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Isolation and Characterization of a Semen-Specific Protein from Human Seminal Plasma: A Potential New Marker for Semen Identification

The identification of semen is of paramount importance in the investigation of rape and other crimes involving sexual assault. The most commonly used procedures for semen identification center on the detection of sperm or the detection of prostatic acid phosphatase activity; methods involving the detection of spermine, choline, or semen antigens are less commonly employed. Unfortunately, none of these procedures is without one or more significant problems. For example, sperm will not be found in the semen of vasectomized or aspermic males; moreover, sperm are mechanically labile and their unequivocal identification in suspected semen stains is often difficult. Also, sperm are cleared from the vagina fairly rapidly and hence may not be found in postcoital vaginal washings. Thus the failure to detect sperm in suspect material by no means counterindicates semen. In the case of the acid phosphatase test, the problems are different. Acid phosphatase is not at all unique to semen or prostatic tissue; this enzyme activity is ubiquitous in nature. Moreover, there is evidence that prostatic acid phosphatase and the acid phosphatase found in normal vaginal secretions are genetically identical and that both are genetically identical to lysosomal acid phosphatase found in most tissues;[2] therefore, the genetic basis of specificity of the acid phosphatase test is in question. The quantitative test can only be based on the extraordinarily high level of acid phosphatase activity in semen; the low levels of activity often found in postcoital vaginal washings are thus equivocal with respect to the question of semen detection. The other tests for semen identification are similarly suspect in reference to their specificity.

From the foregoing it is apparent that it would be desirable to have alternative tests for the detection of semen. A good alternative test should meet several criteria. It should be based on the detection of a semen marker for which the biological basis of specificity could be demonstrated; this condition virtually dictates that the marker should be a protein since protein synthesis is under direct genetic control. To avoid the problem posed by vasectomized and aspermic males, the marker should be a component of seminal plasma. A good marker should be stable in stains and in the vaginal environment. Finally,

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because the effective dilution of semen in the vaginal pool postcoitus may be as much as 1:2000, the marker should be detectable at trace levels.

In this report the identification, purification, and characterization of a seminal plasma protein is described; this protein appears to satisfy the above conditions as a good semen marker.

Material and Procedures

Human semen was obtained from male volunteers in accordance with approved guidelines for the protection of human subjects. Sperm were separated from the seminal plasma by low speed centrifugation (1500 rpm, 10 min, 25°C), and the seminal plasma was subsequently clarified by a high speed centrifugation (36 000 *g*, 20 min, 4°C). Seminal plasma was stored frozen until use.

Electrophoresis on acrylamide gels containing sodium dodecylsulfate was performed as described by Laemmli [*1*]; the gels were stained for protein with Coomassie blue and for glycoproteins with a periodic acid-Schiff stain [*2*]. Immunoelectrophoretic analysis employed 1% agarose gels on glass slides; the tank buffer was 0.076*M* barbital hydrochloric acid, pH 8.2, and the gels contained this buffer diluted 1:4 [*3*]. Chromatographic media for gel filtration (Sephadex G100) and ion exchange (DEAE Sephadex A50 and CM Sephadex C50) were obtained from Pharmacia, Piscataway, N.J.; they were prepared and used as described in the manufacturer's instructions.

Antisera against the purified semen protein were prepared according to the following procedure. Rabbits were immunized subcutaneously with 100 μg of antigen emulsified in Freund's complete adjuvant (Difco); at 4 weeks the first bleeding was taken. At Week 5, the rabbits were boosted with 100 μg of antigen in Freund's incomplete adjuvant (Difco) given by subcutaneous injection; the second bleeding was taken on the sixth or seventh day after the booster. Subsequent boosts contained 100 μg of antigen in saline delivered intravenously and were followed a week later by a bleeding.

