IDENTIFICATION AND INDIVIDUALIZATION OF

SEMEN IN THE INVESTIGATION OF RAPE

DRAFT FINAL REPORT

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I. INTRODUCTION

Within the past decade, the crime of rape has become an issue of national
social concern. Rape entails the physical and psychological humiliatin
of the victim, and no crime, short of homicide, is more feared. Reported
incidences of rape are increasing at a more rapid rate than any other
major crime. At the same time, the arrest and conviction rate is lower
than for any other category of violent crime. It is clear that new tools
are required for rape investigation and prosecution.

Two of the critical questions in rape investigation involve (a) the
determination that a sexual assault has occurred and (b) the identifica-
tion of the assailant. The research objectives of this grant were
centered on these questions.

II. DETERMINATION OF SEXUAL ASSAULT

A. The Problem

The determination that a sexual assault has occurred is indicated
by evidence of semen in the vagina or in the immediate genital area
of the victim. The finding of sperm unequivocally indicates the
presence of semen. However, a significant portion of the male popu-
lation has been vasectimized and have no sperm in their semen.
Moreover, the identification of sperm in vaginal washings and in
semen stains is often difficult. For this reason, many crime
laboratories use the acid phosphatase test as a presumptive test
for semen.

Acid phosphatase is an enzyme secreted by the prostate gland into
human seminal plasma; it is found at higher levels in seminal plasma
than in any other natural source. The acid phosphatase test is usually
considered to be presumptive and not specific because acid phosphatase
activity is also found in other tissues and secretions including human
vaginal secretions. Thus, although high levels of acid phosphatase
activity are usually indicative of semen, low levels are considered
equivocal. This presents a problem, for some rape victims do not
receive a medical examination until many hours have lapsed since the
assault; the acid phosphatase activity may be greatly diminished by
the time the analysis is begun. In addition there is considerable
individual variation in the rate at which acid phosphatase from semen
is degraded in the vagina; in some women, it is lost within very few
hours. If for whatever reason, sperm is not found and acid phosphatase
activity is not high, a charge of sexual assault may appear, incorrectly,
to be unfounded. It is clear then that the acid phosphatase test is
not completely satisfactory.

B. Research Findings

The objective of this phase of the research was to determine the limits
of the specificity of the acid phosphatase test. If possible, we
hoped to develop a completely specific acid phosphatase test. If
such a test were not possible, we sought to develop an alternative
specific test.

In summary, we have determined that prostatic acid phosphatase, the phosphatase found in semen, belongs to a class of tissue acid phosphatases characterized by molecular weight, catalytic properties, and substrate specificities. The vaginal acid phosphatase also belongs to this class. Within this class there is heterogeneity in electrophoretic mobility; the prostatic enzyme can be distinguished from the other tissue acid phosphatases, including the vaginal acid phosphatase, by this criterion. However, the prostatic, vaginal, and several tissue acid phosphatases are immunologically cross-reactive. This finding suggests that they have a common genetic origin and may differ only in a secondary property. Of practical significance, the vaginal and prostatic enzymes may undergo interconversions such that they are indistinguishable. Indeed, we have found that treatment of prostatic acid phosphatase with a bacterial enzyme (such as might be found in the vagina) can alter its electrophoretic mobility such that it appears indistinguishable from the vaginal enzyme. All in all, these observations suggest that the acid phosphatase test has limited specificity and that there is a significant risk of false negatives.

In addition to the acid phosphatase test, immunological tests are also sometimes used for the identification of semen. We have surveyed four commercially available anti-human semen antisera for specificity. None crossreact with blood but all crossreact with other physiological secretions, particularly milk. Thus the commercially available anti-sera for semen identification are not of adequate specificity to be used for forensic purposes.

In order to develop an alternative test for semen that would be semen specific, we have pursued two lines of research. The first involved finding and characterizing a protein specific to seminal plasma. The second involved the characterizing of a fluorescent marker for semen.

The ideal marker for semen would be a seminal plasma specific protein, present at high levels, that would be stable in semen stains and in semen traces collected from the vaginal vault. A systematic charac- terization of human seminal plasma yielded one protein that satisfied these criteria. This protein, which we call protein-30 because its apparent molecular weight is about 30,000 Daltons, has been purified and a high titre antisera has been prepared against it. The tissue specificity of the protein has been verified; it appears to be secreted from the prostate gland into the seminal plasma and has not been found in any other secretion. We have provided a number of laboratories with this antiserum for testing. The tests that have been conducted so far indicate that this antiserum can provide a confirmatory test for semen; further field testing will be required before the technique can be used on a routine basis.

The second line of research toward the development of a specific test for semen grew out of the observation that semen, if allowed to stand in the liquid state, would develop a yellow pigment with an intense yellow fluorescence. We have determined that this pigment is produced

by the action of a bacterium, probably a strain of <u>Pseudomonas</u>
<u>Fluorescens</u>, on some precursor substance contained within seminal
plasma. The identity of this precursor substance is at present unknown;
however, it is known that no other body secretion appears to contain
the precursor compound. Identification and characterization of the
precursor compound may provide a novel test for semen; more work is
needed to develop these observations into a test.

III. IDENTIFICATION OF THE ASSAILANT

A. The Problem

Identifying the assailant in a rape investigation poses unique problems.
In practically all cases, the only witness to the crime is the victim
herself; due to the intense psychological trauma associated with the
assault, the victim cannot be expected to provide a good description
of the rapist. Moreover, due to the nature of the crime and the
trauma suffered by the victim, the reporting of the crime may be delayed
for hours; the police rarely have the opportunity for on-the-scene
apprehension. In addition, although the crime of rape involves the
theft of dignity, there are no stolen goods which can be traced to the
assailant. Finally, rapists are unusually solitary criminals; their
identity is not often broadcast on the informer network. Thus, four
of the major channels of police investigation -- eyewitness descrip-
tion, on-the-scene apprehension, tracing stolen goods, and informer iden-
tification-- are closed off in the investigation of rape. Because
rape is a unique kind of crime, alternatives to traditional channels
of investigation are needed.

A most straightforward appraoch to rape investigation is to make use
of the physical evidence that virtually every rapist leaves behind:
his semen. The semen contains genetic information which may be used
to include or to exclude a suspect from suspicion as a rapist. This
inclusion/exclusion process can be performed as a screening test,
thus circumventing much investigative effort. Moreover, even if no
suspects are in hand, patterns of activity of multiple rapists can
be ascertained. Lastly, the use of this genetic information does not
require the victim to face the suspect; thus the victim is spared this
potential source of anguish.

B. Research Findings

Before the genetic information in semen can be exploited in case
situations, it must be determined what genetic markers are present
in semen and how they are expressed. Recognition of idiosyncracies
of expression is very important. The stabilities of the markers
must be characterized and sources of contamination recognized. These
were the objectives of our research.

The genetic markers of semen can be partitioned into two classes: the cell surface antigens and the soluble protein markers. Apart from noting considerable variability in the expression of the former, our primary attention was directed towards characterizing the protein and enzyme markers.

Over twenty known genetic polymorphisms have been surveyed for their expression in semen. Of these, fifteen were found of which five are present only in seminal plasma, seven are present only in sperm, and three are present in both. In addition, one confirmed new genetic marker in sperm was discovered and another tentative new genetic marker is indicated. The new genetic marker (Sperm Diaphorase) is of particular interest since it is found only in sperm.

In terms of value in the investigation of rape, the protein and enzyme markers in semen can be divided into three groups.

(1) The first group contains those markers for which typing technology is well established and which are present at such high levels in semen that they might be found in a substantial proportion of case material. Enzyme markers in this class include phosphoglucomutase (PGM), peptidase A (Pep A) and phosphoglucose isomerase; of these, PGM has the best discrimination potential and Pep A has value as a marker of racial groups.

(2) The second group contains markers that are found at low to moderate levels in semen and which under some conditions might be typeable in semen stains but which will probably not be typeable in semen traces contained in vaginal washings. This group includes amylase-2, 6-phosphogluconate dehydrogenase, esterase D, Gm, Inv, PGM-3, peptidases C and D, diaphorase, and transferrin. The critical constraint on the use of these markers is the dilution factor and stability; with appropriate developments in the technology of semen trace analysis and with further knowledge concerning stability and contamination effects, more extensive utilization of these markers might be possible.

(3) The third group contains markers which for one reason or another are not currently suitable for use in semen analysis. This group includes alpha-1-anti-trypsin, adenylate kinase, and glucose-6-phosphate dehydrogenase. The situation with adenylate kinase is of particular note. Here one phenotype degrades to give the appearance of another; thus errors in interpretation are possible.

The assignment of markers to these three groups should not be considered binding. In every case, new developments may change things.

IV. IMPLICATIONS FOR THE INVESTIGATION OF RAPE AT PRESENT

(1) Time is of the essence. The victim should seek a medical examination as soon as possible after the assault. Vaginal swabs or washings should be kept refrigerated until the analysis. The analysis should be done as soon as possible to avoid needless loss of information; degradation of semen proteins is particularly rapid.

(2) Neither absence of sperm nor a weak acid phosphatase test should be considered evidence against sexual assault. Analysis of the acid phosphatase activity by electrophoresis can usually distinguish between vaginal and prostatic acid phosphatase. A confirmatory test, such as the immunological test for the semen specific protein, can also be used to detect semen.

(3) Great care should be taken in the collection of semen traces. Semen uncontaminated by vaginal secretions can probably provide more genetic information about the assailant. Thus, in addition to collecting vaginal swabs, the localization and collection of semen stains should be a top priority.

(4) Early analysis of semen traces can yield information of investigative value. A genetic profile of an assailant can be established that can be used to screen suspects. This information also can be used to link different attacks to a single individual.

V. FUTURE RESEARCH AND DEVELOPMENT NEEDS

(1) Detection of Semen.

(a) The acid phosphatase test has value within limits. The critical remaining question is what those limits are; otherwise there is a significant risk of erroneous interpretations. Of particular concern is the possible interconversion of vaginal and prostatic acid phosphatase. It needs to be learned under what conditons this interconversion occurs and how often.

(b) The immunological confirmatory test for semen needs further field testing. The survival of the protein-30 marker post coitus and in stains needs further characterization. Finally, the sensitivity of the immunological test needs to be improved; this is a technical problem.

(2) Individualization of Semen.

(a) The discrimination value of peptidase A could be greatly improved if reliable procedures could be developed to distinguish between the type 1, type 8-1. and type 8 phenotypes. (Current technology separates the type 1, type 2-1, and type 2 phenotypes; type 2 is found in blacks.) This is basically a question of enzymology.

