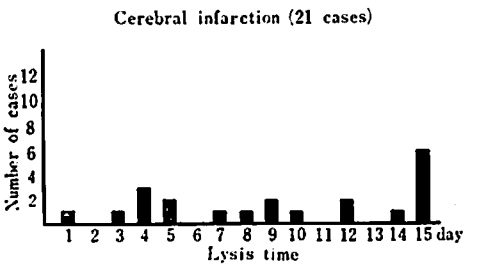


Fig. 4 Comparison of plasmin activity measured by Ratnoff's method between cerebral hemorrhage and infarction within 24 hours after the attack.

cerebral hemorrhage within 24 hours after the stroke, the clot lysis time being within 6 days. Whereas elevated plasmin activity was found in relatively small number of cerebral infarction cases, the lysis time being more than a week for 14 out of the 21 examined cases, especially in 6 cases the lysis not taking place within 15 days.

It was of interest that in cases with the so-called angiospastic encephalopathy (transient disturbance of consciousness, hemiplegia or monoplegia with complete resolution of all signs), blood plasmin activity was not found elevated within 24 hours after the attack (Fig. 5). According to the results by the Ratnoff's method, clot lysis did not take place within 5 days for any of the 9 examined cases of this disease, and 14 days and over for 4 of them. Also by the Macfarlane-Kuroyanagi's method, plasmin activity was shown not elevated in all cases of angiospastic encephalopathy.

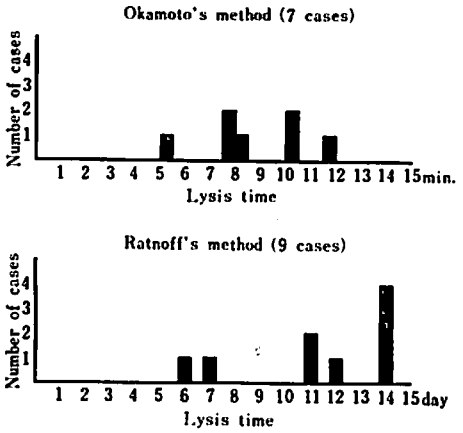


Fig. 5 Plasmin activity of cases with so-called angiospastic encephalopathy within 24 hours after the attack.

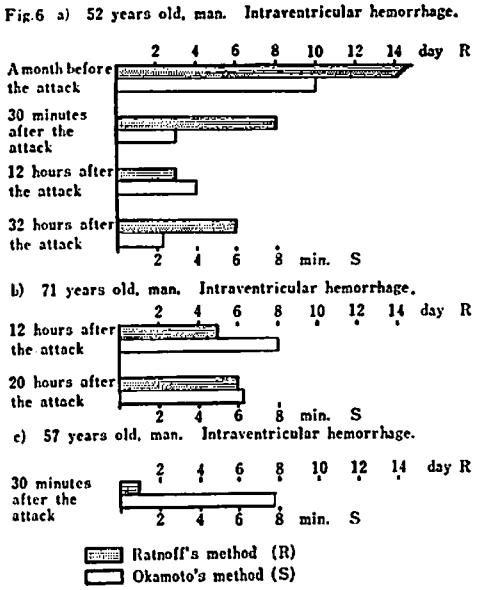


Fig. 6 Fluctuation of plasmin activity during the course of cerebral hemorrhage.

Fig. 6 a, presents results from a patient who died after a course of 36 hours as the result of remarkable massive hemorrhage which took place in the area from the left claustrum to the lateral ventricle. He gave remarkably elevated plasmin activity at 30 minutes after the attack, and died with persisting high activity. At 27 days before the attack he visited our hospital with complaint of dysarthria and incomplete hemiplegia on the left, and was diagnosed as cerebral infarction (autopsy revealed a small infarct in the right thalamus). At this time,

his plasmin activity was not shown elevated at all.

In general, the plasmin activity of cases who died in a relatively short course of the disease and had a large hemorrhagic focus in the brain disclosed by autopsy, was remarkably elevated during the course of the disease (Figs. 6 a, b, c, 9 and 10). In cases who survived more than 10 days after the attack of cerebral hemorrhage and died from the complication of pneumonia and others, plasmin activity showed conspicuous fluctuation, and the enzyme activity tended

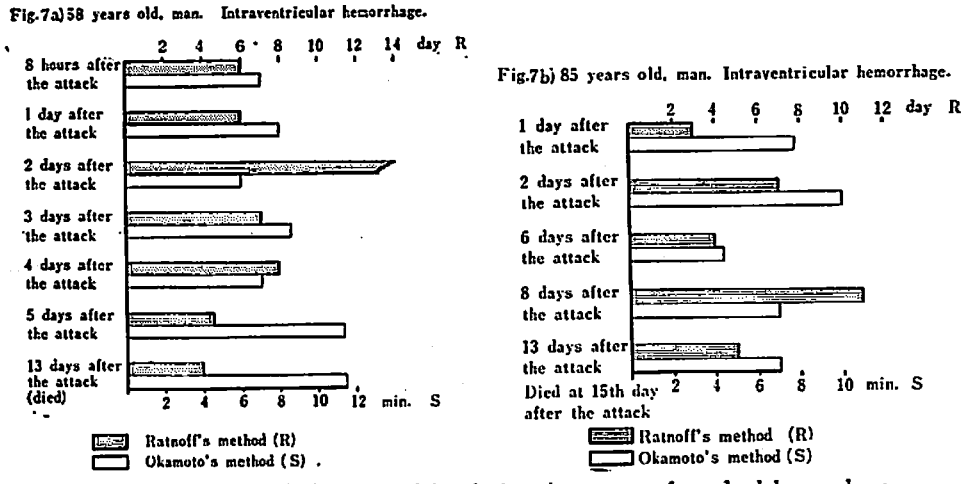


Fig. 7 Fluctuation of plasmin activity during the course of cerebral hemorrhage.

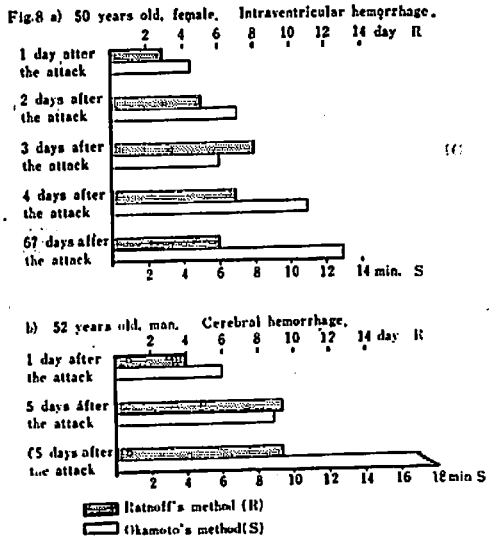


Fig. 8 Fluctuation of plasmin activity in convalescent cases with cerebral hemorrhage.

to increase before the death (Fig. 7, a and b). In these cases with relatively prolonged survival days, hemorrhagic focus was generally not so large as in cases of short survival (Figs. 11 and 12).

In convalescent cases with intracerebral subarachnoid hemorrhage, plasmin activity, even though elevated within 24 hours after the attack, tended to decrease with the lapse of day (Fig. 8).

DISCUSSION

In the field of internal medicine, studies on the plasmin system have recently been investigated by many workers, and plasmin was used by some for the treatment of cerebral infarction (the so-called cerebral thrombosis). In order to elucidate relation between cerebral apoplexy and blood plasmin activity, we performed the determination of this activity in the post-apolectic course, with the following results:

1) In cases with cerebral hemorrhage, blood plasmin activity was elevated within 24 hours after the attack, but in cases surviving over 2 weeks, it was nearly normal. This seems to indicate that blood plasmin activity, like other enzyme systems, when elevated, will induce inhibitory action of antagonistic enzyme, which will eventually bring about the plasmin-antiplasmin equilibrium.

2) In cases of short survival, blood plasmin activity generally remained high until death, and there were always large-sized hemorrhages in their brains. Presumably they died before the establishment of the antagonistic reaction to the plasmin system.

3) Those with long survival showed remarkable fluctuation in plasmin activity, which was considered to be attributed to activation of antiplasmin on one hand, and fever, pneumonia and other complications and therapeutic remedies such as nicotinic acid and chlorpromazine on the other hand (Abe[1]).

4) In many of convalescent cases, plasmin value was elevated within 24 hours after the attack, but even in severe cases of them, it was restoted to normal in about 2 weeks. This is considered to have resulted from stavilization of the plasmin-antiplasmin balance brought about by homeostatic reaction of the plasmin system.

5) In cerebral infarction, plasmin activity was infrequently elevated even within 24 hours after the attack. This indicates conspicuous difference in the plasmin system between cerebral hemorrhage and cerebral infarction. Ooneda[6], on the ground of animal experiments, regarded the activation of plasmin as one of the morphogenic factors of fibrinoid degeneration of arterial wall (angionecrosis) in cerebral arteries, which is the direct cause of cerebral hemorrhage; and

Watanabe[9] reported that the activation of blood plasmin is a cause of increased tendency of bleeding. It is questionable, however, to conclude hastily from our present results that the activation of blood plasmin is the cause of cerebral hemorrhage. There was, for example, a case who died from intraventricular hemorrhage. His plasmin activity was not elevated at the time of apoplectic attack but it was elevated after 3 and a half hours. In this case, plasmin activity was considered to have elevated as the result of bleeding. It is, therefore, a question whether plasmin activity is the cause of cerebral hemorrhage or its result.

6) It was of interest that plasmin activation was seldom observed in cases with angiospastic encephalopathy. It remained obscure whether enzymic activation did not occurred in these cases because of the absence of bleeding, or conversely the absence of enzymic activation is the cause of the recovery after a transient symptom without bleeding.

SUMMARY

1) In cases with cerebral hemorrhage, blood plasmin activity was elevated within 24 hours after the attack, but it tended to fall after 2 weeks.

2) In cases with cerebral infarction, plasmin activity was infrequently elevated even within 24 hours after the attack.

3) In cases who died from massive cerevral hemorrhage in a short time, plasmin activation was especially remarkable.

4) In cases surviving relatively long after the attack of cerebral hemorrhage, plasmin activity showed fluctuation.

5) In cases of cerebral hemorrhage taking a favorable turn, plasmin value was frequently elevated within 24 hours after the attack, but restored to the normal level in about 2 weeks.

6) In cases of angiospastic encephalopathy, plasmin value was not elevated at all within 24 hours after the attack.

The mechanism of plasmin activation in cerebral apoplexy remains in obscurity in many respects on account of rapid reaction of the plasmin system, complexity of factors concerned, as well as difficulty in their assay, and must be further investigated for the elucidation. However, the results of our present study revealed that there was evident relation between cerebral hemorrhage and plasmin system, and it is considered that they will give important suggestions for the diagnosis, prognosis and treatment of cerebral apoplexy.

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PLATE

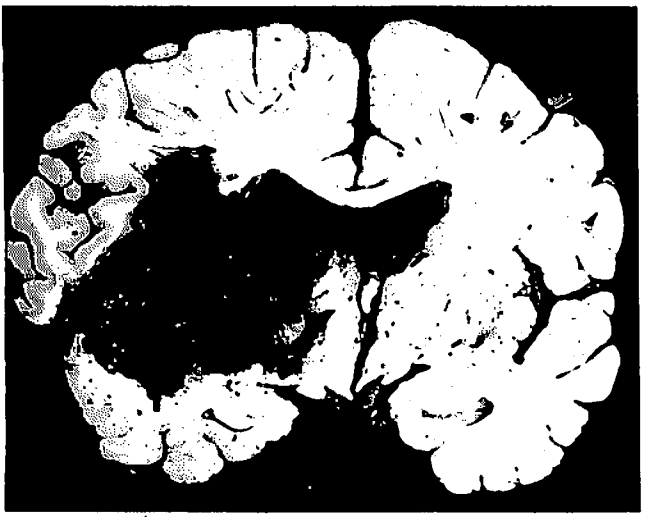

Fig. 9. 57 years old, man (the same case as Fig. 6c). Massive cerebral hemorrhage was observed in the area from the left lenticular nucleus, internal capsule and thalamus to the lateral ventricle. Clinical course: 20 hours.

