On the Examination of Seminal Stains by Exposure to Ultrasonics

2. On the Application of Acid Phosphatase Test to the Examination of Seminal Stains

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INTRODUCTION

The best method on the proof of semen is directly demonstration of spermatozoa and it is the most definite. In the previous paper the authors reported that separation of spermatozoa from seminal stains without destruction by the exposure to ultrasonics and observation microscopically by staining were the easiest and the most clear proof of the presence of semen. However, it may be impossible to prove the spermatozoa when aspermic persons including vasectomy which has recently been increasing and oligospermic persons occur sexal crimes. In this condition it is necessary to prove chemically the semen, and acid phosphatase test is most useful in the high specificity and sensitivity. The authors attempted to exposed to the ultrasonics of the proof of semen according to acid phosphatase test.

MATERIALS AND METHODS

Human semen, human seminal stains and the ultrasonic generator were the same in the report of part 1.[1)]

The acid phosphatase test was performed by King and Armstrong's method and agents of its modified method according to Kaye[3)] and Suyama[8)] was as follows: (1) acidific substrate pH 5.0 (2) phenol agent (Folin-Ciocalten agent) (3) 20 % sodium carbonate. Determination of acid phosphatase as a test for seminal stains was performed by so-called micro-method,[9)] modified method of King and Armstrong. Crushing material put into a small test tube, adding 3 drops of distilled water and was extracted by the exposure to ultrasonics. To a drop of supernatant after centrifugation, 0.2 ml of acidific substrate were

added and warmed at 37.5°C for half an hour (T. acid sample). As controls one tube containing a mixture of acidific substrate and a drop of test solution (C. acid sample blank) and another tube including a mixture of 0.2 ml of acidific substrate and a drop of distilled water (B. acid reagent blank). Into each test tube (T.B. and C.) 0.1 ml of phenol agent and 0.3 ml of 20 % sodium carbonate were added and were left standing for 20 minutes. The solution was detected by macroscopically colorimetric observation. The color judgement was slight blue ± with increase of color density + and ++ unclear blue +++, when compared with the color of B tube.

RESULTS

1) Effect of ultrasonics to the activity of acid phosphatase

On account of determination of frequency, temperature and exposed time of ultrasonics, the authors performed standard test of the 5000 times diluted solution of semen which could be easily colorimetrized macroscopically, and on the most diluted solution showing acid phosphatase positive.

The experimental groups were divided into 64 groups ie., 8 groups by the frequency, 200, 400, 600, 1200 and 2000 kc, 2 groups by the temperature, 10, 37.5°C and 4 groups by the exposed time, 10, 20, 30 and 60 minutes. These groups and unexposed semen as controls were reacted and colorimetrized. As shown in Table 1 when the temperature was 10°C and the frequency was under 200 kc, the activity of acid phosphatase did not decrease, even if the exposed time was an hour, but when the temperature was 37.5°C or frequency was over 400 kc, the activity decreased, when compared with control. (Table 1)

Table 1.

Effect of Ultrasonics on the Activity of Acid Phosphatase
(fresh semen)

temperature		10°C								37.5°C							
exposed time (min)		10		20		30		60		10		20		30		60	
		T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C
frequency (kc)	20	++	±	++	±	++	±	++	±	++	±	++	±	++	±	++	±
	50	++	±	++	±	++	±	++	±	++	±	++	±	++	±	++	±
	100	++	±	++	±	++	±	++	±	++	±	++	±	++	±	++	±
	200	++	±	++	±	++	±	++	±	++	±	++	±	+	±	+	±
	400	++	±	++	±	++	±	++	±	++	±	++	±	+	±	+	±
	600	++	±	++	±	++	±	++	±	++	±	+	±	+	±	+	±
	1200	++	±	++	±	++	±	++	±	++	±	+	±	+	±	+	±
	2000	++	±	++	±	++	±	++	±	++	±	+	±	+	±	+	±
control (unexposure)		++	±	++	±	++	±	++	±	++	±	++	±	++	±	++	±

2) Effect of ultrasonics on the acidific substrate

0.2 ml of the acidific substrate which exposed to ultrasonics under 200 kc for 10-60 minutes (this condition did not change the activity of acid phosphatase according to the result of experimental 1) was added into 5000 times diluted semen and acid phosphatase test was performed at 37.5°C. As a control, unexposed acidific substrate was added into 5000 times diluted semen and acid phosphatase test was performed. Difference between the reaction of exposed cases and unexposed case was not detected, when compared with control. Accordingly ultrasonics did not effect on the acidific substance.

3) Effect of ultrasonics on the activity of phosphatase

To the 5000 times diluted fresh semen, acidific substrate was added. To the solution, ultrasonics under 200 kc was exposed for 10 and 20 minutes at 37.5°C. Acid phosphatase test was performed. On the other hand, acid phosphatase test was performed too on the unexposed control for 10 and 20 minutes. From these two reaction results, reaction time for 10 and 20 minutes was unsufficient.

