Z. Rechtsmedizin 72, 169—173 (1973)

Originalarbeiten — Original Papers

The Electrophoretic Heterogeneity of Prostatic Acid Phosphatase*

Shokichi Ueno and Hiroshi Yoshida

Department of Legal Medicine, Toho University School of Medicine, Tokyo (Japan)

Received September 4, 1972

Summary. Human seminal fluid, when examined by horizontal starchgel electrophoresis using 0.2 M phosphate-acetic acid buffer pH 5.0 and by specific staining of prostatic acid phosphatase (PAP) of the gel using phenolphthalein diphosphate as a substrate, has been proved to show 4 or 5 distinct zones of acid phosphatase activity, thus enabling us the classification of semen into three types, F (fast), S (slow) and 0 (zero), by their patterns of electrophoretic mobility. The frequency of PAP types of unrelated Japanese males is 71.6% S, 15.8% F and 12.6% 0. The typing by this method is independent from the erythrocyte acid phosphatase (EAP) typing. This technique has been proved to be useful in medico-legal practices (in rape cases).

Zusammenfassung. Elektrophoretische Heterogenität der sauren Phosphatase in Prostata. Elektrophoretische Untersuchung von menschlicher Samenflüssigkeit mittels Stärkegelelektrophorese bei 0,2 M Phosphat-Essigsäure-Pufferlösung von pH 5,0 zeigte folgende Resultate: Elektrophoretisch werden die PAP-Aktivität in 4 oder 5 deutliche Bande abgetrennt, je nach den elektrischen Mobilitäten werden F- (schnell laufend), S- (langsam laufend) und 0- (keine Bewegung) Komponente eingeteilt, damit die Identifizierungen bzw. die Einteilungen nach der Heterogenität durch die Untersuchung von Samenflüssigkeit ermöglicht werden. Beim Japaner ist 71,6% S, 15,8% F und 12,6% 0. Die Heterogenität ist unabhängig von derselben der sauren Phosphatase in Erythrocyten. Wir haben auch festgestellt, daß die Technik in der praktischen Gerichtsmedizin (bzw. bei Notzucht) als ein unentbehrlicher Test angewandt werden kann.

Key words: PAP, in human seminal fluid — Prostatic acid phosphatase, electrophoretic heterogeneity.

Prostatic acid phosphatase (PAP) is characterized by the different sensitivity to inhibitors from erythrocyte acid phosphatase (EAP) [1]. In 1963 Hopkinson *et al.* [2] demonstrated 5 EAP phenotypes by starchgel electrophoresis, A, B, BA, CA and CB, and in 1964 by Lai *et al.* [3] a sixth type C. EAP typing is now widely utilized in medico-legal practices.

Our investigation on the electrophoresis of seminal fluids has shown conclusively the existence of heterogeneity for also PAP.

Methods and Materials

Human seminal fluid: Most of the samples were obtained from husbands of patients of the infertility clinic of Toho University Hospital and few others from voluntary donors. The samples are stored in a refrigerator (4° C) before use. For the examination, the supernatant, which obtained by centrifugation of 3000 rpm for 10 min, was diluted up to 1:100.

* Auszugsweise vorgetragen anläßlich des 6. Internationalen Kongresses für forensische Wissenschaften, Edinburgh, 21.—26. September 1972.

Human vaginal secretion: Samples were collected from out-patients of the gynaecology clinic of Toho University Hospital. These are centrifuged, and the supernatants, without being diluted, are used for the examination.

Electrophoresis was carried out on horizontal starch gels (200 — 130 — 3 mm, for 9 samples) which were prepared by solving hydrolysed starch (Connaught Medical Research Laboratories, Toronto, Canada) in gel buffer in 13%, and using Whatman No. 1 filter paper inserts under the condition of a potential gradient of 7 volts/cm for 20 hrs at 4°C. A polyethylene foil was superimposed to prevent drying of the starch gel.

Buffers: The bridge buffer is 0.2 M phosphate-acetic acid buffer, pH 5.0. It contains $Na_2HPO_4 \cdot 2\,H_2O$ (35.628 g/l) and CH_3COOH (15.5 ml/l). For the gels, the bridge buffer is diluted 1:100 and adjusted to pH 5.0. For the dilution of semen, 0.05 M citrate buffer, pH 5.0 was used, which was prepared by mixing 1.051% citric acid and 1.47% sod. citrate in a proportion of 8.2:11.8.

Localization of the enzyme on the electrophoretic gels was carried out almost according to the procedure for the EAP described by other authors [2, 4] with slight modifications stated below. The substrate consists of the pentasodium salt of phenolphthalein diphosphate, 60 mg dissolved in 10 ml of 0.05 M citrate buffer, pH 5.0. This solution is poured over the surface of the gel (not sliced), which is incubated in a covered plastic box at 37°C for 2 hrs. After the solution is poured off, a few drops of ammonia solution (s.g. 0.90) are placed in the box with the gel and the lid is replaced. The ammoniacal atmosphere makes the surface of the gel alkaline, and the areas where phenolphthalein has been liberated from the phenolphthalein phosphate by acid phosphatase are revealed as red zones.

Seminal stains: Fresh drops of seminal fluids with known PAP types were pipetted on glas, cotton and paper, and allowed to dry. These were stored in the Laboratory room at 20—25°C at different period. When used, they were redissolved in a 0.05 M citrate buffer solution, pH 5.0.