Several immunological assay procedures were employed in this study. Routine detection of antigen was by the Ouchterlony double diffusion in gel technique [*3*]; the gels were 1% agar or agarose in 0.14*M* sodium chloride buffered with 10 m*M* Tris HCl [tris(hydroxymethyl)aminomethane hydrogen chloride], pH 7.4 (isotris). Determination of antigen levels in whole semen was achieved by a radial immunodiffusion assay procedure [*4*]. Radial immunodiffusion assay plates were prepared to contain antiserum at an appropriate concentration (5 to 10%) in 1% agarose; the antiserum was mixed with molten agar at 50°C so as not to heat-inactivate the antibody. For "rocket" electrophoresis [*5*], the antisera were incorporated by the same procedure into agarose gels containing the barbital hydrogen chloride for immunoelectrophoresis; the final gel concentration was 1%. Electroimmunodiffusion analysis employed the same gel and buffer conditions as for immunoelectrophoresis.

Results

Identification of Semen-Specific Proteins

The identification of potential semen-specific proteins was accomplished by comparing the proteins of human seminal plasma to the proteins of other physiological fluids by electrophoresis on polyacrylamide gels containing sodium dodecylsulfate (SDS) [*1,6*]. This electrophoretic technique, which separates denatured polypeptide chains on the basis of molecular weight instead of charge [*6*], allows upwards of 40 polypeptides in seminal plasma to be distinguished. This electrophoretic technique possesses two advantages over the conventional electrophoretic and immunoelectrophoretic procedures that have

been previously used in the characterization of seminal plasma and other physiological fluids [*7–10* and references therein]. First, the detection of proteins does not depend on their antigenicity, as is the case with immunoelectrophoresis; thus, proteins that are not antigens can be detected. Second, the method circumvents the vagaries associated with the migration of glycoproteins in conventional electrophoresis; many secreted proteins are polydisperse with respect to charge because of covalently bound carbohydrate. Prostatic acid phosphatase, for example, migrates to produce multiple bands in conventional electrophoresis [*11*] but migrates as a single band in SDS-gel electrophoresis.

The proteins of human seminal plasma and blood plasma as revealed by SDS-gel electrophoresis are compared in Fig. 1; it is clear that the distribution of proteins in the two physiological fluids is quite distinct. The qualitative differences between the patterns

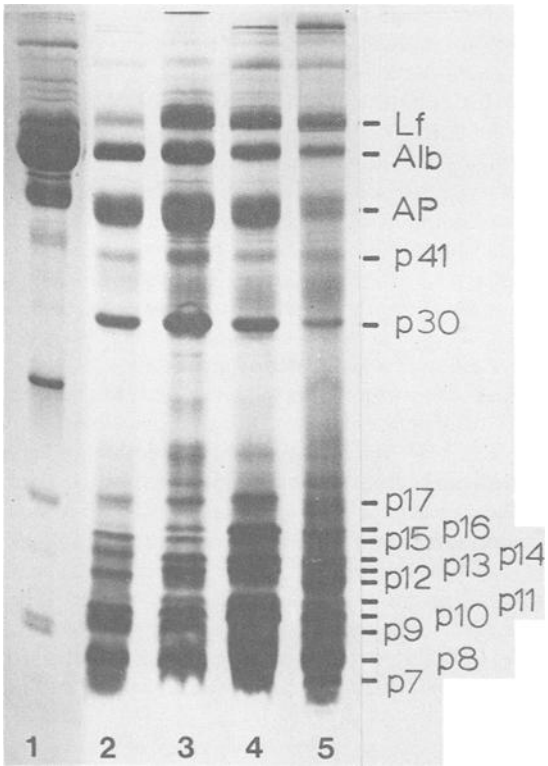

FIG. 1—*Electrophoresis of human blood serum and human seminal plasma on polyacrylamide gels containing sodium dodecylsulfate. The proteins of these two fluids are separated on the basis of* ***molecular weight*** *[6];* ***the largest peptide chains have the lowest mobility and are at the top of the gel. Track 1 contains blood serum****: this portion of the gel has been deliberately overdeveloped to show minor bands. The dominant band in serum is albumin; it constitutes about half of the serum protein. Tracks 2 and 3 contain seminal plasmas from different ejaculates by a single individual. Tracks 4 and 5 contain seminal plasma from different individuals; the individual providing the ejaculate used in Track 5 was vasectomized. The major proteins of seminal plasma, from top to bottom, are lactoferrin* (Lf), *albumin* (Alb), *acid phosphatase subunit* (AP), *and a number of polypeptides of unknown function, designated in order of diminishing molecular weight as p41, p30, p17, p16, p15, p14, p13, p12, p11, p10, p9, p8, and p7.*

of these two fluids and of other secretions such as milk or vaginal secretions were equally marked.