(b) Improved discrimination potential could be gained if methods were developed for separating sperm out of vaginal washings. Many informative genetic markers are contained in sperm. In vaginal washings they are mixed with vaginal cellular material and with mucus; with this contamination, the sperm markers cannot be identified.

(c) The levels of secretor substances in semen need reinvestigation. A number of laboratories have expressed concern about ABO secretor typing of semen stains and vaginal swabs. A survey of the literature indicates that there has been no systematic survey of the variation in expression of ABO secretion in semen. This needs to be remedied if ABO typing of semen is to be relied upon.

PART II: TECHNICAL REPORT

I. INTRODUCTION: PROBLEMS IN THE INVESTIGATION OF RAPE AND SEXUAL ASSAULT

Over the past decade, the crime of rape has emerged as a problem of national public concern. This emergence stems in great part from the belated recognition that the social impact of rape extends beyond the immediate criminal act. Rape involves as no other crime a violation of the victim's physical and psychological person; as a consequence, no crime, short of homicide is more feared. Rape not only traumatizes the victim, it also damages the victim's relationship with family, friends and society; similarly, the fear of rape strains social relationships and raises concern for public security. Thus, it has become recognized that rape and the fear of rape constitute a significant social problem.

The spotlight on rape and rape related issues has illuminated the poor performance of the criminal justice system in dealing with the crime. The Uniform Crime Reports (1975) tell the tale. The incidence of rape is high, from 2 to over 10 per 10,000 population depending upon estimates, and is rising. Only half the founded rapes are cleared; this is lower than for any other crime against a person. Barely over half of those arrested are prosecuted and fewer than half of those prosecuted are convicted of the charged offense; in fact, rape is the only Part I crime for which there are more acquittals or dismissals than convictions. These statistics make it painfully apparent that criminal justice agencies have not been successful in the investigation and prosecution of rape offences. This in turn raises serious questions about the current capabilities and practices of the criminal justice system in dealing with this crime.

The performance of the criminal justice system has certainly been affected by deeply entrenched social and moral attitudes toward rape. Each person involved with a rape case – victim, police, medical personnel, prosecuting and defense attorneys, jurors – will act in accordance with their own subjective moral and social perception of the crime; these perceptions have often worked against the victim and against successful investigation and prosecution. Indeed, one of the great benefits of the recent attention given to rape has been the exposure and discussion of perceptions toward rape and how they contribute to the rape problem. This "sensitization" has resulted in some much needed changes in procedures at local and state levels; in some places laws pertaining to rape and rape prosecution have been significantly redefined. These changes will certainly make the criminal justice system more humane in dealing with rape and rape victims and may well improve its performance.

It must be pointed out however, that these efforts do not really strike to the heart of the rape problem as a criminal justice problem. As a criminal justice problem, the primary concerns are the apprehension, prosecution and conviction of rapists. The achievement of these objectives requires that the information generated in the course of the investigation provide proof that a rape in fact occurred and proof as to the identity of

the assailant; the evidence pertaining to these two questions must be strong enough to sustain an indictment and to convince a jury. It may be inferred from the low rates of success in apprehension, prosecution and conviction that the information gathered as evidence is all too often inadequate; particularly striking in this regard is the low ratio of convictions to acquittals/dismissals in cases prosecuted. At the heart of the problem then, is the quality of the information that is gathered in rape investigations.

If this evidentry information is too often inadequate, it is pertinant to question why: what is there about rape cases that makes good evidence hard to obtain? To answer this question, it is necessary to look at the unique features of rape as a crime and the consequences of this uniqueness for rape investigation.

Rape is typically a quiet crime involving only the victim and assailant; the act is usually done so as not to attract the attention of others. Accordingly police receive relatively few reports of "rapes in progress" to which they can respond in hopes of catching the assailant at the scene. Moreover, because the victim is often physically or psychologically unable to report the offense immediately, the assailant usually has plenty of time to escape from the scene. These two factors greatly diminish the ultimate chance of assailant apprehension since the rate of apprehension drops dramatically as the time delay between the offense and the arrival of first officer increases.

Whether or not a suspect is immediately apprehended, the investigation of rape depends almost solely upon the victim to provide information about the offense and the assailant; if a suspect is in hand, the victim is used to provide the identification. This dependence upon the victim is a direct consequence of the fact that very often, over 75% of the time, the victim is the only witness. (Forcible Rape, A National Survey of the Response by Police, LEAA, 1977). It is thus not surprising that the police center their attention upon the victim and the information the victim can give.

This predominant role for the victim in rape investigation is unsatisfactory in three respects. First, the victim has gone through a morally and psychologically traumatic experience and may not want to talk to the police about the incident nor want to look at suspects for fear of agonizing the trauma; for this reason, many victims simply refuse to participate in the police investigation. Secondly, again because of the emotional trauma involved in a rape, victims may become confused about what happened during the assault or about the identity of the assailant. This is not surprising since witnesses to any crime very often present a confused and inconsistent picture; it is always a greater problem when the witness is part of the action. Finally the victim is a vulnerable witness in court; if the case rests primarily on the testimony of the victim, the defense is likely to attack the credibility of the victim's reconstruction and identification. The dependence upon the victim's participation in rape investigation is indicated by the estimate that at least 60% of arrests in rape cases result from direct victim involvement, e.g., identification by naming or from a line up or photo files (Forcible Rape, ibid).

Once the rape victim is removed from the investigative picture, many of the traditional patterns in criminal investigation become ineffectual. As noted, there are rarely secondary witnesses to provide leads. Files on known offenders are useful only if the assailant is previously. known to the police. Rape is not commonly associated with robbery and hence there are no stolen goods through which an assailant might be traced. For similar reasons, informants are rarely useful. Without victim involvement, rape investigation appears rather random and haphazard.

Even when the victim is willing and able to cooperate fully in the investigation, the victim cannot provide investigative leads to a suspect in a significant number of cases. Forcible rape is a crime in which the assailant is usually unknown to the victim; in about 60% of the reported cases, the assailant and victim are complete strangers and in 30-40% of the cases, the victim is unable even to identify a suspect in a photo or live line up (Forcible Rape, ibid). In these cases, the rape investigation assumes the same random character as when there is no victim involvement.

The potentially conflicting priorities in handling rape victims adds another type of difficulty to rape investigation. The victim is at once a source of information, an item of physical evidence, a physically injured person, and and an emotionally traumatized human being; the investigation cannot begin without the information, the physical evidence is fragile, the injuries require medical attention, the psyche requires attention and consideration. Much of the spotlight on rape results from the fact that this latter priority, the humane concern for the victim's psyche, has been all too often ignored; in the future, this priority should achieve its due recognition. Be this as it may, it must be recognized that there are potential conflicts in these priorities and if, as is proper, humane consideration takes precedence, some aspects of the investigation may be necessarily sacrificed.

A final factor which may be cited as contributing to the problems of rape investigation and prosecution is the difficulty in coordinating the activities of different agencies. The investigation and prosecution of rape involves, perhaps more than for any other crime, the input of diverse agencies. At a bare minimum, a rape report requires the involvement of the police and the medical person who examines the victim. Additional layers of involvement may enter in: hospital administration, clinical testing laboratories, criminalistics laboratories, rape crises counselors, prosecution and defense attorneys, legislators who write new laws. Each entity has its own perception of its function, its own standard operating procedures and its own agenda of priorities; each even has its own language. It is thus difficult to get these diverse entities coordinated. It is not untypical to find questions left unasked because the police think the hospital is doing the asking and the hospital thinks the police are. Evidence does not get examined because the police think the prosecutor will dictate what needs to be done and the prosecutor assumes that the police will take care of everything. The list goes on. The main problem is that the gaps in communication or procedure are between entities and one is not aware that the gaps exist unless one stands back and looks at the rape investigation process as a whole. But because these gaps do exist, information of potential value to investigation and prosecution is lost.

It should be apparent then, that the major problems in rape investigation are a consequence of the nature of rape as a crime, not a result of police or prosecutorial incompetance. Very simply, the nature of the crime renders ineffectual many conventional approaches in crime investigation and places an undue burden on the victim, the one remaining conventional source of information. Is there any solution to these problems? Are there ways to improve the quality and quantity of information of value as evidence in rape investigations? The preceeding analysis suggests several approaches.

First, it is apparent that the gaps between agencies must be closed. This will best be done under the purview of a central overseerer. For without a perspective that takes in the whole process, from the primary rape report to the final adjudication, the gaps will be missed. This suggestion is in accord with and supplements proposals that specialized investigative units dealing with rape be established. (Rape: A Preceptive Package for Police, Hospitals, Prosecutors, and the Public LEAA; 1976). These units would be responsible for coordinating all aspects of the rape investigation leading up to the prosecution; this overview would allow detection and correction of informational gaps. Moreover, such a unit would be better able to integrate information from different sources and thus the efficiency of the utilization of information would be improved.

A second need pertains to increasing the likelihood of victim participation in the investigation and prosecution process. Because the victim is, and always will be, a virtually indispensible source of information and a valuable witness, anything that facilitiates an increased likelihood of victim participation will be beneficial. In this regard, more humane treatment of rape victims by police, by hospitals, and in the courts should yield good returns. It would also be very beneficial to take some of the investigative burden off the victim. If, for example, suspects could be rigorously screened prior to the victim identification step, the victim would be spared having to view suspects unnecessarily. Similarly, victims would be more secure if they knew that the body of evidence against a suspect was more than simply their own word. Anything which decentralizes the role of the victim in the investigative and prosecution processes would reduce the barriers to participation.

These considerations point out a third need: the need for independent sources of information and evidence. Not only would new and independent sources of information decentralize the victim's role, they would also serve to strengthen the foundation upon which the case rests. Moreover, independent sources would allow a rape investigation to proceed failing victim participation and might be very useful in tying together multiple rapes by a single assailant. This need is not easily satisfied; as has been pointed out, the nature of rape is such that most potential sources of information are non-existent. There are, however, two sources of information which could be exploited to a greater degree than at present; these are the medical examination and physical evidence.

Rape is almost the prototype crime for both medical and physical evidence utilization. First of all, there is the obligate physical evidence: semen. Unlike many crimes where the presence of any particular type of physical evidence is unpredictable, in rape there is almost always semen. Secondly, as a crime

against a person, there will almost always be significant medical observations to be made, e.g., marks and bruises, etc. Finally, rape is a kind of crime where there is likely to be a fair amount of physical activity at the scene of the offense: struggling, undressing, the assault itself, redressing, etc; in the course of all these activities, the clothes and bodies of victim and assailant will come into contact with each other and with the scene. There is then, substantial opportunity for trace material to be transferred between victim and assailant, victim and scene, and assailant and scene. The kinds of trace material will vary of course from case to case; the nature of the crime, however, is such that transfer evidence will almost always be present.