Fig. 10. 71 years old, man (the same case as Fig. 6b). Large hemorrhage in the area of the left lenticular nucleus and internal capsules raptured into the lateral ventricle. Clinical course: 20 hours.

Fig. 11. 58 years old, man (the same case as Fig. 7a). Massive hemorrhage in the area of the left lenticular nucleus, a part of the internal capsule and thalamus raptured into the lateral ventricle. Clinical course: 13 days.

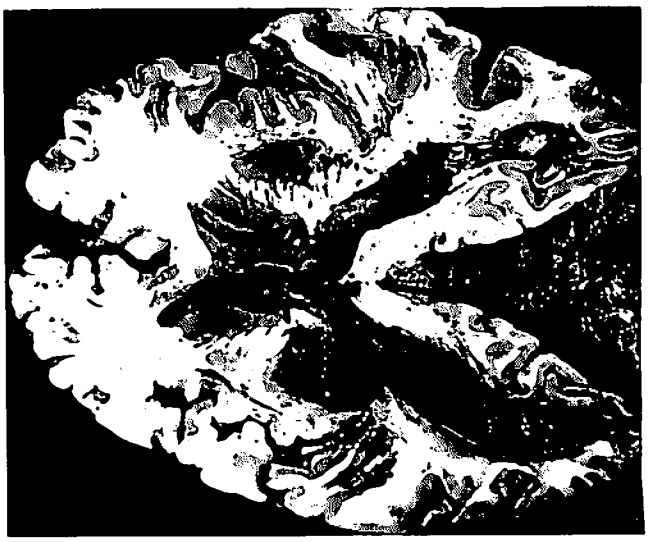

Fig. 12. 85 years old, man (the same case as Fig. 7b). Cerebral hemorrhage destroying the left lenticular nucleus and internal capsule ruptured into the lateral ventricle. Clinical course: 15 days.

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EFFECT OF EPSILON-AMINOCAPROIC ACID AS AN ANTIFIBRINOLYTIC AGENT ON ARTERIAL LESIONS IN HYPERTENSIVE RATS WITH SURGICALLY CONSTRICTED RENAL ARTERIES

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The direct cause of apoplectic cerebral hemorrhage (hypertensive intracerebral hemorrhage) is the so-called angionecrosis in the intracerebral arteries, which is both histologically and histochemically closely similar to fibrinoid degeneration in the systemic arteries found in various hypertensive animals, such as bilateral renal arteries-constricted rats[16] and rabbits[1], rats into which physiological saline was forcibly infused through the carotid artery[15], DOCA-administered rats[19], bilaterally nephrectomized dogs[12] and bilateral renal arteries-ligated dogs[8] (Ooneda[10,11,13]). As to the morphogenesis of the angionecrosis or fibrinoid degeneration, it is considered to be derived from blood plasma, especially fibrinogen, which is insudated into the arterial intima from arterial lumen owing to increased permeability of blood-arterial wall barrier, and which is deposited there as fibrinoid substance. And as the causes of the increased permeability, hypertension, activity of blood plasma fibrinolysin (plasmin), disturbance in electrolytes metabolism and others have been mentioned in animals with experimental hypertension (Ooneda[10,11,12]). On the other hand it has been known that the activity of plasmin, one of the above-mentioned causes, is generally inhibited by the administration of ε-aminocaproic acid** (ε-ACA, Okamoto[9]). In view of this, effect of the administration of ε-ACA on arterial lesions in hypertensive rats was investigated.

MATERIAL AND METHODS

Twenty one male rats of Wistar strain (70–90 g), whose renal arteries were bilaterally constricted with silver clamps[16], were divided into the following

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** The ε-aminocaproic acid was supplied by Daiichi Seiyaku Co., Ltd., Tokyo.

two groups, and they were given through the whole course of experiment 1% saline solution ad libitum as drinking water.

ε-ACA-administered group: 11 animals. From the 4th day after the constriction of bilateral renal arteries, ε-ACA, dissolved in physiological saline solution to 1 M (13.1% solution), was subcutaneously injected in a dose of 0.5 cc once daily for 7 weeks.

Controls: 10 animals. Instead of ε-ACA, physiological saline was given in the same way as the above.

Systolic blood pressure was measured without anesthesia at 3, 5 and 7 weeks after the operation by the electronic indirect method (Nakao[7]). At 7 weeks after the operation, all the animals were killed by exsanguination from the abdominal aorta. Using the blood obtained at the time of killing, fibrinolysin (plasmin) activity in blood plasma was measured by Kuroyanagi's[3,4] modification of Macfarlane's method. Autopsy was performed immediately after death. As a fixative, neutral buffered 10% formalin (Lillie[5]) was used. From each tissue block, paraffin sections were prepared. Stainings and histochemical methods were as follows: Hematoxylin and eosin stain; Weigert's stain for elastic fibers; Mallory's collagen stain; phosphotungstic acid hematoxylin; Pap's silver impregnation; and Rinehart's[14] colloidal iron stain for acid mucopolysaccharides.

Eighteen organs (the heart, liver, kidneys, spleen, brain, lung, pancreas, stomach, small intestine, large intestine, urinary bladder, testes, aorta, mesenteric artery, mesenteric lymph nodes, pituitary, adrenals and bone marrow) of each animal were histologically investigated. Arterial fibrinoid degeneration of each organ was graded as− (0), 0.5+ (0.5), 1+ (1), 2+ (2) or 3+ (3), and the sum of these numbers of all the organs was regarded as the degree of the fibrinoid degeneration of each animal.

Further, the following experiment was carried out in order to know the effect of ε-ACA on blood pressure of rats: Three normal male rats (140–240 g) and 2 (100 and 160 g) with hypertension induced by the above-mentioned method were intravenously given, under intraperitoneal urethan anesthesia, 0.5 cc of isotonic saline containing 1 M ε-ACA. Blood pressure was recorded continuously from the cannulated carotid artery. In this case, 0.5 cc of dextran sulphate was intravenously injected as anticoagulant.

RESULTS

1. *Blood pressure.* At 3 weeks after the constriction of renal arteries, the majorities of the both groups already showed hypertension of above 150 mmHg. Later, with the lapse of day, the blood pressure was elevated increasingly higher,

and at 7 weeks after the operation the ε-ACA-administered group and controls giving averages of 216 and 230 mmHg, respectively. And there was approximately parallel relation in the both groups between the degrees of hypertension and fibrinoid degeneration excepting only one case of the control group, which did not show any fibrinoid degeneration despite hypertension of high degree (Fig. 1).

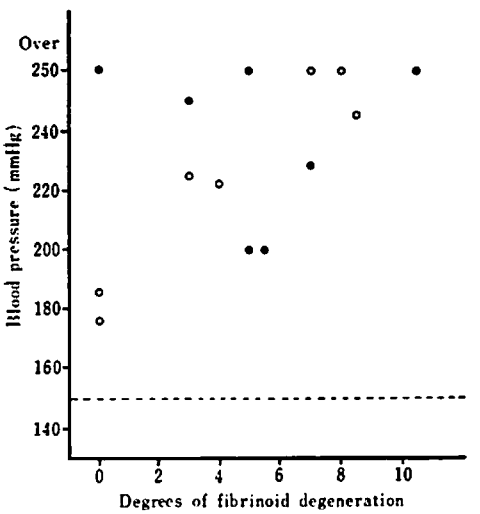

Fig. 1 Relation between blood pressure and fibrinoid degeneration (seven weeks after surgical procedures).
•=Controls
○=ε-ACA-administered animals

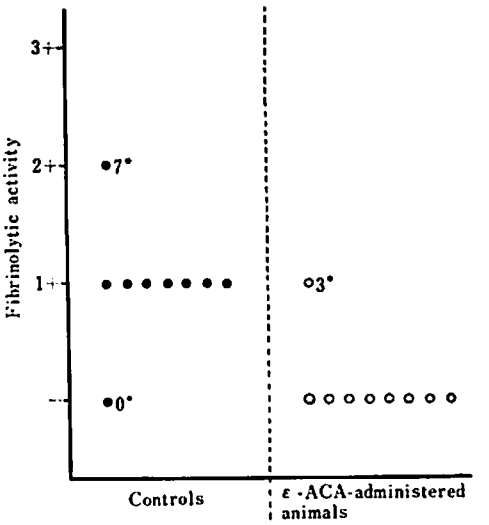

Fig. 2 Fibrinolytic activity of blood plasma in controls and ε-ACA-administered animals.
* Degrees of fibrinoid degeneration

2. *Plasmin activity (fibrinolytic activity of blood plasma).* Plasmin activity was observed in all the controls excepting one animal which did not show fibrinoid degeneration. In contrast with this, plasmin activity was not found in any of the ε-ACA-administered animals except one with fibrinoid degeneration (Fig. 2).

3. *Autopsy findings.* 1) *Nodose lesions along the course of the mesenteric arteries (periarteritis nodosa).* In controls excepting 2 animals, multiple occurrence of miliary nodose lesions was observed along the mesenteric arteries. In the experimental group (ε-ACA-given group), animals without nodose lesion were more numerous than in the control group (six animals out of the 11 experimentals did not show nodose lesions). 2) *Intracerebral hemorrhage.* In 2 of the 10 controls, millet-seed-sized hemorrhagic foci were seen in the cerebral cortex, whereas in the ε-ACA-treated group such was not observed.

4. *Histopathologic findings.* In all the control animals except two, fibrinoid

degeneration of varying degrees was observed in various organs (Fig. 3). The commonest site of the most intense fibrinoid degeneration was the mesenteric artery, followed by the pancreas, large intestine and small intestine in the descending order. In the aorta, it was confined to the intima at the bifurcations. The findings in the mesenteric arteries were as follows: In the intima, there was seen fibrinoid substance either in homogeneous appearance or in appearance of conglomeration of trabecular substances, stained intensely red with hematoxylin and eosin stain, red with Mallory's collagen stain, and dark blue with phosphotungstic acid hematoxylin, and frequently invading the media through dissolved areas of the internal elastic membrane. The majority of smooth muscle cells of the media underwent remarkable regressive and lytic changes. In the adventitia and the surrounding tissue, proliferation of fibroblasts, formation of capillaries, and infiltration of neutrophils and eosinophils were prominent, presenting a picture of periarteritis nodosa (Figs. 4 and 5). Arteries with advanced fibrinoid degeneration occasionally showed aneurysmal dilatation, and in the lumen were formed fibrinous thrombi.

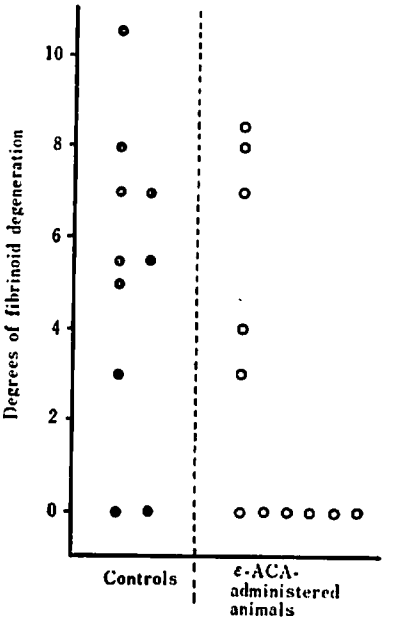

Fig. 3 Degrees of fibrinoid degeneration in controls and ε-ACA-administered animals.

