LABORATORY ASPECTS OF *TRICHOMONAS VAGINALIS**†

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The observations on which this paper is based were made during a recent investigation into *Trichomonas vaginalis* infections in the male. My remarks therefore mainly concern the laboratory diagnosis of male infection. Trichomonad infections in the male are almost always coital in origin. It may be opportune at this moment to point out that there are no grounds whatsoever for holding that *T. vaginalis* infections in the female may originate from the alimentary tract. This site is not the habitat of *T. vaginalis*, which cannot be implanted successfully in the human intestine. Conversely, the intestinal trichomonad, *T. hominis*, has been found incapable of infecting the vagina. Stabler, Feo, and Rakoff (1940) inoculated fifty patients intravaginally with cultures of *T. hominis* and failed to produce a vaginitis ; in these same patients subsequent vaginal inoculations with *T. vaginalis* produced a typical vaginitis. Furthermore, reports published in the U.S.A. on the examination of 200 women for the relative incidence of *T. vaginalis* in the vagina and *T. hominis* in the bowel, showed a 23 per cent. infection with *T. vaginalis* as against a 1 per cent. infection with *T. hominis*. I have drawn attention to these facts, as even to-day erroneous conceptions concerning the origin of *T. vaginalis* and hence the relative importance of trichomonad infestations in the male are still widespread.

There is little agreement on the incidence of *T. vaginalis* infections in the male. The figures reported differ greatly. This may be because the incidence does, in fact, vary, or because the means of diagnosis are unreliable. The incidence of female infections reported varies little from country to country, and failure to obtain similar constant figures for male infections is therefore probably due to defects in laboratory diagnosis. During my investigation an opportunity arose to study the various methods of laboratory diagnosis in common use today.

At first, it was hoped that careful history taking might provide some indication of the possible presence of the flagellate and hence aid laboratory procedures. No specific symptoms, signs, incubation period, or other circumstances were, however, noted. The combination of urethritis and balanitis, especially if the latter tended to be recurrent, appeared at one time to suggest a trichomonad origin of the infection but this was not confirmed by later studies. The further the investigation proceeded, the more one became convinced that diagnosis of these infections depended on the efficient carrying out of a fixed laboratory routine. One very valuable sign which suggested an underlying trichomonad infection soon came to light. This was the close association of a heavy trichomonad urethral infection with a urine which was hazy and contained many fine, light, short threads. These threads on microscopical examination are found to consist mainly of epithelial cells. This type of urine is not present in light infections, but when noted it is an indication for repeated and, if necessary, prolonged search for the flagellate. I attached so much importance to this sign and found it of such value that on one occasion because of its presence I continued examinations of the patient for a period of 27 days, when the organism was eventually identified. As far as could be ascertained no coitus had occurred during the period of investigation ; this not only illustrates the importance of the sign, but stresses the inadequacy of our present methods of investigation. I at first described this urine as " mossy ", but later found that this description did not convey to my colleagues an accurate picture. Because of this I have since dispensed with the term but to me it does still appear aptly descriptive. Lydon (1945) has similarly noted the significance of this type of urine.

Apparatus

Microscopical examination is best carried out with high-power objective and a × 10 eyepiece. The Cooke 1/16 in. achromatic objective is possibly the most suitable as it provides a wider and clearer field for observation. The condenser should be lowered. For routine use, darkground examinations are not necessary, but they may be usefully employed when there is an occasion for a

more detailed investigation. The use of safronin has its advocates but I find it of little advantage.

