A COMPARATIVE STUDY OF THE CITRATE AND LACTATE CONCENTRATIONS IN STAINS FROM SEMEN, VAGINAL SECRETION AND MIXTURES OF THE TWO USING ISOTACHOPHORESIS

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INTRODUCTION

In the scientific examination of sexual assault cases the forensic scientist is continually faced with the inability to reliably determine the presence or absence of vaginal secretion particularly in mixtures with semen.

It has been established that vaginal secretion has a high level of lactic acid (1) and that citiric acid is a good indicator of androgenic stimulation (2).

The objective of this study was to determine whether these two carboxylic acids could be used to determine the presence of semen, vaginal secretion or mixtures of the two from a dried stain.

In the sexually mature woman there is always an excess of glycogen in the vaginal epithelial cells (3) and this is, in some way, metabolised by the lactobacilli and fermented to lactic acid. Lactic acid is also present in semen (4) but in much lower levels than that found in vaginal secretion.

One of the characteristic components of seminal plasma is citric acid (5). The specific function if the citrate ions is not known but they may be present as activators of prostatic acid phosphatase and may also be involved with the coagulation and liquefaction of semen (6). The concentration of citric acid in semen is much higher than in vaginal secretion.

We have attempted to determine quantitatively the levels of citrate and lactate in semen and vaginal secretion as an aid to the identification of these fluids when encountered in sexual assault cases.

We used capillary isotachophoresis because the samples require no prior treatment, very small quantities can be assayed, it is a relatively rapid technique, simultaneous estimations can be made and the results are reproducible.

MATERIALS AND METHODS

Forty-four vaginal swabs taken at various times after intercourse were obtained from seven donors within the Metropolitan Police Laboratory. Each swab was extracted with vortexing into 1 ml of distilled water. The extract was centrifuged to remove any debris and the supernatant stored at -15^{o}C until used.

Ten semen-free post-coital swabs were provided by donors who used a contraceptive sheath during sexual intercourse. These swabs were extracted and stored as above.

Twenty semen samples were obtained from a fertility clinic and stored at -15^{o}C until used.

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Stains were prepared from each of ten samples on clean cotton sheeting and allowed to dry at room temperature. Each complete stain was extracted with vortexing into 200µl of distilled water and centrifuged to remove any extraneous material.

Urine samples were prepared in the same way as the semen stains.

Oral swabs were extracted and stored in the same manner as the vaginal swabs.

All extracts were assayed for citrate and lactate ions using capillary isotachophoresis.

Total protein estimations were obtained by a modification of the Lowry method (7).

Acid phosphatase assays were kindly supplied by A. Davies and S. Wotherspoon of the Metropolitan Police Laboratory.

All estimations were made from 15µl injections of each sample. Separations were performed at a constant current of 150µA, with a chart speed of 0.5mm/sec and a 200mV input.

Leading Electrolyte: 10mM HCl adjusted to pH 2.5 with β-alanine.

Terminating Electrolyte: 10mM Propionic Acid adjusted to pH 4.0 with 1M NaOH.

Initially 15µl of ech sample were injected and assayed. This was followed by a second injection of 15µl of sample which was mixed with 5µl of a standard solution containing 4mM citric acid, 4mM lactic acid and 4mM trichloroacetic acid.

Trichloroacetic acid was chosen as an internal standard because it separates clearly using these electrolytes and does not form mixed zones with either citrate or lactate ions.

A full account of the theory of isotachophoresis has been provided by Holloway and Trautschold (8).

RESULTS AND DISCUSSION

The citrate and lactate concentrations obtained from seminal stain extracts are shown in Fig. 1. Both of the ions show variation from sample to sample but the citrate levels are always considerably higher than those of lactate.

There does not appear to be any relationship between the protein concentrations and either of the carboxylic acids; therefore the ratios of protein : citrate could not be used as an indication of the amount of semen in a particular stain.