As is evident from Fig. 1, seminal plasma contains several major protein species. The two major bands at the top of the gel are lactoferrin (Lf) and albumin (Alb); they have molecular weights of 80 000 and 68 000 daltons, respectively. These two proteins are found in many secretions. The band with the molecular weight of about 50 000 daltons is the subunit of acid phosphatase (AP); the native enzyme is a dimer with a molecular weight of about 100 000 daltons. The assignment of this band to AP was verified by SDS electrophoresis of the purified enzyme. There is a band of secondary intensity at a position corresponding to about 41 000 daltons and, below that, a strong band indicating a protein of about 30 000 daltons; these proteins, which did not appear to be present in other fluids or secretions, were designated p41 and p30, respectively. Apparent seminal plasma specificity was also indicated for some of the peptides in the 10 000 to 20 000 dalton range (designated p17, p16, and so forth, peptides). With the exception of these low molecular weight peptides, the proteins described above showed little sample or individual variation; all were present in vasectomized individuals.

The apparent semen specificity of p30 as indicated by SDS-gel electrophoresis suggested that this protein would be a likely candidate for further testing as a semen marker. In support of the proposition, in an earlier characterization of seminal plasma antigens by Li and Shulman [*9,10*], a protein with a molecular weight of about 31 000 daltons had been identified as semen specific; this protein was designated E_1, according to its mobility in conventional electrophoresis. By using an antiserum generously provided by Dr. Li [*12*], the identity of p30 and the E_1 protein was confirmed: the anti-E_1 antiserum reacted with a substantially pure preparation of p30. Because the p30 designation was originally used in this study, it will be adhered to in the remainder of this report.

Purification and Characterization of p30

The purification scheme for p30 currently in use entails two ion exchange chromatography steps followed by one or two gel filtration chromatography steps; this procedure differs from that used by Li and Beling [*12*] and is described below. All purification steps were performed at 4°C, and p30 was detected during the purification by reaction with anti-p30 antisera. A typical purification began with about 100 ml seminal plasma. This was dialyzed against a buffer of 10 m*M* potassium phosphate, pH 6.9, and applied to a column of carboxymethyl Sephadex C50 (2.5 by 36 cm) that had been equilibrated against that buffer. About 90% of the seminal plasma protein was not absorbed and passed through the column. The p30 protein was retained and was subsequently eluted with a linear salt gradient to 0.5*M* sodium chloride; p30 emerged under a major protein peak. The fractions containing p30 were pooled, dialyzed against the 10 m*M* potassium phosphate buffer, and applied to a column of DEAE Sephadex A50 (3.5 by 23 cm) equilibrated against that buffer. Again p30 was retained and was eluted as a major protein peak with a salt gradient to 0.75*M* sodium chloride. The fractions containing p30 were pooled, dialyzed against distilled water, and lyophilized. The lyophilized protein was dissolved in a small volume of 0.5% ammonium bicarbonate and applied to a Sephadex G100 gel filtration column (3.3 by 48 cm) equilibrated against the bicarbonate buffer; the p30-containing fractions were pooled, lyophilized, and rechromatographed on the Sephadex G100 column. The result was a single protein peak containing the p30 protein. For estimation of molecular weights, the G100 column was calibrated with molecular weight standards: bovine serum albumin (70 000 daltons), ovalbumin (45 000 daltons), horseradish peroxidase (40 000 daltons), trypsin (23 800 daltons), and cytochrome C (11 600 daltons).

