EPIDEMIOLOGY OF INFECTIONS WITH *TRICHOMONAS VAGINALIS* IN THE LIGHT OF IMPROVED DIAGNOSTIC METHODS*†

BY

M. JOAN WHITTINGTON

The Whitechapel Clinic, The London Hospital

Human genital trichomoniasis has received a good deal of attention in recent years, but there are still many aspects of this disease which need fuller investigation. This paper, based on work carried out at the London Hospital during the past 2½ years, is offered as an aid in elucidating certain of the more important of these problems—namely, the incidence, mechanism of infection, cause of relapses in women, and measures directed to control of the disease. I shall deal first with the laboratory methods of diagnosing *Trichomonas vaginalis* infections, and then with the epidemiology of the disease, the study of which is dependent on the effectiveness of the diagnostic technique.

Laboratory Methods of Diagnosis

Three laboratory methods are generally employed for detecting *T. vaginalis* in secretions:

- (1) Examination of fresh smears;
- (2) Examination of fixed, stained smears;
- (3) Culture of the parasites in artificial media.

(1) The first method, the examination of fresh smears, has the advantages of simplicity, speed, and the fact that it can be carried out on the spot, provided the necessary equipment is available. However, with specimens in which the parasites are not immediately apparent, it may be necessary to search carefully for perhaps 20 to 30 minutes before the presence of *T. vaginalis* can reasonably be excluded, and the clinician is seldom able to spare the requisite time and patience for such prolonged searches.

(2) The examination of stained smears has been advocated by many workers, including Schmid and Kamniker (1926), Liston and Lees (1940), Peter and Jirovec (1950), Fowler (1953), Sorel (1954), and Coutts and Silva-Inzunza (1954). Although I have repeated their methods carefully, I have been unable to substantiate the claims made for the superiority

of this diagnostic technique. None of the stains used has proved completely reliable, and therefore this method has not been used in the present investigation.

(3) For the third method, the culturing of secretions, a liquid liver medium, devised by Feinberg (Feinberg and Whittington, 1957), was suggested as a likely diagnostic medium, and experience has proved its value. It fulfils the necessary requirements for a good diagnostic medium, for it is simple, cheap, easily made, keeps for at least 3 months when stored in a refrigerator, and supports a good growth of trichomonads. It is easily the best medium for *T. vaginalis* that I have used. *Candida albicans* and other moulds and yeasts grow luxuriantly in this medium, but their presence does not appear to hamper the growth of the trichomonads. For these reasons I regard microscopic examination of fresh smears and culture of secretions as the diagnostic methods of choice, and have employed them in the investigations to be described.

Material from Female Patients.—Vaginal secretion was obtained in a small, sterile mustard spoon (Jackson, Malleson, Stallworthy, and Walker, 1948), the handle of which was gripped in a sponge holder. After the specimen had been taken, the spoon was placed immediately in a sterile, screw-capped, airtight 25-ml. bottle, and the material was thus prevented from drying. Within 2 hours a loopful of the vaginal exudate was mixed with a drop of saline on a slide and examined microscopically under a cover slip. During the examination, the slide was warmed at intervals over the pilot light of a Bunsen burner, or the flame of a spirit lamp. This procedure is designed to activate any trichomonads present and so to facilitate their recognition. When cold, the parasites become motionless and spherical and may be mistaken for pus cells. Cultures were made by transferring two or three loopfuls of the secretion from the spoon to a 6 × ½" test tube containing about 7 ml. liver medium previously warmed to body

* Received for publication March 29, 1957.

† Paper read before the M.S.S.V.D. on November 30, 1956.

heat. Voided urine was collected in a specimen glass and about 15 ml. was transferred to a sterile centrifuge tube and spun for 5 minutes at 1,600 r.p.m. About 0.5 ml. of the deposit was withdrawn with a sterile Pasteur pipette, and placed in a small tube ($3 \times \frac{3}{8}$ ") of the culture medium, except for the last drop which was used for a fresh smear. The inoculated medium was incubated at $+36^{\circ}\text{C}$. for 4 or 5 days and examined on three occasions during that time—usually on the first, second, and fourth day after inoculation. The culture fluid was centrifuged for 5 minutes, and a specimen was then taken from the bottom of the tube for microscopic examination. This is a desirable precaution, for if only a few trichomonads are present they may escape detection in a sample taken without previous spinning.

