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Genetic Markers in Human Semen: A Review

The utilization of genetic typing in the analysis of body fluids and secretions is an important objective in forensic science; however, before this can be done several questions must be answered. It must be known what genetic markers are present in the fluid of interest and what kinds of nongenetic variability enter into the phenotypic expression of these markers. The stability of the markers must be known, and procedural problems specific to the analysis of a particular fluid must be recognized. It is only after these questions are satisfactorily answered that protocols of analysis which are relatively reliable can be developed.

In this brief review we consider the current state of knowledge regarding the expression of genetic variation in human semen. Basically the genetic markers of semen can be partitioned into two categories: the cell surface antigens and the proteins and enzymes. These categories reflect the chemical nature of the markers and the methods used for their detection. The cell surface antigens, for example, are detected by immunological procedures, usually agglutination reactions or some variation thereof. In the context of this discussion, the soluble ABO antigens found on the soluble secretor substances are considered "honorary" cell surface antigens. Genetic variation of proteins and enzymes, on the other hand, is usually detected by electrophoretic procedures. Both intracellular and extracellular proteins and enzymes are included in this category. Also included are the immunoglobulin markers Gm and Inv, for although they are detected by immunological procedures, they are carried on soluble proteins. Neither category of genetic variation is without unresolved problems. We hope that in this review some of these problems will be clarified; this should serve as a guide for further research.

Cell Antigen Markers in Semen

Semen has been examined for the presence of a number of cell antigen markers; these are listed in Table 1. Considerable attention has been given to the expression of the

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TABLE 1—*Antigen systems in human semen.*[a]

Antigen System	Seminal Plasma	Sperm	Reference
ABO	+	...	1,2, many others
ABO	...	+	6,7,8,9,10,11,12,18
ABO	...	−	14,15,16
Rh D	...	+	20
Rh D	...	−	9,16,21
MN	...	+	12,16
Tja (P system)	...	+	16
Lewis	+	...	23
HL-A	+	...	22
HL-A	...	+	19,37,38

[a] (+) indicates the antigen system was present; (−) indicates the antigen system was absent.

ABO antigens in sperm and in seminal plasma; other antigens have been studied to a lesser extent.

The presence of the secreted A and B antigens in seminal plasma was established in 1926 by Yamakami and Shirai [*1*]; they demonstrated the potential usefulness of this genetic marker system in medicolegal investigations by showing that these antigens could be detected by hemagglutination inhibition procedures in saline extracts of semen stains that were up to four months old. The titer of secreted ABO(H) antigens is generally equal to or greater than the titer in saliva [*2,3*]; unfortunately, further quantitative information seems to be lacking. It is not known, for example, how much quantitative variation there is in the levels of A and H secreted substance in the semen of A secretor individuals. This information is pertinent since it has been shown by Clarke et al [*4*] that in saliva there is significant variation in the ratios of A:H and B:H in A and B secretors. They note that this variation might account for cases of "aberrant" secretion types in which an individual of blood type A may be classified as an O secretor due to an extremely low A:H secretion ratio. In a similar vein, McNeil et al [*5*] noted significant variation in the levels of A substance in A_2 and A_2B secretor individuals. It is clear that further study is needed to determine whether similar problems are associated with the ABO(H) secretion in semen.

The ABO typing of semen traces of secretors by hemagglutination inhibition procedures should not lead to difficulty. However, because the secretor substances are water soluble, attempts to type secretor positive semen by absorption-elution procedures may result in false negatives; the soluble antigens may be discarded during the washing stages of the procedure or the absorbed antibody and antigen may elute together, thus inhibiting agglutination of the test cells.

Although the existence of soluble ABO antigens in the seminal plasma of secretors is not in question, there is considerable controversy concerning the expression of these antigens on the surface of spermatozoa. The first report that the ABO antigens were to be found on sperm was made by Landsteiner and Levine in 1926 [*6*]; the possibility that ABO expression might be related to secretor status was not recognized at that time. However, in the many subsequent reports describing the presence of the ABO antigens on sperm, the role of secretor status has been a point of contention. In some reports it has been asserted that the expression of the ABO antigens on sperm is independent of secretor status [*6–12*]. This is consistent with the fact that ABO expression in most tissues is also independent of secretor status [*13*]. However, other investigators report that the ABO antigens are to be found on the sperm of secretors but not on the sperm of nonsecretors [*14–16*]. This observation is accounted for by the hypothesis that the

ABO antigens are not integral to the sperm membrane, but rather are absorbed from the seminal plasma, much as the Lewis Le^a antigen is absorbed onto the red cells of nonsecretors. This hypothesis is supported by experiments in which sperm from nonsecretors were shown to absorb ABO antigens after incubation in secretor seminal plasma. Thus the relation of secretor status and ABO expression on sperm remains somewhat ambiguous.