Obtaining Specimens

In any one subject, specimens for examination should be obtained from all the usual sites of infection, *i.e.* the subpreputial sac, the urethra, the prostate, and vesicles. It is well to remember that the organism may be present in one or all these sites, even though there is no evidence suggestive of its presence. If immediate examination is possible, specimens should be obtained by gently scraping with a stiff platinum loop of, say, about s.w.g. 18, but if some delay is unavoidable, a cotton-wool swab is perhaps best employed. A dry swab will rapidly lead to drying of the specimen, and to prevent this the swab should be moistened before use with Ringer's solution containing serum or with bacteriological broth, unless it can be well soaked in the discharge. A word of warning is necessary about the use of cotton-wool swabs. Some batches of wool are injurious to the protozoa and may result in diminution or cessation of movement. Care should be taken, therefore, in the selection of wool for this purpose. If it is necessary to delay examination still further, other measures are indicated and the following method has been found satisfactory. The inoculum is collected with a pipette and placed in a screw-capped bottle containing 5 ml. Ringer's solution plus 1 part inactivated bovine or horse serum. If the bottle is then placed in an incubator at 37° C., the trichomonads can be kept alive for periods up to one week. In ordinary room or vest-pocket temperatures, the organisms will remain viable in this solution from 36 to 48 hrs, and its use in routine hospital and clinic work is of obvious value. For sending specimens of *Trichomonas vaginalis* by post, this solution can be successfully employed, but it is generally advisable to incubate the specimen on arrival for 3 to 4 hrs at 37° C. before examination.

It is important that urination should not have taken place for at least 2 hrs before specimens of urethral discharge are taken for examination. I have rarely demonstrated the trichomonad in a urethral discharge when the patient had emptied the bladder recently. Examination of urethral discharge, however, is of limited value, and better results are generally obtained from the examination of urethral scrapings. These may be obtained by gently curetting the lower part of the urethra with a platinum loop. Recent micturition does not appear to affect the efficacy of this method, and even if a urethral discharge is present, scraping will still be found to be a far more reliable method of investigation.

When obtaining specimens from the subpreputial sac, it is important to scrape as widely as possible, paying particular attention to the folds of mucous membranes adjacent to the frenum. Trichomonads are often recoverable from these folds of mucous membrane even when all signs of inflammation have disappeared and scrapes from other areas are negative. The orifices of Tyson's ducts should also be included in the examination as these may be infected, especially if they tend to be patulous. Prostatic specimens should be obtained in the usual manner, but, since the vesicles may also be infected, care should be taken to strip these at the same time

Examination

Specimens should be examined with as little delay as possible. Slides should be warmed before use and the specimens mixed in a few drops of warm Ringer's solution already on the slide. A hurried examination is useless and a detailed and prolonged search is usually required. The recognition of active *T. vaginalis* presents no difficulties, and even under low-power examination its appearance cannot easily be mistaken. The jerking movements, being most characteristic, attract attention even in a packed and crowded field. The spindle-shaped body of *T. vaginalis* may be difficult to distinguish from epithelial cells, but the flagella, especially in motion, are distinct and diagnostic ; in general it is probably unwise to identify *T. vaginalis* without observing flagella or undulating membrane. A difficulty arises at times in distinguishing rounded motionless specimens of trichomonads from pus cells. Under adverse conditions the trichomonad very rapidly becomes rounded in appearance, and the flagella less active and sometimes closely adherent to the body of the organism. It is possibly at such times that errors in identification are most common, more especially if one accepts the existence of an encysted or resting phase. It is also possible that mistakes made at this juncture are the cause of the conflicting claims for the incidence of male infection. Further errors in diagnosis may be caused by motile spermatozoa attached to pus or epithelial cells. The resulting movements can be highly suggestive of trichomonad activity. In the examination of balanitic exudates, the occasional occurrence of fine spirillae should be borne in mind, as these, when lying close to pus cells, may easily be mistaken for flagella. I have twice known this to cause faulty identification by a reliable laboratory technician.

Though I consider that the examination of fresh unstained specimens is the best method, there are others who prefer examination of stained specimens. Liston and Liston (1939) used Leishman's stain and claimed, in their own words, that :

the trichomonads are easily recognized and could only be confused by a careless observer.