Material from Male Patients.—This consisted of (i) secretions from the urethra, and (ii) voided urine. Lanceley (1954) found that trichomonads were demonstrated more frequently in material obtained from the urethra by gently stroking the walls with a platinum loop than from urethral discharge, and the former method was therefore used in the present work.

The first loopful of urethral material was placed in a small tube of the culture medium. From the second loopful a fresh smear was made and examined microscopically. The cultures were examined in the manner described above.

Comparison of Smears and Cultures.—1,704 samples from men and women were examined for *T. vaginalis*, and there was agreement between the direct microscope findings and the cultures in 1,556 (91.3 per cent.) (Table I). In 32 (1.9 per cent.) of the 1,704 samples, fresh smears were positive but cultures were negative, while in 116 (6.8 per cent.) of the 1,704 specimens, positive cultures were obtained when the fresh smears were negative. The total number of samples positive for *T. vaginalis* was 492; in 116 (23.5 per cent.) instances the parasites

TABLE I
COMPARISON OF SMEARS AND CULTURES IN
DIAGNOSIS OF *T. VAGINALIS*

Total Samples	Results							
	Smear — Culture —		Smear + Culture +		Smear + Culture —		Smear — Culture +	
	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
1,704	1,212	71.1	344	20.2	32	1.9	116	6.8
	1,556 (91.3 per cent.)							

All percentages are of total number of samples.

grew in culture although they could not be detected in the fresh smears (Table II). Thus, nearly one quarter of the samples containing trichomonads would have been dismissed as negative had the cultural method not been used. These results indicate the general superiority of cultures over fresh smears, but the fact that more trichomonads were found in the fresh smears than in the cultures made from male urethral samples shows the desirability of using both methods in the diagnosis of trichomoniasis.

Stuart's Medium as a Transport Medium for T. vaginalis.—Stuart's medium, originally designed as a carrier medium for the transport of specimens to be examined for the gonococcus (Moffet, Young, and Stuart, 1948), was suggested as a possible carrier medium for *T. vaginalis*, which would be valuable when laboratory facilities were not available for examining samples as soon as they were taken from the patient. Experiments showed that vaginal trichomonads remain viable in Stuart's medium for 3 days at room temperature and for up to 9 days in the refrigerator. To find out if the organisms survive journeys through the post, swabs dipped in vagina secretions from women with trichomoniasis were inoculated into bottles of Stuart's medium and posted to the laboratory. On receipt, the swabs were removed and placed in tubes of the liver culture medium which were incubated and examined for

TABLE II
COMPARISON OF SMEARS AND CULTURES IN DIAGNOSIS OF *T. VAGINALIS*

Patients		Samples		Total Positive Samples	Smear + Culture +		Smear + Culture —		Smear — Culture +	
No.	Sex	No.	Site		No.	Per cent.	No.	Per cent.	No.	Per cent.
200	M	354	Urethra	90	62	69	17	19	11	12
207	M	378	Urine	109	80	73	4	4	25	23
563	F	747	Vagina	165	129	78	6	4	30	18
70	F	225	Urine	128	73	57	5	4	50	39
Totals 1,704		..	..	492	344	70	32	6.5	116	23.5

trichomonads in the usual way. Eight out of nine samples from patients at Queen Mary's Maternity Home, Hampstead, gave cultures positive for *T. vaginalis*; in one of these, 4 days had elapsed between posting and delivery. Four of the six samples from Whitechapel Clinic patients, and six out of fifteen samples from women attending the Special Clinic at St. Mary's Hospital, Paddington, subsequently gave positive cultures. This quick and effective means of dealing with material from patients suspected of harbouring *T. vaginalis* is commended to all who are faced with a time-lag between the taking and examining of specimens.