This controversy has obvious significance for the forensic analysis of semen. If the ABO antigens are on sperm regardless of secretor status, then it should be possible, at least in principle, to type semen for ABO independent of secretor type. In typing the cell surface antigens, however, the soluble antigens in secretors may interfere. If, on the other hand, the expression of ABO in semen is dependent on secretor status, then the typing of semen should be directed toward the detection of soluble antigens. That there is a controversy at all about the expression of ABO antigens on sperm indicates that there are significant methodological problems.

A second controversy surrounding the expression of the ABO antigens on sperm centers on the question of haploid expression. Normal human somatic cells are diploid; that is, they contain two homologous sets of chromosomes, two copies of every gene. Sperm, on the other hand, contain a haploid chromosome set, one copy of every gene. Thus, in a type AB individual, half the sperm will be carrying the A gene and half will be carrying the B gene. If there were haploid expression, then those sperm carrying the A gene would have only A antigen on their surface; similarly, the sperm carrying the B gene would have only B antigen on their surface. The implication of haploid expression to the forensic analysis of semen is apparent; it would be possible to distinguish an individual of genotype AO from an individual of genotype AA, thus improving the discrimination power of the ABO system.

Whether in fact there is haploid expression of sperm antigens is a point of controversy (see Beatty, Ref *17*, for a review of this subject). There are several reports in the literature that present evidence for haploid expression of sperm antigens [*7,8,10–12,18,19*]; however, these observations have not been confirmed by other investigators [*14–16*]. Moreover, there are fundamental biological and biochemical arguments against haploid expression [*17*]. For these reasons the question of haploid expression should be treated with considerable caution.

For the most part the other antigenic systems have received less attention than the ABO polymorphism; perhaps for this reason, there is less controversy regarding their expression. Majsky and Hraba detected the D antigen of the Rh system [*20*], but their results could not be confirmed by three other groups [*9,16,21*]. The M and N antigens of the MN system have been found on sperm by two groups [*12,16*]. Vulchanov and Popivanov suggest the haploid expression of MN [*12*]; their claim should be taken with the same caution as the claims for haploid expression of ABO. This reservation should also be applied to the claim of haploid expression for the HL-A antigens [*19*]. The HL-A antigens also appear to be carried on soluble substances in the seminal plasma; the origin of these soluble substances is not known [*22*]. There are single reports that the Tj(a) antigen is on sperm [*16*] and that the Lewis antigens are present in seminal plasma [*23*]; this latter finding is not surprising given the relationship of Lewis antigens with ABO and secretor.

It is apparent that there are ambiguities relating to our knowledge of the expression of the blood group antigens in semen, particularly with respect to the antigenic markers on sperm. These ambiguities may stem from several sources. One source is that the procedures used for the detection of sperm antigens differ considerably in their degree of specificity, limits of detection, and sensitivity to interferences. Moreover, procedures developed for the detection of antigens on blood cells may not be completely appropriate for the detection of antigens on sperm. Sperm are notoriously sticky; they will non-

specifically self-agglutinate and can absorb proteins from protein solutions. This stickiness may lead to the nonspecific absorption of small amounts of antibody which might account for some of the discordant findings. Also, as has been noted, seminal plasma may contain significant levels of soluble antigens, and some of these may be difficult to wash off sperm preparations. In addition, the presence of soluble antigens may interfere with antibody absorption tests. Lastly, investigators may have failed to recognize possible interferences by the nonsperm cellular debris which may comprise up to 35% of the cells in semen [*24*].

In summary, our state of knowledge is incomplete regarding the expression of blood group antigens in semen. The situation with the secreted ABO(H) antigens is relatively settled; the primary deficiency is the lack of extensive quantitative data. There is much more uncertainty regarding the expression of antigens on sperm; until there is a more satisfactory resolution of these uncertainties, it is wise to be cautious.

Enzyme and Protein Polymorphism in Semen

Over the course of the past decade it has become apparent that a great many proteins and enzymes in blood plasma and in red cells are genetically polymorphic. We have recently completed a survey of semen for the presence of these known polymorphisms. More than 25 polymorphic loci have been investigated in the course of this survey; some 15 are expressed in semen. An account of this work was presented at the International Meeting of Forensic Sciences in Zurich [*25*]. To establish that proteins known to be genetically polymorphic are present in semen, we followed a two-step procedure. The first step was to determine whether a protein with the activity of a known polymorphic protein was present either in the plasma or sperm fractions of semen. This has been accomplished either by immunological reactivity or by the detection of specific enzyme activity. Absence of activity at this step was taken as evidence that the protein under consideration was not present in semen.

