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STUDIES ON POSTMORTEM BLOOD IN SUDDEN DEATH
PART I. APPLICATION OF IMMUNOELECTROPHORESIS

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1) The identification of the blood in sudden death and its differentiation from normal healthy blood and the blood containing soft coagulum or buffy coated clot were studied and satisfactory results were obtained when used the postmortem blood taken more than three hours after the death.

2) The immunological test was profitable for the differentiation of fibrinolysis from fibrinogenolysis.

3) The difference between fibrinogen of human and of bovine was demonstrated by the immunoelectrophoretic pattern. With the immune reaction, they were distinguished with considerable clarity.

INTRODUCTION

Seligman and Grabar[1], during the course of the investigation on the potential utility of immunochemical techniques for the study of fibrinolysis, found that they could identify degradation products of fibrinogen immunoelectrophoretically. They were also able to detect degraded derivatives of fibrinogen by treating with plasmin. Caspary[2], in 1956, reported some preliminary notes on a similar experiment with human fibrinogen. Salmon[3] used rabbit antisera in immunoelectrophoretic experiments for fibrinogen analysis. He found that the products of fibrinolysis and of fibrinogenolysis can be precipitated by anti-fibrinogen serum. However, few studies have been undertaken concerning the identification of human blood in sudden death by means of immunoelectrophoretic method. For the differentiation of the products of fibrinolysis from those of fibrinogenolysis, no satisfactory method has yet been found up-to-date despite of a number of investigations. The fluidity of the blood in sudden death and its biological significance has come to acquire an importance in the legal medicine. The present paper deals with the identification of blood in sudden death and the differentiation of fibrinolysis from fibrinogenolysis.

In the legal medicine, the identification of the source of a blood is often needed as a practical problem. The authors further studied and succeeded in demonstrating species specificity.

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MATERIAIS AND METHODS

1. Antisera: Anti-bovine fibrinogen, anti-human fibrinogen, and anti-human euglobulin serum were obtained by immunization of rabbits weighing approximately 3.5 kg with bovine fibrinogen, human fibrinogen, and human euglobulin. The immunization was accomplished by five intravenous injections of 20 mg of antigens at three-day intervals. The bleeding of the animals was performed by means of a heart puncture seven days after the last injection. The sera were separated and stored at 0°C until use.

Prior to use, antisera were purified as follows. To 9 ml of antisera was added 1 ml of normal healthy blood serum. The mixture was incubated at 37°C for one hour, and then centrifuged (See Fig. 1). The supernatant was inactivated by heating it at 56°C for thirty minutes.

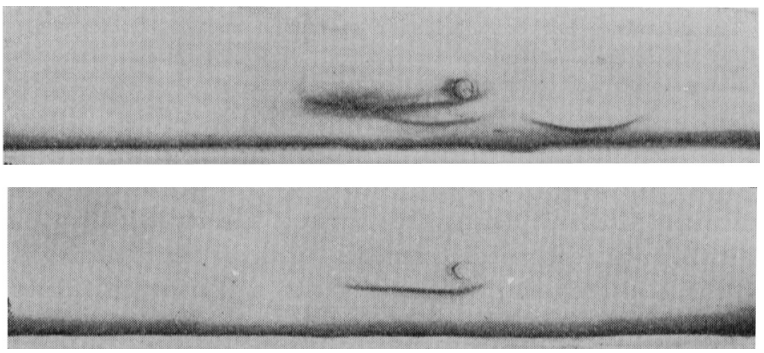


FIG. 1. Electropherograms of antiserum before and after the purification.

2. Degradation products of fibrinogen: For the fibrinolysis, 0.1 ml of varidase (1000 u/ml) was added to 0.5 ml of bovine fibrinogen (4 mg/ml) or of normal healthy human plasma. After mixing, two drops of thrombin (100 u/ml) was added. Visible clots formed within three minutes. The mixture was put aside for 24 hours at 37°C and then centrifuged. The clear supernatant was served as antigen. For the fibrinogenolysis, fibrinogen samples were incubated in the presence of varidase but without thrombin for 24 hours at 37°C. The samples were centrifuged and the supernatants were served as antigens.

