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Post mortem activation of human blood fibrinolytic enzyme in sudden and natural deaths

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Abstract

With the purpose to elucidate the cause and difference of blood fluidity in sudden death and natural one, we have observed the fibrinolysis of the blood in medico-legal and pathological autopsies by means of Fibrin Plate Method, a routine method devised in our laboratory. As the result it has been found that in the blood serum of sudden death and in some of natural deaths from tumors, leukemias, etc., the decrease in fibrinolytic activity is equivalent to the amount of proactivator that combined with the SK-like substance liberated into blood. On the other hand, in the blood of most of natural deaths, and in that bled from vessels and stored in body cavities, no natural fibrinolysis is observable and the same fibrinolytic activity with SK as normal one is demonstrated. Thus it is concluded that the cause of blood fluidity in sudden death is due to the fibrinolysis.

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POST MORTEM ACTIVATION OF HUMAN BLOOD FIBRINOLYTIC ENZYME IN SUDDEN AND NATURAL DEATHS

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It is well known that the blood in sudden death, asphyxia or shock[1], and in the menstrual blood[2], *etc.* shows the fluidity but no coagulability. As for the exact cause of this phenomenon opinions are divided.

By means of Fibrin Plate Method we have been studing the mechanism of fluidity of blood centering around fibrinolysis caused by a proactivator (also called a human factor by some), which is thought to be one of enzyme proteins specifically abundant in human blood[3-5]. In this report we present the findings of *post mortem* fibrinolytic activity of the human blood in sudden and natural deaths from medico-legal and pathological autopsy cases.

MATERIALS AND METHODS

The blood of 78 medico-legal and pathological autopsy and inquest cases shown in Table 1, served as the material. The blood was directly taken from the ventricle, auricle, aorta and vena cava. The blood bled into the body cavities by the stabbing injuries was also taken as the material. After the observation of the fluidity of the blood the serum was seperated quickly. The serum so separated was mixed with physiological saline solution to a series of dilution from 1 : 1, 1 : 5, 1 : 10, 1 : 50, 1 : 100, 1 : 500, 1 : 1,000, 1 : 2,000, 1 : 4,000, 1 : 8,000, 1 : 10,000, 1 : 20,000, 1 : 40,000, 1 : 80,000, 1 : 160,000, 1 : 320,000, 1: 640,000, 1 : 1,280,000 to 1 : 2,560,000, and the other series of identical dilution was prepared with addition of 100 mg. % streptokinase (SK) in the proportion of 3 : 1, and one drop each of these diluted sera was dropped in the center of fibrin plate, and fibrinolysis was estimated. For this estimation we employed the routine Fibrin Plate Method devised in our laboratory and already described in the previous paper[1-5].

Table 1 Fibrinolytic activity of ***postmortem*** blood in autopsy cases

Cases of death	Number of cases	Sudden death	Complete fluidity in heart blood	Appearance of natural fibrinolysis without SK	Decrease of fibrinolytic activity with SK
Asphyxia	8	○○○ ○○○ ○○	○○○ ○○○ ○○	○○○ ×○○ ○○	○○○ ×○○ ○×
Loss of blood due to stabbing or cut injuries	13	○○○○○ ○○○○ ○○○○	×○○○× ○○×× ○○○○	×○○○○ ○○○× ○○○○	×○○○○ ○○○○ ○○○○
Intoxication	3	○ ○ ○	○ ○ ○	○ ○ ○	○ ○ ○
Traffic accident	3	○ ○ ○	○ × ×	○ ○ ○	○ ○ ○
Freezing	1	×	×	×	×
Injuries or hemorrhage of central nervous system	8	○×○ ○○○ ×○	××× ××× ×○	○○○ ○○○ ○○	○○× ○○× ○○
Tumors (except leukemia)	14	××××× ××××× ××××	×××○× ×××○× ×○×○	×××○○× ×○○○○ ×○×○	○×○○× ○○○○× ×○○○
Blood disease (included leukemia)	9	××× ××× ×××	×○× ○○× ×○○	××× ○○× ×○×	○×○ ○○ ××
Inflammation	7	××× ×× ××	××× ○× ××	××○ ○○ ××	○×○ ○○ ××
Liver cirrhosis	3	× × ×	○ × ×	○ × ×	○ × ×
Heart failure and circulatory disturbances	9	×○× ××× ×○○	×○○ ○×○ ×○×	×○× ○×○ ×○○	×○○ ○×○ ×○○