If the activity was found in semen, then the second step was to show that the activity was the product of a genetically polymorphic locus. This question is pertinent because some proteins and enzymes exist in both genetically variable and genetically monomorphic forms; phosphoglucomutase (PGM), for example, is polymorphic at two loci (PGM_1 and PGM_3) and monomorphic at a third (PGM_2). In several cases it was demonstrated that the activity found in semen did not include the genetically polymorphic form. This situation is illustrated in the case of pepsinogen (Fig. 1). Group I pepsinogens are found in urine and have been shown by Samloff and Townes to be genetically polymorphic [*26*]. The pepsinogen found in seminal plasma, on the other hand, belongs to Group II; these pepsinogens are genetically monomorphic [*27*]. Thus, although there is pepsinogen activity in semen, that activity cannot be used as a genetic marker for individualization. Similar considerations apply in the case of acid phosphatase; the acid phosphatase activity found in seminal plasma and in sperm is quite distinct from the polymorphic acid phosphatase activity found in red cells [*28*].

The presence of genetically variable proteins in semen was verified by comparing the phenotypic expression in blood. We have been able to compare blood and semen from individuals of different phenotypes for almost every polymorphism; concordance of phenotypic expression was considered proof that the marker was present.

Table 2 lists the genetic markers that we have found to be absent from human semen. In each case we have determined our limits of detectability with known amounts of the proteins in question. If any of these markers are present at levels lower than these limits, genetic typing would be impractical.

Table 3 lists a number of the polymorphic proteins which have been found in seminal plasma or sperm. There are 16 genetic markers listed in this chart; of these, five are

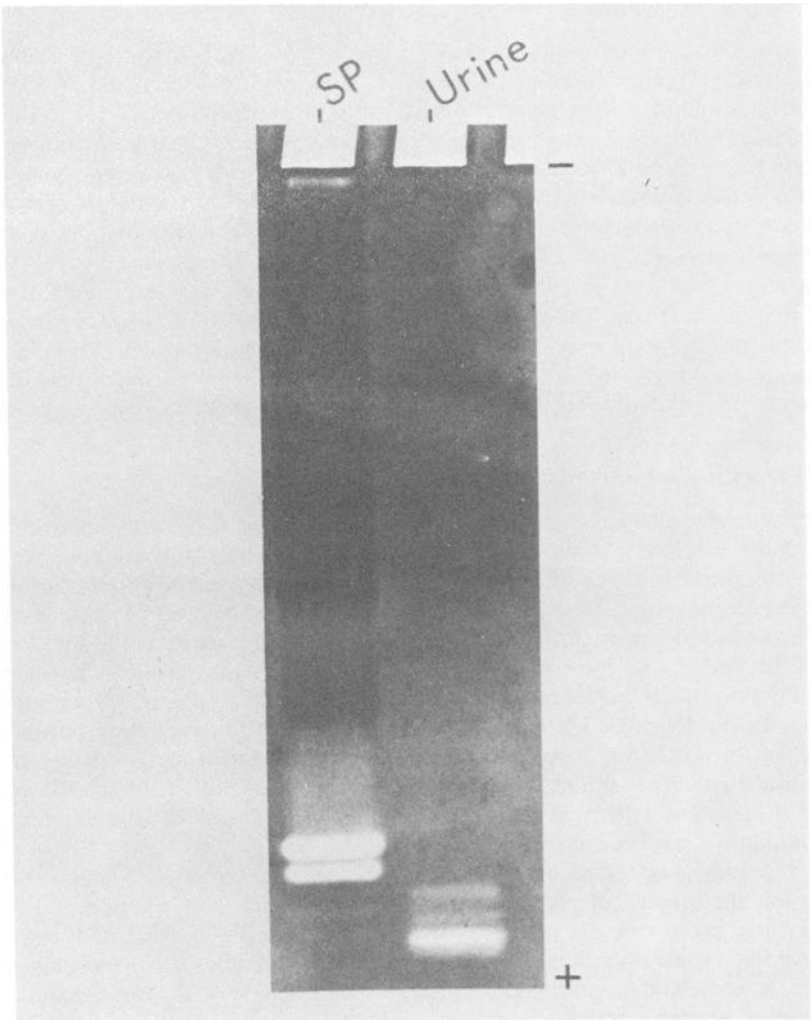

FIG. 1—*Electrophoretic demonstration of the seminal plasma (SP) and urinary pepsinogens. The seminal pepsinogen is characterized by a double banded pattern and a slower mobility than the urinary enzyme. The pepsinogens found in urine and in seminal plasma are the products of different genetic loci; the urinary enzyme is genetically polymorphic, whereas the seminal plasma enzyme is monomorphic.*

TABLE 2—*Protein genetic markers which are not found in human semen.*

Acid phosphatase (red cell)	Glutathione reductase
Adenosine deaminase	Group specific components (Gc)
Alcohol dehydrogenase	Haptoglobin
Alkaline phosphatase	Malic enzyme (mitochondrial)
Ceruloplasmin	Pepsinogen (locus 1)
Complement, third component	Pseudocholine esterase

found exclusively in seminal plasma, seven are found predominantly in sperm, and three are found in both. A discrimination index for each polymorphism is listed at the right of the table; these discrimination indexes apply to a Caucasian population base.

