Elsevier Sequioa A.A., Lausanne – Printed in The Netherlands

THE PERSISTANCE OF SEMINAL CONSTITUENTS IN THE HUMAN VAGINA

ANNE DAVIES and ELIZABETH WILSON
Metropolitan Police Forensic Science Laboratory, London (Great Britain)

SUMMARY

The persistence of spermatozoa, seminal acid phosphatase, choline and seminal blood-group antigens in the human vagina after sexual intercourse has been studied and the following results obtained.

1. Spermatozoa were usually found up to 3 days after intercourse and were occasionally found up to 6 days afterwards. Smears without spermatozoa were obtained from swabs taken as early as 28 hours, but remained rare until 2 days after intercourse.
2. Seminal acid phosphatase sometimes remained detectable up to 3 days after sexual intercourse. The test was most useful on swabs taken within 1 day and rarely useful after 2 days.
3. Choline was usually only detectable on swabs taken within 1 day of intercourse and even within this time many negative results were obtained. The probability of positive results declined swiftly after 14 hours.
4. Seminal blood-group antigens were only detected at a useful level on swabs taken within 48 hours of intercourse. The chances of obtaining a positive result decreased swiftly with an increasing time interval after intercourse.

INTRODUCTON

The persistence of seminal constituents in the vagina is frequently of considerable importance in alleged offences involving sexual intercourse. Interpretation of the tests currently used in this laboratory to determine the presence of semen is often difficult due to variations and contradictions in the published literature.

Semen is usually identified by the presence of spermatozoa. There are widely varying estimates of the time that spermatozoa survive in the vagina. Pollack[1] in his survey of the literature gives various estimates from 30 minutes to 17 days. More recently Sharpe[2] stated that non-motile spermatozoa are usually found for 1 to 12 hours after intercourse, exceptional1y for 18 to 24 hours, and in one unique case they were found 3 to 4 days

after intercourse Morrison[3] stated that they could be found up to 9 days after inter-course.

Two other tests for demonstrating the presence of semen utilise acid phosphatase (AP) and choline, both abundant in human semen. The detection of the enzyme AP has been comprehensively discussed by Kind[4]. There is some confusion in the literature as to whether AP occurs in vaginal secretion. Walther and Hohn[5] quote values for levels of AP activity in vaginal secretions; however, Rupp[6] ignores the possibility of vaginal AP. The Florence Iodine Test[7-9] detects free choline, which increases in amount after ejaculation

Immunological techniques for the identification of semen have been investigated in this laboratory, but are not considered entirely satisfactory, particularly when the semen is mixed with vaginal secretions.

The present study was undertaken because of the contradictions in the available literature and because of the lack of data specifically referring to cotton-wool swabs used by police surgeons for vaginal sampling. Results of the tests on these swabs can sometimes be discussed in great detail in ensuing court cases.

The three main aspects of this work have been the study of the survival of spermatozoa and choline and a critical appraisal of the AP test. Some work has also been carried out on the survival of seminal blood-group antigens but, due to lack of donors, this work is merely indicative of possible trends.

All the female donors were staff of this laboratory, since absolute accuracy in timing was essential, as was the veracity of donors providing semen-free swabs. However, this limited considerably the number of donors available to us. Particular difficulties were encountered in obtaining swabs taken a long time after intercourse and also swabs suitable for grouping purposes. Consequently, statistical analysis of our results has been considered but rejected. However, the data collected are fairly extensive and informative and should be of some value to other forensic scientists.

METHODS AND MATERIALS

Cotton-wool swabs, inserted as high as possible into the vagina at known intervals after sexual intercourse, have been used in this survey. Semen-free vaginal swabs, anal swabs, some plant material, fungi and contraceptive creams have also been tested to aid interpretation of the AP test results.

Spermatozoa

The presence of spermatozoa on a vaginal swab was determined by making and examining one smear from each swab. Each smear was made on a glass slide In 1 drop of distilled water. It was air dried, heat fixed and stained with haematoxylin and eosin. The frequency of spermatozoa was denoted using the following symbols: ++++, many in every field; +++, many or some in most fields; ++, some in some fields, easy to find; + hard to find; 0, none.

