

Venereal trichomoniasis: role of men

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SUMMARY It has been suggested that high zinc concentrations found in men may prevent *Trichomonas vaginalis* from being established in the male reproductive tract. In this investigation *T vaginalis* was readily killed at concentrations of zinc that occur in the prostatic fluid of healthy men (minimum trichomonocidal concentration (MTC) of 6.4 mmol/l). *T vaginalis* was also shown to be killed by human prostatic extracts as well as by human seminal fluid, even when the zinc content was much lower than the MTC for *T vaginalis*. It seems likely, therefore, that there are at least two antitrichomonal mechanisms in the male reproductive tract, one being zinc dependent and the other not relating to zinc content. *Tritrichomonas foetus*, which causes venereal trichomoniasis in cattle, was unaffected by bovine seminal fluid and was killed by zinc only at concentrations far higher than those found in the prostatic fluid in the bull (MTC 200 mmol/l).

Venereal trichomoniasis may be caused by two flagellates, *Trichomonas vaginalis* in man¹ and *Tritrichomonas foetus*, which infects cattle.² In man, women have long been recognised as the reservoir of *T vaginalis*,^{1,3} whereas men are seen to serve merely as the short term vector of the organism.⁴ In symptomatic women the disease is often characterised by severe inflammation and a malodorous seropurulent vaginal discharge.^{1,3-5} Men suffering from *T vaginalis* infections most often exhibit no symptoms, although mild cases of urethritis, prostatitis, and epididymitis have been associated with venereal trichomoniasis.^{4,6,7}

The role of the male in bovine trichomoniasis appears to be far more important than it is in human venereal trichomonad infections. The bull serves as both the reservoir and the vector of *T foetus*, the organism tending to be localised in secretions in the prepuce and around the penis.^{8,9} Unlike men, who often seem to be only transiently infected, the bull, if untreated, usually remains infected and infective for long periods, if not for life.⁹ The cow, however, tends to suffer from short term trichomonad infections, which are often fairly mild. They can occur as fulminant infections if the cow is pregnant, however, in which case early abortion of a poorly developed foetus often results.⁸⁻¹⁰ Most of the trichomonads are

expelled with the aborted foetus, the remainder disappearing within one to two weeks, probably as the result of a hypersensitivity reaction.⁸

The obvious differences in the epidemiology and course of trichomoniasis in men and bulls raises several questions concerning the nature of the antitrichomonad mechanisms in both animals, as well as the ability of the parasites to persist in the urogenital tract of the male. We investigated these aspects of venereal trichomoniasis to assess the actual and perceived role of the male, particularly the human male.

Materials and methods

CULTURES

Isolates of *T vaginalis* were obtained from clinical specimens received at the North West Pathology Laboratory, Tasmania from various general practitioners. Cultures were maintained in Oxoid *Trichomonas* medium at 30°C and subcultured every two to four days. The *T foetus* culture was a laboratory adapted strain donated by the Mount Pleasant Research Laboratories, Tasmania, and was grown in improved Schnieder Medium¹¹ at 30°C and subcultured every 10-14 days. Both cultures were stored at 30°C as they were found to remain viable for longer periods at this temperature.

PROSTATIC EXTRACT AND SEMINAL FLUID

The bovine seminal fluid was provided by the Tasmanian Herd Improvement Organisation and was

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kept at -196°C until required. Human seminal fluid was obtained through the Male Infertility Clinic attached to the Royal Hobart Hospital, Tasmania. The bovine and human seminal fluids were analysed for zinc content by diluting 0.1 ml of the fluid in 10 ml of deionised water.

Prostatic tissue was obtained from cadavers and extracted by homogenising with a buffer (0.25 mol/l sucrose, 1 mmol/l edetic acid (EDTA), and 1 mmol/l TRIS, pH 7.2) 4 ml/g tissue, filtering through muslin, and centrifuging the filtrate for 30 minutes at $250 \times g$ at 4°C . The supernate was decanted and stored at -70°C until needed. A 10 ml aliquot of the supernate was used to assess the zinc content of the extract.

ZINC CONCENTRATIONS

The concentration of zinc in the samples were measured spectroscopically by the Tasmanian Government Analyst.