At the present time, medical and physical evidence is relied upon almost exclusively to provide evidence of sexual penetration, one of the elements in the proof of rape. All rational protocols for the examination of rape victims includes provisions for the collection of vaginal fluid samples which can subsequently by analysed for the presence of semen; these protocols also direct attention to the possibility of physical trauma in the genital area which would provide evidence of assault. Despite the recognized value of physical and medical evidence in this area, however, there is a surprising lack of sophistication. Samples are all too often collected routinely as per protocol directions with little regard to the facts of the particular case which might dictate collection of additional samples. Analysis of samples in the laboratory is not uncommonly deferred until police or prosecutor decide it is necessary for the case and by the time the analysis is finally done, the semen traces in the sample have degraded and the test is negative. The results of the semen test may be over-or-under interpreted; in either case, this results from the lack of a sufficient foundation of knowledge upon which to base interpretation. Of particular concern in this regard, it is not always appreciated by police and prosecutors that a negative test for semen is often obtained even though there has been recent sexual activity; this confusion may lead to the incorrect unfounding of rape reports. It is thus clear that even within this well recognized area of physical and medical evidence utilization, there is room for more fundamental knowledge, for more education about the interpretation and value of the evidence, and for better coordination between the different agencies that use the evidence.

Physical and medical evidence is not commonly applied to the question of consent, another element of the crime of rape, except in the most obvious cases, e.g., when the rape victim has been physically assaulted. Two reasons may be cited for this situation. First, rape victims are rarely examined by persons with appropriate training in forensic medicine; as a consequence, subtle telltale marks and bruises are often overlooked. Moreover, most medical examinations of rape victims focus on the genital area; yet, with respect to the question of consent, far more is to be learned from looking at the clothes and the rest of the body (Paul, Medicine, Science and the Law 15, 154, 1975). The second reason is that the search for evidence pertaining to this question, its subsequent analysis, and the consequent reconstruction would be time consuming and thus would be expensive; the examination of both victim's clothing and the crime scene would be entailed. Despite all this, there is evidence from experience indicating that if people with appropriate training examine rape victims and if the evidence examination is complete, a significant contribution can be made to the problem of corroborating lack of consent.

With respect to the identification of the assailant, physical and medical evidence is rarely used; current practice places the identification almost entirely upon the shoulders of the victim. Two types of evidence might be applied to this question. The first is transfer evidence. As noted earlier, no person can disrobe, perhaps struggle, certainly engage in physical activity, all in ---- place, and not leave some trace. Finding that trace may be difficult and it may require searches at multiple scenes to get enough information to make use of; nevertheless, the evidence will be there if it is looked for. The second kind of evidence is biological evidence: semen, blood, tissue. It is possible to genetically type biological material; this allows suspects to be screened for genetic match and when matches are found, their probative value is readily assessed. Analysis of semen traces in vaginal samples collected from victims within a short time after the assault may be expected to yield useful genetic information in about 50% of cases. Fresh uncontaminated semen can be typed much more extensively; in this case it is potentially possible to distinguish two different males with a probability of 99 out of 100 or better (Blake and Sensabaugh, J. For. Sci. 21, 784, 1976).

From an operational standpoint, the physical and medical evidence described in the foregoing paragraphs can be partitioned into two classes. In the first group is the evidence that can be routinely collected; this includes samples collected during the medical examination of the victim: vaginal swabs or washings, pubic hair, semen traces, victim's underclothes, etc. The collection of this material requires little effort beyond that usually taken in routine examinations. In contrast, the second class of evidence does require extensive effort; this class includes the evidence collected at the scene and from the victim's clothing. To collect this evidence would require a crime scene search and an impounding of the victim's clothes for laboratory examination. This second level of effort obviously would require a strong commitment on the part of the investigating agencies; many agencies may want to use this second level only in special cases, e.g., in the investigation of a multiple rape series.

If physical and medical evidence is to be used to its best advantage, whether at the first or second level of commitment, three conditions must be met. First, the medical examination of the victim must be done by an appropriately trained person. It goes without saying that the examination of the victim and the quality of physical evidence collected can be no better than the person doing the job. The situation is getting much better than it was a few years ago; as a consequence of the attention given to rape, there has been a burst of training for emergency room personnel. However, the situation is far from what it could be; much of what is currently done has been developed out of a vacuum. In this regard, it is worth noting that emergency room personnel see most of the victims of crimes against persons, rape included: these personnel are not at all well trained in the forensic area and as a result, many medico-legal questions are not answered that might be. Given that emergency room medicine is a developing specialty area in medicine, it would be good to push for training programs in forensic medicine for these personnel; emergency room physicians are potentially the forensic clinicians of the future.

The second condition is that the physical evidence be examined by the crime laboratory immediately upon receipt. There is a fundamental biological reason for this; biological materials tend to undergo degradation and the longer the interval before the analysis, the greater the risk that the analysis will yield

meaningless results. From the standpoint of the police investigation, the immediate analysis of evidence has great advantages also. The results of the evidence examination provide additional information that can be useful in the investigation. For example, genetic typing of semen evidence from several cases can be used to associate or dissociate cases where otherwise information was incomplete. Similarly, information of this sort can be used to tie together cases from different jurisdictions which otherwise might not have been associated. Moreover, when cases can be tied together, whether by the physical evidence or by other evidence, the little pieces of unique information associated with each case can be pieced together to yield a more complete picture of the rapist. Finally, as has been noted, possession of genetic information or other identifying bits of information prior to the apprehension of a suspect allows preliminary screening for identity; this relieves the victim of the burden of viewing suspects needlessly.

This leads to the third condition, which is that the medical and physical evidence findings be integrated into the MO or pattern files; if this evidentry information is kept separate, it is of little value at the investigatory level. Moreover, it would be good also to maintain a centralized set of records on rape; this crime transcends jurisdictional boundaries, and associations between offenses in different jurisdictions are apt not to be made without some form of centralized data bank. In this regard, crime laboratories sometimes serve this function if their services extend to multiple jurisdictions.

It should be clear then, that physical and medical evidence can contribute to the investigation and prosecution of rape offenses to a far greater degree than is presently the case. The advantages associated with the utilization of this kind of evidence are many; the examples noted in the foregoing paragraphs are from real life and show that jurisdictions that have exploited physical and medical evidence have benefited from it. In terms of the "success index" described in the first part of this paper (the rates of clearance, prosecution, and conviction), the experience of one jurisdiction is worth noting. In this jurisdiction, the examination of victims is done by a small staff of specially trained physicians and the physical evidence arising from the victim examination is analysed immediately and extensively. The clearance rate for this jurisdiction is not available at the time of writing but of the three hundred indicted, 283 pled without trial and 13 of the 17 that went to trial were convicted.

The foregoing is a rough draft of a paper which will be submitted for publication. Before the paper can be completed, some more data on prosecution success needs to be obtained. This paper was an outgrowth of a Symposium on Rape: Investigation, Prosecution, and Physical Evidence presented at the 44th Semiannual Seminar of the California Association of Criminalists, Berkeley, 1974.

II. IDENTIFICATION OF SEMEN

A. The Acid Phosphatase Test

1. Statement of the Problem

The acid phosphatase test for semen was first suggested by Lundquist in 1945. The basis of his suggestion and the traditional basis of the test is the fact that the level of acid phosphatase activity in human semen is a thousand times higher than in any other body secretion. Thus the detection of very high levels of acid phosphatase activity in a suspect stain or on a vaginal swab would indicate the presence of semen. In the hands of most practitioners today, the acid phosphatase test is done semi-quantitatively: the detection of any acid phosphatase activity within a defined period of observation is taken as a presumptive indication of semen. Because of the technical simplicity of the test and the apparent ease of its interpretation, the acid phosphatase test for semen is routinely used in the crime laboratories of the world.

Over the years, the acid phosphatase test has not been without its critics. Basically two criticisms of the acid phosphatase test have been raised. First, it has been pointed out that acid phosphatase activity is found throughout nature; it is found in plant and animal tissues, in fungi and microorganisms. Although nowhere is the level of activity as high as in human semen, this criticism validly points out that simple detection of acid phosphatase activity does not unequivocally indicate semen. The second criticism specifically concerns the acid phosphatase test as applied to swabs or washings from the vagina to provide evidence of recent sexual intercourse. It has been found that the vaginal pool normally contains low levels of acid phosphatase activity and thus the mere detection of acid phosphatase activity in the vaginal pool does not necessarily indicate the presence of semen. These criticisms of the acid phosphatase test point out that the test is not without ambiguities.

One of the principle objectives of the research supported by this grant was to define the ambiguities associated with the acid phosphatase test and, in so far as possible, to find a solution to these ambiguities. To this end, two questions were posed.

1. How valid is the quantitative acid phosphatase test as applied to vaginal swabs or washings as a diagnostic indicator of recent sexual intercourse?

2. Is there a molecular basis of specificity of the acid phosphatase test?

2. Quantitative Acid Phosphatase Test

Prior studies on acid phosphatase levels in the vagina post coitus provide some general guidelines for the interpretation of the acid phosphatase test (McKlosky, et. al., J. For. Sci. 20, 630, 1975; Godwin and Seitz, Med. Ann. D.C. 39, 147, 1970; Willott, J. For. Sci. Soc. 12, 363, 1972; Gomez, et. al., Amer. J. Clin. Path. 64, 423, 1975). These guidelines may be summarized as follows. After sexual intercourse, the acid phosphatase activity found in the vagina is usually, but not always, greatly elevated. Elevated acid phosphatase activity can be found in the vagina for as long

as 2 or 3 days post coitus but usually the decline of activity is fairly rapid and "insignificant" levels of acid phosphatase activity may be found within 8 hours or sooner. Finally, there is some acid phosphatase activity normally present in the vagina, that is, endogenous to the vagina, and generally these levels are lower than those found post coitus. Based on these guidelines, high levels of acid phosphatase activity found in the vagina can be interpreted as indicative of sexual intercourse probably within a relatively recent time period, and low levels (in the absence of douching, etc.) are properly interpreted as neither indicating nor counterindicating coitus at a less recent time. It would be an error to interpret low levels as counterindicating recent coitus because acid phosphatase activity post coitus can drop very rapidly.