3. Blood stains: Drops of blood were blotted on a filter paper and allowed to dry at room temperature for one week being sheltered from direct sunlight. A small piece of the blood stains was cut out and placed in a test tube containing saline and then centrifuged briefly. The extract was examined by immunoelectrophoresis.

4. Preparation of blood sample: Blood was directly taken from the ventricle, auricle, aorta and vena cava. After the fluidity of the blood was observed, the serum was separated quickly.

5. Immunoelectrophoresis: The immunophor standard set attached to the LKB 6800 A immunoelectrophoresis apparatus was employed. The experiments were performed according to the technique of Scheidegger[4]. The details of the precipitin bands could usually became more visible when stained. The staining was accomplished by treating the gels with Amido black 10 B fol-

TABLE 1. Postmortem Blood Condition of Heart

Cause of death	Complete fluidity	Containing soft coagulate	Age	Sex	Time after the death
Heart failure	+	−	80	♂	2.20′
Intoxication (parathione)	+	−	3	♂	17.15′
Intoxication (KCN)	+	−	23	♂	4.40′
Colonar arteriosclerosis	−	+	75	♂	3.30′
Liver cirrhosis	+	−	52	♂	17.00′
Loss of blood	−	+	26	♂	14.50′
Died by a motor car accident	+	−	25	♂	9.20′
Spontaneous pneumothorax	+	−	19	♂	3.00′
Myciline shock	+	−	67	♂	17.20′
Loss of blood (rupture of art. abdominalis)	+	−	31	♀	9.30′

TABLE 2. Components for Immunoelectrophoresis

Component
Electrophoresis apparatus
Power supply
Staining and rinsing tanks
Slide frames
Frame holder
Gel punch set
Levelling table
Micropipette
Suction needle with rubber tubing
Slide lifter
Rayon wicks
Gel knife
Microfix frames

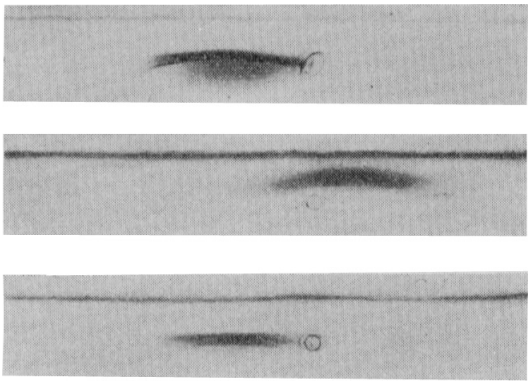
TABLE 3. Experimental Procedure

Standard processing technique	Note
Preparation of chemicals	
1. Buffer solution	Veronal buffer, pH 8.6
2. Buffer agar solution	Difco special agar (1%)
3. Rinsing solution	Consisting of methyl alcohol, acetic acid and water
4. Staining solution	Amido black 10 B
Preparation of the equipment for use	Tank arrangement, Apply agar solution to glass slides
Start	Punch troughs and wells in gel layer Apply sample to be analysed in wells
Electrophoresis run	mA-selector switch is set to "16 mA" 150 V on voltmeter (2.5 hrs.)
Application of immune serum	Make a cut in ends of each trough with gel knife Apply immune serum in troughs
Incubation	Insert holder into humid chamber at room temperature for 20 hours
Washing off excess protein	Set holder down in a bath (1% NaCl solution) for 16 hours
Drying	In order to shorten the drying time a heating lamp can be used
Staining	Place slide frames in a frame holder and immerse it for 5 min. in staining tank
Rinsing	Immerse it in rinsing bath

lowed by washing with saline to remove soluble proteins. After staining, the excess dye was removed by washing with methanol-water-acetic acid mixture (45 : 45 : 10). The details of immunoelctrophoretic procedure shown in Table 3 had been established by Grabar[5], Wieme[6] and Wunderly[7].