Incidence of Trichomoniasis in Women

The prevalence of vaginal trichomoniasis is difficult to estimate. Most investigations into the incidence of the disease have been carried out on gynaecological, ante-natal, or V.D. patients, and general statements based on this selected material are not permissible. Conclusions like those of Trussell (1947) that "one of every four or five adult women harbors the parasite" should therefore be treated with due caution. An attempt to estimate the frequency of *T. vaginalis* infections in the general population was made by examining a group of over 500 women attending a birth control clinic (Whittington, 1951a). The parasite was found in 5.3 per cent. of the whole group, of which 90 per cent. were women with no gynaecological disorders. Among women with gynaecological disorders, the percentage of infection is higher. In the present investigation, 12.8 per cent. of 400 patients attending the Gynaecological Out-Patients' Department of the London Hospital were found to have trichomoniasis. The incidence of this infection among the same number of female patients in the Whitechapel Venereal Diseases Clinic of the same hospital was 21.3 per cent. (Table III). In the latter group, diagnosis was based on the examination of fresh smears only; had the culture method been used as well, it is likely that the percentage would have been still higher.

Relapses in Women

Relapses after treatment for vaginitis due to *T. vaginalis* are very common indeed, and are the source both of distress and anxiety to the patient and of concern to the clinician. Knowledge of the reasons for recurrence of infection should point the way to more satisfactory methods of treatment.

Relapses may be due either to re-infection from an external source, such as an infected male partner, or to parasites, hidden in some extra-vaginal site out of reach of the medicament, which find their way back into the vagina. Concerning the latter

TABLE III
INCIDENCE OF *T. VAGINALIS* IN WOMEN

Author	Date	Type of Patient	No. Examined	No. with <i>T. vaginalis</i>	
				No.	Per cent.
Peterson ..	1938	Obstetric and Gynaecological	5,712	1,405	24.6
Debiasi ..	1939	Obstetric and Gynaecological	1,000	151	15.1
Jirovec and others ..	1942	"Normal" ..	181	26	14.3
		Gynaecological	603	215	35.6
Whittington	1951	Birth Control ..	562	30	5.3
Whittington	1957	Gynaecological	400	51	12.8
		Venereal Disease Clinic	400	85	21.3

possibility, suggested sites for the survival of the flagellates are the urinary tract and that part of the genital tract above the vagina. *T. vaginalis* has been found in the urine of women by a number of observers, including Pattyson (1937), Allen and Baum (1943), and Allen and Butler (1946). The fact that this organism has been recovered from the urine of women in whom no vaginal parasites could be demonstrated (Kean, 1955) suggests the urinary tract as a reservoir of infection. Evidence for this was provided by two women attending the Whitechapel Clinic.

One patient, who had had total hysterectomy 19 months previously, was receiving local treatment for recurrent *T. vaginalis* vaginitis. Trichomonads were found in her urine on two occasions when they could not be detected in the vagina, either by microscopic examination of fresh smears or culture of the exudate. As re-infection from the upper genital tract was ruled out in this case, and the patient denied having had sexual intercourse during the period of observation, it seemed likely that her relapses were due to re-infection from the urinary tract. A course of urethro-vesical irrigations with mercury oxycyanide solution, concurrently with acid sodium phosphate by mouth and local vaginal therapy with acetarsol, has apparently cured this patient, in whom no trichomonads have been found during the 9 months since cessation of treatment.

The other patient attended the clinic as the contact of a man with trichomonal urethritis. The two had been living together for 18 years. The woman complained neither of vaginal nor of urinary symptoms, and no objective evidence of disease was found on examination. She was kept under observation without treatment for 4 weeks, during which period her vaginal secretion and urine were examined five times microscopically and culturally for trichomonads. On the first two occasions, fresh smears and cultures from the vagina were negative for trichomonads, although these organisms were demonstrated in the urine by both methods.