The inadequacy of the existing state of information is compounded by the fact that the several existing studies on vaginal and post coital acid phosphatase activity have employed different analytical procedures and, accordingly, present their results in different terms. For this reason, the data from the different studies are hard to compare and the guidelines derived from them are necessarily very general; it is thus difficult to apply them to specific cases except in the most general way.

Studies to assess the quantitative acid phosphatase test were just beginning at the time the grant terminated; as a result, only two experiments were done. The first involved analysis of vaginal acid phosphatase levels through the menstrual cycle to see if there was cyclic variation. The second was to set up a design for a rigorous measurement of seminal acid phosphatase recovery post coitus.

a. Endogenous vaginal post acid phosphatase through the cycle. Two series of experiments were done. In the first, vaginal post fluids from a single individual were collected daily in a 10 ml saline wash; the collection was done at an Ob-Gyn clinic. In the second series, vaginal post fluids were collected onto a tampon every other day from 3 women; the tampon was washed with 10 ml saline and sequeezed out. The women participating in this series abstained from intercourse during the test. This experiment showed three significant results:

(1) There was no significant variation through the cycle. There was a slight tendency for values to be higher in the backside of the cycle but the increase in values was not significant.

(2) The distribution of values for single individuals was reasonably approximated by a log-normal distribution; the fit to this distribution was better than the fit to the normal distribution.

(3) Average values varied among women; among the four subjects, the range of average values was 3x. This range was not a function of the different sample collection procedures in the two series.

b. Post coital recovery of acid phosphatase activity. No prior study has determined the recovery of seminal acid phosphatase post coitus in a truely quantitatively fashion; as a consequence, there is no good estimate of how

much of the acid phosphatase present in an ejaculate is actually present in the vagina post coitus nor are there good estimates of rates of decay. In this experiment, which was intended as a pilot experiment, the objective was to get some insight into this matter. On 12 occasions, vaginal fluids were collected in a ten ml wash at time intervals 8-12 hrs post coitus; the collection was done at an Ob Gyn clinic. This method of collection was chosen because it is quantitative; collection on swabs, which has been done in prior studies, is not quantitative and would introduce an element of variation due to sample collection. The total acid phosphatase levels in the washes were compared with the average acid phosphatase activity in an ejaculate. It was found that, the recovery of the seminal acid phosphatase was about 1%; that is, at 8-12 hrs post coitus, the total acid phosphatase activity present in vaginal fluids is about 1% of that present in the ejaculate. Moreover, the range of recovered activities was 200x; in two of the twelve cases, the recovered activity levels overlapped significantly with the normal endogenous vaginal acid phosphatase range for that individual. The data from this experiment is summarized in the table below.

Table: Acid Phosphatase Activity (Total Units)

Sample	Mean Activity	Activity Range
Semen: total ejaculate	1000 units	
Vaginal wash:		
8-12 hrs. post coital	9.6 units	0.16 - 32.4 units
endogenous	0.27 units	0.12 - 0.68 units

From this single series of experiments, any conclusions must be tentative. It is apparent that even under fairly well controlled conditions -- quantitative sample collection, a constant time interval, a single subject, etc. -- the level of recovery varies tremendously. This is in accordance with results obtained elsewhere in less well controlled studies. A second observation is that the half life of seminal acid phosphatase activity in the vagina post coitus can be estimated to be in the range 0.5 - 2 hours; this estimate is very tentative, applying as it does to only a single individual. Nevertheless, it does suggest that not much reliance can be placed on estimations of the post coital interval.

c. Need for future work. It is clear that more needs to be done with the quantitative acid phosphatase test; the experiments described above little more than sharpen up the questions remaining to be answered. For example, the threshold between elevated and non-elevated acid phosphatase levels is still not well defined; this is because the range and distribution of endogenous vaginal acid phosphatase activity has not been adequately described although we have made a start here. The range and distribution of post coital acid phosphatase levels as a function of time after intercourse

are also not well described; again we have only made a start. More
importantly, because neither post coital nor endogenous vaginal acid
phosphatase distributions are known, it is not known to what extent
these distributions overlap. From the standpoint of interpretation, it
is very important to know what proportion of acid phosphatase determinations
would be expected to be negative at any given time after intercourse; if
the quantitative acid phosphatase test is negative in a significant number
of cases where intercourse has in fact occurred, then it is possible that
many rape reports are being incorrectly unfounded.

3. Molecular Basis of the Acid Phosphatase Test

a. Approach to the problem. Much effort has been expended to try to improve
the specificity of the acid phosphatase test. A variety of substrates have
been offered as specific for the prostatic enzyme; convincing demonstration
of specificity, however, has been lacking. It has been suggested that the
prostatic enzyme is specifically inhibited by tartrate but there is ample
evidence that the acid phosphatase activity found in the lysosomes of tissues
are also tartrate inhibited. Specific antibodies to prostatic acid phospha-
tase have been claimed but the demonstrations of specificity have failed to
take into account the very low levels of acid phosphatase in tissues
compared to the prostate and semen. Moreover, there is evidence of
cross-reactivity between seminal and vaginal acid phosphatase. Most recently,
it has been shown that the acid phosphatase in semen can be distinguished
from other acid phosphatases by electrophoresis and isoelectric focusing;
of particular significance, the endogenous vaginal acid phosphatase can
be distinguished from the semen enzyme by these methods.

These efforts to improve on the specificity of the acid phosphatase test
side step the central question. This question is that of the genetic
relationship between the acid phosphatases in human tissues. If the
prostatic enzyme is genetically distinct from the other tissue acid phospha-
tases, that is, if it is determined at a separate and distinct genetic locus,
then any differences distinguishing the prostatic enzyme could be considered
real. Moreover, it should be possible to devise new ways to specifically
detect this enzyme; for example, genetically distinct isozymes generally
differ in amino acid sequence and this should be exploitable. If, however,
the prostatic enzyme is not genetically distinct from the other tissue
acid phosphatases, then there is no substantial basis for the assertion
of molecular specificity. Any differences would not be a consequence of amino
acid sequence differences for the sequences would be the same. Rather,
observed differences must arise through secondary modifications to the
polypeptide chain and as such, are potentially reversible. Thus, for example,
the electrophoretic differences between the prostatic and vaginal isozymes
might be the result of differences in attached carbohydrate; the interconver-
sion of these isozyme forms might result from the removal or addition of
carbohydrate by endogenous enzymes or by bacterial action.

The question of specificity is then, ultimately a genetic question. Three
loci for acid phosphatases are currently recognized. The ACP1 locus codes

for the genetically polymorphic erythrocyte acid phosphatase; this enzyme
has a molecular weight of about 15,000 daltons. The ACP_2 and ACP_3 loci
code for the acid phosphatases found in the lysosomes of cells; these
enzymes have molecular weights in the range of 100,000–125,000 daltons and gene-
tic variants are rare (Sensabaugh Isozymes 1, 367, 1975). The prostatic enzyme is
known to have a molecular weight of about 100,000 daltons but its genetic
relationship to the lysosomal enzymes has not been established. The acid
phosphatase in vaginal fluids has not been biochemically characterized and
its genetic status is unknown.

In this work, we attempted to sort out the genetic relationships of the
prostatic and vaginal acid phosphatases with respect to other tissue acid
phosphatases. Both biochemical and immunological characterizations have
been employed.

b. Biochemical comparison of properties. As previously noted, the
molecular weights of the lysosomal acid phosphatases have been determined
to fall in the range 100,000–125,000 daltons. That the prostatic enzyme
also has a molecular weight of about 100,000 is well known. We determined
by gel filtration analysis that the molecular weight of the endogenous
vaginal acid phosphatase had the same molecular weight as the prostatic
enzyme, i.e., about 100,000. This provides evidence that all of these
acid phosphatases belong to a common class defined by molecular weight.

The catalytic properties of the vaginal enzyme and the prostatic enzyme
were compared with those of the lysosomal acid phosphatases from human
kidney and liver. All are about five times more active at pH 5.5 than
at pH 7.0, all are inhibited by tartrate and fluoride, all are not
significantly affected by formaldehyde or Cleland's reagent. The similarity
in these properties reaffirms common classification.

c. Immunological comparison. More definitive evidence pertaining to the
genetic relatedness of the prostatic, vaginal, and tissue acid phosphatases
was sought using immunological procedures. Proteins with very similar or
identical amino acid sequences will be immunologically cross-reactive;
proteins with different sequences will not cross-react. It would thus
be predicted that if the acid phosphatases from different tissues were
genetically identical, they would also be immunologically identical. On
the other hand, if they were genetically distinct, they would not be
cross-reactive. This was put to the test.

Prostatic acid phosphatase was purified from semen; a new purification
scheme was developed which simplified the process and at the same time
allowed other semen proteins to be purified as well. Efforts were also
made to purify the two lysosomal acid phosphatases found in human liver;
these efforts were not successful. Antisera were prepared in rabbits
against the purified prostatic acid phosphatase; the reactivity of each
antiserum with acid phosphatase was verified by immunodiffusion analysis
followed by specific staining of the immunoprecipitate. Immunoelectro-
phoretic analysis indicated that antisera were monospecific for the acid
phosphatase whereas others contained contaminating antibody. The antisera
were titered by titrating their capacity to precipitate one unit of
acid phosphatase acitivity.

The primary experiments to determine genetic relatedness were conducted comparing prostatic acid phosphatase and the acid phosphatases from placenta. The placental acid phosphatases were selected for this experiment because both ACP_2 and ACP_3 loci are expressed in this tissue. The gene products of these two loci are readily resolved by electrophoresis on starch gels at pH 8.6 followed by enzyme staining with the substrate naphthyl phosphate. Under these conditions, up to four bands of acid phosphatase activity are observed; Beckman and Beckman (Biochem. Gen. 1, 145, 1967) have designated these four isozyme bands, A, B, C, and D, from anode to cathode (see figure 1). Isozymes B and/or D are found in most tissues; the C isozyme is found only in placental tissue. The A isozyme is found only in tissues containing the B isozyme and is thought to be a product of B isozyme modification during tissue storage. Genetic variation at the ACP_2 and ACP_3 loci is rare but informative; analysis of the variant patterns indicates that the B and D isozymes are determined at the ACP_3 and ACP_2 loci respectively (Swallow and Harris, Ann. Hum. Gen. 36, 141, 1972). The electrophoretic patterns given by these genetic variants also suggest that the isozymes are dimeric and that the C isozyme represents a hybrid enzyme that contains one B type subunit and one D type subunit.

The acid phosphatase secreted by the prostate gland has an electrophoretic mobility corresponding to that of the A isozyme. In order to determine the relationship of prostatic acid phosphatase (PAP) to the A isozyme of other tissues and to the other tissue acid phosphatase isozymes, we used a procedure in which immunological reactivity was detected by electrophoresis. This approach allows the differential reactivity of the placental acid phosphatase isozymes to be characterized.