The elution position of the p30 peak indicated a molecular weight of about 30 000

daltons for the native protein. The peak fractions were pooled and lyophilized. Analysis of the purified material by SDS-gel electrophoresis showed a single dominant protein band (Fig. 2); by this standard, the degree of purity of several preparations was estimated to

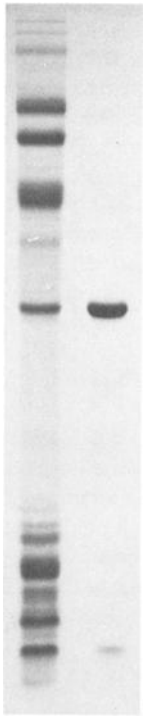

FIG. 2—*Electrophoresis of human seminal plasma and purified p30 on acrylamide gels containing sodium dodecylsulfate. The purified protein is seen as a single dominant band. A trace band corresponding to p8 is also present; this may represent a degradation product from p30.*

be 98% or better. The coincidence of molecular weight estimates on the native protein by gel filtration and on the denatured protein SDS-gel electrophoresis indicates that p30 exists in the native state as a monomer; p30 is not a subunit of a higher molecular weight multimeric protein. Like most seminal plasma proteins, p30 is a glycoprotein; it stains weakly with the periodic acid-Schiff stain on gels and binds to the lectin concanavalin A. Upon isoelectric focusing, the protein splits into several isoelectric isomers with isoelectric points in the range pH 6.5 to 8.0; this behavior is typical of glycoproteins and, in particular, of glycoproteins containing sialic acid. A more complete account of the biochemical characterization of p30 will be presented elsewhere.

Immunological Assay of p30

Whether the p30 protein has any biological activity (for example, enzyme activity) that might be exploited in an assay is not known at this time. Antisera were prepared so that the protein could be detected by immunological assay. Each antiserum was examined for specificity by immunoelectrophoresis with whole human seminal plasma and human blood serum as antigens. In every case, the reaction with seminal plasma resulted in a single major precipitation arc that corresponded to the anti-p30 specificity; there was no corres-

ponding reaction with blood serum (Fig. 3). With some of the antisera, a faint reaction was observed with a blood serum protein with an electrophoretic mobility differing from

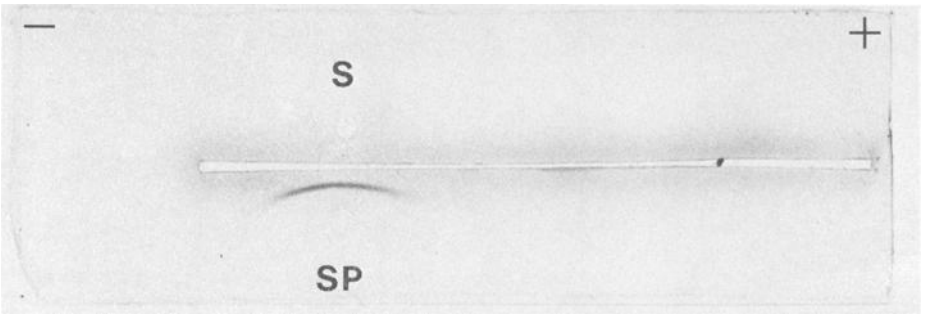

FIG. 3—*Demonstration of monospecificity of anti-p30 antiserum. Human blood serum* (S) *and human seminal plasma* (SP) *were subjected to electrophoresis; the running conditions were 2 h, 4°C, 150 V/cm. At the end of the run, the center trough was cut and anti-p30 antiserum was added; the immunoelectrophoresis plate was then incubated for 24 h at room temperature in a humid chamber during which time the precipitation reaction developed. The gel was then washed in saline, then water, dried, and stained for protein. Note that the antiserum is monospecific, reacting only with p30 in semen. Note also that at the pH of the run, pH 8.2, p30 shows virtually no electrophoretic migration.*

p30; this indicates that the protein preparation used for the preparation of antibody contained a contaminating immunogen. The contaminating antibody could be absorbed by blood serum without affecting the anti-p30 specificity.

