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Identification of human blood by fibrin plate method, a supplemental study

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Identification of human blood by fibrin plate method, a supplemental study*

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Abstract

Following Fibrin Plate Method of SZOLLOSY and RENGEI[2] , and ASTRUP and MULLERTZ[3], the author conducted a series of experiments in an attempt to identify human blood by detecting the proactivator believed to be one of the enzyme proteins contained abundantly in human blood. As the results it has been found that with 0.1 mg. % SK-solution human blood alone responds to the reaction, showing almost absolute species-specificity within 4 hours but not with blood of monkey. In addition, the sensitivity is so high that it responds positively up to the dilution of 1: 8,000 to 1: 10,000 (human blood: physiological saline solution). By means of this method using 0.1 mg% SK-solution it has been clearly demonstated that the identification of human blood is possible in a variety of conditions and states as may be encountered in practical legal medicine such as with blood stains in cloth, wood, stone, leaves of tree even with a trace of blood stain, old human blood stain left standing for 20 to 30 years, old blood mixed with iron rust, blood stains soaked in various oils, and even the blood stained cloth washed thoroughly and left standing in room temperature for 6 months. Therefore, this Fibrin Plate Method seems to be the excellent one for the identification of human blood.

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IDENTIFICATION OF HUMAN BLOOD BY FIBRIN PLATE METHOD, A SUPPLEMENTAL STUDY

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Previously KUMANO[1] has demonstrated the possibility of identifying human blood by the detection of a proactivator, an enzyme protein believed to be almost specific to and abundant in human blood (called a human factor[2]) according to the method of SZOLLOSY and RENGEI[2] and ASTRUP and MÜLLERTZ[3] and stated that this method is an excellent one in practical legal medicine because it is possible to identify human blood with any material whether it is polluted or old blood stain without special treatment but just simply placing the object directly on the fibrin plate and its sensitivity is so high that the human serum in the dilution of 1: 320, 000 to 1 : 640, 000 can respond positively, but specificity of this proactivator in the blood of monkey, dog and cat is much lower, yet it does respond weakly positive.

The author studied the specificity of this proactivator to streptokinase (SK) used in the Fibrin Plate Method for identification of various bloods and found that as far as the human blood is concerned its specificity is nearly absolutely species specific. The result of this study are described in this paper.

MATERIALS

Bloods of human and domestic animals, monkey, dog, cat, rabbit, guinea pig, cow, horse, goat, pig, rat and hen served as the material.

From each blood the serum was separated and diluted to 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 and 1 : 1, 280, 000 with physiological saline solution. Small pieces of cloth were stained with each blood and dried in room temperature. Similarly fish blood stains were collected.

The human blood stains and smears of various types i. e. pieces of cloth with human blood stains left standing for one to thirty years after soaking with

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fresh human blood, pieces of blood stained cloth and blood smeared stone, sand, glass, copper, iron, zinc, wood, and leaf of tree, pieces of blood stained cloths soaked in various oil for one year, and pieces of stained cloths left standing for one year after thorough washing with various kinds of cleanser were collected.

METHODS

Preparation of fibrin plate

For the Fibrin Plate Method a petri dish of 4.5 cm in diameter with lid was used. In a dish 3 ml of 0.2-0.3% fibrinogen solution was put and a drop of thrombin solution was added with the injector and needle in size of 1/2. These were mixed sufficiently by shaking gently for 3—5 seconds. A white gelatin-like plate thus formed was left standing and its surface dried without lid in an incubator at 37° C for 30 minutes before using.