Incubation of a dilute solution of human seminal plasma with the anti-PAP antiserum resulted in complete precipitation of all the acid phosphatase activity; this is demonstrated electrophoretically in Figure 1, tracks a and b. In contrast, incubation of a tissue homogenate of fresh human placenta with the antiserum resulted in only a partial absorption of acid phosphatase activity. Electrophoretic analysis of the unprecipitated activity showed that it consisted entirely of the D isozyme; the A, B, and C isozymes were completely precipitated by the anti-PAP antiserum (Fig. 1, tracks c and d). Similar experiments on acid phosphatase extracts from other tissues and from HeLa cells have given essentially the same result; the A and B isozymes react with the antiserum and the D isozyme does not. Vaginal acid phosphatase (VAP) reacts exactly as prostatic acid phosphatase.

These results indicate that PAP, VAP, and the A, B, and C isozymes of tissue acid phosphatase share antigenic determinants not possessed by the D isozyme. Given the picture of the genetic determination of the tissue isozymes described above, this result provides evidence that the prostatic enzyme and the tissue A isozyme are determined at the same genetic locus as the B isozyme, that is at the ACP_3 locus. The precipitation of the placental C isozyme with the antiserum is consistant with this picture if the C band isozyme is truely a hybrid dimer sharing a subunit with the B isozyme; the C isozyme should then also be precipitated by antibodies against the D isozyme.

These experiments provide strong evidence that PAP and VAP have a common genetic origin with the tissue acid phosphatases encoded at the ACP_3 locus.

Figure 1: Electrophoretic Demonstration of Reaction of
Placental Acid Phosphatases with Antibodies to Prostatic
Acid Phosphatase.

A -
B -
C -
D -

Origin

Placenta   Placenta   Semen   Semen
 + Antibody   + Anitbody

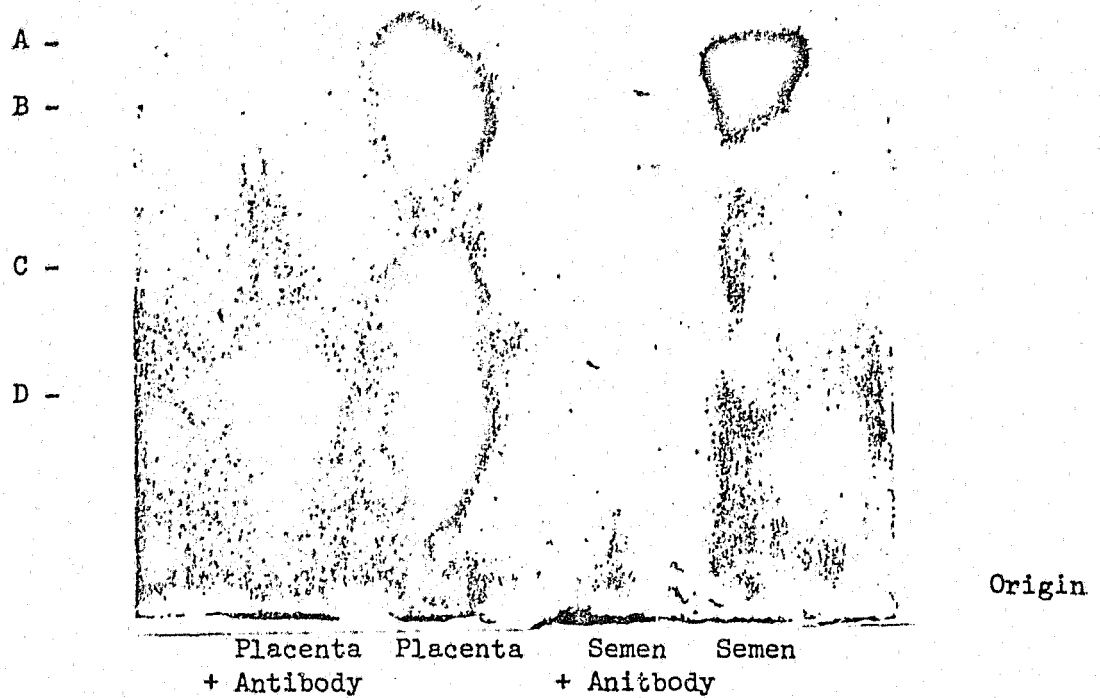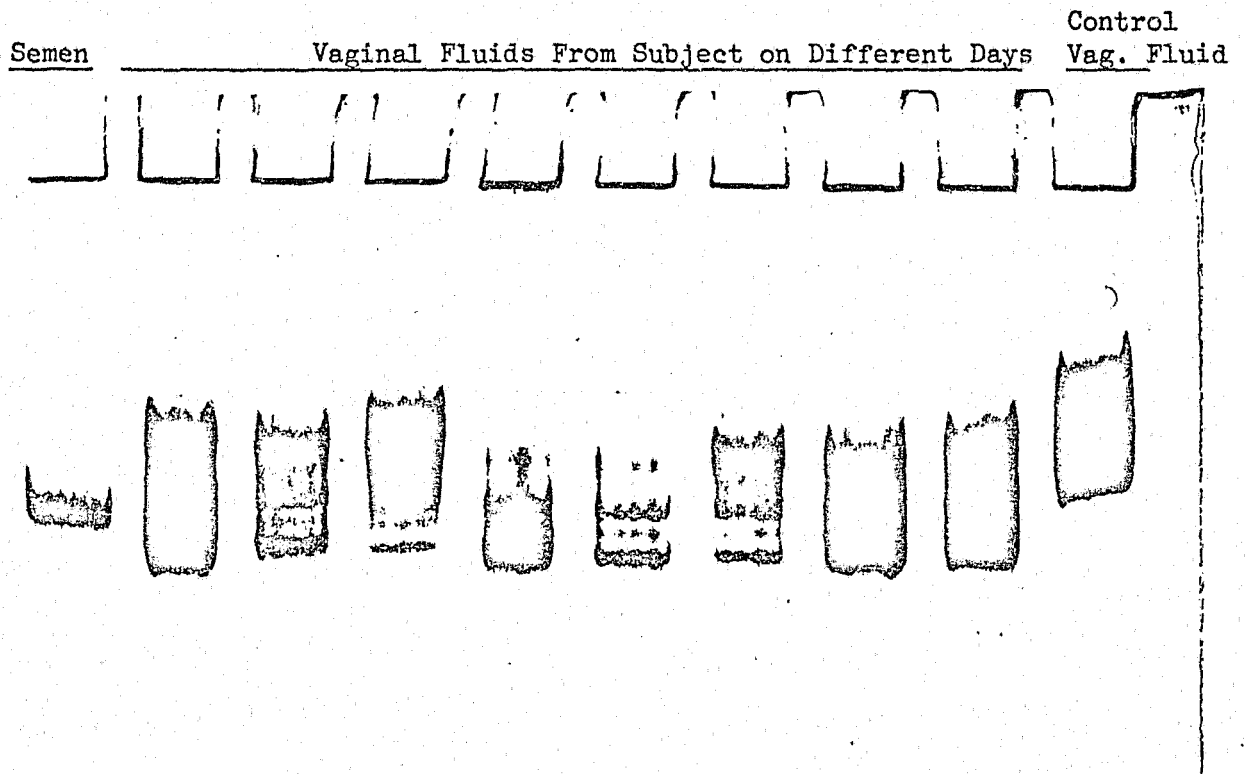Figure 2: Demonstration of Unusual Acid Phosphatase Patterns
in the Vaginal Fluids of a Woman using an IUD for Contraception.

Semen   Vaginal Fluids From Subject on Different Days   Control
                                                                 Vag. Fluid

d. <u>Electrophoretic discrimination of PAP, VAP, and tissue acid phosphatases.</u>
The prior reports of the electrophoretic discrimination of PAP and VAP were
confirmed. However, the situation is not as straightforward as it would
appear at first glance.

On starch gels, PAP has A band mobility which is anodal to VAP which has
B band mobility; on acrylamide, this same mobility relationship is observed.
Given the evidence of the immunological experiments that these enzymes are
genetically identical, it would be predicted that the various isozymes might
interconvert. This was tested <u>in vitro</u> by assessing the effects of sialic
acid removal on the electrophoretic mobility of the enzymes. Treatment with
neuraminadase resulted in a change in the mobility of both VAP and PAP; the
mobility of treated PAP became identical to that of untreated VAP. This
experiment, which was only preliminary, indicates the possibility of
interconversion.

A second complexity was observed when VAP from different women were compared.
It was found that the VAP from some women was represented by a single band
of activity; the VAP from others possessed multiple bands (fig. 2). In the
cases where multiple bands were observed, the additional bands were anodal
to the ordinary VAP band. Since these women had abstained from sexual activity,
there was no question of contamination by semen. Rather the answer seems
to be that women using the IUD for contraception exhibit the extra bands.
These extra bands may well be due to leakage of white cell acid phosphatases
from the uterine space since white cell build up is noted with IUD use.
In this regard, it should be noted that the original report describing the
separation of VAP and PAP was from Britain, a country in which the IUD is
not commonly used.

These findings indicate that the use of electrophoresis for the identification
of PAP is not as straightforward as originally indicated. It is apparent
that much more needs to be done to sort out the biochemistry of the VAP-PAP
electrophoretic difference.

e. <u>Work to be done.</u> The work outlined above provides a foundation for
future work. Several questions remain outstanding. It would be desirable
to test the genetic relationship of PAP, VAP, and the tissue ACP_3 locus
products by additional criteria. It is still possible (although unlikely)
that PAP is controlled at a genetic site different from any of the recognized
acid phosphatase loci; if this were the case, the possibility of a specific
test could yet be held out for. Simply from the standpoint of rigor then,
it would be wise to seek independent verification of the status of the
genetic relationships of the acid phosphatase test. Nevertheless, it appears
that at the molecular level there are very fundamental reservations about
the specificity of the acid phosphatase test.

B. <u>Immunological Tests for Semen</u>

1. <u>Assessment of Commercial Anti-Human Semen Antisera</u>

The possibility of using a semen specific antiserum for the detection of
semen traces was first suggested shortly after the turn of the century and

the idea has been reiterated in many papers since. Anti-semen antisera
are commercially available but the use of these antisera in crime labora-
tories does not appear to be widespread. The primary use for the antisera
appears to be as a confirming test for the identification of semen stains;
the antisera are rarely if at all used for the detection of semen traces in
vaginal material.