Acid phosphatase (AP)

The presence of AP was detected by one of the techniques used in this laboratory which involves the use of sodium naphthyl phosphate as substrate and the diazo dye Brentamine Fast Blue B. The reagents are made up as follows. Solution A:1g Brentamine Fast Blue B; 20 g sodium acetate (hydrated); 10 ml glacial acetic acid; 100 ml water. Solution B: 0.8 g sodium naphthyl phosphate; 10 ml water. The working solution was made up every day and kept in a dark bottle; the following proportions were used: 10 ml solution A; 89 ml water; 1 ml solution B.

A smear was made from a dampened swab onto a piece of filter paper, which was then sprayed in a fume cupboard with the working solution. The time taken for the distinctive purple colour to begin to appear, indicating the presence of AP, was recorded to the nearest 5 seconds.

Choline

Choline was detected using a concentrated solution of iodine in potassium iodide: in the presence of choline characteristic crystals of choline periodide develop. A water extract, obtained by soaking a swab with distilled water until one free drop fell onto a slide, was used for simple extract procedure; also, further concentrated extracts with water were carried out by macerating the swab in distilled water. Any swab used for concentrated extraction for choline was not used for further tests.

Seminal blood-group antigens

Some of the vaginal swabs bearing semen were tested for the presence of seminal blood-group antigens. Only swabs where the blood group reactions of the vaginal secretions could not mask the presence of the seminal blood-group antigens were used for this work.

Grouping was carried out using the techniques of absorption-inhibition and absorption-elution[10]. For absorption-elution, both the sample, a neat extract, and a series of dilutions from this extract were used. Dilution was necessary for this technique because an excess of water soluble A, B and H antigens can result in the absence of reaction with neat stains.

For absorption-inhibition, complete inhibition was accepted as evidence of the presence of an antigen. Partial inhibition was regarded as indicative but not conclusive. Complete correlation of absorption-elution and absorption-inhibition results was necessary for an acceptable result.

Every swab was tested for spermatozoa AP and choline as described. This follows the normal procedure for vaginal swabs received in this laboratory; except that concentrated extracts for the Florence Iodine tests are not routine.

Figs 1—8 summarize the results of this study.

Spermatozoa

Fig. 1 shows the relative percentages of the density of spermatozoa found at different times. There was less obvious variation between individual densities of spermatozoa at different times than there was in the other seminal constituents.

There are sufficient data for the results to be presented graphically up to a time of 96 hours; however, we were only able to obtain a limited number of swabs taken more than 4 days after intercourse. No negative results were obtained before 30 hours; very few negative, results were obtained up to 48 hours, but thereafter they become more frequent. The last positive result occurred at 144 hours (6 days). Between 90 and 156 hours, 41 swabs were taken of which 14 contained spermatozoa; the spermatozoa were extremely difficult to find on all of these. It is possible that spermatozoa could be found on swabs

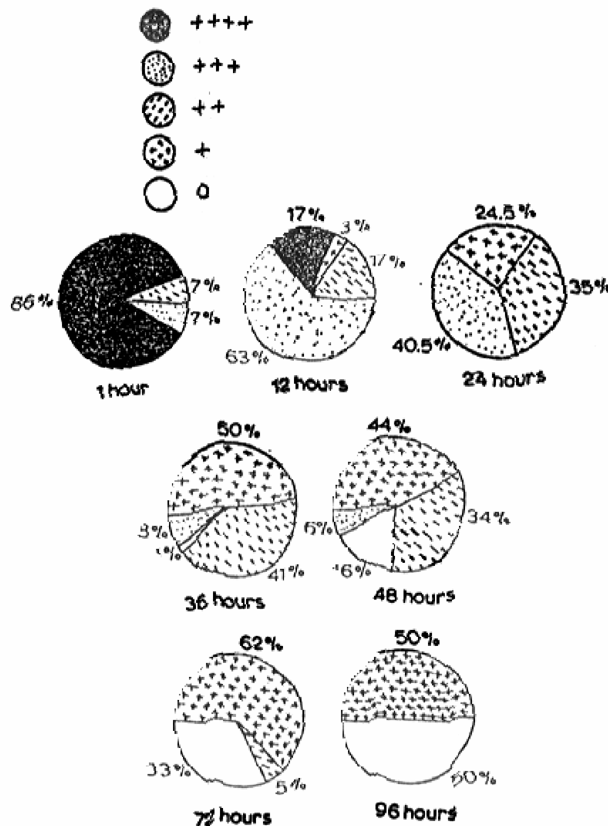

Fig. 1. Relative percentages of the densities of spermatozoa found on smears from vaginal swabs taken at different times after sexual intercourse

taken after 6 days; however, we feel that our data are insufficient during this period for us to make any comment.