TABLE 3—*Protein genetic markers found in human semen.*

Marker	Seminal Plasma	Sperm	Discrimination Index[a]
α_1-Anti-trypsin	+	−	0,19
Adenylate kinase	±	+	0.18
Amylase (Amy_2)	+	−	0.18
Esterase D	±	+	0.31*
Glucose-6-phosphate dehydrogenase	−	+	*
Gm immunoglobulin types	+	−	0.82*
Inv immunoglobulin types	+	−	0.31*
Peptidase A	+ + +	+ + +	0.55*
Peptidase C	−	+	0.02
Peptidase D	−	+	0.04
6-Phosphogluconate dehydrogenase	−	+	0.09
Phosphoglucomutase (PGM_1)	+ + +	+ + +	0.53
Phosphoglucomutase (PGM_3)	−	+	0.55
Phosphoglucose isomerase	+ + +	+ + +	0.02
Transferrin	+	−	*
Sperm diaphorase	−	+	0.59

[a] The discrimination index is defined as the probability that two individuals, selected at random from a large population, will differ with respect to the indicated genetic marker. The data base is a generalized Caucasian population; the asterix (*) designates significantly different values apply to other population groups. In black Americans, for example, the discrimination index for transferrin is about 0.15 and for G-6-PD, about 0.41.

It should be noted that some markers have different discrimination indexes in non-Caucasian populations; transferrin, glucose-6-phosphate dehydrogenase, Gm, and esterase D are notable examples.

The nonenzymatic proteins have been detected by immunochemical means. These proteins include α_1-anti-trypsin, transferrin, and immunoglobulins A and G; the immunoglobulins contain the Gm and Inv markers. Generally, the level of these proteins in seminal plasma is only on the order of 1 to 5% of the level normally found in serum; in addition, there appears to be considerable individual variation in the levels of some of these proteins, particularly the immunoglobulins. These factors contribute to the difficulty in typing Gm and Inv in semen.

The genetically polymorphic enzymes of semen have been characterized principally by electrophoresis. In some cases minor idiosyncratic differences in phenotypic expression are observed between the enzymes found in sperm and red cells. This is illustrated in Fig. 2 for phosphoglucomutase [*29–32*]. The gel on the left shows the PGM patterns of red cell hemolysate and seminal plasma from PGM 1 and PGM 2 individuals. It can be seen that the expression of PGM in seminal plasma differs from that in blood in two respects. First, there is very little expression of the PGM_2 locus in seminal plasma in contrast to that found in red cells. Secondly, in seminal plasma the slower band in the double band pattern is much more intense than the faster; again this is in contrast to red cells in which both bands are approximately equal in intensity.

The gel on the right shows PGM patterns of seminal plasma and sperm from PGM 1 and 2 individuals. As occurs in seminal plasma, the expression of the PGM_1 locus in sperm is much stronger than the expression of the PGM_2 locus. In addition, the sperm samples contain a tertiary band from the PGM_1 locus. This band is approximately in the "d" position for the PGM 1 homozygote and in the "e" position for the PGM 2 homozygote. This phenomenon may lead to difficulties in interpretation and might account for the problems encountered by Rees and Rothwell [*31*]. However, for the most part, PGM typing of semen would appear to be reliable provided this possible interpretational problem is taken into account.