In contrast with this, fibrinoid degeneration was not observed in 6 of 11 animals of the ε-ACA-administered group (Fig. 3). In 5 of these 11 animals,

fibrinoid degeneration was observed, but histologic picture of the arterial lesions was different from that of the controls in the following two points:

1) Though fibrinoid substance was abundantly present in the intima and media, remarkable fibrosis or scar tissue formation was observed in the adventitia, and adventitial cellular proliferation and infiltration were relatively mild (Fig. 7). The ratio of the thickness of the adventitial lesion to the caliber of vessels (measured at the outermost layer of the media in paraffin sections) was 1.9 on an average for the ε-ACA-administered animals against 3.6 for the controls.

2) Only in the ε-ACA-administered group were observed the arteries with the following findings: The intima was thickened owing to the proliferation of fibroblasts and smooth muscle cells, and further fibrinoid substance was deposited in the depths of the intima (Fig. 6). These were not observed in the control group. In the surrounding of the intimal smooth muscle cells, reticular and fine elastic fibers were formed, and in the intimal ground substance, colloidal iron stain-positive acid mucopolysaccharides were increased. In this case, medial and adventitial changes were generally mild.

In the control group, conspicuous fibrinoid degeneration was seen in the cerebral arteries in or adjacent to intracerebral hemorrhagic foci. There was fibrinous network in tissue surrounding arteries that showed fibrinoid degeneration through all the mural layers. Fibrinoid degeneration of the intracerebral arteries was different from that in other organs in that it was not accompanied by conspicuous adventitial cellular infiltration. In the ε-ACA-administered group, however, not only cerebral hemorrhage was absent, but also fibrinoid degeneration was not noticed in the intracerebral arteries.

5. *Effect of ε-ACA on blood pressure of rats.* When ε-ACA was intravenously injected into hypertensive and normal rats, blood pressure immediately showed slight and transient rise. But after a short time, it began to drop, attaining a maximum drop of 50 mmHg in the former and 30 mmHg in the latter. At 5–6 minutes after the injection, blood pressure returned to the initial level.

DISCUSSION

Activation of fibrinolysin (plasmin) is often observed not only in animals with various experimental hypertension (Takatama[16]; Araki[1]; Yoshitomo[19]; Ooneda[12]) but also in human cases of apoplectic cerebral hemorrhage (Goto[2]; Mihara[6]). Plasmin has a capillary permeability-promoting effect in addition to the well-known proteolytic activity (Ungar[17]; Kuroyanagi[3,4]; Watanabe[18]). Takatama[16], who performed the test using rabbit's skin (Menkin and Kuroyanagi's method), confirmed that the serum of rats with surgically constricted

renal arteries frequently showed a capillary permeability-promoting effect. It was consequently assumed that plasmin activity as well as hypertension would be the causes of the increased vascular permeability leading to the development of fibrinoid degeneration. In view of this, ε-ACA, which is an antifibrinolytic agent, was administered to hypertensive rats with surgically constricted renal arteries, and it was found to depress plasmin activity in a remarkable degree (Fig. 2), and also to inhibit the development of arterial lesions (Fig. 3). However, the inhibition was incomplete though appreciable, since in a few animals, despite the absence of plasmin activity, severe fibrinoid degeneration was demonstrated. This indicates that the cause of increased vascular permeability, which induces the arterial lesions, will not be single but plural, and it is considered that not only the plasmin activity, but also hypertension, disturbance in electrolytes metabolism, hypoxidosis and others would participate in it. However, arterial lesions in the ε-ACA-administered group was qualitatively different from those in the control. Namely, in the former were observed conspicuous fibrosis in the adventitia (Fig. 7) and cellular intimal thickening similar to productive endarteritis (Fig. 6).

This adventitial fibrosis would have resulted from periarteritis nodosa, which was produced by highly increased permeability, but which was healed and cicatrized as a result of the inhibition of the increased permeability by the antiplasminic effects of ε-ACA. Despite healing and cicatrization of the adventitial lesion, a large amount of fibrinoid substance was found accumulated in the intima, assumedly because it remained unlyzed owing to decreased plasmin activity which was elicited by the administration of ε-ACA. A portion of such remained fibrinoid substance was shown undergoing organization by proliferating fibroblasts and smooth muscle cells, which were seen beneath the endothelial layer.

Arteries with productive endarteritis-like cellular intimal thickening frequently contained a small amount of fibrinoid substance in the depths of the intima, but change in the media and adventitia was generally slight (Fig. 6). As the reason, it is considered that ε-ACA inhibited plasmin activity to a certain degree, consequently retarding the increased permeability, and inducing cellular proliferation, principally of smooth muscle cells, in the intima. The intimal thickening is also considered to have resulted from resorption of fibrinoid substance in the intima as well as from the increased permeability in a mild degree. Similar intimal thickening was observed by Takatama[16] in ε-ACA-nontreated rats with surgically constricted renal arteries. In the present experiment, however, such was not seen in the control group. Consequently the cellular intimal thickening in this experiment was assumedly due to the influence

of ε-ACA-administration.

In an experiment to investigate the effect of ε-ACA on blood pressure of rats, ε-ACA did not show a persistent hypotensive effect. And at the end of 7 week's experiment, there was no great difference in blood pressure between the ε-ACA-administered group and controls (Fig. 1). Consequently ε-ACA is assumed to have no remarkable effect on the blood pressure.

From the above mentioned results it is clear that the administration of ε-ACA inhibited the development of arterial lesions in hypertensive rats to considerable degree. And this is considered to be ascribed to its depressive effect on plasmin activity.

SUMMARY

1. Hypertensive rats with surgically constricted renal arteries were subcutaneously given 0.5 cc of isotonic saline solution containing 1 M epsilon-aminocaproic acid (ε-ACA; an antifibrinolytic agent) daily for 7 weeks, and it was found that the development of fibrinoid degeneration in arterial wall was considerably inhibited.

2. In the ε-ACA-administered group, though the arterial intima occasionally showed severe fibrinoid degeneration, the adventitia of the arteries showed only slight cellular reaction and conspicuous fibrosis and cicatrization. Further in this group there was sometimes intimal thickening with proliferating fibroblasts and smooth muscle cells and deposited fibrinoid substance. In short, ε-ACA demonstrated an accelerating effect on the healing of arterial lesions in hypertensive rats.

3. The administration of ε-ACA remarkably depressed plasmin activity of hypertensive rats, but did not exert evident effect on blood pressure. It is consequently assumed that the inhibition of the development of arterial lesions and the acceleration of those healing by the administration of ε-ACA would be ascribed to its depressive effect on the fibrinolytic activity of blood plasma (the plasmin activity), which was considered to be one of the causes of the increased vascular permeability leading to the development of the arterial lesions.

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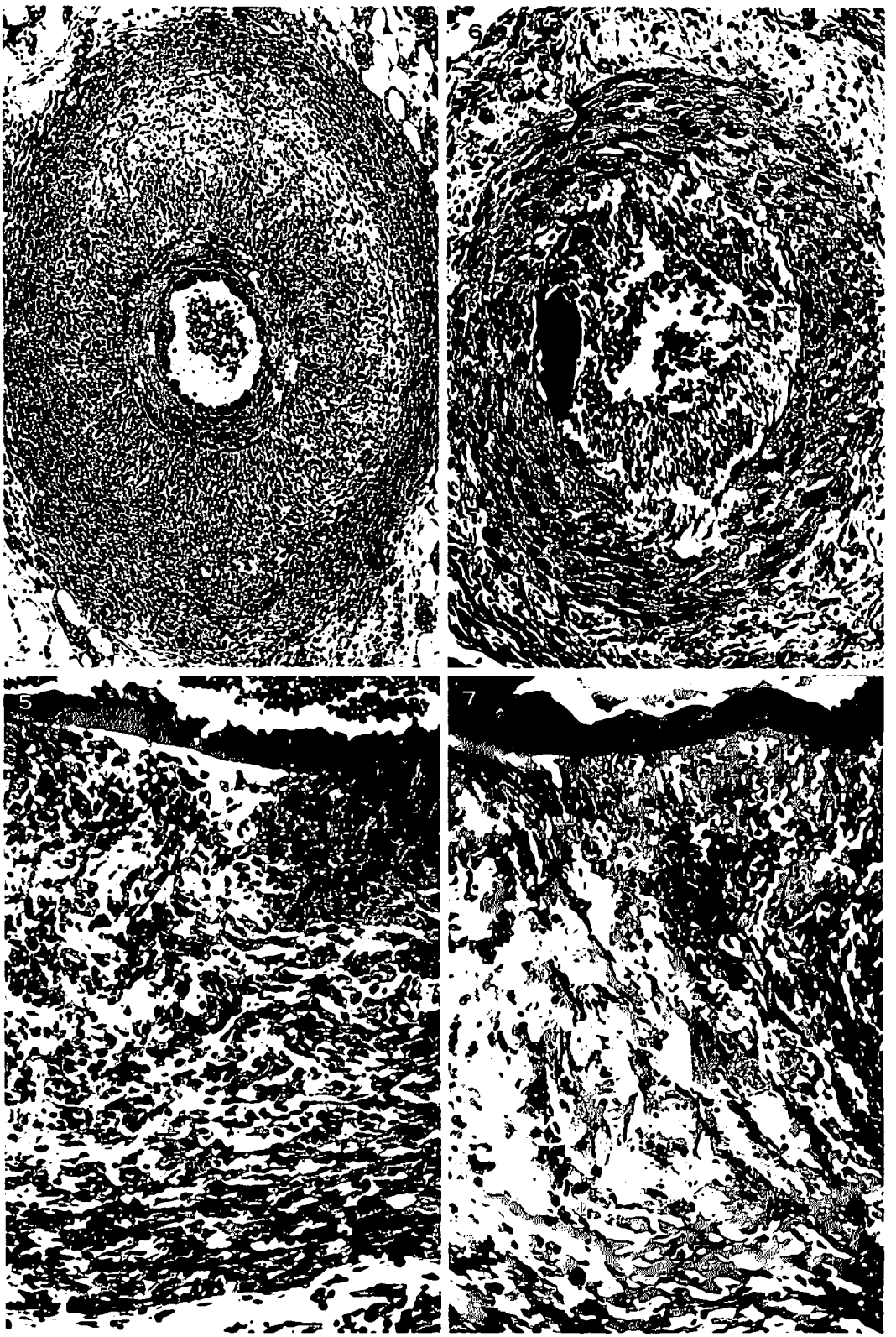
PLATE



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EXPLANATION OF PLATES

Fig. 4. Periarteritis nodosa of the mesenteric artery of the control group. Fibrinoid substance is observed in the intima and media. Medial smooth muscle cells disappeared. Granulation tissue with proliferating fibroblasts and capillaries, and with infiltrating neutrophils and eosinophils is formed in the adventitia and the surrounding tissue. Hematoxylin and eosin stain.

Fig. 5. The mesenteric artery of the control group. Fibrinoid substance is seen in the intima (upper). Infiltrating and proliferating cells and newly formed capillaries are conspicuous in the media and adventitia. Hematoxylin and eosin stain.

Fig. 6. The mesenteric artery of the ε-ACA-administered group. Intimal thickening due to proliferated fibroblasts and smooth muscle cells is observed. There is fibrinoid substance in the depth of the intima. Hematoxylin and eosin stain.

Fig. 7. The mesenteric artery of the ε-ACA-administered group. Fibrinoid substance is seen in the intima (upper). In the media and adventitia more marked fibrosis and less marked cellular infiltration and proliferation are observed in contrast to the control group (Fig. 5). Hematoxylin and eosin stain.