Subsequently, the vaginal secretion remained microscopically negative although the cultures from it were positive for *T. vaginalis*. The urine at the same time showed the parasite both microscopically and culturally. The patient was given urethro-vesical irrigations but defaulted before the results of treatment could be assessed.

Because of the findings in these two cases, it was decided to search more carefully for *T. vaginalis* in the vaginal secretions and urine of patients undergoing treatment for vaginal trichomoniasis. The following procedure was adopted:

Patients with *T. vaginalis* vaginitis were given 2 weeks' local vaginal treatment. At the end of the first week, and on subsequent occasions, the vaginal secretion and voided urine from each patient were examined for trichomonads by the methods previously described. The 32 women investigated in this way can be divided into groups according to the results of the laboratory tests for *T. vaginalis* (Table IV).

TABLE IV
RESULTS OF TESTS AFTER TREATMENT

Group	No. of Patients	Laboratory Diagnosis		Result of Treatment		Defaulted
		Vagina	Urine	Cured	Relapsed	
I	11	<i>T.v.</i> negative	<i>T.v.</i> negative (after treatment)	2	7	2
II	14	<i>T.v.</i> negative	<i>T.v.</i> positive (after treatment)	1	13	0
III	3	<i>T.v.</i> negative	<i>T.v.</i> positive (during treatment)	2	0	1
IV	4 (2) (1) (1)	<i>T.v.</i> positive <i>T.v.</i> positive <i>T.v.</i> positive	<i>T.v.</i> positive (during treatment) <i>T.v.</i> negative <i>T.v.</i> ?	Infection persisted		

Group I.—Eleven of the 32 women appeared to respond to local vaginal therapy (acetarsol in ten cases, Penotran in the eleventh), fresh smears and cultures of the vaginal secretion and urine being negative for trichomonads after treatment. The specimens have remained negative for 6 months in one case and 9 months in another. Two of the eleven women defaulted before the follow-up was complete. The remaining seven patients later suffered relapse of vaginal infection.

Group II.—In fourteen of the 32 women, the parasites were demonstrated in the urine after vaginal treatment, although the latter had apparently cleared them from the vagina. (Acetarsol was used in five cases, and acetarsol and other local treatments in nine cases.) In thirteen of these fourteen cases the vagina later became re-infected with *T. vaginalis*.

Group III.—In three of the 32 patients, trichomonads were found in the urine (although apparently absent from the vagina) during local treatment of the latter. In two of these women the parasite subsequently disappeared from the urine, which has now failed to show *T. vaginalis* for 2½ months in one case and for 2 weeks in the other. The third patient defaulted before a follow-up could be initiated.

Group IV.—Four of the 32 patients (three on acetarsol alone and one receiving various other local remedies as well) failed to make any response to vaginal treatment, trichomonads being found in the fresh smears of the vaginal exudate even during therapy.

Altogether, trichomonads were found in the urine of 25 of the 32 women, and it seems logical to attribute the relapses which occurred in many of the cases, in Group II for example, to re-infection from the urinary tract.

An attempt was made to clear the parasites from the urinary tract by giving the patients courses of seven daily urethro-vesical irrigations with a 1 : 10,000 solution of mercury oxycyanide. Twelve women (one from Group I, ten from Group II, and the symptomless contact referred to on p. 82) were given these irrigations. Vaginal therapy was given concurrently with the irrigations in all but two of them.

One patient defaulted immediately after the irrigations and could not be followed up. The

irrigations appear to have been effective in only one of the remaining eleven cases, no parasites having been demonstrated in the vagina or urine of this patient for 3 months after the irrigations. The remaining ten patients continued to relapse.

Cultures of the secretions from these patients was an important aid in the assessment of treatment; 27·4 per cent. of the urine samples and 14·8 per cent. of the vaginal samples would have been recorded as negative had reliance been placed on fresh smears only (Table V).