Vaginal swabs taken at various times after intercourse were extracted and assayed for citrate, lactate and acid phosphatase. Lactate levels were extremely variable ranging from approximately 1.0 to 10mM with no apparent relationship to post-coital interval (Fig. 2). Citrate concentrations, however, although variable, showed a decrease with time after intercourse similar to that of acid phosphatase (Fig. 3). Gavella (9) has shown a direct correlation between acid

phosphatase and citrate concentrations in semen samples. This relationship was not apparent from the results obtained in this study when semen was extracted from vaginal swabs. This could be due to a variety of reasons; the most likely being a loss by drainage or denaturation. Citrate ions are unlikely to be affected by the vaginal environment but could also be lost by drainage. Vaginal secretion contains very low levels of citrate as shown in the results from post-coital semen-free vaginal swabs (Fig. 4) and from normal vaginal swabs taken more than six hours after intercourse.

Extracts from 15 oral swabs, obtained from donors and from case-work samples, showed levels of citrate which ranged from 0 - 1.0 mM with a mean of 0.34mM. Although these are not high concentrations, it may be necessary to determine whether saliva is present in order to avoid confusion with low levels of semen. Lactate determinations from these swabs showed much lower amounts than those found in vaginal secretion (0 - 0.35mM; mean, 0.08mM) and are unlikely to cause any confusion.

We found negligible levels of lactate and citrate in urine but an elevated level of lactate may be found in cases of bladder infections and after periods of physical activity. Although lactate is not specific for vaginal secretion and citrate is not specific for seminal plasma, quantitative estimations of these two carboxylic acids from dried stains may be of considerable help in the determination of these particular fluids and can also be used to confirm other qualitative assays.

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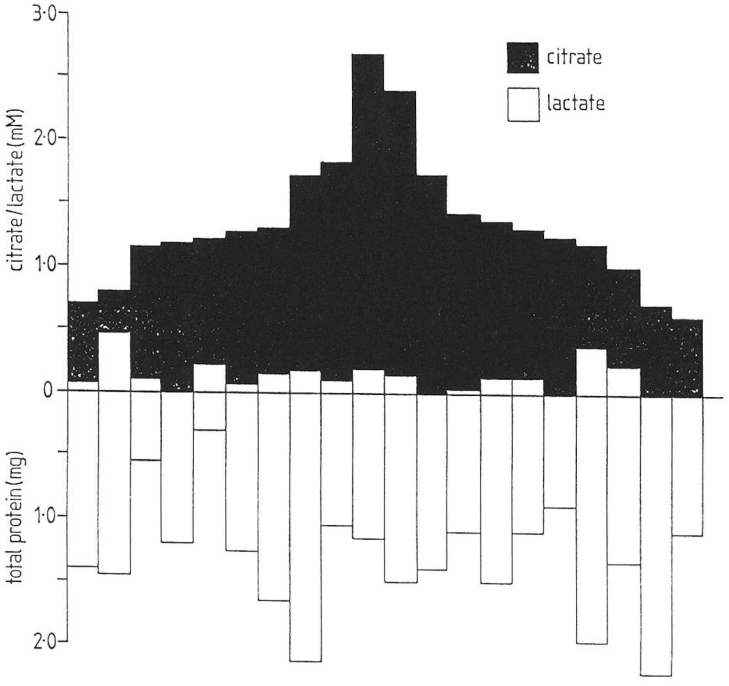

Fig 1 Distribution of citrate and lactate ions and protein concentrations in 20 semen samples

