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The Evidential Value of Peptidase A as a Semen Typing System

REFERENCE: Parkin, B. H., **"The Evidential Value of Peptidase A as a Semen Typing System,"** *Journal of Forensic Sciences,* JFSCA, Vol. 26, No. 2, April 1981, pp. 398-404.

ABSTRACT: Human erythrocyte peptidase A (Pep A) displays a genetic polymorphism in blacks. Its occurrence in human semen was examined for its possible use as a semen typing system. Studies by starch gel electrophoresis, in which the Pep A was located by an improved method, were carried out on semen, semen stains, and vaginal swabs taken at known times after intercourse. In addition, a large number of vaginal swabs, negative for semen, were taken from females throughout their menstrual cycles and examined for Pep A activity. The results indicated that Pep A typing could be carried out on semen and semen stains. However, it was possible to determine the Pep A type on vaginal swabs only when they had been taken within about 3 h after intercourse.

KEYWORDS: pathology and biology, peptidase A, genetic typing

Examination of human erythrocyte peptidase A (Pep A) by Lewis and Harris [*1*] showed it to be polymorphic in black populations, with the three common phenotypes Pep A 1, 2-1, and 2 present in approximately 86.5, 13.0, and 0.5%, respectively, of blacks living in Great Britain. Variation in other ethnic populations is confined to a few rare phenotypes [*1*]. Peptidases A, C, and D were reported to be present in human semen [*2*] and further investigations [*3*] showed Pep A activity in both the spermatozoa and seminal plasma. The Metropolitan Police Forensic Science Laboratories have been using Pep A for some years to type blood and bloodstains of suspected black origin [*4*], and it appeared suitable for use as a marker in semen typing.

The work described in this paper is an investigation into the potential of Pep A as a semen typing system suitable for use in forensic biology.

Occurrence of Peptidases in Semen

The occurrence of Pep A, Pep B, and Pep D in semen samples was investigated by starch gel electrophoresis using the peptides L-valyl-L-leucine, L-leucyl-L-glycyl-L-glycine, and L-leucyl-L-proline, respectively, as specific substrates [*1*]. A number of paired semen and blood samples from the same individuals were used.

In order to determine whether the Pep A phenotype of a male donor could be found from either his blood or semen, Pep A isozymes were examined in 51 paired blood and semen samples from the same individuals. In addition, the seminal plasma and sperm

Received for publication 13 June 1980; revised manuscript received 22 Sept. 1980; accepted for publication 17 Oct. 1980.

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were separated and both fractions were examined by starch gel electrophoresis for Pep A activity.

Semen stains prepared on a variety of materials from previously typed semen samples were stored at room temperature for various lengths of time. Other semen stains, uncontaminated with other body fluids and from donors who could be ascertained with some degree of certainty, were selected from case-work material, and the Pep A phenotype was determined.

Vaginal swabs were donated by 15 females between 20 and 40 years old who abstained from sexual intercourse for the duration of this experiment. The swabs were taken daily throughout a menstrual cycle. The Pep A activity was studied on each swab by starch gel electrophoresis. To confirm the absence of semen, the acid phosphatase level of each vaginal swab was studied by the method of Davies and Wilson (1974) [5]. In addition, each swab was examined visually for the presence of blood, which was confirmed by peroxidase activity.

Other vaginal swabs, taken at known times after intercourse, from 14 different donors, were examined for Pep A activity by starch gel electrophoresis. Each swab was also tested for acid phosphatase and peroxidase as an indication of the presence of semen and blood, respectively.

Samples

Paired blood and semen samples from the same donors were supplied by members of this laboratory and by patients attending fertility clinics.

Vaginal swabs that were free of semen and vaginal swabs taken at timed intervals after sexual intercourse were donated by staff of this laboratory.

Selected case-work material was also used after all other forensic tests had been completed.

Method

Liquid semen samples were introduced into the starch gel on pieces of Whatman 3MM chromatography paper, 0.8 by 0.1 cm. Hemolysates were placed on pieces of cotton thread 0.8 cm long. Threads or pieces of cotton wool were cut from semen-stained fabric or vaginal swabs and moistened with a minimum of gel buffer. Semen stains on nonabsorbent surfaces were swabbed onto cotton threads previously moistened with gel buffer.

For the gel buffer, 0.01*M* maleic acid solution was added to 0.01*M* tris(hydroxymethyl)-aminomethane (Tris) until pH 7.5 was reached. The bridge buffer was made by adding 0.1*M* monobasic sodium phosphate solution to 0.1*M* Tris until pH 7.4 was achieved.