The fibrinogen solution was prepared from bovine serum in the following manner. After adding 0.1 volume of 2.5% potassium oxalate monohydrate solution to bovine blood the mixture was centrifuged at 3,000 r.p.m. for 10 minutes and to 100 ml of the supernatant so obtained 6g of tricalcium phosphate was added and let it mix in a mixer for 20 minutes. The mixture was again centrifuged for 20 minutes at 3,000 r. p. m. and its supernatant was diluted with cold distilled water to a total volume of 200 ml. While stirring this solution, 80 ml of saturated ammonium sulfate solution was added a little at a time and then a white precipitate obtained after centrifugation was dissolved in 50 ml cold physiological saline solution, and to this 100 ml cold distilled water was again mixed. With further addition of 60 ml saturated ammonium sulfate solution the mixture was reprecipitated and by centrifuging for 5 minutes precipitate was obtained. The fibrinogen solution was finally obtained by washing this precipitate once with cold distilled water and by dissolving in 40 ml diethyl barbiturate buffer, and used serveral days after the above treatment.

Streptokinase solution was prepared with Varidase (American Lederle Co.) in concentration of 0.1, 1.0, 10.0, 50.0 and 100.0 mg% with physiological saline solution.

The diethyl barbiturate buffer solution was prepared by mixture 662 ml of 0.1 *M* sodium diethyl barbiturate, 338 ml 0.1 *M* HCl and 320 ml distilled water (pH 7.8).

The thrombin solution was in the concentration of 100 units of bovine thrombin (the product of Mochida Pharm. Co. Ltd., Japan) per one ml of physiological saline solution.

Injection needles used were in size 1/2. It is desirable to have two syringes with such needles, one of which is used for treatment of thrombin solution and

the other for SK-solution.

Techniques for identification of human blood stain

When the materials are liquid, a drop of mixture of the materials and SK-solution in proportion of 3 : 1 is dropped on the center of fibrin plate. When the materials are solid, a small piece of materials with a drop of SK-solution is placed on fibrin plate. As the control, material or SK-solution only is placed on the fibrin plate. Positive result is obtained when the fibrin surrounding material dissolves lucidly. The one which showed dissolution already after two hours is recorded as ##, that after 4 hours as # and that after 8 hours as +, and the showing no dissolution is judged as negative.

RESULTS

Species-specificity and its sensitivity

According to KUMANO[1], each series of diluted sera added with 100 mg.% SK-solution in proportion of 3 : 1 was placed on fibrin plate. In the present experiment after 8 hours the author observed that human blood serum dissolved fibrin up to the dilution of 1 : 640, 000 the serum physiological saline solution, that of monkey up to 1 : 10, 000, that of dog up to 1 : 8, 000, that of cat up to 1 : 4, 000, that of rabbit up to 1 : 2, 000, and that of guinea pig up to 1 : 50, but those of other animals did not even at the undiluted original concentration (Table 1).

Table 1 Fibrinolytic activity of blood with 100 mg% SK-solution

Kinds of animals \ Dilution of blood	initial	5 × 10	1 × 10^2	5 × 10^2	1 × 10^3	2 × 10^3	4 × 10^3	8 × 10^3	1 × 10^4	2 × 10^4	4 × 10^4	8 × 10^4	1.6 × 10^5	3.2 × 10^5	6.4 × 10^5	1.28 × 10^6
human	##	##	##	##	##	##	##	##	##	##	##	#	#	+	+	−
monkey	##	##	##	##	##	##	##	#	+	−	−	−	−	−	−	−
dog	##	##	##	##	##	#	#	+	−	−	−	−	−	−	−	−
cat	##	##	##	##	#	#	+	−	−	−	−	−	−	−	−	−
rabbit	+	#	#	#	#	+	−	−	−	−	−	−	−	−	−	−
guinea pig	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
rat	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
goat	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
pig	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
cow	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
horse	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
hen	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

Pieces of cloths stained with the blood of human, dog, cat, rabbit and guinea pig dissolved fibrin with 100 mg% SK-solution but those stained with

the blood of the other animals proved to be negative.