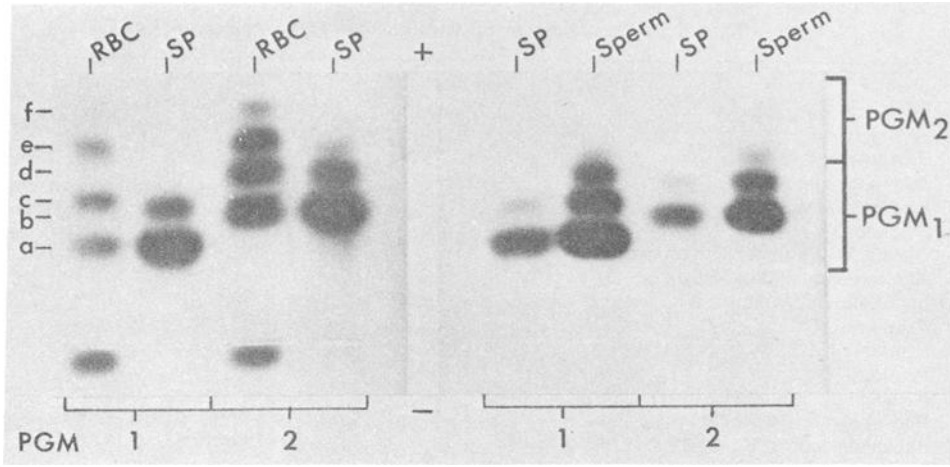

FIG. 2—*The expression of phosphoglucomutase in seminal plasma and sperm differs from the expression of this enzyme in red cells in several respects (see text for details).*

The PGM_3 locus is also present in sperm [*29*]; however, it can only be visualized in concentrated sperm extracts which are allowed to overstain in the PGM_1 region (Fig. 3). This limits the practical usefulness of the PGM_3 locus as a genetic marker.

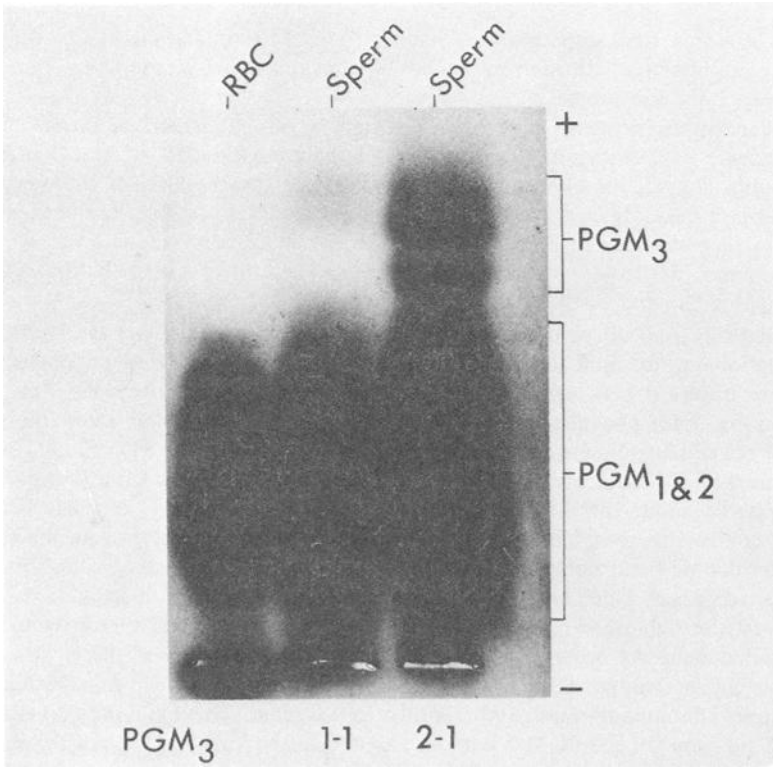

FIG. 3—*The PGM_3 locus is also expressed in sperm, but it can only be visualized in concentrated sperm extracts.*

Idiosyncratic phenotypic expression is also seen with the 6-phosphogluconate dehydrogenase in sperm. When the sperm and red cell enzymes are compared electrophoretically, it is observed that the sperm enzyme has a more rapid anodal mobility (Fig. 4). However, if the red cell hemolysate is incubated for a short time with small