The antisera were assessed for sensitivity by reaction against serial dilutions of seminal plasma in Ouchterlony double diffusion gels. The strongest antiserum gave a visible precipitin reaction at a seminal plasma dilution of 1:128 but not at greater dilutions; based on the mean level of p30 in seminal plasma (see below), this corresponds to an antigen detection limit of about 15 μg p30/ml. Several alternative immunoprecipitation procedures were tested to see if the operational sensitivity of the immunoassay could be improved. Both "rocket" electrophoresis [*5*] and electroimmunodiffusion [*3*] appeared to be less sensitive than the Ouchterlony technique; this is probably due to the low electrophoretic mobility of p30 at the pH employed (see Fig. 3). No improvement in sensitivity could be achieved with either radial immunodiffusion assay [*4*] or Oudin single diffusion [*3*]. Ouchterlony immunodiffusion analysis was used for all subsequent studies.

To determine whether commercial antisera for semen identification possessed antibodies to p30, four such antisera were tested for reactivity with the purified protein. Two possessed weak reactivity with p30 and two did not react. All four reacted strongly with a protein identified to be lactoferrin, a protein found in many secretions and at high levels in milk [*13*]. This investigation will be described in detail in a subsequent report.

Assay of p30 in Semen, Male Reproductive Tract Tissues, and Other Body Fluids

The level of p30 in normal human semen was assayed by a quantitative immunoradial diffusion technique [*4*]. The mean level found was 1.92 mg/ml with a range of 0.24 to 5.5 mg/ml (n = 17). Even at the low end of the range, p30 is present at easily detectable levels.

The double diffusion immunoassay was used to test the tissues of the male reproductive tract to identify the tissue origin of the p30 protein. No reaction was found with extracts of testicular tissue, vas deferens, or seminal vesicle. (The presence of the protein in the

seminal plasma of vasectomized individuals had already precluded testicular origin.) A positive precipitin reaction was obtained with extracts of prostatic tissue (Fig. 4*a*), indicating