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STUDIES ON FIBRINOLYTIC ENZYME SYSTEM IN DERMATOLOGY: LOCAL ANTIFIBRINOLYTIC ACTIVITY IN SEVERAL SKIN DISEASES

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Evidence of pathological fibrinolysis of plasma has been reported to occur in many allergic dermatoses[1-5]. Because of sufficient therapeutic effect of ε-amino-caproic acid (antifibrinolytic substance) on those dermatoses, particularly anti-pruritic effect of localy injected ε-amino-caproic acid, our recent interest in fibrinolytic phenomena was focused on the pathologic physiology of this enzyme system in diseased skin.

BEHAVIOR OF FIBRINOLYTIC ENZYME SYSTEM OF SKIN

Sasaki[6,7] observed the strong fibrinolytic activity in the extract from the mucous membrane of maxillary sinus affected by sinusitis. Our investigation was performed by examining inhibitory activity of skin extract upon the fibrinolytic activity in the extract of maxillary sinus mucous membrane.

1. Materials and Methods

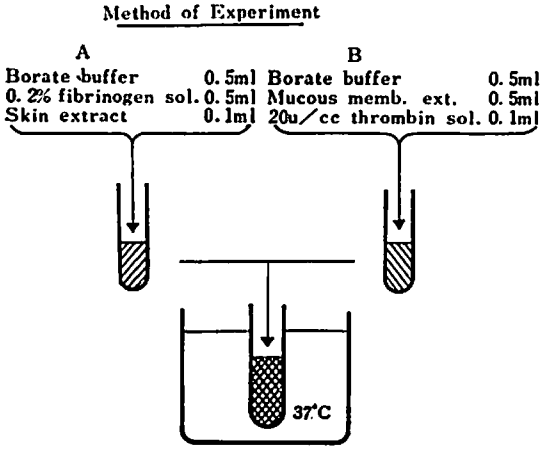
The following test substances were used; (a) extract of maxillary sinus mucous membrane, (b) 0.2% fibrinogen (Cohn Fraction I, Armour Lab.) normal saline solution, (c) 20 u/ml thrombin (Park, Davis) normal saline solution, (d) 0.9% NaCl borate buffer pH 7.45, (e) extract of skin.

Preparation of Extracts:—Both mucous membrane and skin were prepared by using the same method. Skin was prepared with sterile normal saline, and taken by 6 mm punch. The specimen, about 5 mm in thickness, was kept in a deep freezer (−20°C) after washing with cold normal saline. Then it was homogenized by glass-homogenizer with 1 ml of physiological saline to 0.1 g of skin, and centrifuged at 1,000 rpm for 15 min. The supernatant fluid was utilized for our experiments. These process were performed at 0°C.

Performance of Tests:—Each "A" and "B" test tube was prepared as shown in Fig. 1, and then mixed up for making a fibrin clot. The clot was kept at

* Professor of Dermatology.

Fig. 1.



37°C, and the complete clot-lysis time was measured.

Both the normal skin extract and the borate buffer were used as the control of this experiment. Each control was put into tube "A" instead of the extract from affected skin.

2. Results of Experiments

a) Influence of Normal Skin Extract on Fibrinolytic Activity in Extract of Maxillary Sinus Mucous Membrane: Three specimens of mucous membrane, taken from different patients with sinusitis, showed certain fibrinolytic activity in their extracts (Table 1). By adding of the extract of normal skin, prepared

Table 1.

The Influence of Extract of Normal Skin to The Fibrinotytic Activity in The Extract of Maxillary Sinus Mucous Membrane

Number	A Fibrinolytic activity of sinus mucous membrane (minutes)	B Fibrinolytic activity of sinus mucous memb. with normal skin extract (minutes)	B/A
1	75′	66′	0.9
2	255′	255′	1.0
3	130′	115′	0.9
		Mean	0.93

from one healthy adult male, the clot-lysis time revealed no significant change (Table 1). Therefore, B/A ratio was approximately 1.0, i.e., there was no influence of normal skin extract on the fibrinolytic activity in the extract of the mucous membrane.

b) Influence of Affected Skin Extract: Thirty-six patients with common

Table 2.

Diagnosis	with Affected Skin	with Normal Skin
1. Autosensitization dermatitis, Secondary disseminated lesion	1.19 1.27 1.06 1.05	1.14 ± 0.14
Primary lesion	1.00 1.19	
2. Eczema acutum	1.32 1.97 1.14	1.48 ± 0.80
3. Eczema chronicum	2.42 2.65	
4. Neurodermatitis	1.00 1.20 1.10 1.00	1.08 ± 0.13
5. Laque-dermatitis	1.05 1.09	
6. Erythematodes discoides	1.32 1.57 1.11	1.33 ± 0.42
7. Scleroderma en bandes	1.00	
8. Erythroderma subacutum (Wilson-Brocq)	1.19 1.20 1.14	1.18 ± 0.06
9. Urticaria perstans verrucosa	1.09 1.00	
10. Urticaria chronica	1.00 0.95 1.00 1.00 1.07	1.00 ± 0.05
11. Erythema exsudativum multiforme	7.67	
12. Psoriasis vulgaris	2.42 1.52	
13. Pityriasis rosea Gibert	2.17	
14. Dermatitis herpetiformis Duhring	1.14 1.00	

dermatoses were examined, and the results were shown in Table 2. Because of the difficulty to obtain a large specimen of mucous membrane, we could not use the same extract of maxillary sinus mucous membrane for all our series of experiments. Therefore, the results were compared by following method; the extract of normal skin was prepared from same skin, which was used in former experiment. In each experiment the complete clot-lysis time resulted from the fibrinolytic activity of sinus mucous membrane with this normal skin extract was measured, and considered as control-time. On the other hand, the time of complete clot-lysis, which was shown by adding of affected skin extract was

measured, and divided by the control-time. When this ratio was approximately 1.00, we considered no influence of affected skin extract on the fibrinolytic activity. If the ratio became more than it, certain local antifibrinolytic activity of affected skin was suspected. We also expected the acceleration of fibrinolytic activity in case of less than 1.00.

Autosensitization dermatitis: This dermatosis has been considered to be an allergic reaction. The pathological fibrinolytic activity of plasma of this patient was previously recognized[1-5]. However, both secondary disseminated lesion and primary lesion showed no influncee on the fibrinolytic activity.

Acute and chronic eczema: Two cases of chronic eczema revealed significant inhibitory activity in the extracts of their affected skin.

Neurodermatitis: No influence was observed.

Lacque-dermatitis: Though the presence of pathological fibrinolysis of plasma has been observed in lacquer-dermatitis[8], the extract of this lesion did not show any influence.

Erythematodes discoides, Scleroderma en bandes, Erythroderma and Urticaria: No influence was observed.

Erythema exsudativum multiforme: Extremely high anti-fibrinolytic activity was noticed in one case.

Psoriasis vulgaris: The lesion was found to have anti-fibrinolytic activity.

Pityriasis rosea Gibert: There was inhibitory activity in the extract of affected skin.

Dermatitis herpetiformis: No influence was observed.

3. Influence of Extract of Guinea Pig's Skin with Experimental Dermatitis on Fibrinolytic Activity in Extract of Human Maxillary Sinus Mucous Membrane

a) Materials and Methods: Experimental dermatitis was caused by Storck's technique[9] with 209 P strain of staphylococci. The studies in this series were performed by the same method, described above.

b) Results of Experiments: Both experimental dermatitis, resulted from

Table 3.

Experimental Dermatitis (Guinea pig)	with Affected Skin	with Normal Skin
Resulted from Broth Culture of Staphylococcus aureus	1.40 1.28 1.16 1.27	1.28 ± 0.14
Resulted from Sterile Filtrate of Staph. Broth Culture	1.47 1.48 1.57	1.51 ± 0.10

broth culture of the staph. and from sterile filtrate of the staph. broth culture revealed slight inhibitory activity in their extract to fibrinolytic activity of maxillary sinus mucous membrane (Table 3).

The extract of normal guinea pig's skin did not show any influence on the fibrinolysis, as we have observed in cases of human specimens.

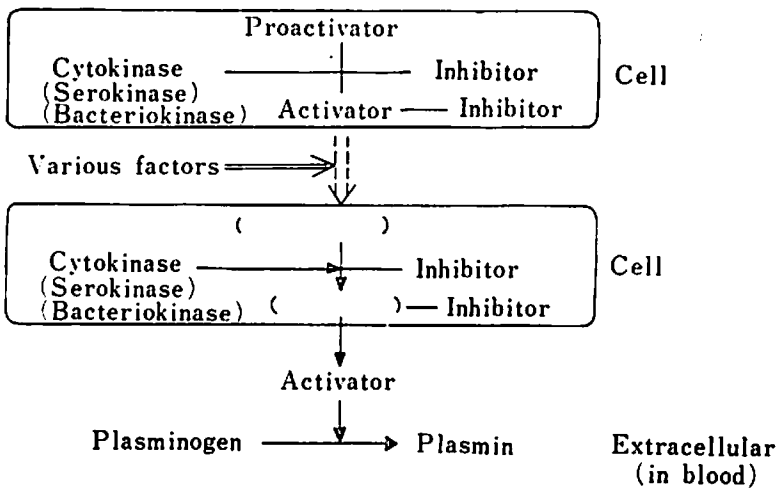
COMMENT

The results obtained in the present clinical and experimental studies point to the existence of local antifibrinolytic activity in the lesion suffered from certain dermatoses.

Consideration of the fibrinolytic enzyme system suggests the existence of inhibitors of activation in cells, and these activator-inhibitor system would be keeping dynamic balance each other (Fig. 2). The frequent demonstration of

Fig. 2.

Presumable Mechanism of Local Antifibrinolytic Activity



plasminogen activator in plasma has focused attention on the mechanisms controlling the release of this substance into the circulation. Allergic reaction is considered as one of these mechanisms. On the other hand, according to the release of activator, the inhibitor in the cells involved in these mechanisms may show its activity. Therefore, the extract of affected skin would be able to contain predominant inhibitors. The fibrinolytic activity in the extract of maxillary sinus mucous membrane is considered to be inhibited by using these

extracts, which are expected to have certain antifibrinolytic activity. We, however, should remark many other factors involved in the fibrinolytic enzyme system and our methods of experiments.

SUMMARY

In the studies herein reported, an attempt has been made to clarify the pathological physiology of local fibrinolytic enzyme system of the skin in patients suffered from several skin diseases. The investigation was performed by the examination of antifibrinolytic activity in the extract of skin of affected area, adopting modified Sasaki's method to measure the inhibitory activity of skin extract upon the fibrinolytic activity in the extract of human maxillary sinus mucous membrane.

The experimental dermatitis, resulted from broth culture of staphylococcus aureus and sterile filtrate of staph. broth culture, in guinea pig was also examined.

The results of experiments showed the existence of anti-fibrinolytic activity in the extract of affected skin of chronic eczema, erythema exsudativum multiforme, psoriasis vulgaris, pityriasis rosea Gibert and experimental dermatitis.

Grateful acknowledgement is made to Prof. Dr. Okamoto, Director of Department of Physiology, Division I, Kobe Medical, Colledge. The assistance of the staff of Department of Physiology, School of Medicine, Keio University, is also gratefully acknowledged.

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UROLOGICAL STUDIES OF FIBRINOLYTIC ENZYME SYSTEM

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Ever since fibrinolysis attracted the attention of both clinical and basic medicine, more and more light has been shed upon fibrinolysin (plasmin) which plays a major role in this phenomenon.

In the field of urology, the prostate, as well as the lung, the heart and the uterus, has been considered to be an important organ rich in tissue activator.

The results of the clinical and experimental studies on various prostatic diseases and idiopathic renal hematuria are reported.

I. Clinical Studies

Table 1.