RESULTS

Degradation products of fibrinogen by streptokinase activated plasmin—Bovine fibrinogen was tested against antifibrinogen serum. Antifibrinogen serum gave one line of precipitation similar to that of α-globulin with bovine fibrinogen



a. bovine fibrinogen (α type)

b. products of fibrinolysis by plasmin (β type)

c. products of fibrinogenolysis by plasmin (α type)

FIG. 2. Electropherograms of fibrinogen and its degradation products

(Fig. 2-a). The same antiserum gave "β-globulin" line with the product of fibrinolysis but no line appeared in the α-globulin region (Fig. 2-b). The "β-globulin" lines seemed to be characteristic for the product of fibrinolysis. On the other hand, the product of fibrinogenolysis gave one line in the α-globulin region (Fig. 2-c), which was very similar to the line obtained with bovine fibrinogen. We are attempting to determine, in the next experiment, which of the two lines in the α-and β-regions corresponds to the blood of sudden death.

Normal healthy blood and postmortem blood—Bloods of sudden death were tested against antihuman euglobulin serum. Anti-human euglobulin serum gave two lines in the β-globulin region (Figs. 3-c, d, e and f) with the plasma in sudden death, whereas the same antiserum gave only one line in the β-globulin region with the plasma from the blood containing soft coagulum (Fig. 3-g). The "β_2-globulin" line which was very similar to the line obtained with the product of fibrinolysis seemed to be characteristic for the plasma of sudden death.

Identification of sudden death with blood stains by means of immunoelectrophoresis—The eluate from blood stains described before was tested against anti-human fibrinogen serum. Anti-human fibrinogen sera gave one line in the β-globulin region with the eluate from the blood stains of sudden death, while on the one hand serum gave one line in the α-globulin region with the eluate from the normal healthy blood stain. The "β-globulin" line which was similar to the line obtained with the product of fibrinolysis seemed to be characteristic for sudden death.

The difference between the fibrinogen of human and of bovine—The product of fibrinolysis was tested against anti-fibrinogen sera. It gave one line in the

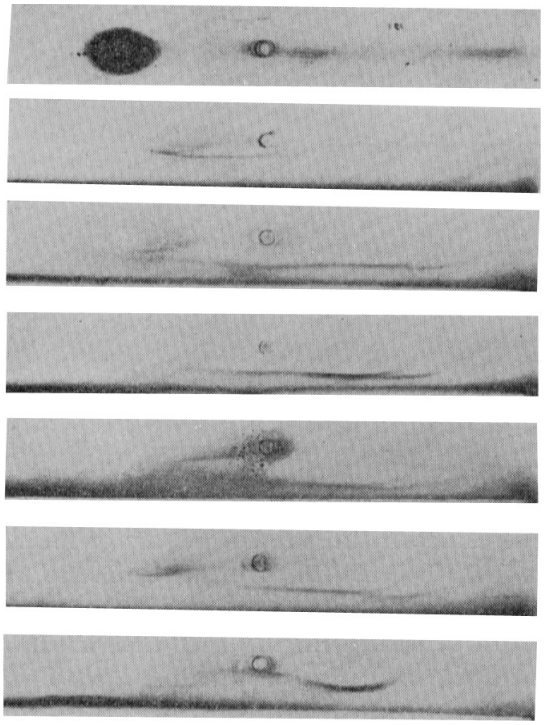


FIG. 3. Immunoelectropherograms of normal human plasma, plasma of sudden death and plasma from the blood containing soft coagulum.