○ : positive cases

× : negative cases

RESULTS

Relationships among the sudden deaths, the complete fluidity of cadaver blood in the heart and the aorta, the appearance of natural fibrinolysis without SK and the decrease of fibrinolytic activity with 100 mg. % SK, are illustrated

Table 1. With a few exceptions they show a close correlation in many cases.

In the cases of natural death the blood generally contained coagulate in their heart, but in those of sudden death a nearly complete fluidity of the blood could be observed in the heart and aorta. In the cases of instant death, however, where the blood was lost by the heart stab or in casualty by a train accident, the heart blood of some contained soft coagulate. Even in those of death from malignant tumor or leukemia, *etc.*, the fluidity of the blood was observed in some of them, on the other hand, no fluidity and coagulate were observable in any normal human blood used as the control.

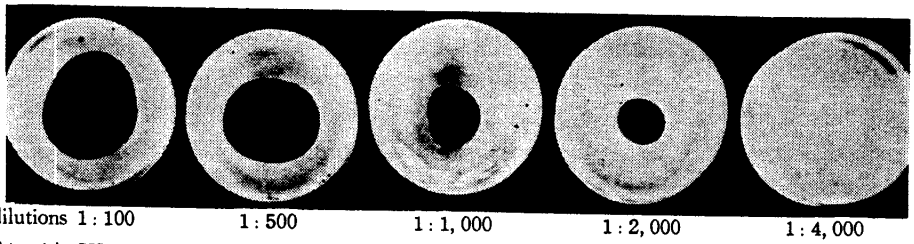
Natural fibrinolysis (without SK) was estimated by the Fibrin Plate Method, and the results revealed that positive spontaneous fibrinolysis was observable in the dilution of 1 : 100 to 1 : 4,000 in sudden death except a few cases of instant death, in which soft coagulate was observed in their heart and aorta (Fig 1). Moreover, this natural fibrinolysis was practically in all (35 cases out of 38) showed complete fluidity, but no fibrinolysis could be observed even in original human blood serum in the cases of normal, instant death or natural death (Figs. 2—4). In a few natural death, however, such as malignant tumors or leukemias, *etc.*, positive spontaneous fibrinolysis was observable (Figs. 5—6).

Next, observation on the fibrinolysis of each blood serum with addition of 100 mg. % streptokinase solution showed fibrinolysis at the concentration of over 1 : 640,000 in the cases of normal, instant death and common natural death (Figs. 2—4).

In the cases of sudden death except instant one and of diseases such as tumors or leukemia, showing the natural fibrinolysis in their blood, however, the fibrinolytic activity with SK was observed generally up to the dilution of 1 : 40,000, revealing a marked decrease and indicating an inverse correlationship between natural fibrinolysis (Figs. 1 and 5—6).

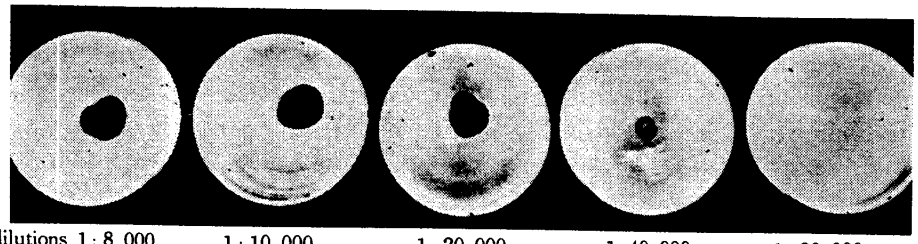
In all the blood of the sudden deaths such as from stabbing, cut injuries or traffic accident, *etc.*, where hemorrhage occurred in the extravascular body cavities—thoracic, abdominal, pericardial, *etc.*, the soft coagulate was included, while on the other hand, the blood in the heart or aorta in most of these cases was in complete fluidity. Estimations of the natural fibrinolysis of both blood without SK showed natural dissolution of fibrin only in uncoagulated blood of the heart and aorta but with the blood accumulated in the body cavities such as abdominal cavity, *etc.*, containing soft coagulate and derived from the same heart, no natural fibrinolysis could at all be observed, while on the addition of SK at last the fibrinolytic activity could be recognized in the same degree as in normal blood (Fig. 7). In this instance, the fibrinolysis with SK of fluid blood in the heart and aorta was found to decrease up to the dilution of less than 1 : 40,000 (Fig. 8).