On decreasing the concentration of SK-solution, the sensitivity was diminished and when the concentration was decreased to 0.1 mg%, it was negative even at the original concentration, except of human serum but that of monkey at the original concentration gave weakly positive result after 8 hours (Tables 2 and 3, Fig. 1). With the SK-solution in the concentration of 0.1 mg% human

Table 2 Fibrinolytic activity of blood with SK-solution at various concentrations

concentration of SK-solution	Kinds of animals	initial	1×10	5×10	1×10^2	5×10^2	1×10^3	2×10^3	4×10^3	8×10^3	1×10^4	2×10^4
100mg%	human	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	dog	++++	++++	++++	++++	++++	++++	+++	+++	+	−	−
	cat	++++	++++	++++	++++	++++	+++	+++	+	−	−	−
50mg%	human	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	dog	++++	++++	++++	++++	++++	++++	+++	+++	+	−	−
	cat	+++	+++	+++	+++	+++	+++	+++	+	−	−	−
10mg%	human	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	dog	++++	++++	++++	++++	++++	+++	+++	+++	+	−	−
	cat	++++	++++	++++	++++	+++	+++	+++	+	−	−	−
1.0mg%	human	++++	++++	++++	++++	++++	++++	+++	+++	+++	+	−
	dog	++++	++++	+++	+	+	−	−	−	−	−	−
	cat	+++	+++	+	−	−	−	−	−	−	−	−
0.1mg%	human	++++	++++	++++	++++	++++	+++	+++	+	+	+	−
	dog	−	−	−	−	−	−	−	−	−	−	−
	cat	−	−	−	−	−	−	−	−	−	−	−

Table 3 Fibrinolytic activity of blood with 0.1mg% SK-solution

Dilution of blood / Kinds of animals	initial	5×10	1×10^2	5×10^2	1×10^3	2×10^3	4×10^3	8×10^3	1×10^4
human	+++	++++	++++	++++	+++	+++	+	+	+
monkey	+	−	−	−	−	−	−	−	−
dog	−	−	−	−	−	−	−	−	−
cat	−	−	−	−	−	−	−	−	−
rabbit	−	−	−	−	−	−	−	−	−
guinea pig	−	−	−	−	−	−	−	−	−

serum reacted positively up to the dilution of 1 : 8, 000 to 1 : 10, 000 within 8 hours and 1 : 2, 000 within 4 hours (Fig. 2).