4) Effect of ultrasonics of the extraction of acid phosphatase from preserved old seminal stains

From the above experiment, it was made up clear that low ultrasonic wave such as under 200 kc did not effect on the acid phosphatase test. From 2.5mm length of a fiber or 5 mm length of 10 fibers of gauze with prolongation of preserved time, acid phosphatase was extracted by exposure to ultrasonics under 200 kc. Extraction of unexposed case was performed for 3 hours to 24 hours. As shown in the Table 2, the best condition was extraction by 20-50 kc frequency for 30-60 minutes, exposed time for 20 minutes was too short. Exposure over 100 kc caused the decrease of extraction from the over 3 years preserved seminal stains.

Table 2.

Experiment on the Extraction of Acid Phosphatase from dried Seminal Stains

preservation time		within 3 months				1 year				2 years				6 years				14 years			
exposed time (min)		10	20	30	60	10	20	30	60	10	20	30	60	10	20	30	60	10	20	30	60
frequency (kc) 20	T	###	###	###	###	###	###	###	###	###	###	###	###	###	###	###	###	±	±	±	±
	C	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
50	T	###	###	###	###	###	###	###	###	###	###	###	###	###	###	###	###	±	±	±	±
	C	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
100	T	###	###	###	###	###	###	###	###	###	###	###	###	###	###	###	###	±	±	±	±
	C	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
200	T	###	###	###	###	###	###	###	###	###	###	###	###	###	###	###	###	±	±	±	±
	C	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±

5) Effect of ultrasonics on the preserved time and amount of material

The minimum amonut of semen which showed acid phosphatase positive was determined on the unexposed semen. On the same amount of exposed semen, acid phosphatase test was performed. As shown in the Table 3 on the semen preserved for 3 months, the minimum amount was 2.5 mm length of one fiber of gauze and difference between exposed and unexposed cases was not detected, but on the semen preserved for 3 and 6 years, the detection of acid phosphatase of exposed cases were better than unexposed cases. The amount of expossed semen was 4/5 of unexposed cases preserved for 3 years and 2/3 of unexposed cases preserved for 3 and 6 years. The phosphatase test was negative in both exposed and unexposed cases preserved for 14 years.

Table 3.

Experiment on the Requisite Amount of Material

preservation time			within 3 months	1 year	3 years	6 years	14 years
length of gauze fiber			2.5mm×1	5 mm×1	5 mm×3	5 mm×3	5 mm×10
experimental method	exposed case	T	##	##	##	##	±
		C	±	±	±	±	±
	unexposed case	T	##	##	##	##	±
		C	±	±	±	±	±

DISCUSSION

A large number of acid phosphatase is contained in the secretion of the prostate. Determinaton of acid phosphatase as a test for seminal stains was first applied in the medicolegal investigation by Hausen (1946),[2] Riisfeldt (1948),[7] Landquist (1950),[6] Kaye (1947-1951)[3][4][5] and in Japan first introduced by Suyama (1954).[8] In the previous report the authors[1] reported that the exposure to the ultrasonics to seminal stains was excellent to free the spermatozoa. In this report the exposure to ultrasonics to semen was applied to the extraction of acid phosphatase.

From the above experiment it is verified that the exposure to ultrasonics under 200 kc at 10°C did not effect the activity of acid phosphatase, but over 400 kc and 37.5°C was decreased. Decrease of activity of acid phosphatase may be due to oxidation of ultrasonics in water and considerating from the enzyme preperty, the exposure at 37.5°C for 30 minutes may be altered the ferment structure. On the other hand, the ultrasonics does not bring to any effect on the acidific substrate. From the above experiment, it is verified that the exposure

to ultrasonics in a certain condition may be applied on the examination of seminal stains, so it is applied on the extraction of acid phosphatase. Acid phosphatase from seminal stains was extracted by the exposure to ultrasonics under 200 kc at 10°C for 30–60 minutes. In this condition oxidative effect of ultrasonics in water was few and strong vibration may be effective to the extraction of acid phosphatase.

The require amonut of semen for acid phosphatase test become larger with long preservation, but clear determination of semen was possible even on 6 years case. Difference between exposed and unexposed cases was not detected within 3 months, but in other cases the require amount for acid phosphatase test by exposure was 4/5 in a year and 2/3 in 3 and 6 years of the amount of unexposed cases.

CONCLUSION

Determination of acid phosphatase test in seminal stains become easier and shorter and more clear by the exposure to ultrasonics. Extraction of acid phosphatase by the exposure to ultrasonics is best condition at 20–50 kc, for 30–60 minutes.

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