Fibrinogen, Plasmin and Serum Acid and Alkaline Phosphatase in Prostate Cancer

No.	Age	Treatment	Fibrinogen (mg/ml)	Ratnoff's Plasma Clotolysis Test (days)	Whole Plasmin (unit)	Euglobulin Lysis Test (unit)	Acid Phosphatase in Serum	Alkaline Phosphatase in Serum
1	67	Honvan Castration Suron	2.2	12	**33.33**	0.56>	1.3	7.2
2	54		3.9	4	**33.33**	0.56>	1.1	5.5
3	56		4.2	6	**38.76**	0.83	2.0	4.0
4	60		2.9	7	19.60	0.56>	12.7	0.7
5	61	Suron	3.0	9	**38.76**	0.67	1.18	8.4
6	74		3.5	7	**22.22**	0.67	4.1	5.4
7	73		3.2	3	**33.33**	0.56>	4.3	6.1
8	83		0.7	12	**25.02**	0.56>	1.3	5.2
9	71		2.4	14<	**22.22**	0.56>	0.73	5.7
10	57	Suron	3.1	14<	**22.22**	0.56>	0.87	5.7
11	54		3.5	14<	**22.22**	0.56>	3.3	6.1
12	57		1.7	14<	13.88	0.56>	1.78	5.38
13	72		3.3	11	21.50	0.56>	1.33	7.03
14	68		4.5	14<	19.59	0.56>	1.64	6.02

Suron (4, 4-dihydroxy-rs-diphenyl-n-hexane, TEIKOKUZOKI)
Honvan (diethyldioxystilben diphosphate, ASTAWERKEA)

* Professor of Urology.

The results obtained after examining the plasmin systems in 14 cases of prostatic cancer are shown in Table 1. In 10 out of these cases plasmin activation was noted. In benign prostatic hypertrophy (BPH), however, it was noted in 7 out of 12 cases (Table 2).

Table 2.
Fibrinogen and Plasmin in BPH.

No.	Age	Fibrinogen (mg/ml)	Ratnoff's Plasma Clotolysis Test (days)	Whole Plasmin (unit)	Euglobulin Lysis Test (unit)
1	57	1.9	6	20.83	0.56>
2	80	2.1	6	20.83	0.56>
3	67	2.7	6	20.83	0.56>
4	68	4.2	6	17.54	0.56>
5	78	2.7	6	15.15	0.56>
6	58	2.2	7	25.64	0.56>
7	74	4.1	6	28.98	0.56>
8	58	4.7	6	23.81	0.56>
9	54	3.0	4	22.22	0.56>
10	75	2.5	4	22.22	0.56>
11	64	3.5	5	25.02	0.56>
12	74	3.1	12	27.29	0.56>

Many authors have reported that plasmin activation takes place during transurethral resection of the prostate (TURP). The writers examined 29 cases of TURP on the variation of the fibrinogen and plasmin and found 20 positive cases (68.9%) (Fig. 1 and 2).

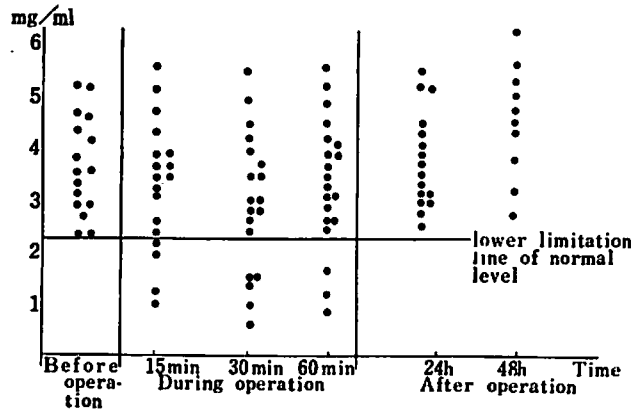

Fig. 1 Fibrinogen in TURP.

ε-aminocaproic-acid (Ipsilon-Daiichi Seiyaku), an antiplasmin agent, was injected in 7 cases during and after the operation and only one case was found

to have plasmin activation.

Fig. 3 shows the time changes of plasma fibrinogen, plasmin in blood,

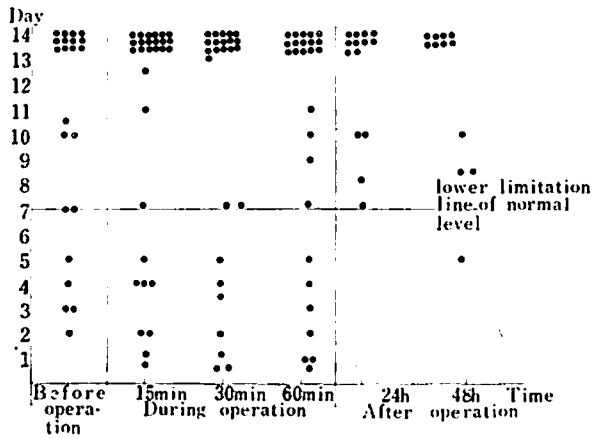

Fig. 2 Ratnoff's plasma clotolysis test.

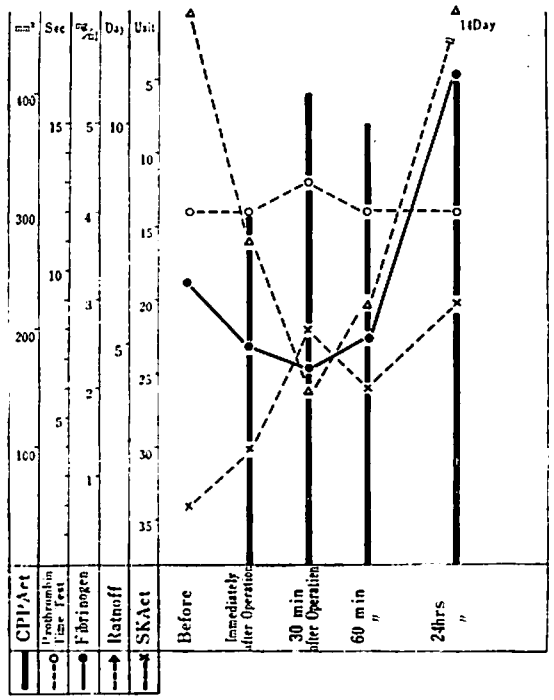

Fig. 3 Fibrinogen, Plasmin, CPP action and Prothrombin time test in the TURP (53 year old).

capillary permeability promoting action (CPP action),[1] and prothrombin time test in Case No. 1 of the TURP group. In the early stages during TURP, the plasmin level increased significantly and plasma fibrinogen level decreased. After these changes, plasmin was restored to normal within 24–48 hours, but, on the contrary, the fibrinogen level tended to increase. In this case, there was little change in prothrombin time, CPP action became positive as soon as surgery began, and the plasmin level was restored almost back to normal within 24–48 hours, but, CPP action remained positive.

II. Idiopathic (Essential) Renal Bleeding and Plasmin System

Activation of plasmin in blood was noticed in 26 of the 33 cases (78.8%) having this disease. As a result of these tests, 25 cases (75.5%) were found to be positive when tested by the streptokinase (SK) activation test (Whole plasmin).[2] Again CPP action was recognized in 17 of the 19 cases (89.5%) (Table 3).

Table 3.
Fibrinogen, Plasmin and CPP action in idiopathic renal bleeding

No.	Sex	Age	Fibrinogen (mg/ml)	Ratnoff's Plasma Clotolysis Test (days)	Plasmin (unit)	Euglobulin Lysis Test (unit)	CPP Act
1	♂	24	not coagulated	not coagulated	41.67	0.56>	++
2	♂	27	1.7	6	21.49	0.56>	++
3	♀	21	2.0	4	12.30	0.56>	++
4	♂	44	2.8	4	30.30	0.56>	++
5	♂	23	1.5	6	22.22	0.56>	+
6	♂	20	2.4	4	30.30	0.56>	+
7	♀	26	1.5	4	31.70	0.56>	++
8	♂	32	2.1	7	30.30	0.56>	−
9	♂	20	1.7	4	33.33	0.56>	++
10	♂	29	3.4	6	25.64	0.56>	+
11	♂	20	2.5	8	23.8	0.56>	+
12	♀	27	2.3	4	22.22	0.56>	+
13	♀	30	impossible	impossible	23.81	0.56>	+
14	♀	13	2.4	12	33.33	0.56>	+
15	♂	22	2.3	7	27.77	0.56>	+
16	♂	27	2.5	5	41.67	0.56>	+
17	♂	34	1.6	5	23.53	0.56>	+
18	♀	32	2.5	5	29.72	0.56>	−
19	♂	25	2.9	5	23.81	0.56>	+

Eighteen patients were treated with ε-aminocaproic-acid as antiplasmin therapy, as shown in Table 4, of which 10 showed effective results and 8 non-

effective. Among these were two cases of renal bleeding which had resisted various therapies but was cured with only one injection of Ipsilon. This therapy has had an instant effect, and after a course of 5–6 injections hematuria diminished. Thus, if after a course of this therapy hematuria remains unchanged, some other therapy should be instituted.

Table 4.
Effect of ε-Amino-caproic Acid on essential renal bleeding

No.	Sex	Age	Fibrinogen (mg/ml)	Ratnoff's Plasma cloto lysis test (days)	Whole plasmin (unit)	Euglobulin test (unit)	Treatment
1	♂	29	22	5	27.77	0.56>	Completely healed after 1 injection
2	♀	32	21	7	23.81	0.56>	〃
3	♀	38	27	7	23.81	0.56>	Completely healed after 3 injections
4	♂	48					Completely healed after 4 injetcions
5	♂	19					Completely healed after 5 injections
6	♂	28					Completely healed after 10 injections
7	♂	33	32	7	27.77	0.56>	〃
8	♂	24					Hematuria diminished after 10 injections
9	♂	27	22	7	22.2	0.56>	〃
10	♂	31	21	6			〃
11	♂	20					Ineffective after 10 injections
12	♀	48	20	4	12.43	0.56>	〃
13	♂	23	15	4	30.30	0.56>	〃
14	♂	23					〃
15	♀	26					〃
16	♀	21					〃
17	♀	44	28	4	30.30	0.56>	〃
18	♂	27	45	5	35.10	0.56>	〃

III. Experimental Studies

Various theories on the etiology of idiopathic renal bleeding have been presented in the past. Recently, however, with the effective application of chlorpromazine, a part of this disease seems to be explained by the disturbance of the autonomic nerves, aside from the allergic theory. As there is a close connection between allergy and the autonomic nervous system it is difficult to distinguish between the two. The following experiments were conducted to clarify the above mentioned relationship.

1. Irritation Syndrome, Reilly: Eight rabbits were used in this experiment. The results obtained with the appearance of hematuria are presented in Table 5.

Table 5.

Fibrinogen, Plasmin and CPP action in renal bleeding in rabbits irritated with croton-oil to plexus renalis

No.	Treatment		Fibrinogen (mg/ml)	Ratnoff's Plasma Clotolysis Test (days)	CPP Action
1.	Before (from heart)		3.0	7	+
	1 hr after irritation	from V. jug.	2.7	3	+++
		from V. renal.	2.6	3	+++
2.	Before (from heart)		5.1	8	−
	1 hr after irritation	from V. jug.	3.0	3	+++
		from V. renal.	2.7	3	+++
3.	Before (from heart)		3.1	6	+++
	1 hr after irritation	from V. jug.	2.1	4	+++
		from V. renal.	2.1	5	+++
4.	Before (from heart)		3.3	7	++
	1 hr after irritation	from V. jug.	2.3	5	++
		from V. renal.	2.8	4	+
5.	Before (from heart)		2.9	5	+
	2 hrs after irritation	from V. jug.	2.3	4	+++
		from V. renal.	1.9	3	+++
6.	Before (from heart)		2.5	6	+
	2 hrs after irritation	from V. jug.	2.2	5	+++
		from V. renal.	2.1	5	+++
7.	Before (from heart)		3.5	7	−
	3 hrs after irritation	from V. jug.	3.2	4	++
		from V. renal.	3.2	4	++
8.	Before (from heart)		4.5	7	−
	immediately after Anesthesia and Treat.	from V. jug.	4.5	7	++
	1 hr	from V. jug.	3.6	4	+++
	2 hrs	from V. jug.	3.7	4	+++
	5 hrs	from V. jug.	3.8	6	+++
	10 hrs	from V. jug.	4.1	7	+++
	24 hrs	from V. jug.	7.4	7	+++

2. Inhibition of Reilly's Phenomenon by the Use of Chlorpromazine (CP): As soon as three intravenous injections of 1 mg/kg of CP were given every thirty minutes to 5 rabbits, Reilly's phenomenon was produced by the Sac Jugulaire method. The appearance of hematuria, plasmin in blood, fibrinogen and CPP

action were all inhibited in all of the cases (Table 6).