Human blood stain and smear on various objects

Fig. 1 The species specificity of human blood with 0.1 mg% SK-solution

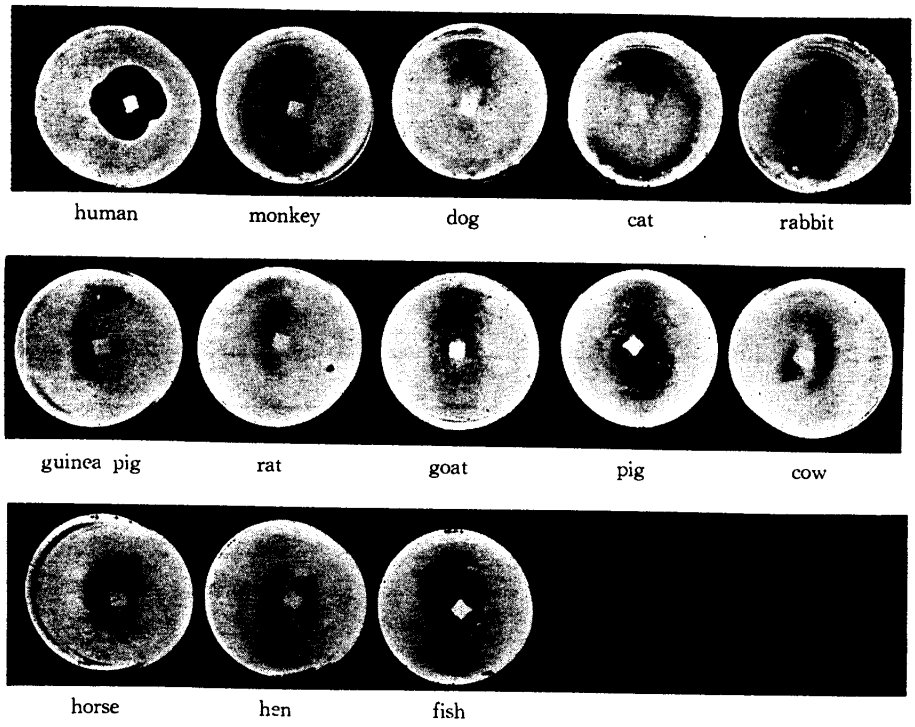
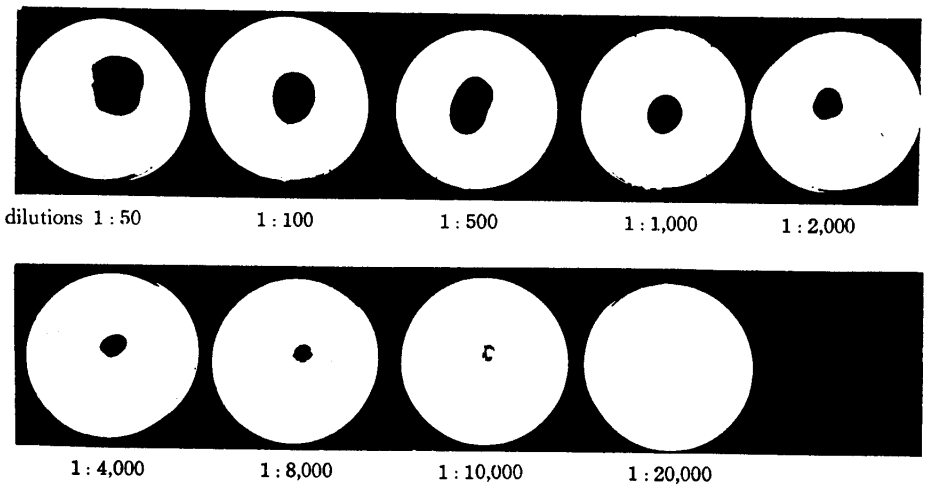


Fig. 2 The sensitivity of human serum with 0.1 mg.% SK-solution

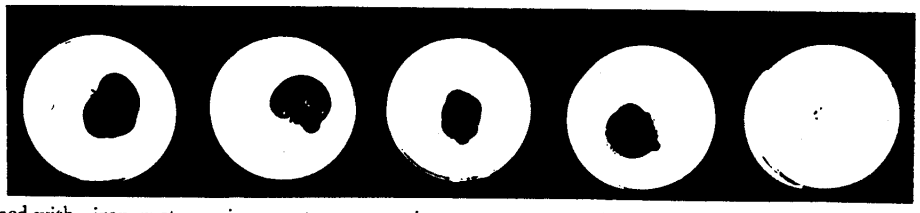


Positive result is observed up to the dilution of 1 : 8,000 of human serum

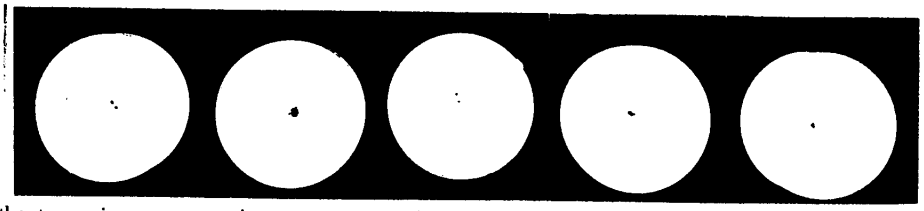
Fig. 3 The fibrinolysis of human blood smeared on various objects, with 0.1 mg% SK-solution (8 hours after the test)

stone sand glass wood leaf

Fig. 4 The fibrinolysis of human blood mixtures in various rust with 0.1 mg% SK-solution