A method at present used at the Liverpool School of Tropical Medicine can usefully be adapted for the staining of trichomonads. The specimens are first mixed with a little plasma and then spread as for a blood film. This is dried in the air and fixed and stained in the normal way with Leishman's stain. I have no personal experience of this method, but it is claimed that worthwhile results can be produced. Allison (1943), using Sellar's Negri stain, claimed that the trichomonads were thus made easily identifiable. Fowler (1953) has claimed good results from the use of a staining method with methyl

violet. In spite of these claims, however, it is felt that the successful interpretation of stained slides calls for a high and even specialized degree of skill and experience, both in staining and microscopical reading. The main value of staining is that immediate microscopical examinations are not necessary.

The examination of centrifuged deposits from freshly passed urine yields good results. Though inferior as a method of investigation to the examination of urethral scraping, its routine use should always be employed. During my own investigation it was found unnecessary and even inadvisable to wash the urine deposits, as this appeared to make the organisms less active and hence to mask their identity. In a series of twenty patients suffering from previously recognized trichomonad urethritis, the organism was recoverable from eighteen specimens of freshly-passed urine. As the protozoa becomes inactive in stale urine, centrifuging and examination should be carried out at once.

Culture Media

Before the use of antibiotics was known, it was difficult to obtain bacteria-free cultures, but this is now relatively easy. With these protozoa it is impossible to achieve growth in a solid medium. A fluid medium has its disadvantages but here it is a necessity. Johnson, Trussell, and Jahn (1945) used a medium containing cysteine hydrochloride, peptone, liver, and maltose, to which the name C.P.L.M. was given. This medium contained a small quantity of agar which made difficult the collection of the organisms. McEntegart (1952) modified this medium and omitted the agar. Feinberg (1953) in turn modified McEntegart's medium and this modification has proved to be the most successful yet devised. For ordinary clinical use we added to this medium 1,000 units penicillin and 500 units streptomycin per ml. In this way it was hoped to provide a medium for *T. vaginalis* which might prove as valuable for diagnostic purposes as the various media used in gonococcal investigations. The highly interesting findings of Whittington (1951) encouraged this hope. She, it may be remembered, cultured the seminal fluid of 26 men whose wives were or had recently been infected with *T. vaginalis*. The organisms were found in seven of the 26 specimens ; six of these were positive culturally, but only in two of the specimens could flagellates be observed microscopically. In my own investigation one hundred urethral scrapings from patients with non-specific urethritis were inoculated on to Feinberg's medium ; four of these were positive microscopically. Positive culture results were obtained only with inocula previously found microscopically positive. Further investigations with subpreputial, prostatic and vesicular specimens gave similar findings. These disappointing results are no im-

provement on those published by Magath (1938), who, using a simple pre-antibiotic medium, found that with microscopically positive specimens he could invariably obtain positive culture results. My findings are perhaps in conflict with the results of recent experiments *in vitro* which have shown that even with inocula containing very small numbers of trichomonads (such as 5 to 10 protozoa per ml.) the medium will permit growth. One possible cause for the negative findings may be that for the greater part of the investigations inoculated media were discarded after 4 days if growth had not occurred. Observations later showed that at least 7 days should be allowed to elapse before discarding any specimens. It was interesting to note the profuse growth of yeasts isolated on the inoculated media. As the yeasts interfered with the trichomonad growth, it was found necessary to add small quantities of methyl or gentian violet to the medium. At first a concentration of 1 in 15,000 was used but 1 in 7,500 was later found more satisfactory. There were no grounds for considering any possible relationship between the presence of yeasts and trichomonad infections of the male urethra. This limited investigation suggests that at present nothing is to be gained by the use of cultural methods in trichomonad investigations in the male and that microscopic examinations are equally as efficient and at the same time far less troublesome.