Tails are most frequently found attached to spermatozoa on swabs taken within 1 hour of intercourse. They are commonly found up to 16 hours; thereafter spermatozoa with tails occur rarely but have been found as late as 72 hours.

Acid phosphatase (AP)

I. Vaginal acid phosphatase

Most vaginal swabs from our donors displayed some vaginal acid phosphatase activity causing colour to develop within 40-200 seconds. Other vaginal swabs examined in the laboratory have developed colour as early as 30 seconds. These were known to contain only vaginal acid phosphatase as they were tested electrophoretically using a technique that distinguishes between vaginal AP and seminal AP[11]. However, these fast reaction times are rare; times under 65 seconds are relatively uncommon, the average being 90-100 seconds.

Swabs from pregnant women gave a fast AP test reaction time as did swabs with smears exhibiting unusually large numbers of bacteria. The implications of these observations have not been further investigated. Some work was done to ascertain whether vaginal AP activity showed any cyclical variation but none was observed, possibly due to the imprecision of the method and the low concentrations of the enzyme involved.

2. *Seminal acid phosphatase*

There was considerable variation in seminal AP activity between individuals and to a lesser extent between ejaculates from the same individual.

A reaction time of less than 30 seconds was considered a very good indication of the presence of semen as no vaginal AP reacted within this time. The graphs In Fig. 2 show the percentage of swabs taken at various times after intercourse, that reacted at 5, 10, *15,* 20 and 25 seconds; Fig. 3 summarizes these results. It can be seen that there is a decreasing likelihood of obtaining a reaction time of less than 30 seconds with an increasing time interval after intercourse, the outside limit being just under 48 hours. The initial dip in this graph is of great interest. The implications of this are considered in the discussion at the end of this paper.

A reaction time of less than 65 seconds was considered a fair indication of the presence of semen, although this must be confirmed by other methods since vaginal AP occasionally reacts within the time range 30-65 seconds. Fig. 4 shows the percentage of swabs obtained at various times which reacted in under 65 seconds. The curve is not continued beyond 72 hours because of lack of results, but all swabs obtained after this time having a reaction time of less than 65 seconds were tested electrophoretlcally and found to contain only vaginal AP.

Reaction times greater than 65 seconds have been obtained from swabs with plentiful