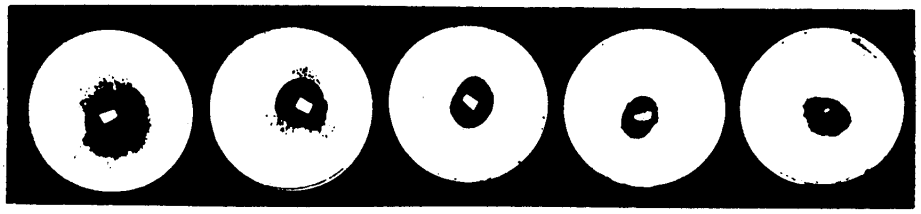
stained with human blood: iron rust, iron rust, zinc rust, zinc rust, patina



without human blood: iron rust, iron rust, zinc rust, zinc rust, patina

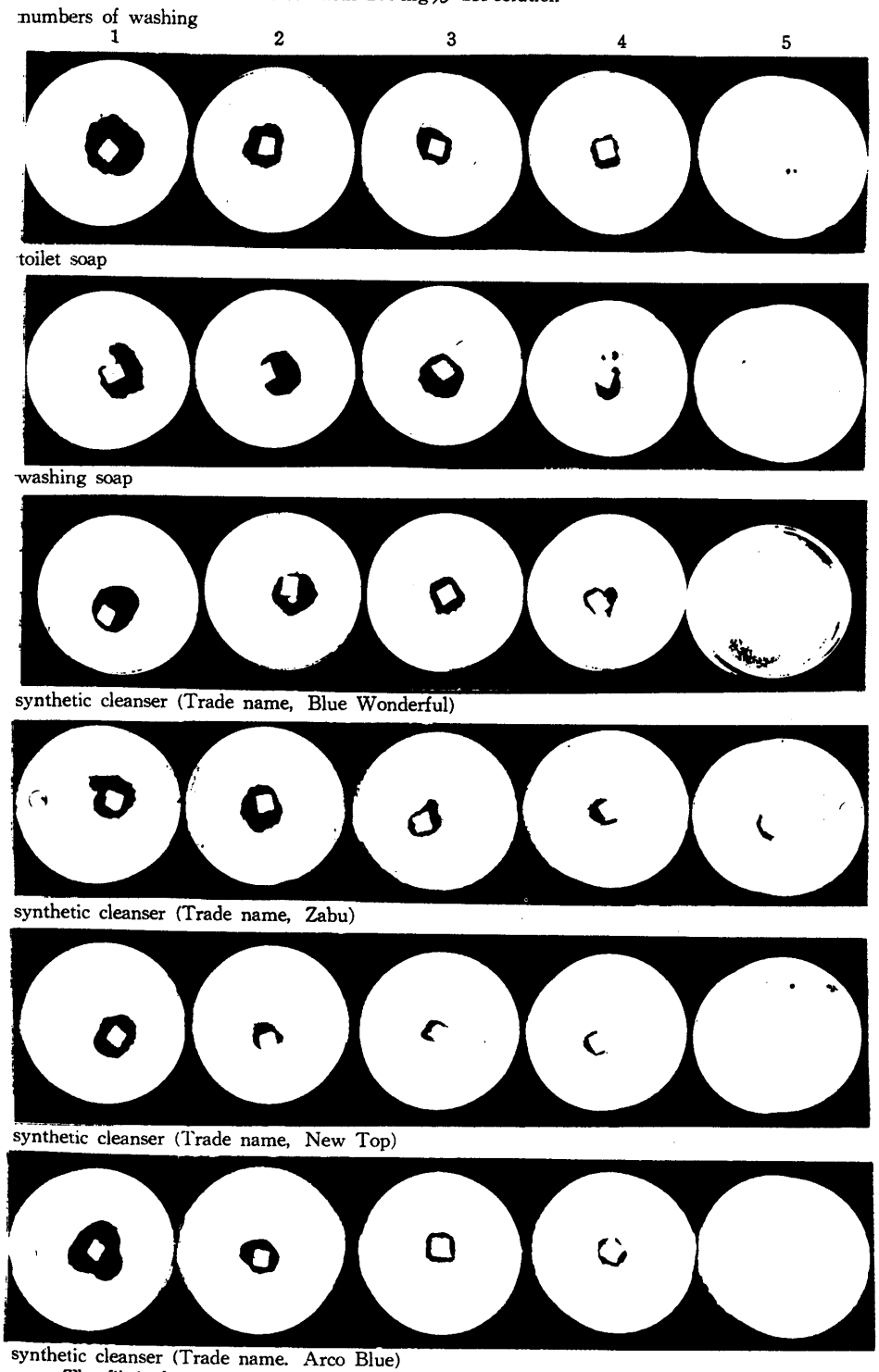
Positive result is observable in iron and zinc rust, but not in patina