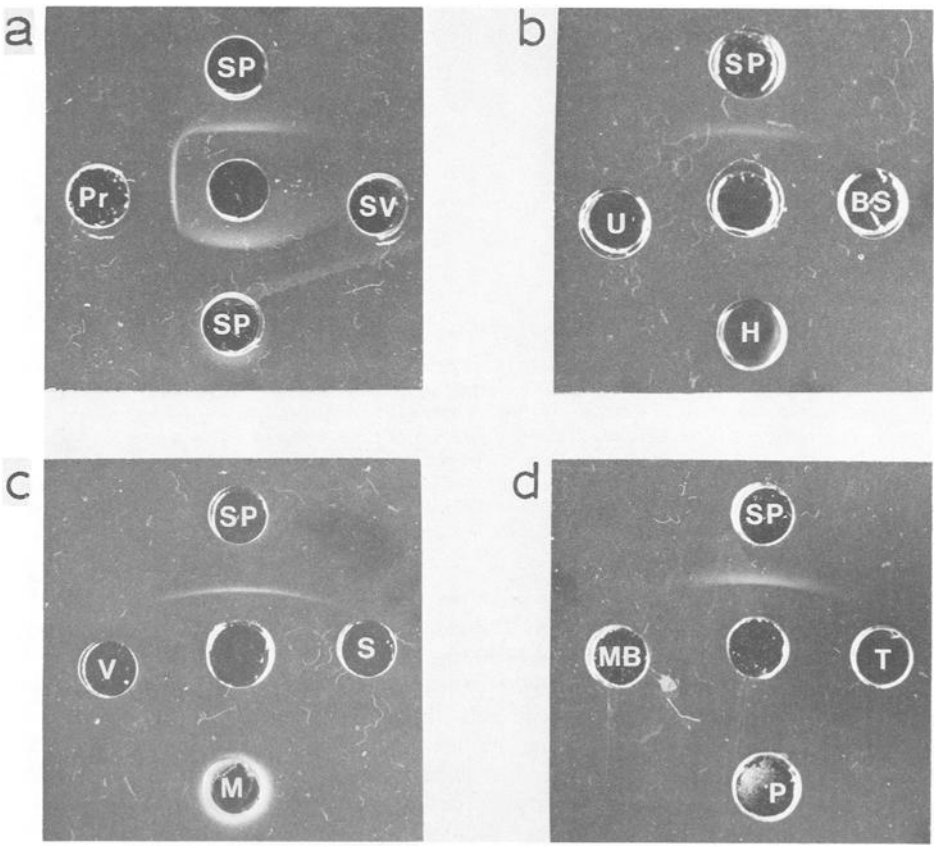

FIG. 4—*Tissue origin and specificity of p30. Various fluids have been assayed for p30 by immunodiffusion assay. The top well in each figure contains seminal plasma at a dilution of 1/50 (SP/50); the center well contains anti-p30 antiserum.* (a) *Reading counterclockwise, SP/50, prostate tissue extract* (Pr), *seminal plasma undiluted* (SP), *and seminal vesicle extract* (SV). (b) *SP/50, urine concentrated about 50 times* (U), *whole blood hemolysed by freezing and thawing* (H), *and blood serum* (BS). (c) *SP/50, vaginal fluid concentrated four times* (V), *human milk* (M), *and saliva* (S). (d) *SP/50, menstral blood* (MB), *perspiration* (P), *and tears* (T).

that the prostate gland is the likely tissue of origin. Li and Beling [*12*] suggested that p30 was "probably" non-prostatic in origin; however, they did not test prostatic tissue directly as was done here and the method they did use may not have been sensitive enough to detect the antigen in prostatic tissue. The double diffusion assay showed the level of p30 in the prostatic extract to be 1 to 2% of that found in semen.

Assay of various human physiological fluids with the most sensitive antiserum indicated that p30 is not present at detectable levels in blood serum, red cell hemolysate, tears, perspiration, saliva, milk, menstral blood, urine, or vaginal fluids (Figs. 4*b–d*). These findings do not unequivocally indicate the specificity of this protein to the prostate gland and semen for it is possible that a more sensitive assay, immunological or otherwise, might detect the protein in other tissues or secretions. However, if p30 is present in any of the tested fluids, it is present at less than 1/100 of the level found in seminal plasma.

The species specificity of p30 was examined by reacting anti-p30 antiserum against whole seminal plasma from boar, bull, and ram. The antiserum gave no reaction with the bull and ram seminal plasmas. Boar seminal plasma nonspecifically precipitated the test antiserum; the nonspecificity of this precipitation was demonstrated by the parallel precipitation of several protein solutions including nonimmune rabbit serum, normal human serum, and human seminal plasma. These results confirm the results of electrophoretic analyses of the three seminal plasmas on SDS acrylamide gels; none of the three contain a protein migrating at the same molecular weight position as human p30.

Application to Forensic Case Situations

The problem of semen identification arises primarily in two contexts: the analysis of suspect stains and the analysis of vaginal washings or swabs. The immunoassay for p30 has been tested in both contexts. The protein has been detected without difficulty in extracts of semen stains up to 1 year old; although no other stains were tested, p30 appears to be fairly stable and should probably survive in older material. The success with postcoital vaginal washings has been less consistent; p30 has been detected in some but not in others. This appears to be due to the effective level of semen in the vaginal pool. As noted above, the strongest antiserum used in this study did not detect p30 in seminal plasma diluted more than 1:128; accordingly, p30 would not be detected in vaginal washes containing semen at a greater dilution. When the postcoital vaginal washings were assessed for effective semen concentration (by measurement of the acid phosphatase levels in washings) it was found that those washings containing semen at less than 1:100 dilution gave a positive p30 test and those with an effective dilution greater than 1:100 gave negative results. Thus more sensitive antisera or more sensitive tests will be needed to detect p30 at the greater dilution often encountered in vaginal washings collected in rape cases.