Results and Discussion

Fig. 1 shows examples of three different patterns of PAP activity so far been distinguished in different individuals. Three types will be referred to as S or "slow", F or "fast" and 0 or "zero", according to their patterns of 4 or 5 zones of enzyme activity, starting from the "origin", "first", "second", "third", "fourth" and "fifth", which migrate towards the anode just contrary to this

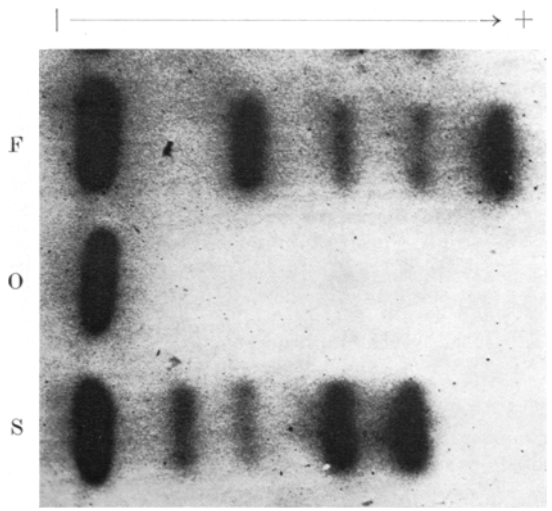

Fig. 1. Starch gel electrophoretic patterns of prostatic acid phosphatase with three types

Table 1. *Description of the three PAP electrophoretic patterns*

PAP-type	Zone of enzymatic activity						
	0	1	2	3	4	5	6
S or "slow"	+	+	+	+	+	—	—
F or "fast"	+	—	+	+	+	+	—
0 or "zero"	+	—	—	—	—	—	—

Table 2. *Incidence of PAP type*

PAP-type	Number	%
S	68	71.6
F	15	15.8
0	12	12.6
Total	95	100.0

order (Table 1). If samples do contain blood, EAP in the blood migrate towards the cathode (!!) under the above-mentioned condition of electrophoresis, and yet showing the characteristic bands which indicate their EAP types.

In almost all instances samples have been subjected to electrophoresis on more than one occasion, and in some of voluntary donors more than one sample were obtained on different occasions. The classification was always in agreement.

The appearance of above-mentioned bands of enzyme activity was totally inhibited by an addition of one drop of saturated D(+) tartrate to the substrate, however, no obvious changes in the patterns by the addition of formalin were observed.

Storage of samples for longer period in a refrigerator sometimes resulted in some modification of the pattern, with the appearance of one or more bands moving more rapidly than the fifth zone. The first zone, which is not altered by storage is most essential for the classification, and by observing this characteristic one will find no difficulty in classifying human semen.

The frequency of PAP types of 95 unrelated Japanese males, who were hitherto examined, are shown in Table 2.

Accordingly, the S type is most common, F and 0 type relatively rare, and yet these two are in more than 10% observed.

The problem of any possible relations between EAP and PAP typing attracts our interest. Blood were drawn from almost all the voluntary donors of semen, for whom EAP as well as PAP typing was performed. Table 3 shows the result of such for 22 persons which indicates clearly the independence of EAP and PAP classification (Table 3).

It is a well-known fact that the sera of prostatic cancer patients contain PAP. Three serum samples, which contained total AcP, ranging from 57 to 69, and PAP, ranging from 52 to 65, showed universally 0 type PAP. In one patient, whose

Table 3. *Independence of PAP and EAP typing*

PAP-type	EAP-type			Total
	B	BA	A	
S	4	2	3	9
F	1	2	1	4
0	5	3	1	9
Total	10	7	5	22

prostatic secretion was obtained by massage of the gland, the results for two specimens (serum and prostatic secretion) coincided with each other.

EAP of blood stains was successfully typed by some authors [5—8]. A small series of experiment for the determination of PAP type of artificially produced seminal stains were undertaken. Seminal stains on various materials, which were stored in our Laboratory room at 20—25°C for 1 or 2 weeks, have reduced their solubility and at the same time their enzyme activity to half or to one third of those originally contained, but still retained enough potency to give their correct PAP types.

Vaginal secretion was secured from out-patients of the gynaecology clinic of Toho University Hospital. Our attempt of PAP typing for these 20 samples, however, unsuccessful, giving no traces of coloration even on the origin of the electrophoretic gels. The determination of King-Armstrong unit for possibly contained PAP showed, in most of them, under 50, which is the lower limit of sensitivity of our method. This may possibly explain the above-mentioned outcome. We succeeded, however, in the typing of 3 victims out of 4 different rape cases, which lately successively broken out in this district of the city. Two were typed as S and one as 0. In one case, which showed S type, time had elapsed 7 hrs before she was brought to a clinic by a police to get the specimen. It is now our great concern to which type the culprits, who are still at large, would be belonged, and how to secure their consent for obtaining specimens, when they were arrested. These experience made us confident that the determination of PAP type of vaginal content in rape-murder case, where seminal fluid can persist in far longer period in vagina of the deceased, may be far more easy, although we are encountered by the same problem stated above.

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Professor Dr. Sh. Ueno
Department of Legal Medicine
Toho University School of Medicine
Omori, 143 Tokyo
Japan