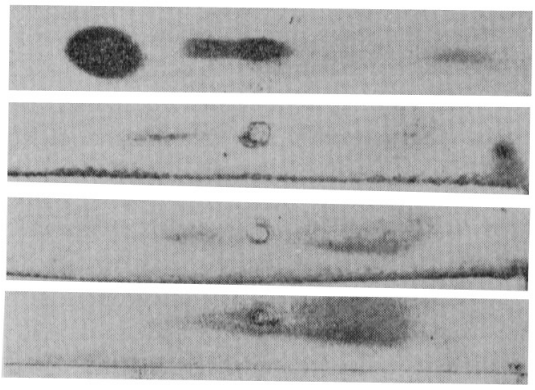
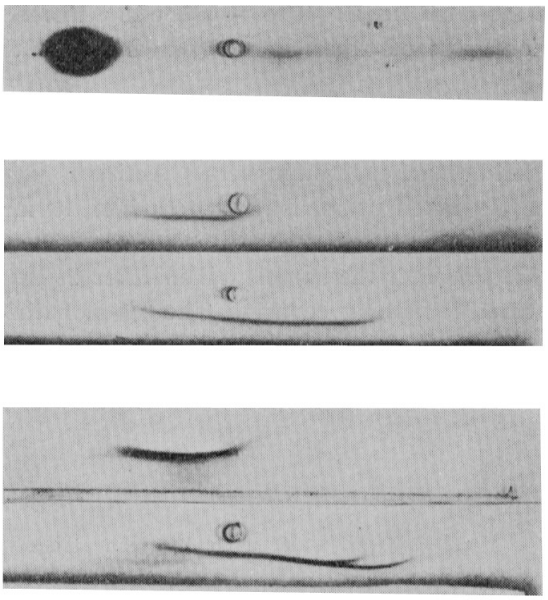


FIG. 4. Immunoelectropherograms of antisera obtained from blood stains.

β_1-region with anti-bovine fibrinogen sera whereas it gave two lines in the β-globulin region (β_1, β_2) with anti-human fibrinogen sera. This result can be applied to distinguish the source of anti-fibrinogen sera.



a. normal human plasma

b. normal human plasma (α type)

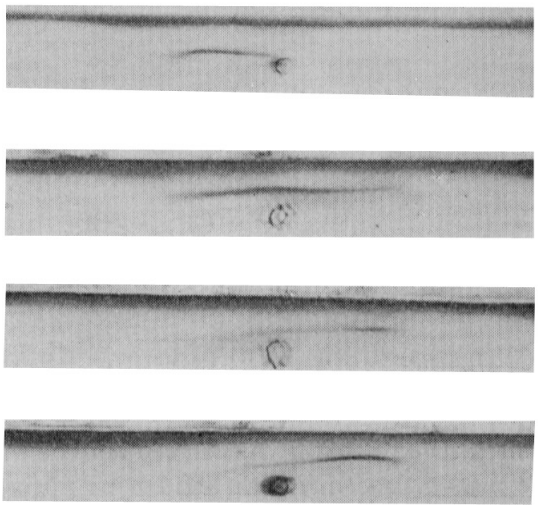
c. products of fibrinolysis (β_1 type)

d. normal human plasma (α type)

e. products of fibrinolysis (β_1, β_2 type)

FIG. 5. The difference between the fibrinogen of human and of bovine.

Postmortem blood pattern in sudden death—Postmortem blood samples taken from the heart at the periods of 3, 9, and 15 hours after the death were tested against anti-bovine fibrinogen sera. Antisera gave two lines in α-and β-regions with blood taken from the heart at 3 hours after the death: the same



a. normal human plasma (α type)

b. 3 hours after the death (pneumothorax)

c. 9 hours after the death (motor car accident)

d. 15 hours after the death (loss of blood)

FIG. 6. Patterns of blood in sudden death

antiserum gave only one line in the β-globulin region with the blood taken at the periods of 9 and 15 hours after the death. The "β-globulin" line seemed to be characteristic for postmortem blood taken from the heart more than 3 hours after the death.

Effect of heparin—Fibrinolytic and fibrinogenolytic reaction mixtures with heparin were tested against anti-human euglobulin sera. Antisera gave one line in β_1-region with the sample taken at incubation period of 10 minutes from the reaction mixtures containing one-tenth volume of heparin. The same antiserum gave one line in β_2-region with the sample taken after 25 minutes' incubation of the reaction mixtures containing heparin. The "β_2-globulin" line which is similar to the line obtained with the postmortem blood taken from the heart at over 3 hours after the death seemed to appear after the long time incubation.