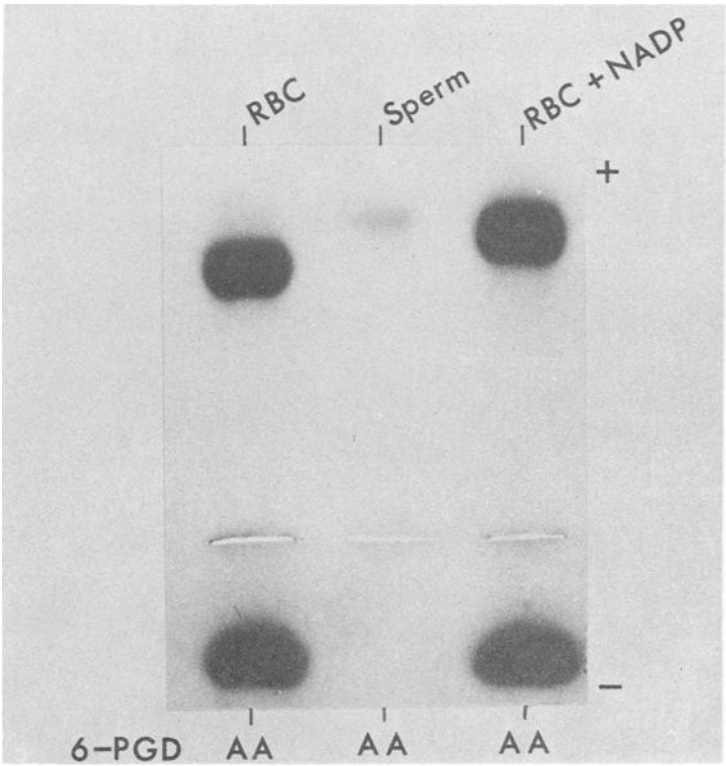

FIG. 4—*The coenzyme NADP binds to the 6-phosphogluconate dehydrogenase of sperm in vivo, shifting its electrophoretic mobility towards the anode. The same effect can be achieved in hemolysates by incubating them with NADP prior to electrophoresis.*

amounts of nicotinamide-adenine dinucleotide phosphate (NADP), the two enzymes have the same mobility. This clearly shows that the initial differences in mobility are the result of differences in coenzyme binding; the sperm enzyme in vivo is saturated with NADP whereas the red cell enzyme is not. A greater change in mobility is seen in the AA homozygote pattern than in the CC homozygote pattern; this may indicate that the "A" enzyme binds NADP more strongly than the "C" enzyme. This anodal shift is distinct from the appearance of cathodal bands described by Carson and his co-workers [*33,34*]. This anodal shift should not cause any interpretive problems if NADP is included in the gel, as is the practice in many laboratories.

A difference between semen and blood is also seen in the expression of the adenylate kinase (AK) polymorphism. In this case the difference is the marked instability of one of the AK phenotype patterns (Fig. 5). The upper gel shows red cells and freshly prepared seminal plasma and sperm from Type 1-1 and 2-1 individuals. One can see that most of the AK activity is found within the spermatozoa and that the pattern for the sperm enzyme matches the red cell enzyme for both phenotypes. However, when these same samples were stored 24 h at 4°C and re-analyzed by electrophoresis, there is a marked decrease in the activity of the "2" band in the heterozygote relative to the other

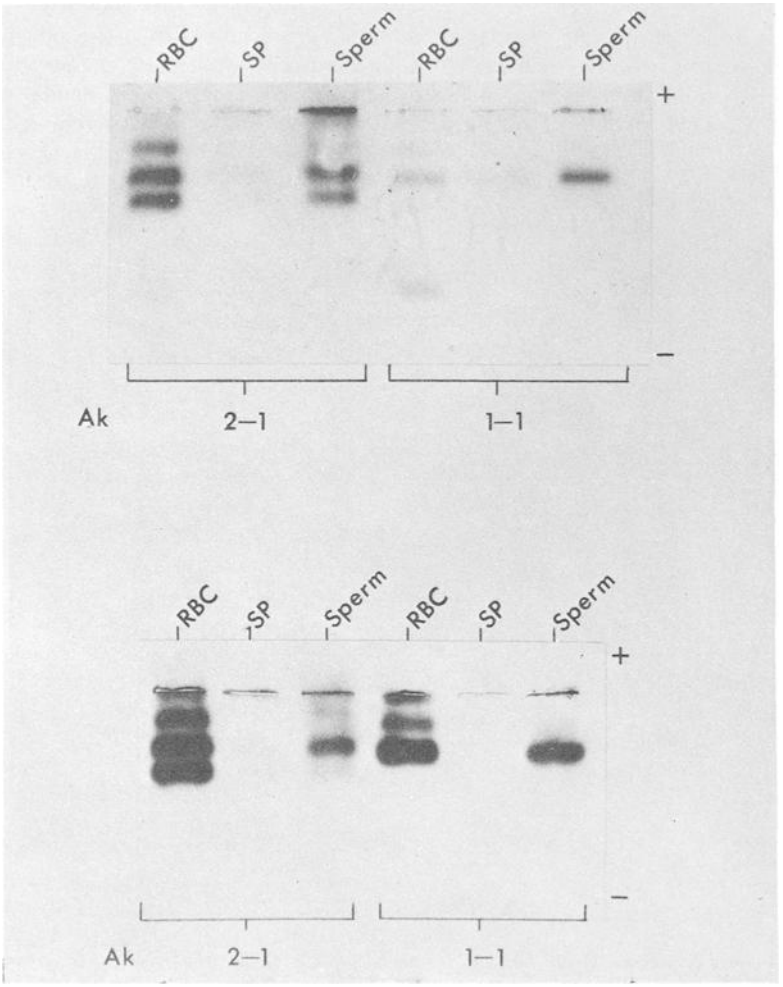

FIG. 5—*The adenylate kinase "2" isozyme is very labile in lysed sperm. The upper gel shows the pattern obtained from freshly collected samples. The lower gel shows the pattern of these same samples after they have been stored for 24 h at 4°C.*

bands. This same phenomenon is not observed in red cells, suggesting that there is a protease or peptidase in spermatozoa that preferentially degrades the "2" enzyme. This finding will of course limit the usefulness of this marker for typing sperm. This is most unfortunate since AK is, under most other circumstances, one of the most stable of the enzyme genetic markers.