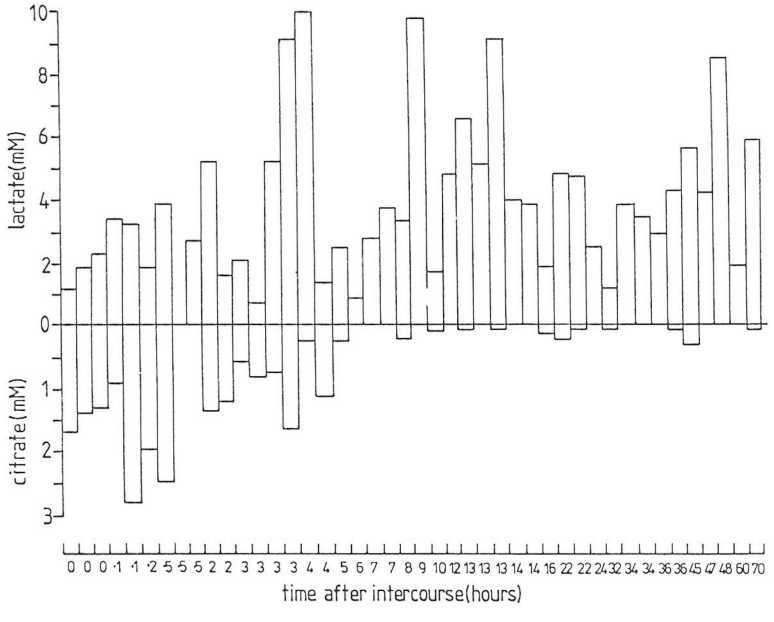

Fig 2 Concentration of citrate and lactate ions from vaginal swabs taken at various times after sexual intercourse

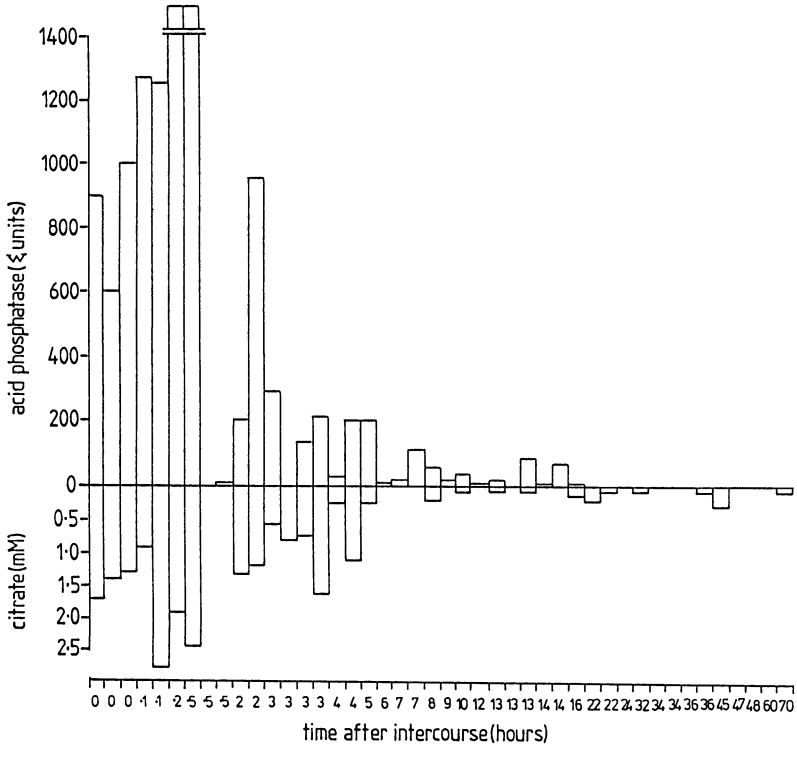

Fig 3 Decrease in the level of citrate ions and acid phosphatase activity from vaginal swabs taken at increasing times after sexual intercourse

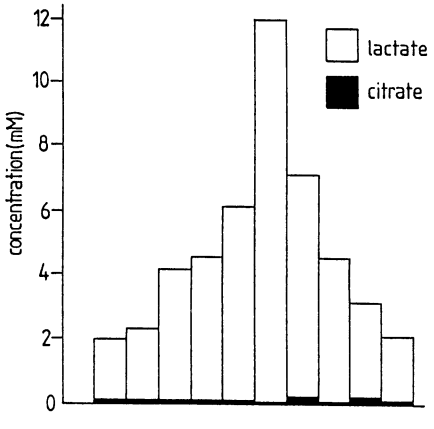

Fig 4 Distribution of citrate and lactate ions in extracts from 10 semen-free post-coital vaginal swabs