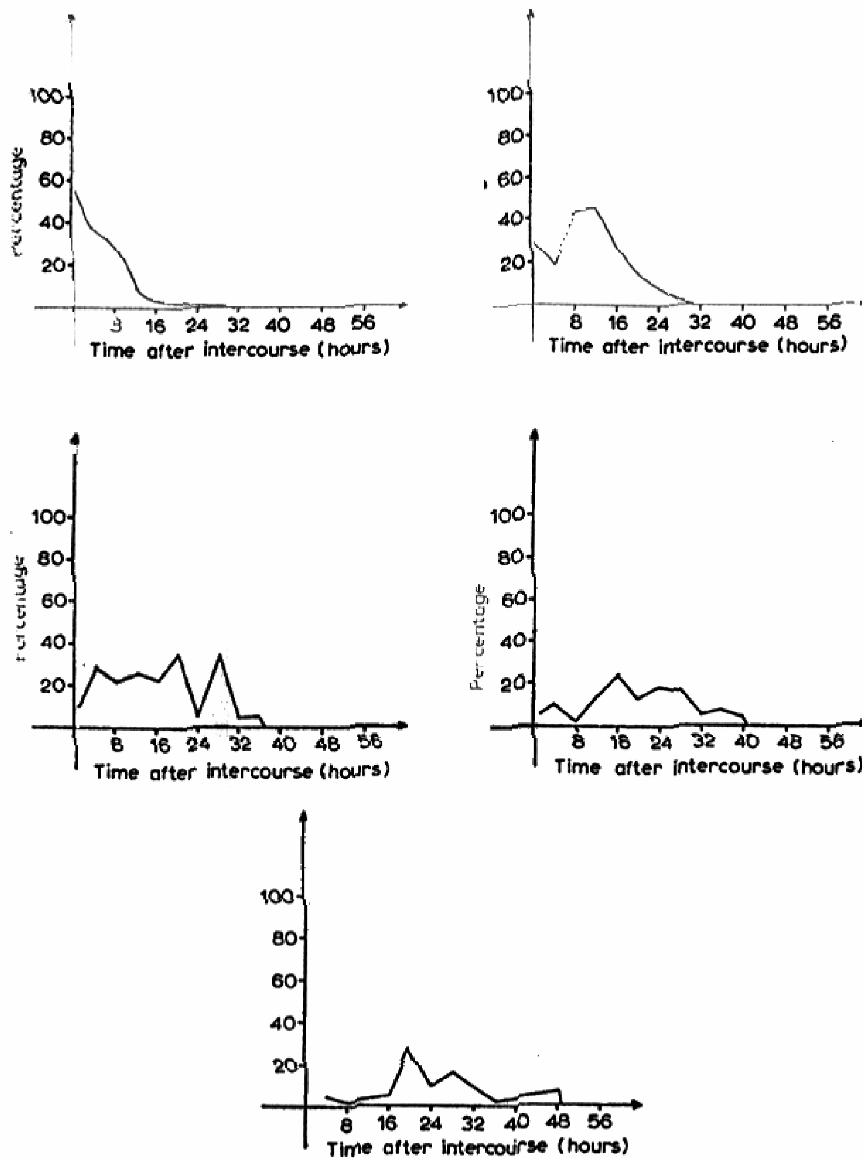

Fig. 2. The five graphs show the percentages of vaginal swabs, taken at various times after sexua1 intercourse, reacting at 5 (A), 10 (B), 15 (C), 20 (D) and 25 (E) seconds with theAP test solution.

spermatozoa taken as recently as 16 hours after intercourse, so although this sort of result is rare a slow reaction time need not necessarily indicate lack of semen.

3. PositiveAP test reactions from other sources ‘

Some work was carried out on certain vegetables, fungi and contraceptive creams and the results obtained were similar to those quoted by Kind[4]. Vegetable extracts appeared to contain low concentrations of AP but fungi showed faster reaction times. Some contraceptive creams, particularly those containing hexyl resorcinol, gave false positive results.

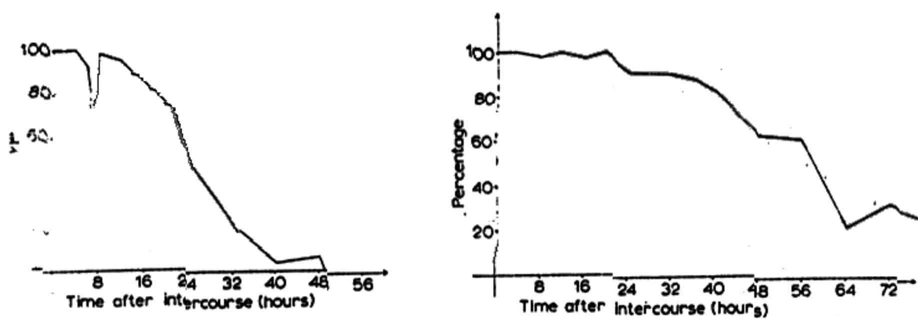

Fig. 3 (left). Percentage of vaginal swabs, taken at various times after sexual intercourse, reacting within 25 seconds with the AP test solution.

Fig. 4 (right). The percentage of vaginal swabs, taken at various times after sexual intercourse, reacting within 65 seconds with the AP test solution.

However, these were clearly distinguishable due to the somewhat different colour that developed, usually a more pink-brown colour. When suspected, these false positives can be confirmed by repeating the test, and not using naphthyl phosphate[4].

Five anal swabs from different donors were tested for AP activity and gave colour reaction development times of between 60 seconds and 100 seconds.