Fig. 5 The fibrinolysis of old blood stained cloths, left standing 5—30 years with 0.1 mg% SK-solution (8 hours after the test)



5 yrs. 10 yrs. 15 yrs. 20 yrs. 30 yrs.

Fig. 6 The fibrinolysis of human blood stained cloths washed immediately with various cleansers with 100 mg% SK-solution



The fibrinolysis occurs after 4 washings in average (24 hours after the test)

Pieces of cloth, stone, sand, glass, wood and leaves left standing for one year after stained or smeared by human blood and placed directly on the fibrin plate dissolved fibrin with 0.1 mg% SK-solution but did not without SK-solution. However, SK-solution alone as the control dropped on the fibrin plate did not dissolve fibrin at all (Fig. 3).

Pieces of the blood stain mixed with iron or zinc rust, left standing for one year gave positive results with 0.1 mg% SK-solution, but blood smear mixed with patina and treated similarly did not dissolve fibrin (Fig. 4).

The old human blood stain

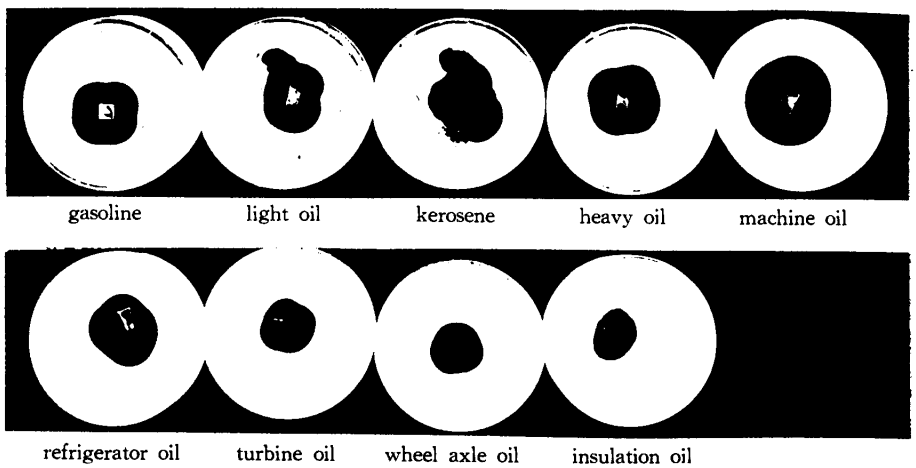
The old human blood stains left standing for one to five years showed positive results within 4 hours and very rare old human stains left standing for 20 to 30 years proved to be positive within 8 hours (Fig. 5).

The washed human blood stained cloth

Human blood stained cloth dried in room temperature was washed thoroughly with soaps or synthetic cleansers and repeating this process, samples were collected after each washing and tested after 6 months.