a) without SK



dilutions 1 : 100 1 : 500 1 : 1, 000 1 : 2, 000 1 : 4, 000

b) with SK



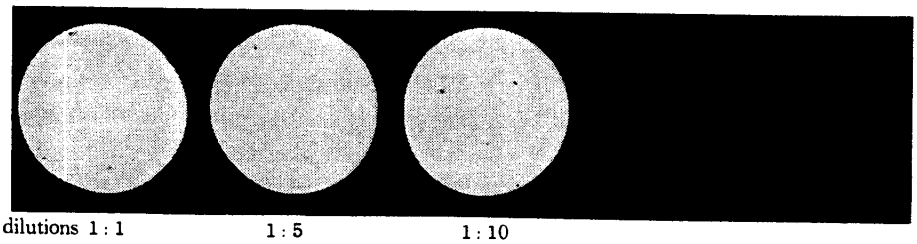
dilutions 1 : 8, 000 1 : 10, 000 1 : 20, 000 1: 40, 000 1 : 80, 000

Fig. 1 Fibrinolytic activity of heart blood in case of sudden death, subarachinoideal hemorrhage (66 years, female; dark red, complete fluid blood; 24 hrs. after the test)

a) Dissolution of fibrin plate can be recognized in the serum without streptokinase (SK) up to the dilution of 1 : 2, 000.

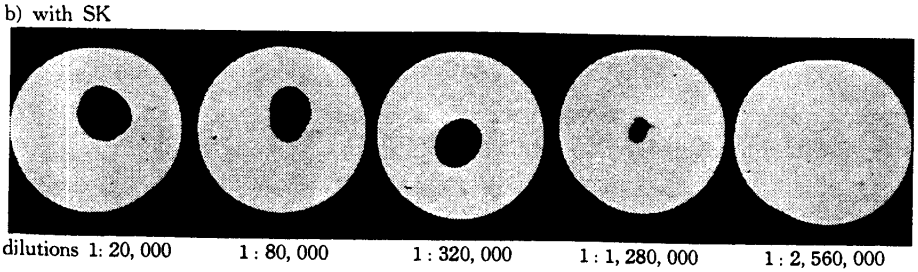
b) In the serum with SK up to the dilution of 1 : 40, 000 the dissolution of fibrin plate can be seen.

a) without SK



dilutions 1 : 1 1 : 5 1 : 10

b) with SK



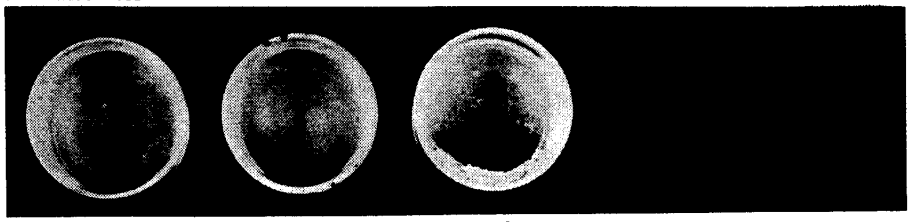
dilutions 1: 20, 000 1 : 80, 000 1 : 320, 000 1 : 1, 280, 000 1 : 2, 560, 000

Fig. 2 Fibrinolytic activity of venous blood in normal healthy adult (33 years, male, one of the authors, 24 hrs. after the test)

a) No dissolution of fibrin plate can be recognized in the serum without SK.