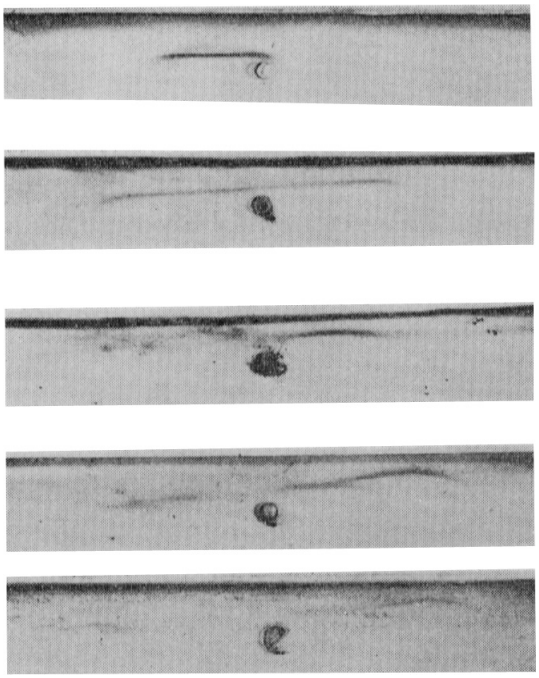


FIG. 7. Effect of heparin

DISCUSSION

The results obtained are summarized in Table 4. As can be seen, the serum of sudden death gave a positive reaction not only with anti-bovine fibrinogen sera but also with anti-human fibrinogen sera. The major constituent had im-

TABLE 4. Immunoelectrophoretic Patterns of Fibrinogen and Its Degradation Products

Antibodies	Antigens	Immunoelectrophoretic patterns
Antibovine fibrinogen sera	Bovine fibrinogen	α_2 region
〃	Products of fibrinolysis by plasmin	β_1 〃
〃	Products of fibrinogenolysis by plasmin	α_2 〃
〃	Human blood plasma	α_2 〃
〃	Human blood serum	—
〃	Blood serum in sndden death (completely fluidal)	Decrease in α_2 region, increase in β_1 region
Antihuman fibrinogen sera	Human blood plasma	α_2 region
〃	Human blood serum	—
〃	Blood plasma in sudden death (completely fluidal)	Decrease in α_2 region, increase in β_1 and β_2 regions
〃	Blood plasma in sudden death (containing soft coagulum)	Decrease in α_2 region, increase in β_1 region
〃	Products of fibrinolysis by plasmin	Decrease in α_2 region, increase in β_1 and β_2 regions
〃	Products of fibrinogenolysis by plasmin	Decrease in α_2 region, increase in β_1 region

munoelectrophoretically the same mobility as β-globulin. Both fibrinolysis and fibrinogenolysis induce increases of β_1-globulin. In view of recent observation that the major component of serum may be β_1-globulin, it is interesting to notice that the concentration of this globulin always decreases in an acute attack and rises again during remission: the acute attack seems to be accompanied by serious cellular destruction, an effect probably requiring a complement (Grabar and Burtin[8], Seligman and Hanau[9]). Serum from the blood of sudden death shows an increase of β_2-globulin; β_2-globulin precipitin arc, which is ordinarily scarecely visible, can redily be demonstrated in normal sera only if these sera are concentrated before the electrophoresis, is very prominent (Burtin *et al.*[10], Scheiffarth and Gotz[11]). According to Ryback[12], immunoelectrophoretically plasmin appears in two zones in the β-region.

The comparison of the blood in sudden death and the blood containing

soft coagulum or the normal healthy blood was done according to antigenic criteria. The fact that the blood in sudden death does not clot is well known. This failure of clottability might be due to the fibrinolytic activity of the blood in sudden death. As a matter of fact, with anti-human fibrinogen sera or with anti-human euglobulin sera, blood of sudden death gives a characteristic line in β_2-region which must be due to fibrinolytic products. Applying the immuno-electrophoresis, it became possible to differenciate the blood in sudden death from the blood containing soft coagulum and from the normal healthy human blood.

As to the species specificity, application of immunoelectrophoresis made possible to distinguish fibrinogen of human from that of bovine, as described above. The selection of an appropriate antiserum is important for this purpose.

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