Despite their low frequency of use in the crime laboratory, immunological
tests for semen do offer an alternative that should not be ignored. In
principle, immunological tests can be made very specific and very sensitive.
Therefore, we sought to assess commercially available anti-human semen
antisera for their specificity and sensitivity. Four commercial antisera
were assessed; two were from separate manufacturers and two were different
lots from the same manufacturer.

The specificity of an antiserum depends upon the antigens it recognizes.
A semen specific antiserum should contain antibodies that recognize
antigenic moieties which are uniquely found in semen; it should not contain
antibodies to antigens found in other tissue material as well as in semen.
Therefore, to assess the specificity of the four commercial antisera, we
attempted to identify the antigens in human semen that were recognized by
each antiserum.

Immunoelectrophoretic (IEP) analysis showed that each of the commercial
antisera recognized several antigens in human semen; none recognized antigens
in human blood plasma. The semen protein antigens reacting with these
antisera were tentatively identified from their IEP patterns. Three of the
four antisera reacted with prostatic acid phosphatase, and all four reacted
strongly with a protein which appeared to be lactoferrin; other minor
semen proteins which could not be identified also reacted with the four
antisera. The reaction with "lactoferrin" was the dominant reaction in
every case. This protein is found in many secretions -- milk, sweat, tears,
but not blood plasma -- and it is thus important to know for sure whether
the commercial anti-semen antisera reacted with this protein; if they did,
then the non-specificity of these antisera would be manifest.

To verify that the reaction was with lactoferrin, two approaches were taken.
First, it was shown that the commercial anti-semen antisera reacted with
milk and other secretions known to contain lactoferrin. The second, and
more rigorous approach, was to show that the commercial antisera reacted
with purified human lactoferrin. Lactoferrin was purified from human milk
by an established procedure and was shown to be homogenous by several
criteria. The reaction of the commercial antisera with the lactoferrin
preparations was demonstrated both by double diffusion analysis and by
immunoelectrophoresis.

The reactivity of the commercial anti-human semen antisera with lactoferrin
demonstrates their lack of specificity. This is not a condemnation of the
use of immunologically based tests; indeed, such a test has been developed
on this project and will be described below. Rather, the problem identified
here has its roots in the manufacture of antisera. All too often the
manufacturer prepares non-specific antisera and then attempts to make it

specific by absorbing out cross-reacting antibodies. A better approach
would be to start with specific proteins and make antibodies against these;
the specificity of the antisera would follow the specificity of the antigens.
The problem of defining specificity is compounded because the average crime
laboratory analyst uses commercial antisera as test reagents without
critically examining the basis of specificity. This aspect of the problem
of specificity is not limited to this one situation; species typing
antisera are not critically examined either.

In summary, the results of this investigation undercut the use of
commercial anti-human semen antisera in immunological tests for semen
identification. It is paramount that before any such preparation be used,
its specificity be critically examined.

A manuscript describing this work is in preparation.

C. Investigations on Alternative Approaches to Semen Identification

1. Introduction

In this section, we describe three approaches to the development of an
alternative test for semen. This work has been guided by the following
considerations.

A good test would be one based on the detection of a seminal constituent
which could be shown to be unique to semen. It should be possible to
demonstrate specificity both empirically and from biological first
principles; this condition suggests that the presence of the marker compound
should be genetically determined. To avoid the problems 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, because the effective dilution of semen in the vaginal pool post
coitus may be as much as 1:2000, the marker should be present in semen
at high levels and/or be detectable at trace levels.

2. Semen Fluorescence Factors

It has been recognized for many years that semen fluoresces (Pollak, Arch.
Path. 35, 140, 1943) and semen phosphorescence has been exploited for the
localization of semen stains (Calloway, et. al., J. For. Sci. Soc. 13, 223,
1973). It has also been noted that liquid semen allowed to stand for several
days often develops an intense yellow or green fluorescence. If any of the
fluorescent factors present in semen were specific to semen, they potentially
could be exploited in a test for semen. Accordingly, we set out to identify
the various fluorescent factors in semen so that their specificity could
be assessed. This work will be described in more complete detail in the
Master's thesis of Robert Garbutt.

a. Native fluorescence of fresh semen. The fluorescence spectrum of
fresh seminal plasma is typical of protein solutions; the excitation
maximum is at 345 nm. Separation of seminal plasma components by gel
filtration chromatography shows that most of the fluorescence resides
in the protein fraction. Some additional fluorescence is observed in low

molecular weight fractions; chemical analysis shows that this fluorescence is produced by free tyrosine and free tryptophan. These two amino acids are the primary fluors in proteins and their presence in the free state in seminal plasma is probably a consequence of protein degradation. The fluorescence of fresh semen thus is typical of protein solutions and is not at all specific to semen.

b. Fluorescent factors in semen stains. As semen stains age, they develop visible fluorescence; fully developed, this fluorescence is blue-white to green in color. A typical fluorescence spectrum of a semen stain extract shows a broad excitation band (350-450 nm) with a peak at about 420 nm and a broad emission band (410-500 nm) with a peak at about 450 nm. Thus semen stains have quite different fluorescence properties than whole liquid semen. To learn more about the fluorescent factors that develop in semen stains, semen stain extracts were fractionated by gel filtration chromatography. The dominant visible fluorescence was associated with the protein fraction; the fluorescence spectrum of this fraction was virtually identical to that of the whole stain extract. This protein associated visible fluorescence could be a result of chemical modification of seminal plasma proteins or of protein binding of some non-protein fluorescence factor. To test these alternatives, fresh semen was fractionated, the fractions were dried, and the development of fluorescence in each fraction was monitored. It was found that fluorescence developed in non-protein fractions and this fluorescence had the same excitation and emission spectra as observed with the whole stain extract. The fractions in which fluorescence developed were further analysed by thin layer chromatography; at least ten fluorescent compounds were separated by this technique. Thus the visible fluorescence that develops in semen stains appears to be the result of the conversion of a non-protein precursor compound (or compounds) to several fluorescent products. The conditions of stain fluorescence developed were investigated to learn more about this process. It was found that fluorescence development was fastest at water activities greater than 0.65; oxygen did not seem to be required since fluorescence development occurred in the absence of air provided water vapor was present. The development of fluorescence in seminal plasma fractions not containing protein indicates that the conversion of precursor to fluorescent product is not an enzyme dependent process.

An attempt to isolate and chemically characterize the fluorescent products was made; not enough material of sufficient purity could be obtained for analysis.

c. Fluorescent factors in standing liquid semen. Whole semen or seminal plasma allowed to stand at room temperature or in the refrigerator sometimes develops an intense fluorescence; this is accompanied by the development of a yellow color in the semen. We had not noticed this phenomenon with blood serum or any other physiological secretion; the possibility thus existed that the "yellow fluor" was unique to semen. We set out to characterize the "yellow fluor" and to discover the mechanism of its production.

Two distinct fluorescence spectra were observed for standing seminal plasma. One spectrum exhibited an excitation peak at about 400 nm and an emission maximum at 460 nm; the second showed an excitation peak at 420 nm and an emission peak at 500 nm. In studying the development of this fluorescence, it appeared that the 400/460 fluorescence showed up first and sometimes, but not always, converted to the 420/480 fluorescence.

Both 400/460 and 420/480 fluorescence factors were characterized by thin layer chromatography and gel filtration chromatography. These analyses showed that there were at least five separable compounds exhibiting the characteristic fluorescence spectrum and that none of these compounds were protein in nature. It was possible to separate out of fresh seminal plasma fractions which, if allowed to stand, developed the characteristic fluoresence. It was not possible to purify enough of the precursor or of the fluorescent products to establish their chemical identity.

The mechanism underlying the development of the yellow fluorescent compount was shown to be biological: the yellow fluors developed as the by-product of bacterial contamination. This was shown in a series of experiments in which seminal plasma made sterile by millipore filtration or heat treatment exhibited no fluorescence development until innoculated with untreated seminal plasma: seminal plasma containing antibiotics showed no fluorescence development either. It was subsequently possible to isolate the bacterium and show that it was indeed responsible for the production of yellow fluor in standing semen. The bacterium was identified as a strain of Pseudomonas fluorescens; the strain type was identified by its nutritional requirements.

To determine whether the production of a fluorescent factor by this bacterium could be exploited in a test for semen, we investigated the conditions of fluorescence development. It became apparent that although only semen contained the precursor that turned into the characteristic fluorescent factor, others physiological fluids were acted upon by the bacterium to produce fluorescent compounds with different fluorescent spectra. Thus it would appear that a simple fluorescence development test system mediated by the bacterium is not immediately practical.

d) Conclusions and Prospectus for Future Work. We have shown that the fluorescence in stains and in standing semen develops as a consequence of the conversion precursor compounds into the fluorescent factors. Because the fluorescence that develops in both cases appears to be unique to semen, it is possible that the precursor compounds are semen specific; if this indeed proves to be the case, then it still may be possible to develop a test for semen based upon their detection. Although ih this study we were not able to chemically identify the precursor compounds, we were able to learn quite a bit about them; the foundation is laid for future study.

3. Seminal Ribonuclease

A test based upon the detection of an enzyme activity offers many advantages. There are several accounts in the literature describing sperm specific enzymes; the best known is the testicular isozyme of lactate dehydrogenase (LDHx) (Goldberg (1975) Acta Endocrin. 194 Suppl., 202). However for the reason stated above, seminal plasma specific enzymes would be of greater value in

the identification of semen. Unfortunately, there is relatively little information in the literature pertaining to seminal plasma specific enzymes although there are several enzyme activities for which specificity has been suggested (Mann, Biochemistry of Semen and of the Male Reproductive Tract). It has been reported that bull seminal plasma contains a specific ribonuclease of seminal vesicle origin (Alessio, et.al., (1972) Eur. J. Biochem. 26, 153, 162). We determined that human seminal plasma contained high levels of ribonuclease activity and that the activity can survive long periods of time (up to 15 years) in semen stains. We therefore instituted studies to characterize the human seminal ribonuclease so that it might be determined whether it was specific to semen. Ribonucleases catalyse the hydrolysis of ribonucleic acid (RNA). Enzymes from different sources exhibit different specificity in the site of cleavage, differences in pH optima, and differences in susceptability to inhibitors. The characterization of the ribonuclease activity in human semen suggested that several isozymes were present. This characterization also suggested that the seminal ribonucleases were distinct from ribonucleases found in other tissues. This, however, does not provide an adequate base for the development of a test for semen useful in the forensic context; other tissues do possess ribonuclease activity (albeit at much lower levels) and an array of assays are required to demonstrate the distinctness of the seminal enzymes. In addition, the assay procedures themselves are rather inconvenient and would not easily lend themselves to routine analysis in a forensic lab.