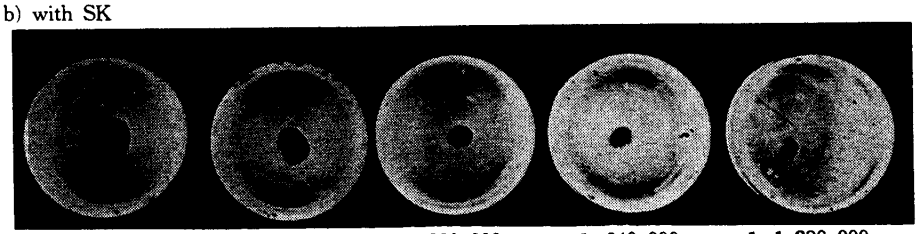
b) In the serum with SK up to the dilution of 1 : 1, 280, 000 the dissolution of fibrin plate can be seen.

a) without SK



dilutions 1 : 1 1 : 5 1 : 10

b) with SK



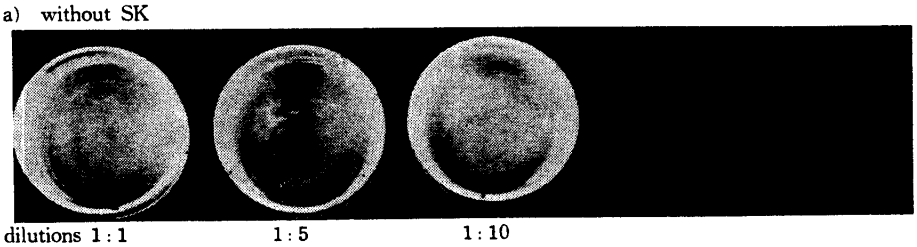
dilutions 1 : 80, 000 1 : 160, 000 1 : 320, 000 1 : 640, 000 1 : 1, 280, 000

Fig. 3 Fibrinolytic activity of aorta blood in case of instant death suffered by heart and lung stab injuries (21 years, male; containing small, dark red, soft coagulate in the aorta; 24 hrs. after the test)

a) No dissolution of fibrin plate can be recognized in the serum without SK.

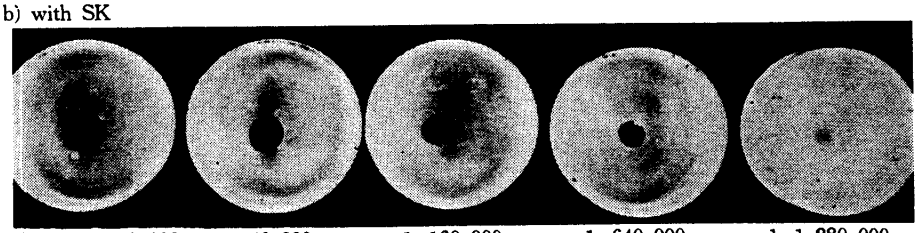
b) In the serum with SK up to the dilution of 1 : 1, 280, 000 the dissolution of fibrin plate can be seen.

a) without SK



dilutions 1 : 1 1 : 5 1 : 10

b) with SK



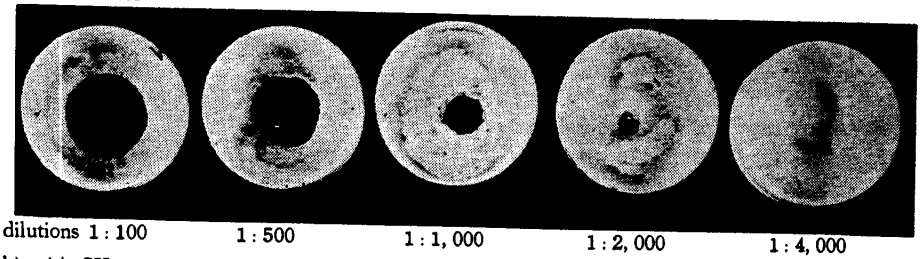
dilutions 1 : 10, 000 1 : 40, 000 1 : 160, 000 1 : 640, 000 1 : 1, 280, 000

Fig. 4 Fibrinolytic activity of heart blood in case of natural death, encephalitis (2 years, male; containing dark red, soft coagulate in the heart and aorta, 24 hrs. after the test)

a) No dissolution of fibrin plate can be recognized in the serum without SK.