Table 6.
Fibrinogen, Plasmin and CPP action in Reilly's Phenomen inhibited by the use of chlorpromazine

No.	Sex		Before	Immediately after 3 Inj.	1 hr after	3 hrs after	5 hrs after	24 hrs after
43	♂	Fibrinogen (mg/ml)	4.3	not coagulated	4.0	3.2	2.7	5.4
		Ratnoff's Plasma clotolysis Test (days)	14<	not coagulated	14<	14<	14<	10<
		Whole Plasmin (unit)	16.22	16.66	16.22	16.22	15.79	16.66
		CPP action	—		—	—	—	++
45	♂	Fibrinogen (mg/ml)	3.2	2.4	2.4	2.7	2.4	6.1
		Ratnoff's Plasma clotolysis Test (days)	14<	14<	14<	14<	14<	14<
		Whole Plasmin (unit)	16.66	16.66	16.66	13.89	17.09	17.09
		CPP action	—		—	—	—	++
46	♂	Fibrinogen (mg/ml)	2.1	1.9	1.8	1.8	1.6	6.4
		Ratnoff's Plasma clotolysis Test (days)	14<	14<	14<	14<	14<	14<
		Whole Plasmin (unit)	17.54	20.02	18.76	18.76	20.02	22.22
		CPP action	—		—	—	—	+++
48	♂	Fibrinogen (mg/ml)	1.7	2.0	2.2	2.3	2.3	dead
		Ratnoff's Plasma clotolysis Test (days)	14<	14<	14<	14<	14<	dead
		Whole Plasmin (unit)	21.45	16.66	20.02	20.02	20.02	dead
		CPP action	—		—	—	—	++
49	♀	Fibrinogen (mg/ml)	1.6	1.8	1.6	1.3	1.5	4.5
		Ratnoff's Plasma clotolysis Test (days)	5	6	6	6	5	5
		Whole Plasmin (unit)	20.02	20.02	18.84	20.02	20.02	20.02
		CPP action	+		—	—	—	++

3. Plasmin System of Renal Tissue Undergoing Reilly's Phenomenon: Renal tissue undergoing Reilly's phenomenon, when compared with healthy renal tissue showed the fibrinolysis of human maxillary sinus mucous membrane to be markedly inhibited. When irritation was applied to one side of the kidney, the inhibitive action upon fibrinolysis of sinus mucous membrane was stronger on this side than on the side on which irritation was not applied (Table 7).

4. Anaphylactic Shock by Egg-white Albumin: Hypodermic injections of 0.1 ml of 1% egg-white albumin solution were given to 8 guinea pigs weighing approximately 500 g twice a week for 3 to 4 weeks for a total of six to eight injections. Three weeks later following the last injection 1.0 ml of the same

Table 7.
Plasmin system in the renal tissue of rabbits irritated by croton oil to their plexus renalis sinistra

		Fibrinolytic Time		Inhibition to fibrinolysis of Human Maxillary Sinus Mucous membrane (H. M. S. M. M.)	
Healthy kidney		135 minutes		60 minutes	
	Side / No.	Left kidney	Right kidney	Left kidney	Right kidney
After 1 hr	89	110 min	110 min	190 min	110 min
After 1 hr	90	110	110	210	120
After 1 hr	91	110	110	160	125
After 3 hrs	92	115	120	195	135
After 3 hrs	93	110	115	170	130
After 3 hrs	94	110	115	175	130

solution was injected intravenously to produce anaphylactic shock. Then passive cutaneous anaphylaxis was studied at the same time. The results are shown in Table 8.

Table 8.
Anaphylactic shock produced by egg white albumin

No.	Fibrinogen (mg/ml)	Whole plasmin (unit)	Heated fibrin plate (mm)	CPP Act.	P. C. A.
1	1.8	11.90	12×45	+	+
2	1.6	8.33	13×14	±	±
3	2.3	8.33	8×8	+	±
4	1.9	22.22	15×10	±	±
5	1.1	13.33	11×10	±	±
6	1.2	8.53	8×9	+	+
7	3.0	8.33	7×9	−	−
8	1.6	8.33	10×10	±	±
control	3.1	22.22	5×4	−	−

A significant activation of plasmin was obtained by the heated fibrin plate method, but by the SK activation method (Whole plasmin), on the contrary, a tendency toward prolongation was observed. The results of both CPP action and passive cutaneous anaphylaxis corresponded to each other in a similar way.

5. Inhibition of Anaphylactic Shock by Chlorpromazine: Thirty minutes after an injection of 20 mg/kg of chlorpromazine was given to a guinea pig which had been previously sensitized with egg white albumin, an attempt was made to produce anaphylactic shock without success. In only one case fibrinogen value was found to be zero, but in all the other cases it was within the normal

range. Plasmin in blood also was not activated by the heated fibrin plate method except in one case. In the SK activation test, all of the cases showed prolongation, but both CPP action and passive cutaneous anaphylaxis were negative (Table 9).

Table 9.
Inhibition of anaphylactic shock by chlorpromazine

No.	Fibrinogen (mg/ml)	Whole Plasmin (unit)	Heated Fibrin Plate (mm)	CPP Act	PCA	Result
1	0	<8.33	11×12	+	−	Anaphylaxy is not revealed
2	2.62	<8.33	5×4	−	−	〃
3	2.10	<8.33	7×7	−	−	〃
4	2.60	<8.33	5×5	−	−	〃
5	2.31	<8.33	5×5	−	−	〃

6. Plasmin in the Renal Tissue under Anaphylactic Shock Method: As shown in Table 10, fibrinolysis in a healthy guinea pig's kidney was observed on an average of 3 hours; in a kidney removed immediately after death from anaphylactic shock, fibrinolysis occurred on an average of 75 minutes later, and in a kidney which was removed from a guinea pig 10 minutes after CP inhibition from anaphylaxy, fibrinolysis occurred on an average of 80 minutes later. The inhibitive action on fibrinolysis of the maxillary sinus mucous membrane took place about 60 minutes later on an average in a healthy kidney, and in about 40 minutes later in a kidney which had had CP inhibition, but in a kidney which had been removed from a guinea pig which died of anaphylaxy, fibrinolysis began to take place about 90 minutes later on the average and completely ended

Table 10.
Plasmin in the renal tissue itself under anaphylactic shock guinea pig

	Fibrinolytic Time	Inhibition to Fibrinolysis of H.M.S.M.M.
Human Maxillary Sinus Mucous Membrane (H.M.S.M.M.)	27 min.	
Healthy Kidney	200 180	65 min 55
Kidney under Anaphylactic shock	70 80 80 65	80 90 110 107
Kidney under Inhibition of Anaphylactic Shock by Chloropromazine	85 80 100 120	46 40 30 45

2 hours later on the average, which was considered to be a marked prolongation (Table 10).

7. Inhibition of the Trypanblue Accumulating Action by Ipsilon: The rabbits which have been given an intravenous injection of 5 ml of 5% Ipsilon 30 minutes previously were examined with regard to CPP action and, passive cutaneous anaphylaxis. The exudate from inflammatory skin lesions was then examined with regard to Menkin's Leukotaxin. All these trypanblue accumlating action by all of these tests was found to be inhibited.

DISCUSSION AND SUMMARY

In 1930, Jürgens[3] recognized a serious bleeding tendency and hypofibrinogenemia in cases of prostatic cancer with metastasis. Since then Marder (1949)[4], Seale et al (1951)[5], Tagnon et al (1953)[6], Stefan et al (1959)[7] and others have confirmed that the bleeding tendency was due to hypofibrinogenemia. Since Huggins et al[8] reported in 1942 that proteolytic enzymes were made in the prostate and the presence of this enzyme maintained the semen in its liquid state, the importance of prostatic fibrinolysis has been stressed by Chwalla et al (1956)[9] and Tagnon (1954) and others.

We examined patients with BPH and cancer of the prostate, but could find no difference in plasmin activation between them. The fact that the percentage of positive results in SK activation test (whole plasmin test) was high led us to conclude that there was a good supply of the SK activator in the prostate, and both by BPH and prostatic cancer.

Thus it can safely be said that, if some kind of kinase is involved, fibrinogenemic purpura may take place. Further, there have been many reports of a tendency toward bleeding while undergoing TURP due to fibrinolysis and hypofibrinogenemia since Scott[10] reported his two cases first in 1954. There have been various discussions on the tendency toward bleeding due to plasmin activation while the prostate is undergoing surgical procedures. To treat this bleeding due to plasmin, Scott et al (1954) administered estrogen; Tagnon et al, diethylstilbesterol; Lombardo[11], touluidine blue and fibrinogen; Neat et al (1961), an intravenous injection of fat emulsion and Fetter et al (1961), an intravenous injection of ε-amino caproic acid.

As has already been stated there have been a large number of reports of the studies on how essential renal bleeding is produced, but at the present time its causes have not been explained precisely. It was Klemperer who first noted the relationship between the abnormal autonomic nerves and the disturbances

in the renal blood flow. Then Reilly[12] observed the relationship between the autonomic nervous system and the onset of renal inflammation. Sarre u. Moench (1951)[13], Griessmann, Eufinger (1952)[14] and others have succeeded in producing a disturbance similar to renal inflammation by stimulating the autonomic nerves in various ways.

On the other hand, since Bergman reported the close relationship between the autonomic nervous system and the plasmin system, there have been a large number of reports along this line. As studies on plasmin developed, theories in which they tried to attribute the factor producing anaphylaxy or anaphylaxy-like phenomenon to plasmin have been advanced. Possibilities of allergic renal bleeding were reported by Adelsberger, Tzank et al, Cottet and Rhodes. In general, fibrinolysis is observed in adaptation syndromes such as mercuric poisoning and other substances, a large volume of blood transfusions, malignant tumors, surgical operations, burns, shocks and allergic diseases. Thus, plasmic activation can be observed not only by allergy and anaphylaxy but also by stress. Therefore it is considered that it should be regarded as an attendant phenomenon which is seen in shocks in a broad sense.

There are many interesting points in the relationship between Reilly's phenomenon and allergy and this relationship is considered to have a close connection with peripheral vascular failure. Menkin extracted a substance from inflammatory exudates which increases capillary permeability and called it Leukotaxin. Kuroyanagi discovered that inactivated serum has Leukotaxin-like action promoting capillary permeability in certain diseases and called it capillary permeability promoting action. This CPP action has a close relation with fibrinolysis, and as is seen in the above mentioned results, Ipsilon seems to act not only on plasmin specifically, but also on the CPP factor.

We have confirmed the role of the plasmin system (including the CPP factor) as a factor which produces renal hematuria from the clinical and experimental results.

CONCLUSION

A tendency toward plasmin activation was observed in prostatic cancer as a problem of plasmin in the field of urology. Plasmin activation was markedly seen in TURP and also the appearance of hypofibrinogenemia and CPP factor were seen.