Attempts to characterize the seminal plasma ribonucleases by electrophoresis were for the most part unsuccessful. We were able to visualize the enzyme activity on acrylamide gels but could not get high resolution separation. Moreover, the stability of the activity on the electrophoresis gels was not constant. We also attempted to characterize the ribonuclease activity by gel filtration analysis; these efforts were without marked success.

Our inability to satisfactorily characterize the seminal ribonuclease after a year of work led to the abandonment of this project; it was apparent that the degree of characterization required for the development of a forensic test could not be completed in the remaining period of the grant. However, the seminal ribonuclease does offer potential and should not be discounted.

4. Other Enzymes

Several other enzymes were investigated as possible seminal plasma markers; these included acetyl-hexoseaminidase, sorbitol dehydrogenase, and neutral proteases. The hexose-aminidase and protease experiments were suggestive but were not followed up due to time constraints.

5. p30 Protein

The electrophoretic characterization of human semen (Section III, below) indicated the presence of a protein of 30-32,000 molecular weight which was

present in semen at very high levels. This protein, designated p30, was singled out for further study. The results of this work have been accepted for publication in J. Forensic Science; the preprint is enclosed as an appendix: "Isolation and Characterization of a Semen Specific Protein from Human Seminal Plasma. A Potential New Marker for Semen Identification."

III. CHARACTERIZATION OF SEMINAL PLASMA PROTEINS

A. Rationale

A complete characterization of seminal plasma proteins has been undertaken with several objectives in mind. (a) The characterization may lead to the discovery of new semen specific markers; the protein p30 was first noted as part of this seminal plasma protein characterization. (b) Differences between individuals observed during the course of the characterization will provide leads to individualization studies. (c) The characterization will provide the basic parameters for overall persistence studies. (d) If systematic changes occur with time in a stain or with time in the female reproductive tract, then it may be possible to develop tests for the determination of time of ejaculation or intercourse. (e) Semen possesses considerable lytic activity, and it is important to know which components of seminal plasma are stable during storage and which are labile.

B. Characterization

1. Basic Findings

Seminal plasma proteins have been characterized by five criteria: electrophoretic mobility in an anodal acrylamide gel system, electrophoretic mobility in a cathodal acrylamide gel system, isoelectric point by isoelectric focusing, molecular weight as determined by gel filtration and subunit molecular weight as indicated by SDS gel electrophoresis. Seminal plasma proteins have been fractionated by electrophoresis and by gel filtration and then characterized. In addition, each fraction has been assessed for its carbohydrate content. These studies yield the following information. Seminal plasma contains several very acidic proteins and a great number of very basic proteins; these acidic proteins and basic proteins are not present in blood plasma. These acidic and basic proteins are for the most part of low molecular weight and do not contain significant amounts of carbohydrate. There are also a number of "neutral" proteins, proteins with isoelectric points in the range pH4-8; these tend to have molecular weight of 20,000 daltons or greater and tend to be glycoproteins. There are also in semen high molecular weight aggregates of low molecular weight subunits; these have a considerable amount of bound carbohydrate.

2. Changes with Aging

With this basic information in hand, we investigated the storage properties of the seminal plasma proteins. Seminal plasma samples from the same individual, stored frozen for periods of days to months, were shown to have very different patterns; the difference resulted primarily from the loss of the very basic proteins. This suggested that the disappearance

of the basic proteins might serve as a marker for time of intercourse, and several experiments have been run to test this. Seminal plasma from different individuals were incubated at body temperature for various times after ejaculation; it was found that the very basic proteins disappear at a regular rate. This is illustrated in Blake and Sensabaugh (1976 J. For. Sci. 21,784). This phenomenon requires further study; estimation of time of ejaculation or time of intercourse may be possible.

The time dependent degradation of seminal plasma proteins has a distinct operational ramification. This is that the analysis of seminal plasma must be conducted rapidly and under conditions that are inhibitory to the degradation processes. Improper collection techniques at the crime scene or storage procedures in the crime laboratory could result in the loss of information or in the generation of artifact. Either result could lead to error.

IV. INDIVIDUALIZATION OF SEMEN

A. Statement of Problem

The utilization of genetic typing in the analysis of semen is an important objective; genetic information about a rapist gained from analysis of his semen can provide significant corroborating evidence as to his identity. Before this objective can be practically realized, several questions must be answered. It must be known what genetic markers are present in semen and what kinds if nongenetic variability enter into their phenotypic expression. The amount and stability of each marker must be known as must any procedural problems specific to the analysis of semen. It is only after these questions are answered that reliable protocols of analysis can be developed.

The work described in this section is partitioned into two parts. The first deals with the identification of genetic markers in semen and their phenotypic expression; this demonstrates the theoretical potential of semen individualization. The second part deals with the quantitation of each marker present in semen and detection limits; this demonstrates the practical potential of semen individualization.

B. Identification of Genetic Markers in Semen

1. Survey of Known Genetic Markers

Human sperm and seminal plasma was surveyed for the presence of twenty five known genetic marker proteins and enzymes. This work has been described in detail in two publications (Blake and Sensabaugh, (1976) J. For. Sci. 21, 784 and Intl. Microform. J. For. Med. 10, 21, and in the doctoral thesis of E. T. Blake);both publications are included as appendices.

2. Identification of New Genetic Markers

During the course of this work, a new genetic marker in sperm was identified: sperm diaphorase. This work has been published (Caldwell, et.al.(1976) Science 191, 1185) and the publication is included as an appendix.

Another possible new genetic marker was noted; this was seminal plasma lactoferrin. Variation in the electrophoretic mobility of this seminal plasma antigen was detected by immunoelectrophoretic analysis; about 100 individual seminal plasma samples were analysed by this technique. The variable protein was identified as lactoferrin using a monospecific anti-serum prepared against purified milk lactoferrin. Because the typing procedure required considerable interpretation, we sought to develop an alternative typing procedure; acrylamide gel electrophoresis and isoelectric focusing were tested but neither gave satisfactory results. Lactoferrin has bound sialic acid and we thought perhaps this charged carbohydrate was the problem. We thus did experiments in which sialic acid residues were striped off lactoferrin of different types; the result was that the variation between types was obscured. This suggested that the source of the variation might be in the number of sialic acid residues attached to the lactoferrin; to test this hypothesis would require biochemical analysis beyond the scope of the grant and accordingly, work on this potential polymorphism was halted.

3. Gene Frequencies of Genetic Markers

In order to assess the discrimination potential of any genetic marker, it is necessary to know phenotype frequencies. This necessitates a population study. In the course of this project we did two such studies. The first was on the sperm diaphorase polymorphism and has been reported (Caldwell, et al., (1976) Science 191; 1185). The second was on Esterase D in orientals; this study was also published (Golden and Sensabaugh (1976) Humangenetik, 35; 103).

C. Quantitation of Genetic Markers in Semen

The ease of detection of a genetic marker in semen depends upon the amount of the marker initially present in semen and upon its stability. We have determined the levels in sperm and seminal plasma of many of the markers described above; to put this data into context, we have compared the levels of each marker in semen and in blood. We have also determined the threshold limit of detection required for the typing of most of the markers; this allows relative estimates of the amount of semen required for a typing determination. Several significant observations were made in this study.

Comparison of enzyme activities in sperm and seminal plasma showed that many enzymes usually thought of as intracellular, eg, glucose-6-phosphate dehydrogenase, adenylate kinase, phosphoglucomutase, etc., were found at higher levels in seminal plasma than could be readily accounted for by leakage from sperm. This interpretation was verified by finding comparable levels of these enzymes in the seminal plasmas of non-vasectomized and vasectomized males (Table 1). A consequence of this observation is that genetic typing can be done on semen of vasectomized males.

Several enzymes, notably peptidase A, phosphoglucose isomerase and phosphoglucomutase, are present in seminal plasma at 10-100 times the levels in sperm; 80% or more of the activity of these three enzymes in whole semen is from the seminal plasma. For these three enzymes, the level of activity in seminal plasma allows typing on very small amounts semen; 1 ul or less of whole semen provides enough enzyme activity for typing (Table II). In contrast, some of the other genetic markers are not present in high enough concentrations in seminal plasma for convenient typing; e.g., 50-100 ul of whole semen would be required for a typing analysis. However sperm constitute a more concentrated source of enzyme and if sufficient sperm could be collected, a typing analysis could be done on the lysed sperm. Thus, for example, about $5x10^6$ sperm contain enough 6-phosphogluconate dehydrogenase for typing; this number of sperm can be collected from about 50 ul of whole semen.

It is possible to assess the effective dilution of a semen sample by a quantitative measurement of its acid phosphatase activity. Thus, if an average ejaculate contains 1000 units/ml acid phosphatase activity and a vaginal wash post coitus contains 10 units/ml, then the effective semen dilution is 1:100. By corrolating the activity of the genetic markers to the effective dilution, it is possible to predict whether a typing operation will be successful. Thus at an effective dilution of 1:100, it should be possible to type peptidase A but it probably would not be possible to type phosphoglucomutase unless sperm could be separated out of the wash.

These analyses lead to the conclusion that the genetic markers of semen can be partitioned into three categories: (a) those markers which are present at such low levels that they have little, if any, practical value at the present time; (b) those markers present at low to moderate levels can be typed only when sufficient amounts of relatively undiluted semen are available for analysis; and (c) those markers present at high enough levels to justify routine typing analysis.