The method of Wraxall and Culliford [6] was used to prepare the 1-mm-thick 10% starch gel. Electrophoresis was carried out at 2.5 V/cm for 16 h in a cold room at a temperature of about 4°C. The use of cooling plates in this technique produced inferior results.

The zones of Pep A activity were located by a modification of the method of Suguira et al [7]. A variety of dipeptides can be used as substrates for the location of Pep A [1]. Most are hydrolyzed by other peptidases, but L-valyl-L-leucine appears to be specific for Pep A and therefore was used.

The reaction mixture consisted of 10 mL 0.05*M* Tris-hydrochloric acid buffer (pH 8.0), 10 mg peptide (L-valyl-L-leucine), 5 mg L-amino acid oxidase (Sigma Chemicals) (crude, dried venom from *Crotalus admanteus*, approximately 0.3 units per milligram), 5 mg 3-(4,5-dimethyl thiazolyl-2)-2,5 diphenyl tetrazolium bromide (MTT), and 1 mg phenazine methosulfate (PMS). The reaction mixture was mixed with 10 mL 2% aqueous agar solution that had been boiled and then cooled to about 55°C. This mixture was poured over

the gel between the origin and anode and was incubated at 37°C for about 1 h. Blue zones appeared at the sites of Pep A activity.

Results and Discussion

By the use of selected dipeptides and tripeptides, it was found that Pep A and Pep B occurred in semen with the same electrophoretic mobility as the corresponding erythrocyte enzymes (Fig. 1). Semen Pep A had a higher activity and semen Pep B a lower activity than the corresponding erythrocyte enzymes. No Pep D was found in the semen samples examined. This finding is in agreement with that of Rapley et al [*8*], who reported that Pep A occurs in several tissues with a higher activity than that in erythrocytes, Pep B with less activity, and Pep D, although present in all tissues, at low levels. The tissue enzyme Pep S [*8*] was found only occasionally in semen samples and is not evident on any of the photographs shown in this paper.

In the 51 paired samples tested, the erythrocyte Pep A of 49 donors was typed as Pep A 1 and of 2 individuals, as Pep A 2-1. No donors of Pep A 2 were found in this survey. In each case, the Pep A phenotype obtained from the semen sample was the same as that of the corresponding erythrocyte sample (Fig. 2, Samples 5 to 8). Thus it appears that Pep A in semen has the same phenotype pattern as in the corresponding erythrocytes. This is an important finding as it eliminates the necessity of obtaining a semen sample from a suspect of a sex crime when the Pep A phenotype has been determined from a blood sample.

When samples of lysed spermatozoa and seminal plasma that was free of spermatozoa

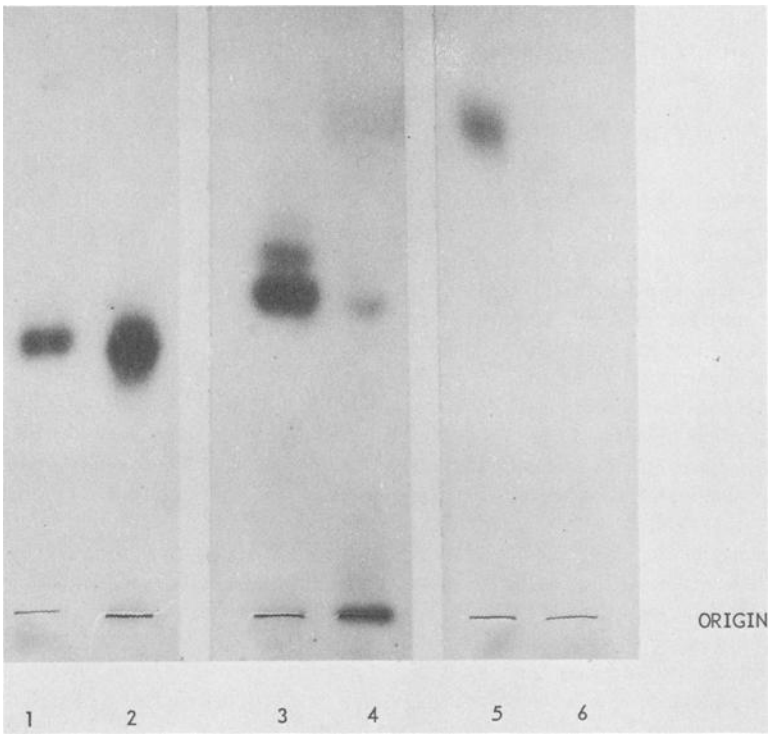

FIG. 1—*Peptidases A, B, and D in semen and erythrocytes. Samples 1, 3, and 5, lysed erythrocytes; 2, 4, and 6, liquid semen. Substrate for Samples 1 and 2,* L*-valyl-*L*-leucine; for 3 and 4,* L*-leucyl-*L*-glycyl-*L*-glycine; and for 5 and 6,* L*-leucyl-*L*-proline.*