Serological investigations of trichomoniasis in man have been scanty compared with the extensive work on bovine infections with *T. foetus*. Prompted by the observations of Muniz (1950), McEntegart (1952) used the technique of agglutination of sensitized red cells for the detection of circulating antibodies in human trichomoniasis. By this means it was possible to demonstrate serum antibody in a large proportion of infected women. No similar investigation of known infected males was carried out but it was noted that the sera of 6 per cent. of apparently normal men contained serum antibodies. With these observations in mind it was decided to apply the test to male patients attending venereal disease clinics, and to check the results by clinical examination. Sera from 174 male patients, most of whom were suffering from non-specific urethritis, were tested ; nineteen (approximately 10 per cent.) gave positive reactions. No correlation, however, between these results and the clinical condition of the patients was established ; in fact, none of those found clinically positive gave positive serological readings. A closer relationship between the results of the haemagglutination tests and the clinical condition was noted in women patients suffering from trichomoniasis, but discussion of this is outside

the scope of this paper. A short time later, serum antibody readings taken before and after infection from males who were experimentally infected with *T. vaginalis* confirmed the previous negative findings. It is probable that serological tests of any sort are unlikely to be of assistance in the diagnosis of male trichomonad infections. Complement-fixation tests and intradermal injections of bacteria-free organisms were investigated by Trussell (1947), but the results were similarly disappointing and they now appear to be of no practical importance.

The inevitable impression gained from all these methods of diagnosis is that they are very much " hit and miss ", and, even when applied collectively, leave a large margin of error. It is felt that until more is known about the whole subject of both male and female trichomoniasis, little real progress in diagnosis, treatment, or prophylaxis will be forthcoming. Sufficient has been written in medical journals during recent years to show the wide interest that exists in *T. vaginalis* infections. This interest is no doubt stimulated by the poor response to treatment of trichomonas vaginitis and the uncertainties regarding its origin. It is now generally accepted that a considerable proportion of these infections are coital in origin, but the exact proportion arising in this way remains unknown. All of us here have met the innocent type of infection, such as the case of a married woman whose conduct is above suspicion, where there is no reason to doubt that of her husband. What is the origin of this infection ? The spread of the organism in gynaecological wards and clinics through imperfectly sterilized gloves, instruments, or bedpans requires no explanation, but it is more difficult to account for those infections occurring not so obviously in closed communities. What of the girl who develops a trichomonad vaginitis after a weekend spent in a seaside boarding house ; suspicions may be aroused but are they warranted ? What part does the lavatory seat or the communal towel play in these infections ? Opinions differ and there is little agreement on the subject. That the trichomonad can initiate a urethritis in the male has recently been demonstrated, but is it possible that the organism's effects can be more far-reaching ? Kolesoff (1950) demonstrated an antagonism between *T. vaginalis* and spermatozoa and further observations are necessary to discover if there is any relationship between trichomonad infections and male sterility. Does the infection spread beyond the vagina in women, and are the uterus and tubes ever invaded ? Trichomonad infection in cows may cause sterility or abortion ; is there a possible parallel in humans ?

The answer to all these and other problems will not be found, I believe, with the present unsatisfactory methods of examination, treatment, and investigation of trichomonad infections. These have led and can only lead to stalemate. The real solution lies in the setting up and running of special " discharge " clinics, in which the gynaecologist and venereologist would work in close collaboration. If such clinics were run by the venereologist, he could then carry out the comprehensive investigation which is long overdue. His work should also include a study of the other causes of vaginal discharge, and of monilial infection in particular. The answer to some of the problems of non-specific urethritis in the male may well be found here. The gynaecologists would hardly object to this arrangement, as in the main, their chief interest lies in the surgical aspect of their specialty. These clinics would not be venereal disease clinics, nor in any way connected with them, for experience shows that it is difficult and indeed often impossible to obtain the attendance there of this type of patient ; they should be part of a General Hospital service, and with facilities for male and female examinations, and careful and intimate history taking, and adequate laboratory resources immediately at hand. There are many indications that pertinent and sustained interrogation of patients might yield much valuable information.

The idea of a " special discharge clinic " is by no means original, indeed such clinics are already functioning in a few places, but I am sure that its wider adoption with close co-operation between gynaecologists and venereologists, would be immensely rewarding.

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