In the first category are those genetic markers which can be typed in semen only with considerable difficulty, either because the activity is very low or because the typing methodology is not readily accessible. The markers in this category are Gm, Inv, alpha$_1$-antitrypsin, sperm diaphorase, phophoglucomutase (PGM$_3$), peptidase D, and glutamate-oxalo-acetate-transaminase. Typing Gm and Inv in semen is restricted by the

Table I

Sepcific Activity of Enzymes in Sperm and Seminal Plasma

Enzyme	Sperm	Nonsperm Cells	Specific Activity (milliunits/mg protein)	
			Seminal Plasma	Seminal Plasma (vaseatomized)
Adenylate kinase	330.8 ± 177.3(12)		1.58 ± 1.04(11)	1.96(1)
Glucose-6-phosphate dehydrogenase	107.7 ± 10.5(7)	+	0.114 ± 0.066(8)	0.087(1)
Peptidase A	997.2 ± 589(6)	+	155.9 ± 129(13)	248.1 ± 256(3)
Phosphoglucomutase	525.9 ± 263.2(13)		14.5 ± 8.7(17)	18.7 ± 1.1(2)
6-Phosphogluconate dehydrogenase	163.3 ± 114.5(10)	−	0.207 ± 0.1(16)	0.135(1)
Phosphoglucose isomerase	2,592 ± 1,987(6)	−	104.8 ± 47.6(9)	62.8(1)
Amylase (Amy$_2$)	−	−	24.1 ± 9.8(20)	31.8 ± 19.3(2)

Table II

Estimates of Minimum Quantity of Semen for Typing Determinations

Genetic Marker	*Minimum Amount Detectable	Whole Blood (ul)	Whole Semen (ul)	Seminal Plasma Alone (ul)	Sperm Alone Equivalent vol. of whole semen (ul)	Number of Cells (10^6)
Phosphoglucose isomerase	0.5	0.1	0.1	0.1	0.6	0.05
Peptidase A	0.25	1	0.04	0.04	1.3	0.1
Phosphoglucomutase (PGM$_1$)	0.5	1	1	1	13	1
Amylase (Amy$_2$)	5	5	5	5	-	-
Adenylate kinase	0.5	0.01	5	10	13	1
6-Phosphogluconate dehydrogenase	0.5	1	25	60	40	3
Glucose-6-phosphate dehydrogenase	0.5	1	40	90	60	5
Transferrin	5	5	50	50	-	-
Gm	10 (IgG)	2	100	100	-	-
Inv	10 (IgG & IgA)	2	100	100	-	-
Alpha$_1$-Antitrypsin	10	10	150	150	-	-
Sperm Diaphorase		-	150	-	150	13
Phosphoglucomutase (PGM$_3$)	0.5	-	400	-	400	35

*Minimum amounts are estimates and are assumed to be the same for all proteins which are dete ted by a similar mechanism (e.g., coenzyme linked tetrazolium stain). For enzymes, the number refers to the number of milliunits applied to a gel in order to obtain an adequate typing response. For nonenzymatic proteins, the number refers to ug protein required for a typing response.

the low level of IgG in seminal plasma, difficulties in obtaining typing
antisera, and the complexity of the typing procedure. This is unfortunate
since Gm has the best discrimination index of all the protein genetic markers
in semen. More sensitive methods for typing Gm will increase the usefulness
of this marker for typing semen; however, typing will be restricted to
laboratories which type Gm on a routine basis. The practical utilization of
sperm diaphorase is limited because it is expressed only in sperm and testi-
cular tissue. Therefore, there is little point in attempting to type this
locus unless reference material can be obtained from a suspect. The activity
of the other markers is sufficiently low that typing will be almost impossible
unless large quantities of semen are available for analysis. The cumulative
identity index for genetic markers in this category is 0.018; Gm and Inv
contribute significantly to this value.

Genetic markers with moderate activity and which have limited potential for
typing are amylase, transferrin, adenylate kinase, 6-phosphogluconate
dehydrogenase, glucose-6-phosphate dehydrogenase, and esterase D. Of these
only amylase and transferrin are found at significant levels in seminal plasma.
Therefore, the others cannot be typed unless sufficient quantities of sperm
are present. The stability of amylase is not known and its level in seminal
plasma is not far from the limit of detectability; accordingly, if seminal
plasma is significantly diluted by the collection procedure, some concentra-
tion step will be required to detect amylase activity. Transferrin typing
in seminal plasma would not be justified unless it is alleged that the semen
came from a Black; in any case, due to the relatively low level in semen,
genetic typing would require an autoradiographic procedure. The other enzymes
will require significant amounts of sperm in order for typing to be possible;
however, sample can be conserved by typing multiple loci on a single electro-
phoretic gel. Esterase D and adenylate kinase can be typed together as can
6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase. As is
the case with tranferrin, there is little point in attempting to type glucose-
6-phosphate dehydrogenase unless a Black is suspected.

There are three genetic markers in the last category: phosphoglucose isomerase,
peptidase A, and phosphoglucomutase. The activity of each of these genetic
markers is very high in both the plasma and sperm fractions of semen. Phospho-
glucose isomerase does not have a high discrimination potential; variants of
this enzyme are rare. Peptidase A has a good theoretical discrimination
potential; unfortunately there is difficulty in distinguishing the products
of the Pep_A^1 and Pep_A^8 alleles. Until procedures are developed which can clearly
make this distinction, typing of peptidase A will be limited to detecting the
products of the Pep_A^2 allele which occurs only in Black populations. At the
present time phosphoglucomutase is the best single protein genetic marker in
semen. It is not difficult to type in dried or liquid semen as long as one
is aware that its phenotypic expression in semen is somewhat different from
that in blood.

It should be noted that this categorization of genetic markers is not
binding. Should, for example, a new procedure for adenylate kinase typing
be developed that increased the sensitivity of detection 20 fold; this

would reduce the threshold amount of semen required for typing from 5 ul to 0.25 ul. Use of this hypothetical super sensitive procedure then would shift adenylate kinase into the routine category. This illustrates the importance of assessing detection limits quantitat; it allows one to see how much improvement in sensitivity is required to make routine typing practical.

A manuscript describing this work is in preparation.

PART III   APPENDICES

I.   GRADUATE STUDENT TRAINING

1.   Edward T. Blake (in part), D. Crim., Dec. 1976.
     Dissertation Topic: Genetic Markers in Human Semen.

2.   Robert Garbutt, M. Crim., Dec. 1977.
     Thesis Topic: Fluorescence in Human Seminal Plasma.

3.   Keith Inman, M. Crim., Dec. 1977.
     Thesis Topic: Genetic Relationship of Acid Phosphatases.

II. PRESENTATIONS

1.   Symposium on Rape: Investigation, Prosecution, and Physical Evidence.
     1974.
     Participants: G.F. Sensabaugh (Moderator); Bay Area Women Against Rape;
     Alameda County District Attorney's Office; Alameda County Public Defender;
     Berkeley Police Department; Administrative Assistant, 12th Assembly
     District.

2.   Esterase D Polymorphism in Blood Stains. Edward T. Blake and George F.
     Sensabaugh. 44th Semiannual Seminar of the California Association of
     Criminalists, 1974.

3.   Differential Expression of Amylase Loci (Amy_1 and Amy_2) in Human Body
     Fluids and Secretions. Edward T. Blake and George F. Sensabaugh.
     45th Semiannual Seminar of the California Association of Criminalists,
     1975.

4.   Molecular Evolution and the Immunological Determination of Species.
     George F. Sensabaugh. Seventh International Meeting of Forensic Sciences,
     Zurich, 1975.

5.   Protein and Enzyme Polymorphisms in Human Semen. Edward T. Blake and
     George F. Sensabaugh. Seventh International Meeting of Forensic Sciences,
     Zurich, 1975.

6.   Assessment of Individuality. G.F. Sensabaugh and C.V. Morton. 46th
     Semiannual Seminar of the California Association of Criminalists, 1975.

7.   Sperm Diaphorase: Genetic Polymorphism of a Sperm-Specific Enzyme in
     Man. Kevin Caldwell, Edward T. Blake, and George F. Sensabaugh. 46th
     Semiannual Seminar of the California Association of Criminalists, 1975.

8.   Individuality of Blood. G.F. Sensabaugh. Pacific Conference on Chemistry
     and Spectroscopy, American Chemical Society, 1975.

9.   Biochemical Genetics of Semen and the Investigation of Rape. E.T. Blake
     and G.F. Sensabaugh. Pacific Conference on Chemistry and Spectroscopy,
     American Chemical Society, 1975.

10. Considerations on the Individualization of Blood. G.F. Sensabaugh, C.V. Morton, E.T. Blake, M. Grubb. 28th Annual Meeting, American Academy of Forensic Sciences, 1976.

11. Expression of Genetic Markers in Human Semen. E.T. Blake and G.F. Sensabaugh. 28th Annual Meeting, American Academy of Forensic Sciences, 1976.

12. Rape, Reproduction, and the Biochemical Genetics of Semen. G.F. Sensabaugh. Seminar, Department of Genetics, University of California, Berkeley, 1976.

13. Origin of a Fluorescent Factor in Human Semen. R. Garbutt and G.F. Sensabaugh. 47th Semiannual Seminar of the California Association of Criminalists, 1976.

14. Biochemical Characterization of a Protein Specific to Semen. G.F. Sensabaugh. 47th Semiannual Seminar of the California Association of Criminalists, 1976.

15. Immunological Cross-Reactivity of Prostatic Acid Phosphatase. Keith Inman and George F. Sensabaugh. 47th Semiannual Seminar of the California Association of Criminalists, 1976.

16. Biochemical Genetics of Semen. G.F. Sensabaugh. Seminar, Department of Biochemistry, University of California, Berkeley, 1976.

17. Purification and Characterization of a Semen Specific Protein. G.F. Sensabaugh. 29th Annual Meeting, American Academy of Forensic Sciences, 1977.

18. Rape, Reproduction, and the Biochemical Genetics of Semen. G.F. Sensabaugh. Seminar, Division of Medical Genetics, University of Washington, Seattle, 1977.

19. Biochemical Genetics of Semen and the Investigation of Rape. G.F. Sensabaugh. Northwest Association of Forensic Scientists, 1977.

III. PUBLICATIONS

1. Sperm Diaphorase: Genetic Polymorphism of a Sperm-Specific Enzyme in Man. Kevin Caldwell, Edward T. Blake, and George F. Sensabaugh. Science 191 1185-1187, 1976.

2. Genetic Markers in Human Semen: A Review. Edward T. Blake and George F. Sensabaugh. J. For. Sci. 21, No. 4, 784-796, 1976.

3. Protein and Enzyme Polymorphisms in Human Semen. E.T. Blake and G.F. Sensabaugh. International Microform Journal of Legal Medicine 10, No. 4, Article 21, 1975.

4. Molecular Evolution and the Immunological Determination of Species. G.F. Sensabaugh. International Microform Journal of Legal Medicine 11, No. 2, Article 219, 1976.

5. Esterase D Typing in Blood Stains. E.T. Blake and G.F. Sensabaugh. Forensic Serology 1, No. 5, 1-4, 1976.

6. Medical Protocol for Victims of Sexual Assault - Bay Area Hospital Conference on Sexual Assault, Queens Bench Foundation, San Francisco.

7. Esterase D Polymorphism in Chinese and Japanese. V.L. Golden and G.F. Sensabaugh. Human Genetics 35 103, 1976.

8. Isolation and Characterization of a Semen Specific Protein from Human Seminal Plasma. A Potential New Marker for Semen Identification. G.F. Sensabaugh. J. For. Sci. (in press).

END