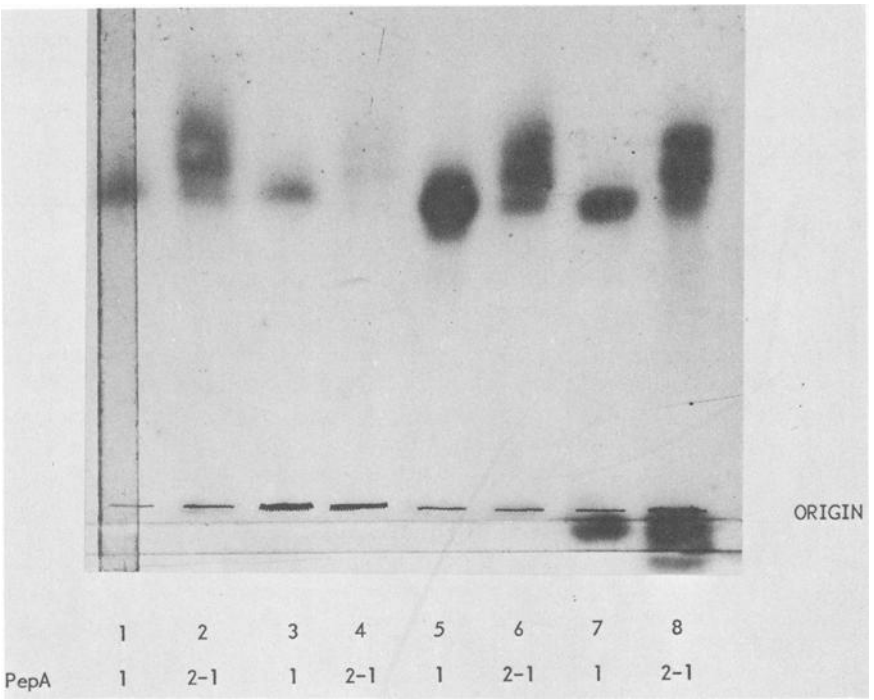

FIG. 2—*Peptidase A in semen stains (Samples 1 to 4), liquid semen (Samples 5 and 6), and lysed erythrocytes (Samples 7 and 8).*

were examined by starch gel electrophoresis, the seminal plasma gave strong Pep A activity but the lysed spermatozoa gave no activity. Thus, Pep A can be determined in semen samples that contain no spermatozoa.

The Pep A typing of 40 semen stains made in the laboratory and stored at room temperature showed that the phenotype of these stains could be determined during periods of storage up to six weeks (Fig. 2, Samples 1 to 4), which defines the survival time of the more labile Pep A 2-1 phenotype. The Pep A 1 phenotype can be easily typed up to three months (Table 1).

As the stain ages, the bands of Pep A activity fade evenly, with no preferential loss of any band in the 2-1 phenotype. No additional bands form during this aging and hence the risk of mistyping aging semen stains is minimized. The extended survival time of the Pep A 1 phenotype is presumably due to the greater concentration of enzyme in its major component than in any of the bands in the Pep A 2-1 phenotype.

A further 46 semen stains, uncontaminated with other body fluids and from donors who could be ascertained with some degree of certainty, were selected from case-work material and the Pep A phenotype was determined. These stains were also accurately typed up to six weeks (Table 1).

No acid phosphatase activity was found on any vaginal swab during this experiment. Peroxidase activity was found only on swabs bearing visible traces of blood, indicating menstruation.

For semen-free vaginal swabs, Pep A activity was found only on the swabs visibly stained with menstrual blood. On no occasion did a blood-free, semen-free vaginal swab show any

TABLE 1—*Peptidase A typing results on a variety of selected semen stains.*

Type	*n*	Pep A 1[a]	Pep A 2-1[b]	Pep A 2	No Activity	Number Wrongly Typed
Laboratory-made stains	40	29	5	0	6	0
Case semen stains	46	31	5	0	10	0

[a] Up to three months old.
[b] Up to six weeks old.

Pep A activity. This result indicates that Pep A activity is absent from vaginal secretions and occurs in the vagina only as a component of blood during menstruation.

Examination of Pep A activity on vaginal swabs taken at known times after sexual intercourse showed (Fig. 3) that only swabs taken within 3½ h after sexual intercourse showed any Pep A activity and that no Pep A activity was found on any swab taken later than 3½ h after intercourse.

