Post Coital Changes in Semen Glyoxalase I (GLO)

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Introduction

The frequencies of the commonly occuring GLO phenotypes are sufficiently high in all races to be used as blood markers in forensic science. (Emes and Parkin, 1980). Since GLO is also present in semen it has further use in the investigation of sex crimes.

Studies of human vaginal secretions showed that, apart from menstrual blood, GLO was absent from the unstimulated vagina.

The examination of post coital vaginal swabs from couples of different GLO phenotypes showed that the semen GLO activity was no longer detectable after a period of about 1 to 2 hours post intercourse, being replaced by GLO of the female phenotype (Fig. 1). This female GLO persisted for variable lengths of time up to about 10 hours post coitus, after which no GLO activity could be detected in the vagina.

The production of vaginal GLO appears to be due to sexual stimulation as it has been observed on vaginal swabs taken just before intercourse. Studies into the rapid loss of seminal GLO are described as this is not clearly understood.

Experimental

Three possible mechanisms have been investigated, (i) Breakdown by vaginal or seminal proteinases, (ii) inactivation by acid pH, (iii) semen drainage from the vagina.

(i) Vaginal and seminal proteinases

Acid and neutral proteinases have been described in semen and vaginal secretion (Lundquist 1952, 1955; Denker, 1977)

Acid proteinases:

The concentration of acid proteinases in semen were found to be about 15 times greater than in vaginal swab extracts. Acid proteinase was purified from pooled semen by the method of Ruenwongsa and Chulavatnatol (1975). The pH stability of this acid proteinase shows maximum activity at about pH 4.5 with a rapid loss of activity as the pH increases.

Neutral proteinases:

Neutral proteinases were partially purified from the pooled eluates from semen free vaginal swabs by sephadex G-200 column chromatography,

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yielding a major and two minor proteinase fractions. Each of these fractions had optimum pH activity at about pH 8.0.

These proteinase fractions were separately concentrated and incubated with semen aliquots at 37°C for 24 hours. No apparent loss of activity occurred when the semen was examined by electrophoresis.

(ii) **pH studies**

GLO was purified from pooled semen by DEAE sephacel and S-hexylglutathione affinity chromatography. The maximum activity of this GLO was found to be at about pH 6.5 to pH 7.0, with a fairly rapid decline of activity to pH 4.0 (Fig. 2).

The stability of semen GLO to pH shock was determined by incubation in the range pH 4.0 to 8.0 for 2 hours then, after adjusting the pH, assaying the GLO at pH 7.0. The results showed about 90% loss of activity after 2 hours incubation at pH 6.0 and about 97% loss at pH 5.0 (Fig. 3).

The pH changes of the semen pool in the vagina after intercourse were determined by taking serial vaginal swabs and measuring the pH at intervals up to 14 hours post intercourse. A rapid fall in pH occurs over the first 2 to 3 hours, when the pH falls from about pH 8.0 to about pH 5.5. After this initial period the decline in pH is much less rapid (Fig. 4).

(iii) **Semen drainage**

Davies and Wilson (1974) reported that the loss of seminal constituents from the vagina was mainly due to drainage, with the residual seminal components being diluted with vaginal secretions. Martin (personal communication) investigated the loss of acid phosphatase activity and citrate ions from post coital semen in the vagina. His results showed that both the citrate ion concentration and acid phosphatase activity fell by approximately 60% in the first 2 hours after intercourse (Fig. 5 and 6). This may be interpreted as being due to drainage.

Discussion

The rapid loss of semen GLO activity after intercourse severely restricts its use as a marker in cases of sexual assault. This loss of activity is unlikely to be due to the action of seminal or vaginal proteinases.

Concentrated neutral proteinases from the vagina did not produce any observed affect on semen GLO and acid proteinases demonstrated very little activity at the pH range recorded in the semen pool (pH 8.0 to pH 5.5) during the first 2 hours after intercourse.

The pH of the semen in the vagina falls to about pH 5.5 in the first 2 hours after intercourse and at this pH the activity of GLO falls by about 60%. Incubation at this pH followed by a readjustment to pH 7.0 for assay or electrophoresis, further reduces the activity due to pH shock. Over the time period up to 60% drainage of semen from the vagina has been noted.

My conclusions are that a combination of drainage and loss of activity due to low pH are the main agents in producing loss of semen activity after intercourse.