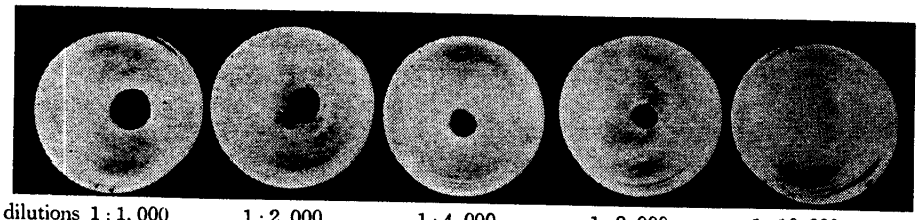
b) In the serum with SK up to the dilution of 1 : 640, 000 the dissolution of fibrin plate can be seen.

a) without SK



dilutions 1 : 100 1 : 500 1 : 1, 000 1 : 2, 000 1 : 4, 000

b) with SK



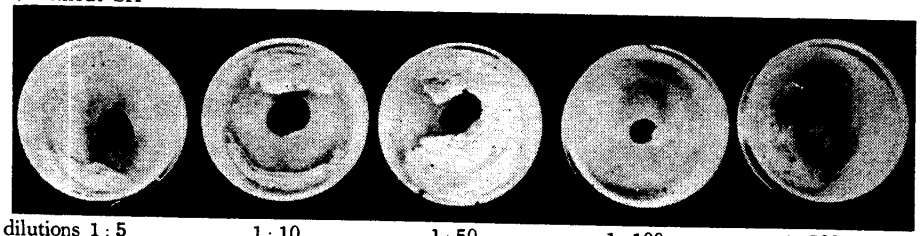
dilutions 1 : 1, 000 1 : 2, 000 1 : 4, 000 1 : 8, 000 1 : 10, 000

Fig. 5 Fibrinolytic activity of heart blood in case of malignant tumor, ovarial carcinoma and its metastasis (59 years, female; containing soft coagulate in heart, 24 hrs. after the test)

a) Dissolution of fibrin plate can be recognized, using the serum without SK, up to the dilution of 1 : 2, 000.

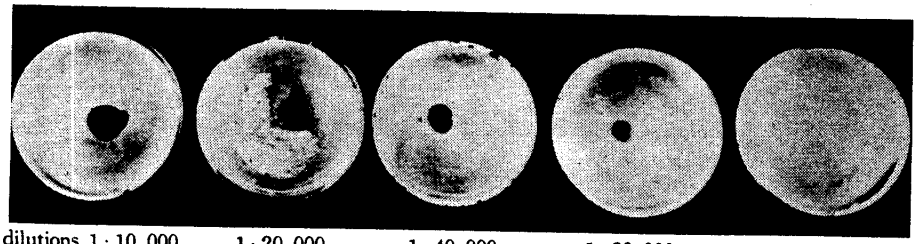
b) But in the serum with SK up to the dilution of 1 : 8, 000, showing a marked decrease of fibrinolytic activity, the dissolution of fibrin plate can be seen.

a) without SK



dilutions 1 : 5 1 : 10 1 : 50 1 : 100 1 : 500

b) with SK



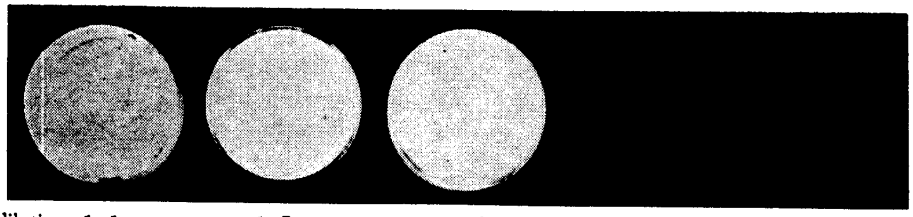
dilutions 1 : 10, 000 1 : 20, 000 1 : 40, 000 1 : 80, 000 1 : 160, 000

