Acta Medica Okayama

Volume 21, *Issue* 1 1967 *Article* 2

FEBRUARY 1967

Identification of semen from criminal materials by means of paper chromatography–a forensic-medical study

Kohichi Satoh[*] Hideo Ishizu[†] Toshio Habara[‡]

Nobuo Akiyama[**] Seishi Ueno[††] Taro Kiyotani[‡‡]

Masaru Kondo[§] Mikio Yano[¶]

[*]Okayama University,
[†]Okayama University,
[‡]Okayama University,
[**]Okayama University,
[††]Okayama University,
[‡‡]Okayama University,
[§]Okayama University,
[¶]Okayama University,

Identification of semen from criminal materials by means of paper chromatography–a forensic-medical study*

Kohichi Satoh, Hideo Ishizu, Toshio Habara, Nobuo Akiyama, Seishi Ueno, Taro Kiyotani, Masaru Kondo, and Mikio Yano

Abstract

In the present experiments attempts were made to identify semen from various specimens such as the semen itself, spots of semen on clothes, putrefied semen or semen contaminated with blood, menstrual blood, vaginal fluid, according to the techniques of LEVONEN. As the result it has been clarified that in every instance it is possible to isolate and detect the spots of choline by spraying Dragehdorff's reagent.

*PMID: 4229055 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 21, 9~14 (1967)

IDENTIFICATION OF SEMEN FROM CRIMINAL MATERIALS BY MEANS OF PAPER CHROMATOGRAPHY — A FORENSIC-MEDICAL STUDY —

Kohichi SATOH, Hideo ISHIZU, Toshio HABARA, Nobuo AKIYAMA, Seishi UENO, Taro KIYOTANI, Masaru KONDO and Mikio YANO

Department of Legal Medicine, Okayama University Medical School, Okayama, Japan (Director: Prof. Y. Mikami)

Received for publication, December 12, 1966

The identification of semen as the material evidence for crime is extremely important in legal medicine. It is most desirable to detect the spermatozoa in its morphologically-intact form, but in actual practices it is quite difficult to identify spermatozoa themselves by the light microscope. Added to this, there are difficulties in the identification of such materials as semen adhered to a few sheets of paper and the cases where criminals prove to be azoospermia, oligospermia or asthenospermia, making it practically impossible to detect the spermatozoa. In such instances, it is desirable to detect the semen by a physical or a chemical method specific to the fluid. In the past the ultraviolet irradiation method has been employed as a physical reaction test, and the methods by FLORENCE (1896), BARBERIO (1905), and PURANEN (1936), and the acid phosphatase method as chemical reaction tests. Of these methods, the acid phosphatase test is most extensively used, and there are many reports[2-7] based on the investigations conducted by this technique. While this method is an excellent one because of its high specificity, there is a danger of the seminal phosphatase losing its activity by heat (for example by ironing) as the seminal phosphatase itself is an enzymatic protein. Consequently, to overcome this disadvantage, it is sometimes necessary to combine this method with some physico-chemical methods other than the enzymatic reaction for the identification of semen.

We conducted the identification of semen by using the mixtures of semen and blood, semen and menstrual blood, semen and vaginal fluid, and putrified semen by developing each of these specimens on the paperchromatography according to the technique of EEVA LEVONEN[8] with choline and spermine derived from the semen by means of spotting.

MATERIALS AND METHODS

Materials were prepared in the following manner: 1) Semen itself obtained from the organ, 2) 3 ml semen smeared on cotton cloth, 20 cm^2 in

size and dried in a warm room, 3) semen kept in a test tube to allow it putrify in a warm room, 4) 3 ml semen mixed with 3 ml venous blood aspirated from the elbow vein and smeared on a cotton cloth, 20 cm^2 in size and dried in a warm room, 5) blood aspirated from the elbow vein, 6) a ball of absorbent cotton, 1 cm^3 in dimensions, soaked with menstrual blood on which 1 ml semen was smeared, 7) menstrual blood smeared on a ball of absorbent cotton, 1 cm^3, 8) the mixture of vaginal fluid and semen, 9) vaginal fluid alone, 10) for the control choline chloride solution, and 11) the semen taken from the vagina of a raped woman and the semen adhered to the clothes of a young girl after raping. These materials served for the paperchromatographic study.