TABLE V
COMPARATIVE VALUE OF SMEARS AND CULTURES IN DIAGNOSIS OF *T. VAGINALIS*

Site	Results					Total No. of Samples
	Smear and Culture +	Smear + Culture --	Smear -- Culture +		Smear and Culture --	
			No.	Per cent.		
Vagina	29	1	16	14·8	62	108
Urine	50	2	31	27·4	30	113

Site of Infection in the Female Urinary Tract

To find out, if possible, in which part of the urinary tract the trichomonads were located, patients in whose voided urine the parasite had been detected were subjected to the following investigation:

The area round the urethra was thoroughly cleansed with a dry cotton-wool swab, and the terminal quarter of an inch of the urethra cleaned with a sterile swab stick. This was intended to remove all traces of vaginal material from the urethral orifice. A sterile platinum loop was then passed into the urethra to obtain material from this site. The terminal part of the urethra was then again cleaned with a swab stick and massaged to express material from Skene's ducts, the resulting secretion being removed with a platinum loop. A rubber catheter lubricated with mercury oxycyanide paste was passed into the bladder, and about 15 ml. urine collected in a sterile centrifuge tube. This was spun and the deposit examined for trichomonads. Cultures and fresh smears were made from the material obtained from the three sites (urethra, Skene's glands, and bladder), and also from samples of the vaginal secretion which were taken in most of the cases.

Fifteen patients were investigated in this way, and trichomonads were isolated from the urethrae of two out of twelve, from Skene's glands of seven out of the fifteen, and from the urine of three out of the fifteen cases. Trichomonads were found in the vaginal secretions of four out of eleven of the women, but could not be demonstrated in seven (Table VI).

TABLE VI
SITES OF INFECTION WITH *T. VAGINALIS* IN 15 FEMALES

Patient No.	Vagina	Urethra	Skene's Glands	Urinary Tract (Catheter Urine)
1	—	+	+	+
2	+	—	+	+
3	+	—	+	—
4, 5	—	—	+	—
6	—	+	—	—
7, 8	+	—	—	—
9	NS	—	+	+
10	NS	NS	+	—
11, 12	NS	NS	—	—
13, 14, 15	—	—	—	—

NS=No specimen

From this small number of observations it is not possible to draw definite conclusions, but Skene's glands appear to be a common focus of infection.

Incidence of Trichomoniasis in the Male

Although *T. vaginalis* infestation is regarded primarily as a disease of women, there is increasing evidence for its occurrence in men as well (Young, 1949; Whittington, 1951b; Lanceley, 1953; Durel, Roiron-Ratner, Siboulet, and Sorel 1954; Sorel, 1954; and Jíra, Rössler, and Svejcar 1955). The relative

mildness of symptoms in men with trichomoniasis, together with the small number of parasites present in the secretions in most cases and the correspondingly greater difficulty in finding them, may account for the fact that, until fairly recently, infections with *T. vaginalis* in men were not widely recognized. A survey of the incidence of this infection in male patients attending the Whitechapel Clinic of the London Hospital, and in a small number of husbands of women with vaginal trichomoniasis attending the Gynaecological Out-Patients' Department of the same hospital (402 men in all) has yielded the results shown in Table VII.

TABLE VII
T. VAGINALIS INFECTIONS IN MEN

Category of Patient	N.S.U.	Treated Gonorrhoea	<i>T.v.</i> Contacts	NV.D.	Symptomless	Total
No. Examined	326	30	24	11	11	402
No. with <i>T. vaginalis</i>	50 (15.3 per cent.)	5	8	0	0	63

As the Table shows, the patients were divided into five groups:

- (1) Men with so-called non-specific urethritis.
- (2) Men under treatment for gonococcal infection.
- (3) Men who had been asked to attend the clinic as contacts of women with vaginal trichomoniasis.
- (4) Patients described as having "non-venereal disease".
- (5) A control group of men without symptoms of uro-genital disorder attending for "check-up" examination.