Fig. 6 Fibrinolytic activity of heart blood in case of eosinophilic leukemia (62 years, male, complete fluid blood, 24 hrs. after the test)

a) In the serum without SK dissolution of fibrin plate can be recognized up to the dilution of 1 : 100.

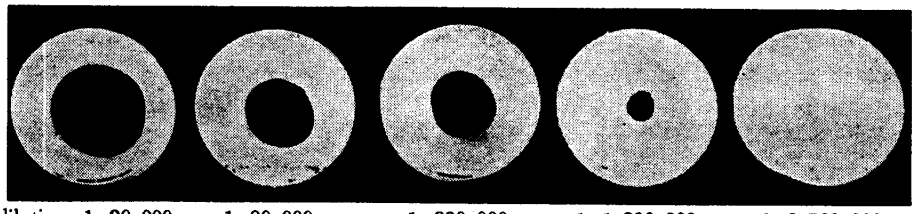
b) In the serum with SK up to the dilution of 1 : 80, 000, showing a marked decrease of fibrinolytic activity, the dissolution of fibrin plate can be seen.

a) without SK



dilutions 1 : 1 1 : 5 1 : 10

b) with SK



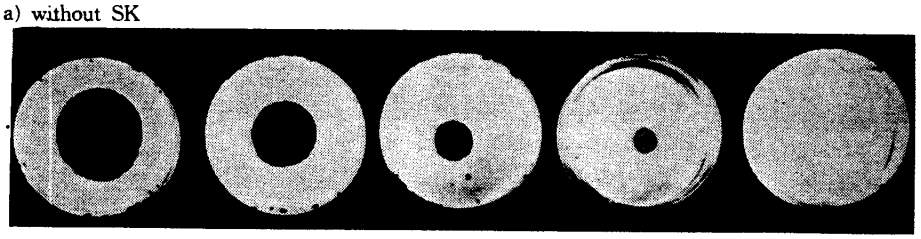
dilutions 1 : 20, 000 1 : 80, 000 1 : 320, 000 1 : 1, 280, 000 1 : 2, 560, 000

Fig. 7 Fibrinolytic activity of bled blood in the thoracic cavity from traffic accident (33 years, male; much, dark red, soft coagulate in the thoracic cavity; 24 hrs. after the test)

a) No dissolution of fibrin plate can be observed using the serum without SK.

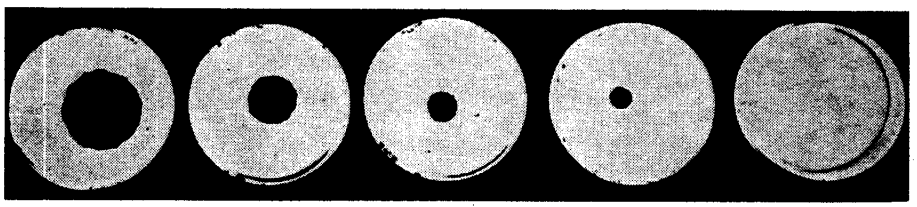
b) On the addition of SK the fibrinolytic activity can be recognized up to the same dilution of 1 : 1, 280, 000 as in normal blood.

a) without SK



dilutions 1 : 50 1 : 100 1 : 500 1 : 1, 000 1 : 2, 000

b) with SK



dilutions 1 : 4, 000 1 : 10, 000 1 : 20, 000 1 : 40, 000 1 : 80, 000

Fig. 8 Fibrinolytic activity of the blood from the picked heart containing very small soft coagulate in same case as Fig. 7 (24 hrs. after the test)

a) In the serum without SK dissolution of fibrin plate can be recognized up to the dilution of 1 : 1, 000.

b) Fibrinolytic activity with SK of this blood is found to decrease up to the dilution of 1 : 40, 000.