Methods : We followed the techniques of EEVA LEVONEN. Namely, we used 0.5 ml of semen, 1 ml of blood, and 2 cm^2 of the cotton cloth smeared with the material, and 1 cm^3 size-absorbent cotton ball smeared with the material. Each of these materials is placed in a centrifuge tube and 1～1.5 ml of 20% trichloroacetic acid are added. This treatment is done for the purpose to precipitate the protein that interferes with the paperchromatography, and trichloroacetic acid can be removed later with ether. Fifteen minutes afterwards the material in the trichloroacetic acid is stirred well and the cloth or absorbent cotton ball is squeezed well, and then the liquid layer is centrifuged at 3,000 rpm for 15 min and the supernatant is placed in a funnel. Next, 1 ml water and 2 ml ether are added and shaken well, left standing and ether is removed. Again 2 ml ether are added and repeat this washing twice. After the ether washing, the water layer is put in an evaporating dish and placed over a hot water bath to dry and solidify the residue. This residue is dissolved in 1～2 drops of 0.5 N hydrochloric acid and a drop of this solution is put on the filter paper (Tôyo Roshi No. 50) at 2.5 cm from the lower margin of the paper of 2×40 cm^2 in dimensions. For the control choline chloride dissolved in 0.5 N hydrochloric acid is placed on the filter paper for a similar paperchromatography. For the solvent isopropylalcohol, glacial acetic acid and water in proportion of 5 : 1 : 4 (v/v) was used. After placing the specimen and choline chloride on the filter paper the paperchromatography was conducted for 5～7 hours in a closed chamber by the ascending method. After the development the filter paper is dried in a warm room, and by spraying Dragendorff's reagent over the paper, spots of coloration are obtained.

As for the Dragendorff's reagent, we prepare A solution consisted of 0.85% bismuthsubnitrate, 40 ml distilled water, and 10 ml glacial acetic acid, and B solution containing 8 g potassium iodide and 20 ml distilled water. When we use both A and B solutions, we take 5 ml of each solution and by adding 20 ml of glacial acetic acid and distilled water make the final volume 100 ml.

RESULTS

The day after obtaining semen: 0.5 ml of it is developed on the paper chromatography for 5～7 hours according to the method of LEVONEN. By this method two spots derived from choline and spermine respectively appear at Rf 0.74 and 0.32, but our results yielded a purple spot of a round to kidney shape near Rf 0.79. This purple spot was verified to be that of choline by the simultaneous development of the choline chloride. On the other hand, we failed to obtain clear-cut spot for spermine but there appeared an indistinct spot of irregular shape that seemed to be of spermine. However, we are not certain whether it was truly a spot of spermine or not because we could not carry out

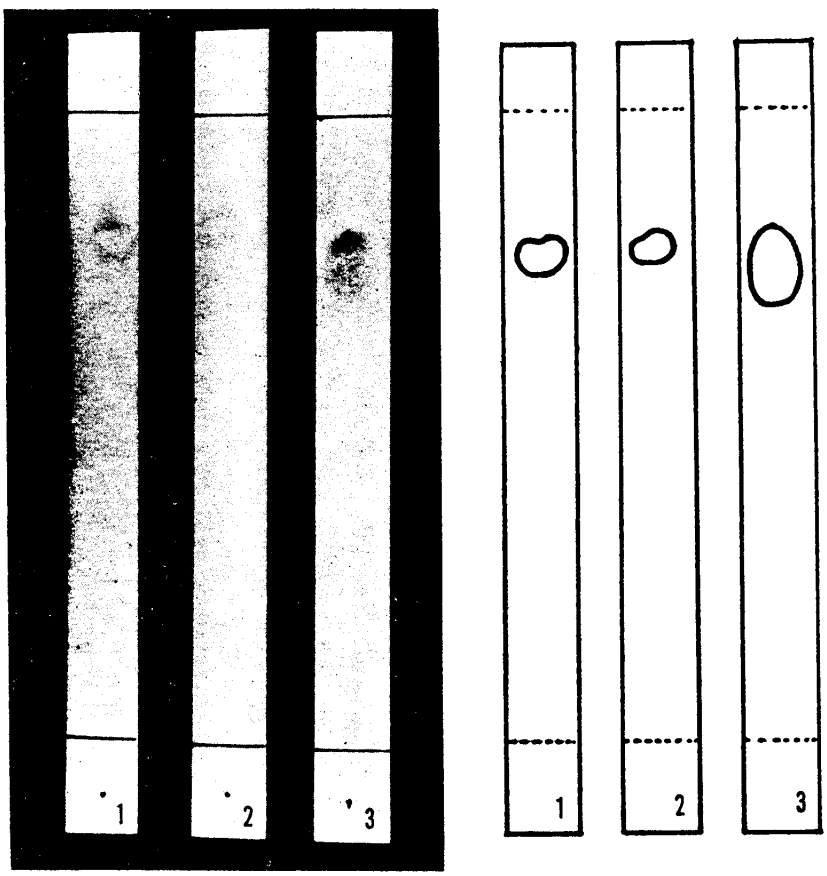

Fig. 1 Paperchromatograms of Semen
1 : Semen
2 : The mixture of vaginal fluid and semen
3 : Choline choloride as control

the simultaneous development as we did not procure the specimen of spermine. Therefore, in the subsequent experiments we followed only the spots of choline.