EFFECT ON VENEREAL TRICHOMONADS

The time kill microtitre plate method used in this investigation was that of Krieger and Rein,^{12,13} although in our study both *T vaginalis* and *T foetus* were used. Forty eight hour cultures of either of the venereal trichomonads were washed three times in saline at $250 \times g$ for 10 minutes, counted in a haemocytometer, and adjusted to give (10^5 to 6×10^5) organisms per ml of culture medium. The wells of a microtitre plate were inoculated with 0.1 ml volumes of the trichomonad cultures. An extra 0.1 ml of medium was added to each well. A 0.05 ml volume of inorganic salts, prostatic extract, human seminal fluid, or bovine seminal fluid were also added to the plate to give a total fluid volume of 0.25 ml.

PREPARATION OF INORGANIC SALTS

Serial twofold dilutions of the inorganic salts, ranging from 0.2 to 25.6 mmol/l for *T vaginalis* and 25 to 800 mmol/l for *T foetus* were prepared by inoculating the first two rows of a microtitre plate with 0.05 ml of zinc chloride, magnesium chloride, zinc sulphate, or magnesium sulphate (64 mmol/l in 0.85% saline for *T vaginalis* and 4 mol/l in 0.85% saline for *T foetus*). Rows two to seven also had 0.05 ml of culture medium added in addition to the inorganic salts. A serial doubling dilution series was then performed using a microdiluter. A control of saline and culture medium was included. Plates were incubated anaerobically in Brewer jars at 30°C for six hours and examined by phase contrast microscopy. The minimum trichomonacidal concentration (MTC) of the inorganic salts was defined as the lowest concentration at which all the trichomonads were killed.

Thus trichomonads were incubated anaerobically for six hours in the presence of serial doubling dilu-

tions of the inorganic salts zinc chloride, magnesium chloride, zinc sulphate, or magnesium sulphate. We also incubated *T vaginalis* with human prostatic extract and human seminal fluid and *T foetus* with bovine seminal fluid.

Results

The results obtained showed a remarkable difference between the *T foetus* strain and the clinical isolates of *T vaginalis*. Figures 1 and 2 show that the magnesium salt solutions did not appreciably alter the survival of *T foetus* or *T vaginalis* in vitro after incubation for six hours under anaerobic conditions. The zinc salts, however, did kill the organisms, giving a MTC of 200 mmol/l of zinc for the bovine trichomonad, whereas *T vaginalis* was shown to have a MTC of 6.4 mmol/l zinc.

A trichomonacidal effect was also observed when incubating the human trichomonad with human prostatic extracts and seminal fluid. The zinc concentrations in the human prostatic samples were similar to those in the normal human prostate (2.3–15.3 mmol/l¹²), but the seminal fluid had a much lower zinc concentration, yet *T vaginalis* was killed by both the prostatic homogenate and the seminal fluid. No such phenomenon was seen with *T foetus*; the trichomonad remained unaffected by seminal fluid after incubation for six hours.

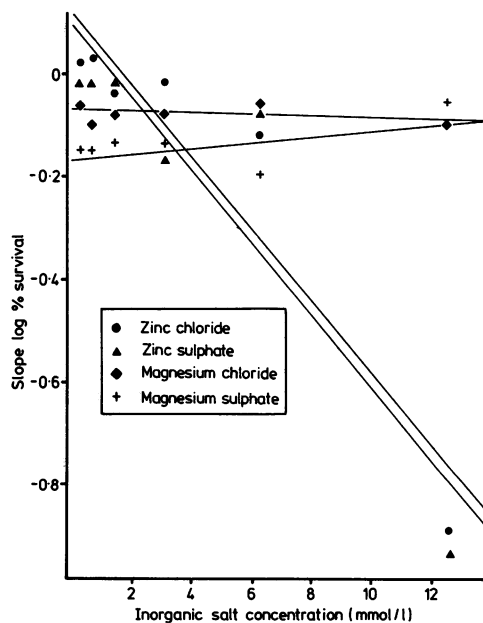


Fig 1 Survival of *Trichomonas vaginalis* in Oxoid No 2 *Trichomonas* medium with added inorganic salts. Each point represents the mean of six triplicate experiments.