In 26 out of 33 cases of spontaneous renal bleeding, plasmin activation was observed, and in 17 out of 19 cases of the same disease, CPP action was observed.

Plasmin activation and CPP factor appear under renal bleeding due to the

abnormal autonomic nerves and anaphylactic shock.

CPP factor is one of the causes that produces shock. And in the kidney undergoing shock, inhibitive action upon fibrinolysis of the maxillary sinus mucous membrane becomes markedly stronger.

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STUDIES ON PLASMIN AND FIBRINOGEN BY NEW METHOD WITH ε-AMINO CAPROIC ACID

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In the previous studies the authors reported a close relationship between hemorrhagic tendency and elevated plasmin activity in the patients with hemorrhagic disorders. At present time, however, the method by which plasmin levels are measured, is the most controversial problem and the various methods were applied simultaneously in one individual and results are synthetically evaluated.

The results obtained by these various methods usually vary rather considerably and these differences are considered to be induced not only by difference of the reagents to be used but also depending on whether only naturally existing plasmin levels or whole plasmin levels are measured by adding streptokinase into the tested plasma. In any event, collective evaluation of the results obtained by various methods is generally accepted attitude to measure plasmin levels at present time.

ε-Amino Caproic Acid (ε-ACA) recently synthesised by Okamoto and others, has been proved to be a powerful antiplasmin substance in the numerous experiments[1,2]. This substance was successfully used for a treatment of the patients with hyperplasminic hemorrhage in our previously reported studies. Recently Fukutake reported his new method to measure plasmin activity in which ε-ACA of various concentrations was added into the tested plasma and whole plasmin activity was expressed by the minimum concentration of ε-ACA enough to inhibit fibrinolysis in vitro[3]. Streptokinase was also added in the plasma in order to measure whole plasmin levels by converting whole plasminogen into plasmin in the presence of Streptokinase. The modified Fukutake's method was devised by authors and human plasmin levels were measured in various conditions such as in the stored plasma or the plasma from the individuals who

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administered ε-ACA in the various period of time prior to the test in order to standardise the mode of administration of this valuable new drug, ε-ACA.

Fibrinogen levels were also measured simultaneously. Some interesting interrelationships among the Plasmin, Antiplasmin and Fibrinogen encountered in the present studies were also reported.

MEASUREMENT OF PLASMA WHOLE PLASMIN LEVELS

A) Reagent

1) 0.85% normal saline
2) 1 mg/ml ε-ACA solution in normal saline
3) 5000 u/ml S.K. solution in normal saline (Lederlly, Varidase)
4) 200 u/ml Thrombin solution in normal saline (Park Davis, thrombin topical)

B) Method

Approximately 5 ml of oxalated plasma were collected as materials. Eight test tubes were prepared and the reagents were placed in each tube as shown in table 1. Thrombin solution was added as the last procedure with immediate

Table 1

An order in placing reagent into series of test tubes, and concentration of ε-ACA in each tube

Test tube No. / Reagent	1	2	3	4	5	6	7	8
Normal saline	0.5 ml		0.5	0.5	0.5	0.5	0.5	0.5
1mg/ml ε-ACA sol. in normal saline		0.5	0.5	↘ 0.5	↘ 0.5	↘ 0.5	↘ 0.5	↘ 0.5→discarded
5000 u/ml SK sol. in normal saline		0.1	0.1	0.1	0.1	0.1	0.1	0.1
Oxalated plasma	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
200 u/ml thrombin sol. in normal saline	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Concentration of ε-ACA mg. per 100 ml of plasma	0	100	50	25	12.5	6.25	3.125	0

shaking of the tubes to insure homogeneous mixtures before clots formed at the bottom of the tubes. All test tubes were incubated in a warm bath at 37°C for an hour. (Thirty minutes were not enough to complete the reaction and no significant difference was observed in periods over one hour).

C) Interpretation of the results

Interpretation of the results was made according to the criteria shown in table 2. In this criterion, the results in the No. 2 test tube was chosen as a

control because no fibrinolysis was expected to take place in the No. 2 test tube which contains the highest concentration of ε-ACA 100 mg/100 ml. Practically (±) was considered almost equal to (−), and (+) almost equal to (#) in table 2.

Table 2
Criteria of reaction

(−) A negative reaction, presenting the same appearance as seen in the test tube No. 2.

(±) A doubtful reaction in which a fibrin clot appears slightly samller than the one seen in the test tube No. 2 with either usual clot or gel type coagulation.

(+) Weakly positive reaction, in which a fibrin clot is markedly smaller than the one in the test tube No. 2. or a fibrin clot is brittle and easy to break into peaces by shaking or water clear solution with visible fibrin fragments on shaking is observed.

(#) Positive reaction, presenting complete fibrinolysis with water clear appearance.

Table 3
Whole plasmin activity and plasma fibrinogen levels in normal individuals

Case No.	Sex	Test Tube No. 1	2	3	4	5	6	7	8	Fibrinogen Level mg/dl
1	F	−	−	−	−	±	#	#	#	310
2	F	−	−	−	−	#	#	#	#	260
3	F	−	−	−	±	+	#	#	#	400
4	M	−	−	−	±	#	#	#	#	210
5	M	−	−	−	−	#	#	#	#	375
6	M	−	−	−	±	+	#	#	#	305
7	M	−	−	−	−	+	#	#	#	380
8	M	−	−	−	−	#	#	#	#	410
9	M	−	−	−	−	+	#	#	#	340
10	M	−	−	−	−	#	#	#	#	245
11	M	−	−	−	−	±	#	#	#	310
12	M	−	−	−	±	+	+	#	#	375
13	M	−	−	−	±	+	#	#	#	215
14	M	−	−	−	−	+	#	#	#	270
15	M	−	−	−	±	#	#	#	#	310
16	M	−	−	−	−	#	#	#	#	195
17	M	−	−	−	±	#	#	#	#	280
18	M	−	−	−	−	#	#	#	#	265
19	M	−	−	−	−	+	#	#	#	340
20	M	−	−	−	−	+	#	#	#	425
21	M	−	−	−	−	#	#	#	#	350
22	M	−	−	−	−	+	+	#	#	245

Thus the minimum necessary concentration of ε-ACA per 100 ml of plasma to inhibit fibrinolysis in vitro was calculated according to table 1 which show all concentrations in test tubes. The result in the No. 1 tube in table 1 indicated naturally existing plasmin activity.

D) Fibrinogen level determination

Since fibrinolysis was an essential reaction in this experiment, the fibrinogen level in the tested plasma was determined simultaneously by Shibata's method[4]. One half ml of plasma was required for this test.

NORMAL VALUE

Plasma from 22 healthy individuals was tested by this method in order to determine the normal value with the results as shown in table 3. Inhibition of fibrinolysis was observed in the test tube containing 25 mg of ε-ACA per 100 ml of plasma (test tube No. 6) in the majority of these individuals. Level of fibrinogen and sex of each individual had no significant effect on inhibition of fibrinolysis.

Table 4.

Change in whole plasmin level and fibrinogen level in stored plasma (stored in a freezer at −20°C)

Case A

Days for storage	Test Tube No. 1	2	3	4	5	6	7	8	Plasma fibrinogen level mg/dl
1	−	−	−	−	±	++	++	++	310
2	−	−	−	−	++	++	++	++	305
3	−	−	−	−	++	++	++	++	260
4	−	−	−	±	++	++	++	++	300
5	−	−	−	±	++	++	++	++	300
6	−	−	−	±	++	++	++	++	260*
7	−	−	−	±	++	++	++	++	265*

Case B

Days for storage	Test Tube No. 1	2	3	4	5	6	7	8	Plasma fibrinogen level mg/dl
1	−	−	−	±	+	+	++	++	375
2	−	−	−	−	++	++	++	++	325
3	−	−	−	−	++	++	++	++	310
4	−	−	−	−	++	++	++	++	320
5	−	−	−	±	++	++	++	++	340
6	−	−	−	−	++	++	++	++	295*
7	−	−	−	±	++	++	++	++	305*

* Fibrinogen level determined after second centrifugation.

MEASUREMENT OF PLASMIN LEVELS IN STORED PLASMA

It is of great importance for laboratories to know how long plasma materials can be stored without any significant change in the plasmin values. Plasma storage tests, therefore, were performed with the plasma from 4 healthy individuals who were equally divided into two groups with 2 specimens of plasma kept in the freezer at −20°C and 2 specimens of plasma in an electric refrigerator at 4–6°C. The plasma from both groups demonstrated a slight elevations of whole plasmin levels as shown in tables 4 and 5, after 2 days' storage in the

Table 5.
Change in whole plasmin level and fibrinogen level in stored plasma (stored in a refrigerator at 4–6°C)

Case C

Days for storage	Test Tube No. 1	2	3	4	5	6	7	8	Plasma fibrinogen level mg/dl
1	−	−	−	±	++	++	++	++	215
2	−	−	−	±	++	++	++	++	220
3	±	−	±	+	++	++	++	++	200
4	±	−	±	++	++	++	++	++	230
5	−	−	−	++	++	++	++	++	245
6	−	−	−	++	++	++	++	++	230*
7	−	−	±	++	++	++	++	++	200*

Case D

Days for storage	Test Tube No. 1	2	3	4	5	6	7	8	Plasma fibrinogen level mg/dl
1	−	−	−	−	+	++	++	++	270
2	−	−	−	±	++	++	++	++	230
3	−	−	−	±	++	++	++	++	280
4	−	−	−	+	++	++	++	++	285
5	−	−	±	++	++	++	++	++	290
6	−	−	±	++	++	++	++	++	180*
7	−	−	−	++	++	++	++	++	215*

* Fibrinogen level determined after second centrifugation.

freezer group and more prominent 4 days after storage in the refrigerator. There results indicated the material for this test to be examined on the day it was collected.

The fibrinogen levels in the plasma from both groups which showed a gradual decrease in the first several days of storage, also rose up again above its original level after 4 days' storage.

We considered this unpredicted phenomenon to be due to white precipitates which began to appear in the bottom of test tubes on the fourth day of storage. There precipitates were considered to be some protein substance, probably fibrin. On the 6th and 7th day of storage the plasma was examined after removal of these precipitates by centrifugation resulting in decreased fibrinogen levels as shown in tables 4 and 5.

DETERMINATION OF MINIMUM EFFECTIVE DOSE OF ε-ACA IN MAN

As stated previously, the minimum effective dose (M.E.D.) of ε-ACA to inhibit whole plasmin activity in vitro was determined by the present experiment, from which M.E.D. of ε-ACA in vivo was calculated considering circulating plasma flow to be approximately 50 ml/kg.

Two different ways, intravenously and orally, were used in this experiment to administer ε-ACA and these groups were compared with each other. Eight healthy male adults were equally divided into two groups, A and B, A group with 5% ε-ACA solution intravenously and B group with oral administration

Table 6.

Change in whole plasmin level and fibrinogen level in man after administration of minimum effective dose of ε-ACA intravenously. (Group A_1)

Case 1: WT. 51.4 kg
Administered ε-ACA; 13 ml (650 mg)

	Test Tube No.								Plasma fibrinogen level mg/dl
	1	2	3	4	5	6	7	8	
Before administration	−	−	−	−	+	++	++	++	340
After administration 30 min	−	−	−	−	−	++	++	++	330
1 hour	−	−	−	−	+	++	++	++	310
2 hour	−	−	−	−	+	++	++	++	340
3 hour	−	−	−	−	±	++	++	++	360

Case 2: WT. 62.8 kg
Administered ε-ACA; 16 ml (800 mg)

	Test Tube No.								Plasma fibrinogen level mg/dl
	1	2	3	4	5	6	7	8	
Before administration	−	−	−	−	+	++	++	++	425
After administration 30 min	−	−	−	−	−	+	++	++	345
1 hour	−	−	−	−	+	++	++	++	355
2 hour	−	−	−	−	+	++	++	++	405
3 hour	−	−	−	−	+	++	++	++	455

Table 7.