In this experiment ordinary toilet soap, washing soap and some of synthetic cleansers (Wonderful K, etc.) were used. The results may be summarized as follows: With 0.1 mg% SK-solution, the samples collected after first washing were positive. With 100 mg% SK-solution the samples collected within four washings in average proved positive within 24 hours (Fig. 6). However, no difference in the intensity of fibrinolytic activity between samples with soaps and with synthetic cleansers could be recognized. As the control, pieces of cloth without blood stain were tested after washing with soaps and synthetic cleansers

Fig. 7 The fibrinolysis of human blood stained cloths soaked in various oils for one year with 0.1 mg% SK-solution



and they gave all negative results. With these samples, the Leucomarachit Green Test was performed and it proved to be positive up to the third washing. The precipitation test with anti-human serum precipitin rabbit serum was positive only after one washing.

Human blood stained cloths soaked in various oils

Pieces of human blood stained cloths soaked in various oils for one year showed positive results within 4 hours. In this experiment gasoline, light and heavy oils, kerosene, machine oil, refrigerator oil, turbine oil, wheel-axle oil and insulation oil were used (Fig. 7).

DISCUSSION

KUMANO[1] studied the identification of human blood by means of detecting proactivator according to Fibrin Plate Method of SZOLLOSY and RENGEI,[2] and ASTRUP and MÜLLERTZ[3] and reported the method to be excellent but its specificity remained unclarified.

Concerning the species distribution of proactivator as proven by this method, KUMANO[1] reported that in the sera of human, monkey, dog and cat it was positive but the author found that it to be positive also in the sera of rabbit and quinea pig.

The author studied the species-specificity with this method and succeeded in demonstrating virtually absolute species-specificity by decreasing the concentration of SK-solution to 0.1 mg% and by judging the result within 4 hours. With 0.1 mg% SK-solution human serum was positive up to the dilution of 1 : 8,000 to 1 : 10,000, while the sera of other animals including that of monkey to be negative within 4 hours. Thus with this method, it is possible to identify human blood with blood stains on various objects, old blood stains left standing for 20 to 30 years and blood stained cloths soaked with various oils, important cases to be encountered in traffic accident.

In practical legal medicine it is very important to identify the blood stains on the tool or weapon of crime, so that in this investigation, human blood mixed with iron or zinc rust and left standing for one year was tested. It was found that such blood stain responded positively to the test, but that mixed with patina could not be identified. In the case where the tool of crime proves to be a fish knife, there arises a question whether the knife is stained with fish blood. Therefore, experiments were conducted with several fish blood stains on cloth and left standing for one year in room temperature and tested with 100 mg% SK-solution but the result proved to be all negative.

In the case of cloth suspected of having been already washed, it is desirable to test it with 100 mg% SK-solution and to judge the results 24 hours after the

test, because with 0.1 mg% SK-solution positive result can be obtained only after one washing but with 100 mg% SK-solution it can be identified as human blood even after 4 washings.

From these results, it is obvious that this Fibrin Plate Method has many advantages in practical legal medicine because the materials stained with human blood can be readily used as they are, and therefore, it seems to be the most excellent method available today for the identification of human blood.

SUMMARY

Following Fibrin Plate Method of SZOLLOSY and RENGEI[2], and ASTRUP and MÜLLERTZ[3], the author conducted a series of experiments in an attempt to identify human blood by detecting the proactivator believed to be one of the enzyme proteins contained abundantly in human blood.

As the results it has been found that with 0.1 mg. % SK-solution human blood alone responds to the reaction, showing almost absolute species-specificity within 4 hours but not with blood of monkey. In addition, the sensitivity is so high that it responds positively up to the dilution of 1 : 8, 000 to 1 : 10, 000 (human blood : physiological saline solution).

By means of this methcd using 0.1 mg% SK-solution it has been clearly demonstated that the identification of human blood is possible in a variety of conditions and states as may be encountered in practical legal medicine such as with blood stains in cloth, wood, stone, leaves of tree even with a trace of blood stain, old human blood stain left standing for 20 to 30 years, old blood mixed with iron rust, blood stains soaked in various oils, and even the blood stained cloth washed thoroughly and left standing in room temperature for 6 months.

Therefore, this Fibrin Plate Method seems to be the excellent one for the identification of human blood.

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