These results are rather surprising in that a gradual falloff in the proportion of swabs that can be typed would be expected. There is no obvious reason for this sharp cutoff; it is possible that when swabs from a larger number of donors are examined, a greater spread in the survival time of seminal Pep A may occur.

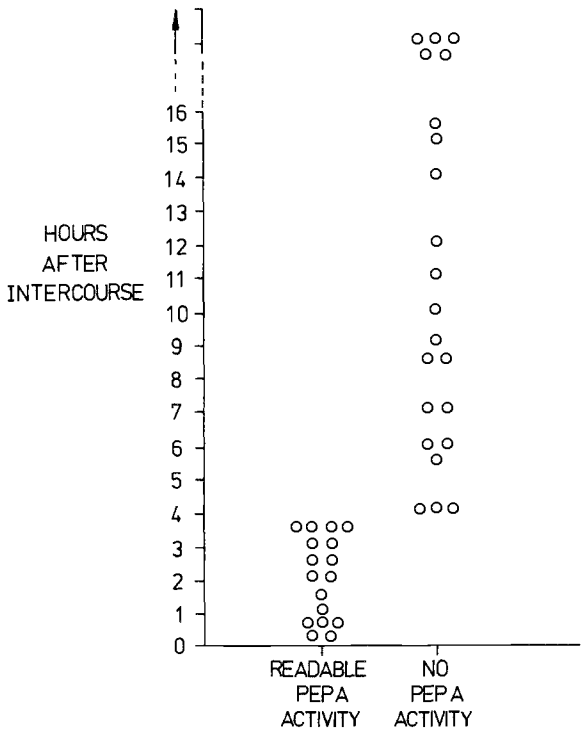

FIG. 3—*Peptidase A results on vaginal swabs taken at known times after intercourse.*

Distribution of Phenotypes

The incidence of Pep A phenotypes in several ethnic population groups living in southeast England, selected from suspects and victims of crimes of violence, has been compiled by the Metropolitan Police Force Forensic Science Laboratories (Table 2). The populations considered were composed of 383 Eastern Mediterranean people (such as Greeks and Turks), 1629 blacks (African and West Indians living in the United Kingdom), 587 South Asians (mainly Indians and Pakistanis), 41 East Asians (mainly Chinese), and 197 Middle Eastern people (Arabs).

In this sample of 1629 blacks, 210 individuals of Pep A 2-1 and 9 of Pep A 2 phenotypes were found. This gave an observed incidence of 12.89% for the Pep A 2-1 and 0.55% for the Pep A 2 phenotype, with a gene frequency of 0.0700 for the Pep A^2 allele. The sample of 197 Middle Eastern bloods showed 4 Pep A 2-1 phenotypes, giving an observed gene frequency of 0.0102 for the Pep A^2 allele. These observed data are in good accord with expectation, given the calculated gene frequencies. The introduction of the Pep A^2 allele into the Arab population may have been the result of gene flow by contact with black races in the past.

No variation was found in the Eastern Mediterranean, South Asian, and East Asian populations examined.

Conclusions

1. Peptidase A occurs in seminal plasma with the same phenotype pattern as in blood, allowing the semen Pep A type to be determined from a blood sample.
2. Semen Pep A can be typed in a dry semen stain, free from other body fluids for up to six weeks.
3. There is no vaginal Pep A detectable by starch gel electrophoresis, but blood (menstrual or resulting from tissue damage) can show Pep A activity.
4. Seminal Pep A could not be detected on a vaginal swab taken later than 3½ h after intercourse.

TABLE 2—*Incidence of Pep A phenotypes in various populations in southeastern England.*

Population Group	Total	Pep A 1	2-1	2	Gene Frequencies	
					Pep A^1	Pep A^2
Eastern Mediterrean (such as Turks, Greeks)						
n	382	382	0	0	1.0000	0.0000
Observed %	...	100	0	0	...	...
Blacks (African, West Indian)						
n	1629	1410	210	9	0.9300	0.0700
Observed %	...	86.56	12.89	0.55	...	...
South Asian (Indian, Pakistani)						
n	587	587	0	0	1.0000	0.0000
Observed %	...	100	0	0	...	...
East Asian (Chinese, Japanese)						
n	41	41	0	0	1.0000	0.0000
Observed %	...	100	0	0	...	...
Middle Eastern (Arabs)						
n	197	193	4	0	0.9898	0.0102
Observed %	...	97.97	2.03	0	...	...

Acknowledgments

I should like to thank Dr. Elizabeth Wilson of this laboratory for her help in organizing the collection of vaginal swabs and Ms. Lynn Altass for technical assistance.

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