A rather unique type of problem is presented by sperm diaphorase, an enzyme which we have recently shown to be genetically polymorphic [*35*]. The three common phenotypes of sperm diaphorase are shown in Fig. 6. The enzyme appears to be fairly stable in whole semen, but once the sperm are lysed rapid degradative changes occur. The problem with this enzyme is that it is found only in sperm and testicular tissue. While this may initially seem to be an attractive feature of this enzyme for forensic purposes, it will be difficult to obtain reference typing material from suspects.

In general, the protein and enzyme polymorphic markers are not subject to the uncertainties associated with the antigen markers. There is no question about their presence. It is important that the phenotypic variation in expression be recognized;

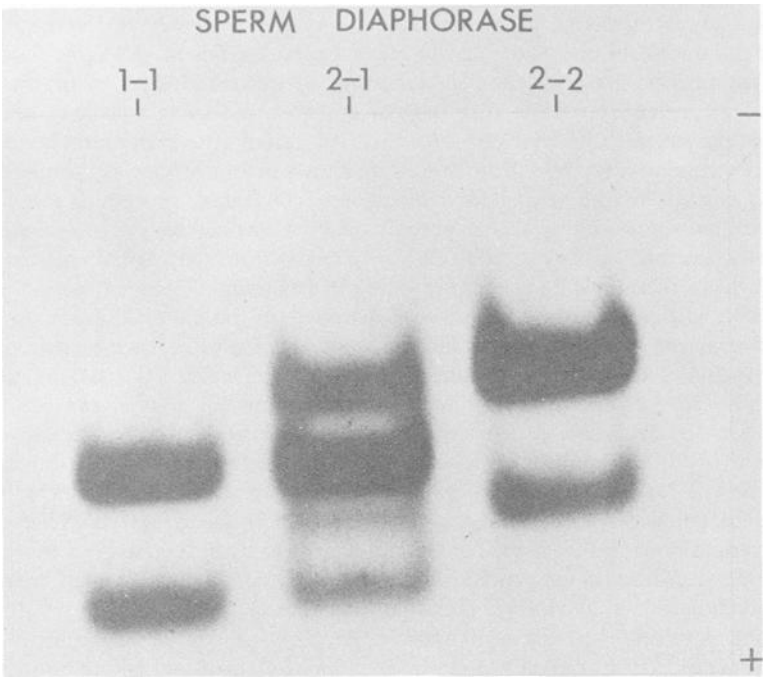

FIG. 6—*Electrophoretic pattern of the three common phenotypes of the sperm-specific enzyme sperm diaphorase.*

otherwise, errors in interpretation might result. Thus, it would seem that the protein enzyme markers provide a great potential for the individualization of semen. However, it must be stressed that there is much more that must be known and much more work must be done before any of the genetic markers, whether antigens or enzymes, can be employed on a practical basis in the crime laboratory.

Practical Problems in the Forensic Analysis of Semen

In the preceding two sections we have outlined what is currently known about the expression of genetic variation in semen and we have raised some of the basic issues and questions. This is but the first step to practical applications. There are a number of intrinsic procedural problems specific to the analysis of semen and even more problems associated with the analysis of semen in the forensic context. These problems must be recognized and overcome before the genetic typing of semen can become a reality in the forensic laboratory.

Perhaps the most fundamental problem concerns the amount of material made available for analysis. As has been noted, many of the most discriminating genetic markers are located in the sperm fraction of semen. Sperm constitute about 5% of the volume of semen; this is about an eighth of the volume fraction of red cells in whole blood. Accordingly, a marker must be present at an eightfold higher level in sperm to give an equivalent typing response. Since this is not the case with many of the markers, there is a need for more sensitive procedures of analysis.