Anti-p30 antisera have been provided to 14 crime laboratories for preliminary testing. The specificity of the antisera was further analyzed by testing for reactivity with a variety of fluids and solutions not tested in this laboratory. None of the tested materials other than semen yielded a positive reaction; included among the nonreacting materials were stomach contents, bile, cow's milk, cat semen, chimpanzee semen, egg yolk, egg white, coffee, cola, Karo® syrup, Fab® detergent, Sea and Ski® suntan lotion, Conceptrol®, Vaseline® Intensive Care® lotion, Breck® setting lotion, Dep®, Alberto Balsam®, and Lensine®.[3] With respect to the detection of semen traces in stains and in vaginal swabs, the reporting laboratories report findings similar to those described above. In addition, the immunological test for p30 has been successfully used as a confirmatory test for the identification of semen stains in several case situations.

Discussion

A good marker for a physiological fluid such as semen should be stable, specific, detectable at trace levels, and present in all individuals. Of these criteria, the question of specificity is the most central and also the most subject to confusion. The specificity of a test for semen can be defined in both empirical and biological terms. Empirical specificity is demonstrated by showing that the semen marker cannot be detected in any materials other than male reproductive tract tissue and secretions. However, empirical specificity is by definition conditional for it is always possible that the marker might be detected in other than male tract material by a more sensitive test or under different test conditions. Moreover, empirical specificity is also relative because it extends only to the materials

[3] S. Williams and M. Kollmar, personal communication.

tested; the possibility exists that the putative marker might yet be found in some material thus far not tested. Defining specificity on biological terms involves different criteria. It should be demonstrable that the putative semen specific marker is synthesized in one of the tissues associated with the male reproductive tract (that is, testes, epididymis, seminal vesicle, prostate gland, and so on) and it should be demonstrable that the synthesis of the marker is restricted to that tissue. Thus, for example, sperm qualify as semen-specific markers because sperm are a unique product of the male germ line tissue in testes. By extension, sperm-specific proteins such as lactate dehydrogenase X [*14,15*] are also semen specific. However, the biological specificity of prostatic acid phosphatase is conditional on its high activity in semen; the enzyme itself appears to be genetically identical to acid phosphatase enzymes synthesized in other tissues, including vaginal tissue. This brief discussion points out that although the demonstration of empirical specificity and the demonstration of biological specificity overlap considerably, the two definitions of specificity are based on different criteria and have different limitations. It should be clear that the overall demonstration of specificity must take both empirical and biological considerations into account.

By these standards, the case for p30 as a semen-specific marker is promising. The protein appears to be synthesized in the prostate gland from which it is secreted into semen; the regulation of its synthesis and secretion is not known and further studies are needed to determine whether its synthesis is restricted to the prostate. At the present limits of detection, p30 has not been found in any other tissue or secretion. The immunological test for p30 is not interfered with by any of the nonbiological materials thus far tested. More sensitive assay methods are needed to verify these indications of specificity.

At the level of specificity thus defined, the immunoassay for p30 can be used in conjunction with the acid phosphatase test as a confirmatory test for the detection of semen. The protein appears stable in semen stains and is present in semen at sufficient levels to be readily detected in stain extracts. Moreover, because of the species specificity inherent in immunological reactions, a positive p30 test would preclude any question of possible vegetable origin for an acid phosphatase positive stain. For the detection of semen traces on vaginal swabs or in vaginal washings, the test for p30 is of limited value because of the restricted sensitivity of the existing immunoassay. The survival of p30 in postcoital vaginal fluids appears to parallel that of acid phosphatase activity, but, because the current immunological assay for p30 is about ten times less sensitive than the acid phosphatase assay, p30 can at present be detected only in postcoital vaginal material that contains a relatively great amount of seminal residue. In the usual case, the effective dilution of semen in the vaginal pool is such that the level of p30 is below current limits of detection. Should more sensitive assay procedures verify the specificity of p30 relative to normal vaginal secretions, the detection of p30 could take on even greater value as a definitive test for the identification of semen traces in the vaginal pool postcoitus.