Change in whole plasmin level and fibrinogen level in man after administration of double dose of M. E. D. of ε-ACA intravenously (Group A_2)

Case 3: WT. 59.2 kg
Administered dose of ε-ACA; 30 ml (1500 mg)

	Test Tube No. 1	2	3	4	5	6	7	8	Plasma fibrinogen level mg/dl
Before administration	−	−	−	−	++	++	++	++	350
After administration 30 min	−	−	−	−	±	+	++	++	235
1 hour	−	−	−	−	+	++	++	++	260
2 hour	−	−	−	−	++	++	++	++	270
3 hour	−	−	−	−	++	++	++	++	365

Case 4. WT. 75.4 kg
Administered dose of ε-ACA; 38 ml (1900 mg)

	Test Tube No. 1	2	3	4	5	6	7	8	Plasma fibrinogen level mg/dl
Before administration	−	−	−	−	+	+	++	++	245
After administration 30 min	−	−	−	−	−	±	+	++	175
1 hour	−	−	−	−	−	+	+	++	195
2 hour	−	−	−	−	+	+	++	++	205
3 hour	−	−	−	−	±	+	+	++	295

of ε-ACA tablet (1 tablet contains 250 mg of ε-ACA). In the intravenously administered group (group A) M.E.D. (group A1) and a double dose (group A2) were given, while in the oral group (group B) a double dose (group B1) and quadruple dose (group B2) were given with consideration for incomplete absorption by the gastrointestinal tract.

Table 8.

Change in whole plasmin level and fibrinogen level in man after administration of double dose of M. E. D. of ε-ACA orally (Group B_1)

Case 1: WT. 50.0 kg
Administered dose of ε-ACA; 5 Tab. (1250 mg)

	Test Tube No. 1	2	3	4	5	6	7	8	Plasma fibrinogen level mg/dl
Before administration	−	−	−	±	++	++	++	++	310
After administration 1 hour	−	−	−	±	++	++	++	++	310
2 hour	−	−	−	−	+	++	++	++	275
3 hour	−	−	−	−	++	++	++	++	270
4 hour	−	−	−	−	+	++	++	++	310

Case 2: WT. 57.2 kg
Administered dose of ε-ACA; 6 Tab. (1500 mg)

	Test Tube No. 1	2	3	4	5	6	7	8	Plasma fibrinogen level mg/dl
Before administration	−	−	−	−	++	++	++	++	195
After administration									
1 hour	−	−	−	±	++	++	++	++	185
2 hour	−	−	−	±	+	++	++	++	175
3 hour	−	−	−	−	+	++	++	++	180
4 hour	−	−	−	−	+	++	++	++	200

In the intravenously administered group (A), as shown in tables 6 and 7, whole plasmin level having once decreased to one half in thirty minutes, returned to its original level in one hour after administration of this drug in all cases. In the group with double dose this phenomenon was more impressive. Fibrinogen levels in all cases showed a tendency to decrease between 30 and 60 minutes and then returned to the former levels or slightly higher 3 hours after administration.

Table 9.

Change in whole plasmin level and fibrinogen level in man after administration of four fold dose of M. E. D. of ε-ACA orally. (Group B_2)

Case 3: WT. 61.0 kg
Administered dose of ε-ACA; 12 Tab. (3000 mg)

	Test Tube No. 1	2	3	4	5	6	7	8	Plasma fibrinogen level mg/dl
Before administration	−	−	−	±	++	++	++	++	280
After administration									
1 hour	−	−	−	−	++	++	++	++	260
2 hour	−	−	−	−	±	+	++	++	285
3 hour	−	−	−	−	+	++	++	++	280
4 hour	−	−	−	±	+	++	++	++	270

Case 4: WT. 51.0 kg
Administered dose of ε-ACA; 10 Tab. (2500 mg)

	Test Tube No. 1	2	3	4	5	6	7	8	Plasma fibrinogen level mg/dl
Before administration	−	−	−	−	++	++	++	++	265
After administration									
1 hour	−	−	−	−	++	++	++	++	255
2 hour	−	−	−	−	+	++	++	++	230
3 hour	−	−	−	−	±	++	++	++	240
4 hour	−	−	−	−	+	++	++	++	260

In the orally administered group, as shown in tables 8 and 9, there was a slight decline of whole plasmin level during 2 hours after administration which did not return to the original level in 4 hours. This phenomenon was also impressive in the group with the quadruple dose. The quite similar result was obtained in group B to that of group A as for fibrinogen levels.

DISCUSSION

1) Criticism against the method used in the present experiment.

Since there is no generally accepted method of measuring fibrinolysis or actual plasmin activity at the present time, various methods were applied simultaneously in one individual.

The results of the test vary greatly in same individual depending on what methods are used. For example, in previous studies on plasmin activity, while the present authors proved a close relationship between plasmin activity and hemorrhage in hemorrhagic diseases by Lewis and Furguson's method with euglobulin, this relationship was not particularly impressive when done by the Okamoto's method measuring whole plasmin level with streptokinase[5]. This, we believe, is because the Okamoto's method is greatly influenced by various values of plasminogen and/or its activator system existing in the plasma, and thus the results obtained by this method naturally do not always go parallel to that obtained by other methods in which already activated plasmin levels only are measured. In this regard the present experiment could be criticised because whole plasmin levels were measured by adding streptokinase.

Five hundred units of S.K. per 0.5 ml of plasma was used in our experiment because it has been shown by Okamoto and others that 50–100 units of S.K. was necessary to activate plasminogen to plasmin in 0.1 ml of plasma[6]. Also 20 units of thrombin was considered enough to coagulate 0.5 ml of plasma which contains approximately 1–3 mg of fibrinogen.

The main difference between the Fukutake's method and ours exists in the kinds of coagulants used to form clot; the former used Calcium Chloride instead of thrombin as used in the latter.

A comparison was made between the Fukutake's method and our's on the plasma from 4 healthy individuals preparing 2 series of 8 test tubes, for each individual. In addition, one series had 1/40 M Carcium Chloride and the other series had thrombin instead. Both series were compared according to our criteria after incubation for one hour at 37°C. No significant difference was observed between the two series of plasma except for only slight superiority

Table 10.
Comparison of the results obtained by our method and modified Fukutake's method

Case	Coagulant	Test Tube No. 1	2	3	4	5	6	7	8
A	Thrombin	−	−	−	−	++	++	++	++
	$CaCl_2$	−	−	−	−	+	++	++	++
B	Thrombin	−	−	−	−	++	++	++	++
	$CaCl_2$	−	−	−	−	+	++	++	++
C	Thrombin	−	−	−	±	++	++	++	++
	$CaCl_2$	−	−	−	±	++	++	++	++
D	Thrombin	−	−	−	±	++	++	++	++
	$CaCl_2$	−	−	−	±	++	++	++	++

in sensitivity recognised in our method in 2 cases out of four (Table 10). The disadvantage in the Fukutake's method is the presence of white sediments (probably of Calcium Oxalate) formed in the test tubes producing difficulty in evaluation of results. On the other hand no sediments were encountered in our method producing water clear solution when complete fibrinolysis took place. The most contraversial point in the present experiment is whether the results were greatly influenced by the amount of fibrinogen. In an extreme case, for example, of congenital afibrinogenemia, there would possibly be a falsely high result by our method indicating powerful fibrinolysis without any actual elevation of plasmin activity. In this regard fibrinogen values in each case were simultaneously measured, however, no significant influence on the results of the test from a little change in fibrinogen levels was observed.

Two different modes of fibrin clot were observed at the end of the reaction; the one forming fibrin clot and the other Gel type coagulation. This difference was considered to depend on the number of platelets rather than the amount of fibrinogen in plasma, since this Gel type coagulation was more frequently encountered when centrifuged for 10 minutes at a speed of 3,000 rpm than it was when done at 2,000–2,500. More evidence in favor of this theory was observed in 4 cases of plasma storage test in which all 4 plasmas having formed fibrin clots in the first several days, finally produced Gel type coagulation as the time for storage passed on.

2) The normal values as determined by this method.

In the experiment done by our method on 22 normal plasmas, 20 cases showed minimum effective dose of ε-ACA to inhibit fibrinolysis in 100 ml of plasma to be 25 mg and the other 2 cases showed 12.5 mg.

When as comparing this value with the one reported by Fukutake in which 12 out of 20 showed the M.E.D. to be 20 mg and the remainder showed less than 20 mg, almost equal results were obtained.

The type of the coagulant used in this experiment was proven to have no significant influence on the result. No difference was observed between both sexes as previously reported by Fukutake.

3) Experiment on stored plasma.

The results obtained by this experiment indicated an elevation of whole plasmin value in both groups of plasma stored in freezer (−20°C) and in a regular refrigerator (4°C–6°C). Higher concentration of the plasma due to vaporization and/or a hypothesis in which only antiplasmin decreases during the period for storage may partially explain this elevation of whole plasmin level, however, satisfactory explanations must wait for further investigations. Fibrinogen levels in the stored plasma demonstrated gradual decreases every day as previously reported.

4) Determination of M.E.D. to inhibit fibrinolysis in vivo.

The experiment was carried out by giving the calculated M.E.D. of ε-ACA to the normal individuals both intravenously and orally.

The intravenously administered group in which minimum effective dose and its double dose were given, demonstrated a decrease in whole plasmin levels to one half of original level in 30 minutes. These levels returned to the original one in one hour except for one case. On the other hand Abe and Nilsson demonstrated a more prolonged effect of ε-ACA lasting approximately 4 hours after administration by their fibrin plate method with euglobuline. This, we believe, is because serially two fold dilution ε-ACA adapted in our experiment was unable to demonstrate small difference. Fibrinogen level once decreased after giving ε-ACA returned to the original level or more in 2 hours, although on the contrary an increase in fibrinogen level was theoretically predicted by giving ε-ACA which supresses fibrinolysis. These unpredicted results may indicate fairly dynamic and complicated plasmin-antiplasmin-fibrinogen mechanism, and by injecting this antiplasmic drug the fibrinogen level was believed to decrease hand in hand with the plasmin level.

In the orally administered group two fold and four fold dose of M.E.D. of ε-ACA were given. ε-ACA level in the plasma reached the maximum in 2 hours after administration and did not return to the original level in four hours. Nilsson reported more rapid absorption of ε-ACA from gastro-intestinal tract (30–60 minutes) and also rapid excretion in urine, suggesting this drug be given every 4–6 hours. From the present study it was concluded that the

effect of this drug was immediate and reliable but short acting when given intravenously, while a slow but long acting effect was observed in the orally administered group. The fibrinogen level in the latter group changed less remarkably but in the same fashon as in the intravenous group.

SUMMARY

1) Whole plasmin level in the plasma with all plasminogen activated by streptokinase was determined by measuring minimum effective dose of ε-ACA necessary to inhibit fibrinolysis.

2) Twenty five mg was determined to be M.E.D. of ε-ACA necessary to inhibit whole plasmin activity in 100 ml of plasma in normal individuals. No difference between sexes was observed.

3) The material must be examined on the day it is collected because an elevation of whole plasmin activity due to unknown cause was noted in the stored plasma.

4) The effect of ε-ACA in the plasma was immediate and sure but short acting when given intravenously, on the other hand when orally given the effect was slow but long acting (more than 3 hours).

5) The fibrinogen level decrease following administration of ε-ACA, although an increase of fibrinogen level along with decreased plasmin activity was theoretically expected.

This rather puzzling, unexpected phenomenon may indicate some dynamic and complicated relationship among plasmin, antiplasmin and fibrinogen, which is of great interest and awaits further investigation.

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