In the case where 3 ml semen were smeared on the cotton cloth measuring 20 cm² and allowed to dry in a warm room: A piece of cloth 2 cm² is taken from the semen stained cloth at the intervals of 1, 2, 5 days and up to two weeks for the identification, and in every case it was possible to obtain choline spots.

In the case where semen was left standing in a test tube to putrify in a warm room : It was also possible to identify the choline spots up to the tenth day but as the material dried up, thereafter no test was carried out.

With the combination of 3 ml semen and 3 ml venous blood smeared on a piece of cotton cloth measuring 20 cm² : Taking a piece of cloth 2 cm² in size at the intervals of 1, 3, 5 days and up to the second week, it was possible to identify the choline spots in every instance.

With the mixture of menstrual blood and semen : In this instance, the choline spots could be identified similarly but in the tests of menstrual blood alone no choline spot appeared.

With the mixture of vaginal fluid and semen : In this case also the choline spots were obtained, but with vaginal fluid alone no such spots appeared. It was also possible to detect the choline spots from both the material obtained from the vagina of the raped woman and that from the spots of the clothes of a young girl after being raped.

As is obvious from these findings, the spots detected in such a way are those of choline derived from semen in every case. This point was verified by the simultaneous development of choline chloride on the paperchromatography.

DISCUSSION

According to EEVA LEVONEN, when semen is developed on the paperchromatography with the solvent of isopropylalcohol, glacial acetic acid and water in the proportion of 5 : 1 : 4 (v/v), and sprayed on with Dragendorff's reagent the spots appear at Rf 0.32 and 0.74. The former spot appears as a pink rectangular one of spermine derived from semen, and the latter is a purple spot of a round to kidney shape derived from choline.

In our experiments we obtained a purple spot near Rf 0.79 and another indistinct pinkish purple spot near Rf 0.5. By the simultaneous development of choline chloride the former was confirmed to be that of choline derived from semen but as to the latter since we could not procure spermine specimen, we are not certain whether these spots were those of spermine or not as we had no way to verify them.

OHKUMA *et al.*[9] conducted according to the method of LEVONEN paperchromatography (Tôyo Roshi No. 51) after passing choline chloride, spermine phosphate and human semen through the ion-exchange resin column, using Dragendorff's reagent for coloration, and found one spot of an orange or reddish orange color at Rf 0.72~0.78 for choline chloride, one spot of an orange to reddish orange color at Rf 0.59 for spermine phosphate, and two spots for human semen, that is, one of an orange or reddish orange color at Rf 0.60 and another of an orange color at Rf 0.76.

Such discrepancies in the values of Rf seem to be due to the difference in the filter paper used by these investigators.

It has been clarified that in the identification of semen by usiug this method even the presence of other contaminants such as blood, menstrual blood or vaginal fluid in semen or in seminal fluid spots, does not at all interfere with the coloration of choline spots.

In the course of the present experiments we obtained the specimens from two cases of rape victims and performed the paperchromatography in the identical fashion. The results revealed that it is possible to detect the spots of choline derived from semen fluid, proving the materials to be semen. In these two cases it was also possible to detect spermatozoa.

CONCLUSION

In the present experiments attempts were made to identify semen from various specimens such as the semen itself, spots of semen on clothes, putrefied semen or semen contaminated with blood, menstrual blood, vaginal fluid, according to the techniques of LEVONEN. As the result it has been clarified that in every instance it is possible to isolate and detect the spots of choline by spraying Dragendorff's reagent.

ACKNOWLEDGEMENT

To Prof. Y. MIKAMI, we are indebted for much valuable advice during this work and we also wish to express our thanks to Assistant Prof. K. HABA for his assistance in many ways.

REFERENCES

1. PURANEN, U. H.: *Deutsche Ztschr. f. d. ges. gerichtl. Med.* 26, 366, 1936
2. KUTSCHER, W., and WOHLBERGS, H.: *Ztschr. f. physiol. Chem.* 236, 237, 1935
3. KAYE, S.: *J. Lab. & Clin. Med.* 34, 728, 1949
4. LUNDQUIST, F.: *Arch. Path.* 50, 395, 1950
5. FISHER, R. S.: *New England J. Med.* 240, 738, 1949
6. SUYAMA, H., ICHINOSE, K., and HIRONAKA, M.: *Kagaku to Sosa* 7, 35, 1954 (in Japanese)
7. KIMURA, Y.: *Jap. J. Legal Med.* 13, 542, 1959 (in Japanese)

8. LEVONEN, E.: *J. Forensic Sci.* 5, 102, 1960
9. OHKUMA, S. and MIYAUCHI, C.: *Reports of the National Research Institute of Police Science* 17, 1, 1964 (in Japanese)