Choline

As with the ÀP reactions there is a marked difference between individuals and between different ejaculates from the same Individuals. Forbes[12] found that the concentration of

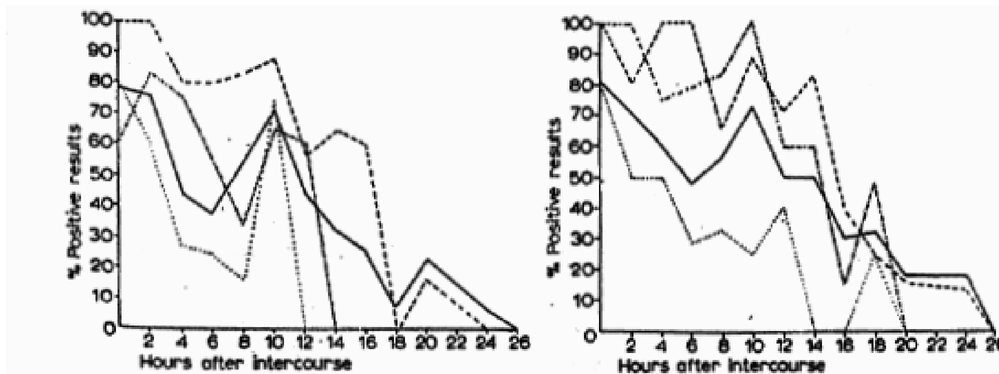

Fig. 5 (left). Choline reaction results, (——) total; (····) donor pair 8; (----) donor pair 13; (-·-) donor pair 15

Fig. 6 (right). Choline (concentrated extract) reaction results, (——) total; (····) donor pair 8; (----) donor pair 13; (-·-) donor pair 15

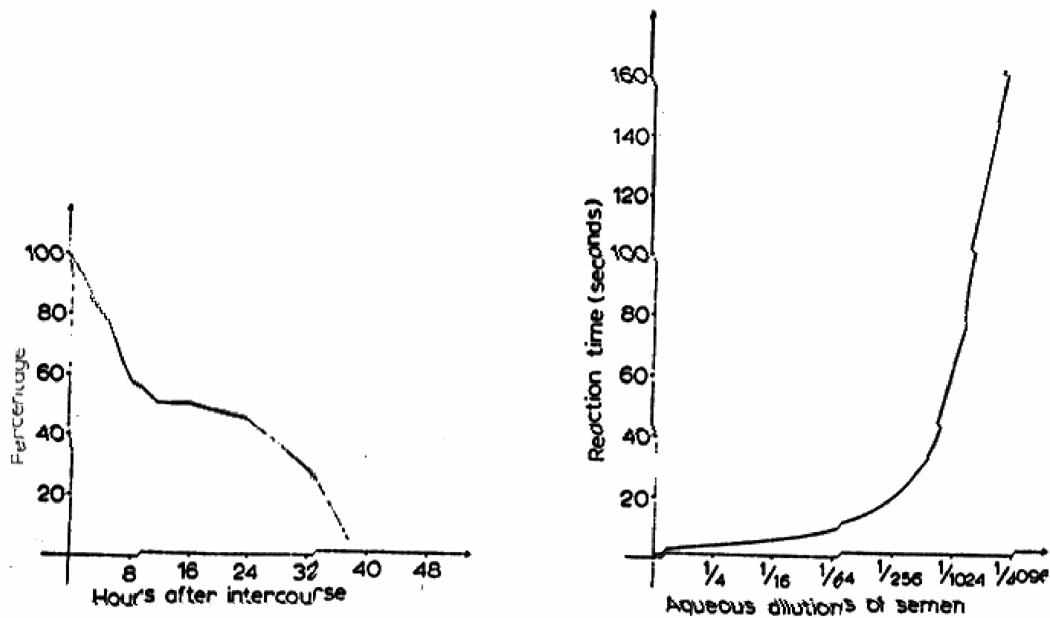

Fig. 7 (left). The percentage of vaginal swabs, taken at various times after sexual intercourse, giving acceptable grouping reactions. The one positive result obtained at 48 hours is not included.

Fig. 8 (right). This graph was prepared by Mrs. S. Renard. Aqueous dilutions of semen were made which were then absorbed onto cotton-wool swabs. These wee then tested for acid phosphatase in the same way as vaginal swabs, and the reaction times noted. The results give an indication of the concentration of semen of the vaginal swabs used in this work.

choline in semen from different individuals varied. Figs 5 and 6 show the reactions obtained from tests for choline using simple and concentrated extractions.