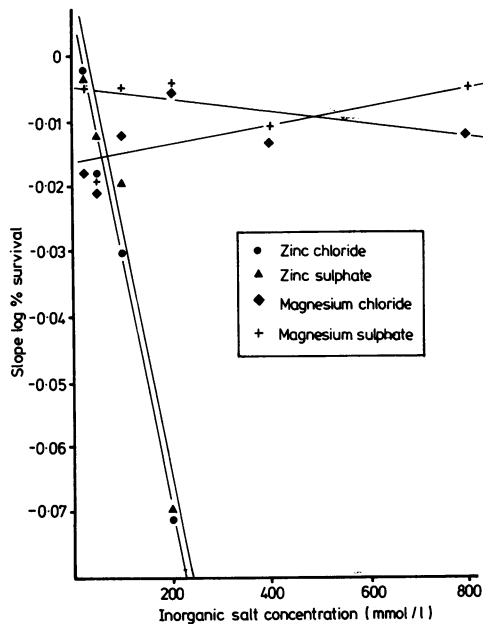


Fig 2 Survival of *Trichomonas foetus* in improved Schnieder medium with added inorganic salts. Each point represents the mean of six triplicate experiments.

Discussion

The role of the male in human trichomoniasis has been somewhat of an enigma since Donné originally described the disease. It is now generally accepted that men are affected by the organism, but usually only with mild symptoms and the infection is often transient. At most, therefore, men usually act merely as short term vectors of *T vaginalis* and not as long term reservoirs of the infection. The bull, however, has a more defined role in bovine trichomoniasis and serves as both the reservoir and the vector of the organism, *T foetus* often being retained within the reproductive tract for long periods.⁹

Men and bulls are known to accumulate zinc in the prostate, which gives rise to higher concentrations in the urinogenital tract than are found in adjacent tissues.¹⁴ Zinc has been found to have bacteriolytic properties,^{15,16} and may therefore also affect the ability of other pathogens to establish in the reproductive tract of the male.¹⁷ We used a method whereby we examined the in vitro effects on *T foetus* and *T vaginalis* of some inorganic salts and of fluids from the reproductive tract. The results confirmed and expanded the original observations of Krieger and Rein.^{12,13}

T vaginalis was found to be sensitive to relatively low concentrations of both zinc chloride and zinc sul-

phate, giving a MTC of 6.4 mmol/l of zinc. No such phenomenon was observed in the control experiments with magnesium salts, the human trichomonad remaining largely unaffected. The trichomonacidal effect observed was thus not due to the presence of the chloride or sulphate ions, nor was it a generalised heavy metal phenomenon, but rather a result of the specific presence of zinc. Similarly, *T foetus* was not affected by the magnesium salts, but gave a MTC of 200 mmol/l of zinc, about 31 times greater than the MTC of zinc for the human trichomonad. This remarkable difference in susceptibility to zinc may be important in explaining the differences in the epidemiology and course of venereal trichomoniasis between men and bulls.

The normally high zinc concentrations in human prostatic secretions (2.3–15.3 mmol/l)¹² appear to be important in preventing the human trichomonad from establishing in the reproductive tract.^{12,13} The MTC for *T vaginalis* of 6.4 mmol/l of zinc, as estimated in this investigation, is well within the normal range of prostatic zinc concentrations recorded for men. Most men would, on this evidence, be unlikely to develop long term venereal trichomoniasis by prostatic establishment as the organism is unlikely to grow at such high zinc concentrations.

T vaginalis was also killed by human prostatic extract and by human seminal fluid, though the zinc concentrations in these fluids were generally lower than is typically found, because of the preparative technique. On these observations, a second trichomonacidal mechanism may be present in men, in addition to the zinc dependent route already noted. This concurs with the hypothesis that clinical presentation of trichomoniasis is dependent on a balance of the sensitivity of *T vaginalis* isolates to zinc and the zinc independent factors in the prostatic fluid.¹³ Though the original study of Krieger and Rein was undertaken using *T vaginalis* and canine prostatic ejaculate, with extrapolation to the human condition, our results with human prostatic extract and seminal fluid do show that a second, zinc independent, antitrichomonad system exists in men.

This is not the case for *T foetus*, however, as the organism was found to be unaffected by bovine seminal fluid after incubation for six hours. In addition, the MTC of 200 mmol/l zinc was far higher than the zinc concentration in the reproductive tract of the bull.¹⁸ It therefore appears that, unlike in men, neither a zinc dependent nor an "alternative" antitrichomonad system is present in the urinogenital tract of bulls, which may result in the ready establishment of bovine venereal trichomoniasis.

Although the results for *T foetus* have to be interpreted with some care, as the organism is a laboratory adapted strain rather than a fresh isolate, this

investigation has shown two important differences between *T vaginalis* and *T foetus*, which may partly explain the often contrasting fates of trichomonads in the reproductive tracts of men and bulls. Further similar comparative studies may prove to be of some benefit when attempting to elucidate the still ambiguous role of men in human venereal trichomoniasis.

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