References

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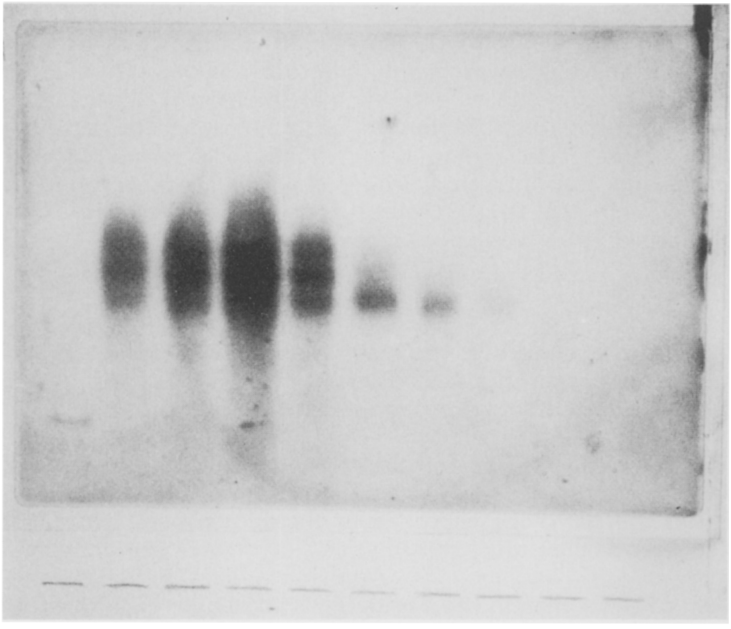

Fig. 1 GLO on vaginal swabs taken (left to right) - Pre-intercourse, immediately, ¼, ½, 1, 2, 3, 4, 6 and 7 hours post intercourse. (Female : GLO 1; Male GLO 2-1).

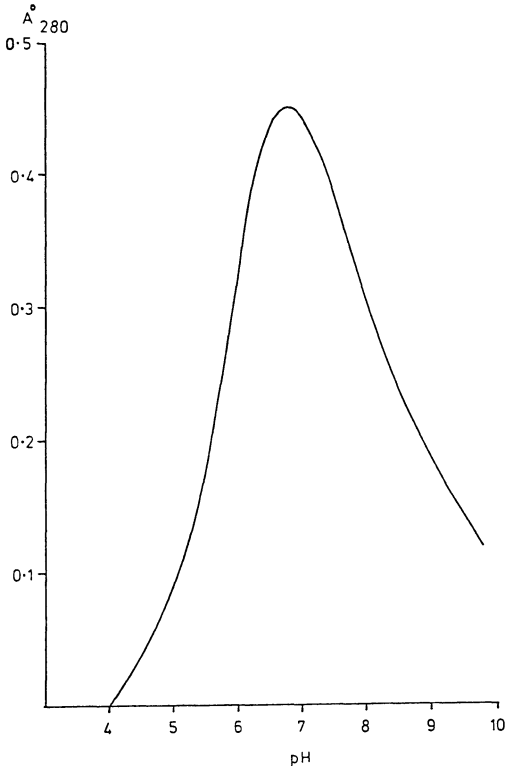

Fig. 2. Semen GLO activity in the range pH 4·0 to pH 9·5

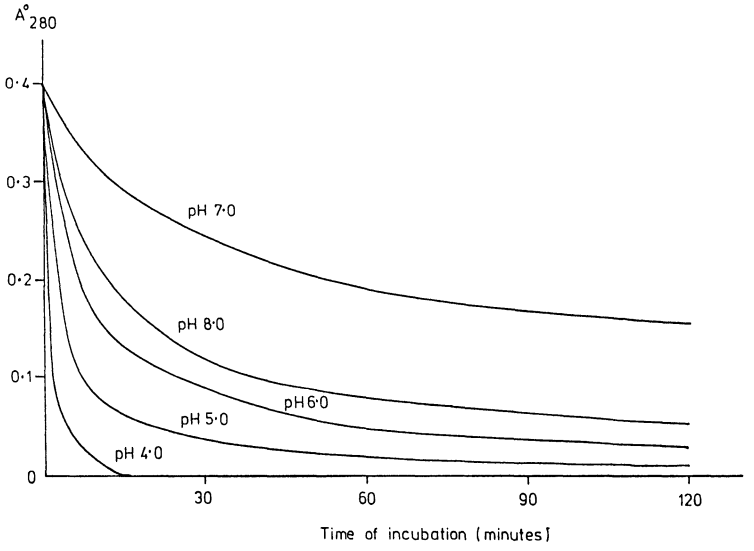

Fig. 3. The stability of semen GLO when incubated at pH 4·0 to 9·0 then readjusted to pH 7·0 for assay