Concentrated extractions do not appear to be anymore likely to yield positive reactions than simple extracts and indeed in some cases, when semen concentration is obviously lower (at later times), it appears that all the choline is extracted during the simple extraction. A few positive results have been obtained from concentrated extracts from swabs taken some time after intercourse (32, 36 and 54 hours). However, after 26 hours negative results are usually obtained from both methods. There is a rapid decline in the number of positive results obtained after 14 hours.

Some work was carried out on the storage of swabs and how this would affect the detection of choline. This appears to be irrelevant provided that swabs have been dried before storage; this also applies to AP activity. Swabs stored at room temperature have given positive reactions up to a year. Some swabs stored undried and left at room temperature did not give positive results. These were compared with swabs taken at the same time which had been dried before storage. A number of vaginal swabs from donors who had not had intercourse, and thus were semen free, were tested and gave no positive choline reaction.

The data on this work are sparse as there were only four pairs of donors with suitable blood group combinations and only three of these donated an appreciable number of swabs. One of these was an O non-secretor female and an O secretor male (pair 4), but these reactions were of limited usefulness as the vaginal secretions of the female donor occasionally give indications of H antigen activity. The percentages of acceptable grouping results obtained at various times after sexual intercourse are shown in Fig. 7.

From pair 1, 8 swabs were obtained covering a range of 8-54 hours; five of these reactions were acceptable, the latest acceptable reactions being at 24 hours.

The largest number of swabs was obtained from pair 2. The majority of these swabs obtained up to 12 hours after intercourse gave acceptable grouping reactions. A few acceptable reactions were obtained from swabs taken between 12 and 36 hours, and some weakly positive reactions were obtained after this and up to 54 hours; however, these could only be regarded as indicative. Grouping reactions were correlated with AP test colour development in this donor and it was found that acceptable grouping reactions normally occurred when the AP colour development time was 30 seconds or less. The implications of this are discussed later.

Most swabs taken from pair 3 within 24 hours of intercourse gave acceptable grouping reactions. One acceptable result was obtained after this at 48 hours. These reactions are better than those obtained from donor 2 and it is interesting to note that donor 3 had faster AP reaction times than donor 2; and the 48-hour swab giving acceptable grouping reactions also gave an unusually fast reaction time of 25 seconds.

Most swabs obtained from pair 4 within 16 hours gave acceptable grouping reactions, thereafter acceptable reactions became rare. As stated earlier the blood-group antigen combinations of this donor are not ideal and later results have therefore been viewed with some caution. The AP reaction time for donor 4 was longer than those obtained from donor 3. This is reflected in the number of acceptable reactions obtained.

DISCUSSION

Since we had to rely on volunteers working in this laboratory, we were short of donors and in fact only had four frequent pairs. Even with these we lacked data at certain times. Due to this no statistical analysis of the results has been made.

The loss of seminal constituents from the vagina appears to be primarily due to drainage, the residual constituents being progressively diluted with vaginal secretions. The large effect of drainage is particularly noticeable In Fig. 3. The majority of donors had intercourse last thing at night, a fact which resulted in most swabs being taken at least 8 hours after intercourse. However, some were taken within the first few hours, during which time the female donors were walking about. The graph in Fig. 3 clearly shows that drainage has a pronounced effect on the amount of seminal AP in the vagina. The swabs

taken during the first few hours when the women were active show a marked loss of seminal AP, whereas those taken from donors who had slept for approximately 8 hours after intercourse show that the AP activity is retained when the woman is in he prone position. Comparison of the results obtained from swabs taken within 6 hours with those taken between 8 and 14 hours, from the graphs of the choline results In Figs 5 and 6, also reveals this difference between sedentary and active female donors.

The effect of drainage is enhanced by menstruation and sometimes by bathing. In contrast to people who are mobile after intercourse, high values for all seminal constituents are obtained from dead victims who have been raped immediately before or after death[2].

Spermatozoa, as the most unequivocal and longest surviving seminal-constituent in the vagina, are the most useful indications of the presence of semen, remaining sometimes for as long as 3-4 days after seminal AP and choline have ceased to be detectable. Therefore, in cases involving aspermic and oilgospermic men, semen detection will be difficult should the swab be taken at any prolonged length of time after intercourse. This is particularly relevant in case work since there is thought to be a higher Occurrence of aspermia and oligospermia in men committing sexual offences than in the normal population[1]. Also of considerable importance in this respect is the increase in vasectomy operations; indeed; the impact of this is already beginning to be noticed in this laboratory, Some work on this has been carried out by Nun *et al.*[13].