The problem of availability of enough material for analysis is especially acute in the case of semen samples collected from the vaginal vault. This is frequently done by vaginal swabbing. However, vaginal swabs may not collect enough of a semen trace

to allow analysis for some of the genetic markers. Two procedures for the collection of seminal traces from the vaginal cavity might be more efficient. A vaginal lavage with isotonic saline has the advantage of collecting a substantial portion of the vaginal contents; unfortunately, it also dilutes these contents and some step may need to be taken to concentrate material for analysis. An alternative procedure is to collect vaginal contents on a tampon. This procedure allows the absorption of virtually the entire fluid content of the vaginal cavity and is a convenient procedure for collecting vaginal contents for studies of the normal cycle of vaginal secretions. Regardless of what method is used in the collection of semen traces from the vaginal vault, it is quite apparent that efforts must be made to capture the maximum number of sperm.

Even if a sufficient quantity of seminal material can be collected from the vagina, a second problem is manifest. Washings or swabbings from the vagina post coitus will contain vaginal secretions and seminal plasma, vaginal epithelial cells, mucosa, and sperm cells. To meaningfully individualize semen from this source, the male genetic markers must be distinguished from the markers of the female. A first step toward this problem involves determining which genetic markers are found in vaginal secretions and cellular material; studies of this sort are underway in several laboratories, including ours. It is apparent that there is considerable individual variation as well as cyclic variation in the composition of the vaginal material. An alternative approach to this problem may be the development of procedures for the differential separation of sperm from the other cellular material collected from the vaginal vault.

A problem intrinsic to semen analysis is the rapid rate of degradation of seminal plasma proteins. Both seminal plasma and sperm contain a number of proteases and peptidases [*36*]; it is believed that these contribute significantly to the degradation process. The rapid degradation of some seminal plasma proteins is illustrated in Fig. 7.

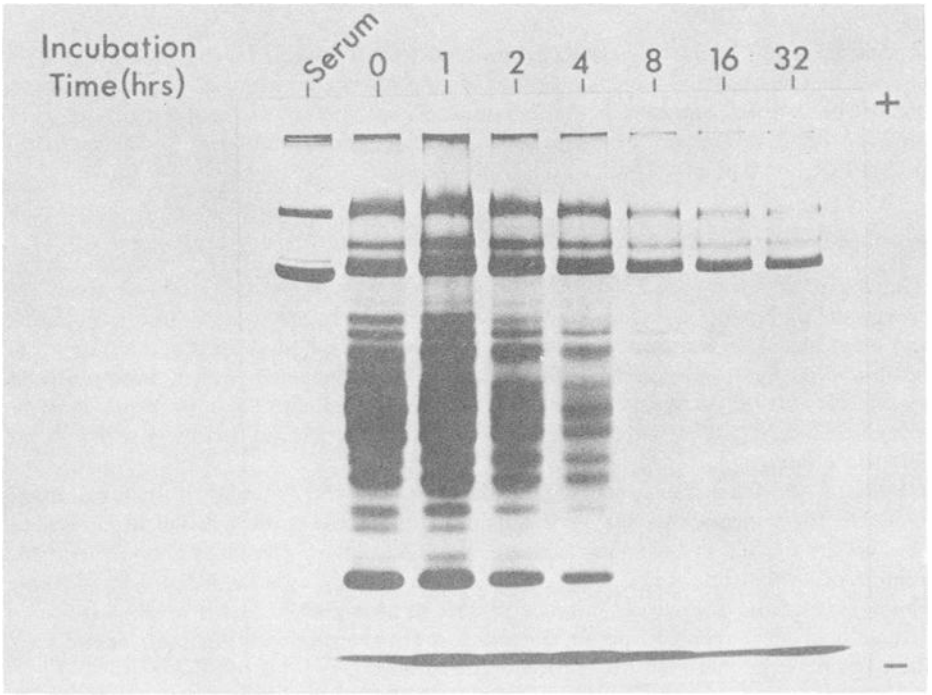

FIG. 7—*Rapid degradative changes occur in the proteins of seminal plasma when they are incubated at 37°C. This is significant since changes of this nature can be expected to take place in seminal plasma proteins in the vagina after intercourse.*

In this experiment, aliquots of fresh seminal plasma were incubated at 37°C for periods of 1 to 32 h; each aliquot was then characterized by electrophoresis. It is quite apparent that many of the protein bands are progressively lost. This same pattern of protein degradation is seen in frozen samples of seminal plasma stored over periods of several months. It is quite probable that degradative changes of this sort occur in the vagina post coitus. Until more is learned about the process of degradation in general and about the susceptibilities of the different genetic markers to degradation in particular, the significance of the phenomenon for the genetic analysis of semen can only be guessed.

This brief discussion illustrates the fact that the genetic typing of semen is not nearly as straightforward as the genetic typing of blood. There are unique problems of sample collection, sample dilution, contamination, and degradation. Recognition of these problems is the first step toward their solution and, with continued research, progress toward the practical utilization of genetic typing of semen will be made.

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