DISCUSSION

It has been well-known for a long time that the blood of sudden death is fluid, and as for its exact cause of this phenomenon some of the opinions available are the theories of the excess of carbon dioxide (Bonne *et* Brouardel, 1897), the formation of some anticoagulant (Corin, 1883), the decoagulation (Brouardel, 1897), the degeneration of fibrinogen (Vogel, 1926), and the wasting of fibrinogen due to fibrinolysis (Satoh, 1954), *etc*[1]. Among them the last one, fibrinogenolysis or fibrinolysis, is at present the most promising theory and in our observations of fibrinolysis by means of Fibrin Plate Method in the autopsy cases, too, it has been recognized that the cause of making the blood of sudden death fluid is due to the fibrinolytic activity.

Even in the blood of natural death except sudden one, such as tumor, leukemia or some of heart failure, the natural fibrinolytic activity was observed, but the cause of making the blood of these natural deaths fluid being so exceedingly complex, it can not be explained by the fibrinolytic activity alone so that the disturbances of coagulation mechanism in most of these cases need to be taken into consideration, and then fluidity of blood in natural death seems not to be parallel with the fibrinolytic activity of these blood, differing from that in sudden death.

There are many reports, however, dealing with the fibrinolytic activity in malignant tumors[6], the diseases of blood[7], collagenous or allergic[8-9], liver cirrhosis[9], pregnancy[9], trauma[9], operation[9-10], anesthesia[7], anxiety[7,11], fear[7,11], pain[7,11], hard labor[7,11], and fatigue[7,11], *etc*., but most of them describe the fibrinolytic activity not of *post mortem* cases but that of living persons. MOLE[12] has observed cases of no fluidity in the cadaver blood of cachexia, and we have also recognized the like in many cases.

The fact that fibrinolytic activity in the blood taken from the heart or aorta of sudden death decreases considerably on addition of SK as compared with that in normal blood seems to suggest an inverse relationship between the former and natural fibrinolysis. This natural fibrinolysis does occur, just as in the case of the fibrinolysis on addition of SK *in vitro*, due to the reaction of the proactivator in blood with an SK-like substance liberated from the vessel wall and the decrease in the fibrinolytic activity of fluid blood on the addition of SK corresponds only to the increase in natural fibrinolytic activity. This fact was also observed in life by MAKI[13], who thought that the abnormal decrease in fibrinolytic activity of euglobulin activated by SK was very significant in the light of clinical symptom because it suggests a considerably marked fibrinolysis to have occurred previously.

The fact that the blood accumulated in the thoracic, or in the abdominal

cavity as in the case of stabbing, or cut injuries of the heart or large blood vessel, contains the soft coagulate and shows no natural fibrinolysis and that the fibrinolytic activity on addition of SK is the same just as the normal one, seems to suggest the necessity of the liberation of an SK-like substance to the heart or vessels to make cadaver blood of sudden death fluid.

And it is suggested that the fibrinolysis of the blood of the heart or the aorta in the cases of instant death due to a large hemorrhage, that is similar to the normal, means the necessity of the movement of heart for the release of an SK-like substance.

CONCLUSION

With the purpose to elucidate the cause and difference of blood fluidity in sudden death and natural one, we have observed the fibrinolysis of the blood in medico-legal and pathological autopsies by means of Fibrin Plate Method, a routine method devised in our laboratory.

As the result it has been found that in the blood serum of sudden death and in some of natural deaths from tumors, leukemias, *etc.*, the decrease in fibrinolytic activity is equivalent to the amount of proactivator that combined with the SK-like substance liberated into blood. On the other hand, in the blood of most of natural deaths, and in that bled from vessels and stored in body cavities, no natural fibrinolysis is observable and the same fibrinolytic activity with SK as normal one is demonstrated. Thus it is concluded that the cause of blood fluidity in sudden death is due to the fibrinolysis.

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