In these abnormal cases the electrophoretic method of distinguishing between vaginal and seminal AP is obviously useful[11]. We have used this method to check some of our results, particularly where an unusually fast AP reaction time was obtained from a vagina1 swab taken some time after intercourse; invariably a high level or vaginal AP was shown. We have used this method rather than an immunological method because we consider it to be more satisfactory. It appears that a high level of vaginal AP often corresponds with a large number of bacteria present on the smear. Clearly this is important in case work, as here there tends to be a larger than normal incidence of women with vaginal infections, which could produce misleading AP results. “Thrush” *(Candida albicans),* a relatively common vaginal infection, is often accompanied by large numbers of bacteria. Thus there is a strong possibility of a particularly fast AP reaction development time occurring on swabs from such women. Pregnancy, with its associated increased vaginal flora, also produced faster vaginal AP reaction times.

There is a great variation between individuals in the seminal AP development time; however, loss of seminal AP activity does not necessarily mean loss of spermatozoa.

The results obtained from the tests for choline indicate the very rapid disappearance of this seminal constituent and support Kind’s findings[4] that testing for choline on vaginal swabs is of limited usefulness.

The results from grouping were disappointing due to the apparent lack of close correlation with tests for other seminal characteristics; however, there were very few donors. When a swab gives a seminal AP reaction time of 30 seconds or under, there is a higher probability of obtaining useful grouping reactions. Since there were exceptions to this,

the possibility of the non-reaction of seminal blood-group antigens must always be considered when evaluating the grouping reactions of the mixture of semen and vaginal secretions of swabs.

Although we have given figures for the probability of detecting various seminal constituents from vaginal swabs taken at different times after intercourse, it appears that there is such considerable variation between individuals that this is of great importance and should always be considered when evaluating results.

REFERENCES

1. O.J. Pollak, Semen and seminal stains, *Arch. Pathol.,* 35 (1943) 140-184.
2. N. Sharpe, The significance of spermatozoa in victims of sexual offence:, *Can. Med. Assoc. J.,* 89 (1963) 513-514.
3. A.J. Morrison, Persistence of spermatozoa in the vagina and cervix, *Br. J. Vener. Dis.,* 48 (1972) 141- 143.
4. S.S. Kind. The acid phosphatase test, in A. Curry (Ed.), *Methods of Forensic Science,* Interscience, London, 1964, pp. 267-288.
5. G. Walther and P. Hohn, Aktivitatsbestimmungen der sauren Phosphatase in verschiedenen biologischen Medien unter Verwendung von *o*-Carboxyphenylphosphat, *Z. Rechtsmed.* 69 (1971) 259-262.
6. C. Rupp, Sperm survival and prostatic acid phosphatase activity in victims of sexual assault, *J. Forens Sci..* 14 (1969) 177-183.
7. A Florence, Die sperme et des tachcs de sperme en medecine legale, *Arch. Anthr'opol. Criminol.Psrchiatr. Med. Leg.,* 10(1895-96) 417-434.
8. K V. Stanek, Uber das cholinperjodid und die quantitativ Sallung von Cholin durch Kalium trijodid. *Z. Physiol. Chem.,* 46 (1905) 280-285.
9. V. Stunek, Uber die quantitativ Prennung von Cholin und Bettain, *Z Physlol. Chem.,* 47 (1906) 83-87.
10. M. Pereira in B.J. Culliford, *The Examination and Typing of Bloodstains In the Crime Laboratory,* U.S. Department of Justice, US. Government Printing Office, Washington, 1971, pp. 67-105.
11. K. Wraxail and E. Adams, Phosphatases in body fluids: The differentiation of semen and vaginal secretion, *Forens. Sd.,* 3 (1974) 57—62.
12. C. Forbes, The scope and fallacies of the Florence reaction for seminal stains, *Police J. (Lond.),* 13 '1940) 162—171.
13. S Nun. I. Musacchio and J.A. Epstein. Variations in seminal plasma constituents from fertile, subtertile and vasectomized azo spermic men, *Fertil. Steril.,* 23 (1972) 357—360.