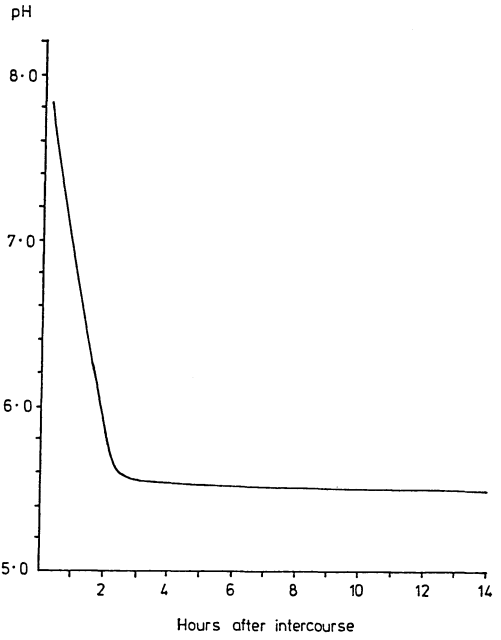

Fig. 4. Changes in vaginal pH after intercourse

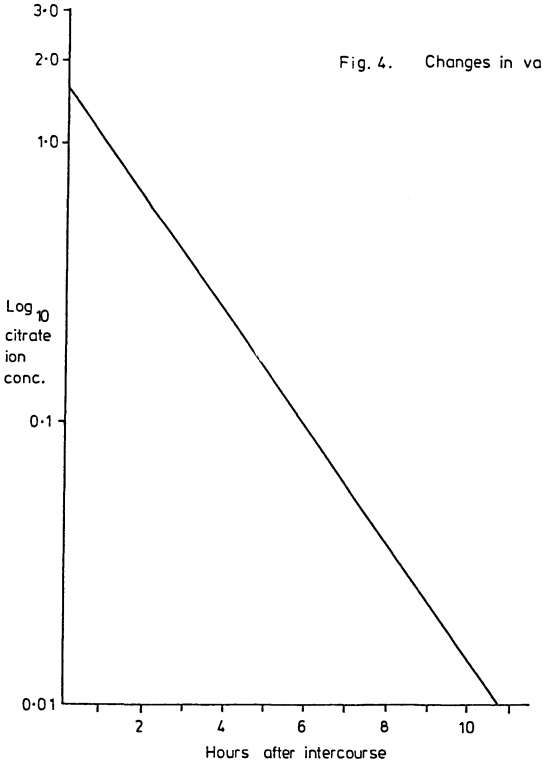

Fig. 5. Loss of citrate ions from semen after intercourse

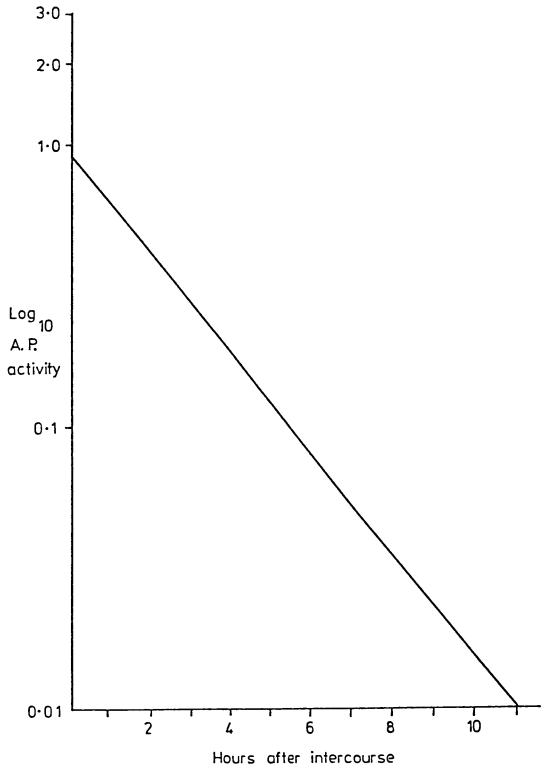

Fig. 6. Loss of acid phosphatase activity from semen after intercourse