It should be pointed out that the immunoassay for p30 differs from the conventional immunological test for semen. The antisera used for the conventional immunological tests are usually antisera prepared against whole human semen that have been absorbed with blood serum to remove antibodies to proteins found in both fluids. The specificities of these antisera are thus defined in terms of the remaining, presumably specific, antibodies, not in terms of the antigens they recognize. The problem with this approach is illustrated in the finding that commercial anti-semen antisera all contain antibodies against lactoferrin, a protein found at negligible levels in blood but found at appreciable levels in semen and other physiological fluids [*13*]. In contrast, the approach used in this study was directed toward identifying proteins potentially specific to semen; the selected proteins could then be subjected to intensive characterization for specificity. By focusing on the potential marker protein, questions concerning both operational and biological specificity can be directly addressed.

Summary

The characterization of an apparent semen specific protein, designated p30, is described; this report lays the foundation for an alternative test for the identification of semen based on the detection of this protein. The development of a more sensitive assay procedure for p30 is required to further define the specificity of the protein. A more sensitive assay would also greatly enhance the value of a p30 test with respect to questions arising in the forensic context. Efforts to develop an assay for p30 with the requisite sensitivity are currently in progress.

Acknowledgments

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References

[1] Laemmli, U. K., "Cleavage of Structural Proteins During the Assembly of the Head of Bacteriophage T4," *Nature*, Vol. 227, No. 5259, 1970, pp. 680-685.
[2] Fairbanks, G., Steck, T. L., and Wallach, D. F., "Electrophoretic Analysis of the Major Polypeptides of the Human Erythrocyte Membrane," *Biochemistry*, Vol. 10, No. 12, pp. 2606-2617.
[3] Crowle, A. J., *Immunodiffusion*, 2nd ed., Academic Press, New York, 1973.
[4] Fahey, J. L. and McKelvey, E. M., "Quantitative Determination of Serum Immunoglobulins in Antibody-Agar Plates," *Journal of Immunology*, Vol. 94, No. 1, 1965, pp. 84-90.
[5] Axelson, N. H. and Bock, E., "Identification and Quantitation of Antigens and Antibodies by Means of Quantitative Immunoelectrophoresis. A Survey of Methods," *Journal of Immunological Methods*, Vol. 1, No. 2, 1972, pp. 109-121.
[6] Weber, K. and Osborn, M., "The Reliability of Molecular Weight Determinations by Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis," *Journal of Biological Chemistry*, Vol. 244, No. 16, 1969, pp. 4406-4412.
[7] Quinlivan, W. L. G., "Analysis of Proteins in Human Seminal Plasma," *Archives of Biochemistry and Biophysics*, Vol. 127, No. 1, 1968, pp. 680-687.
[8] Hermann, G., "Immunoelektrophoretische Unterschungen am Menschlichen Sperma Plasma," *Clinica Chimica Acta*, Vol. 4, No. 1, 1959, pp. 116-123.
[9] Shulman, S. and Li, T. S., "The Analysis of the Antigens of Human Seminal Plasma," in *Immunology of Reproduction*, Bulgarian Academy of Sciences Press, Sofia, 1973, pp. 133-141.
[10] Li, T. S. and Shulman, S., "Immunoelectrophoretic Analysis of Human Seminal Plasma Fractions After Fractionation by Various Methods," *International Journal of Fertility*, Vol. 16, No. 2, 1971, pp. 87-100.
[11] Smith, J. K. and Whitby, L. G., "The Heterogeneity of Prostatic Acid Phosphatase," *Biochimica et Biophysica Acta*, Vol. 151, No. 3, 1968, pp. 607-618.
[12] Li, T. S. and Beling, C. G., "Solution and Characterization of Two Specific Antigens of Human Seminal Plasma," *Fertility and Sterility*, Vol. 24, No. 2, 1973, pp. 134-144.
[13] Masson, P. L., Heremans, J. F., and Dive, C., "An Iron Binding Protein Common to Many External Secretions," *Clinica Chimica Acta*, Vol. 14, No. 6, 1966, pp. 735-739.
[14] Blanco, A. and Zinkham, W. H., "Lactate Dehydrogenases in Human Testes," *Science*, Vol. 139, No. 3555, 1963, pp. 601-602.
[15] Goldberg, E., "Lactic and Malic Dehydrogenase in Human Spermatozoa," *Science*, Vol. 39, No. 3555, 1963, pp. 602-603.

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