Comparing the incidence in men with non-specific urethritis (15.3 per cent.) with the figures obtained by other workers on similar patients, we see that Lanceley (1953) found the incidence to be 5.3 per cent. in 310 men, and Jíra and his collaborators (1955) found 10 per cent. in 765 men. On the other hand, Coutts, Vargas-Salazar, Silva-Inzunza, Olmedo, Turteltaub, and Saavedra (1955) claimed that 68 per cent. of 2,482 of their male patients in Chile were infected with the parasite.

Contact Tracing.—The men attending the Whitechapel Clinic were given contact slips for their female consorts to attend the Clinic so that both partners could be examined and treated concurrently. Table VIII shows the incidence of *T. vaginalis* infection in 118 female consorts.

It is particularly interesting to note the high incidence of trichomonal infection in the female consorts of men suffering from gonorrhoea, even of those men in whose secretions the parasite could not be shown.

TABLE VIII
T. VAGINALIS IN CONSORTS OF MALE PATIENTS

Male Patients		Contact Slips Issued*	Female Contacts			
Category	T.v.		T.v. positive	T.v. negative	Contact did not Attend	No Contact
N.S.U. .. 326	50 positive 276 negative	————→	10 18	5 39	35 218	0 1
Treated Gonorrhoea 30	5 positive 25 negative	←———— ————→	3 15	0 1	2 9	0 0
T.v. Contacts .. 24	8 positive 16 negative	←————	8 16	0 0	0 0	0 0
NV.D. .. 11	All negative	————→	1	1	8	1
Symptomless .. 11	All negative	————→	0	1	10	0

* Arrows show whether the contact slips were given from the men to the women or vice versa.

Incidence in White and in Coloured Men.—The incidence of trichomoniasis was higher in coloured than in white men. The parasite was found in 23 out of 81 (28·4 per cent.) of the coloured, and in 40 out of 321 (12·5 per cent.) of the white patients. This observation agrees with the findings of other workers (Feo, 1944; Roth, 1944).

Symptoms in Men with Trichomoniasis.—It is generally conceded that symptoms of uro-genital disorder in men with trichomoniasis are either mild or lacking altogether (Young, 1949; Whittington, 1951b; Draper, 1955, *inter alia*). Of the 63 men with *T. vaginalis* infections studied in the present survey, twelve were symptom-free and none of the remaining 51 had severe symptoms related to the uro-genital tract.

Persistence of Infection.—Lanceley and McEntegart (1953), who observed the course of trichomoniasis in three experimentally infected men, stated that the disease appeared to be self-limiting, the period during which the protozoa could be found in the genital tract varying from 4 to 94 days.

In order to throw more light on this question, nineteen men with trichomoniasis attending the Whitechapel Clinic were kept under observation while specific treatment for their infection was withheld. Eight of the nineteen patients defaulted while still infected with the parasite. Three of these had continued to show the parasite for 91, 90, and 88 days respectively.

In six of the remaining eleven cases, it was deemed wiser to treat the men for their *T. vaginalis* infections before they, too, defaulted. In these six men, the durations of their infections before treatment were 101, 87, 76, 36, 32, and 20 days respectively. In five of the untreated cases, the trichomonads disappeared spontaneously. Two of these five men had been observed for 8 and for 4 days. In the remaining

three cases, trichomonads were found on one occasion only. The evidence is small, but it does suggest that, in some cases, the disease may be self-limiting.

Mechanism of Infection

There is some conflict of opinion about the infectiousness of this disease and about the method of transference from patient to patient, some observers regarding it primarily as a venereal disease, and others believing accidental contamination to be the common means of infection.

Venereal Transmission.—The evidence for venereal transmission may be summarized as follows: As *T. vaginalis* is a parasite of the human genital tract, sexual intercourse suggests itself as a likely method by which the disease is transmitted. The facts that a not inconsiderable number of men are found to be infected with the parasite, and that the flagellates are frequently found in the consorts of infected men and women, both lend support to this view.

The following clinical evidence also suggests coitus as a method of infection. Drummond (1936) described women with recurrent trichomonal vaginitis who were cured only after the parasites had been eradicated from the genital organs of their husbands. By treating both *T. vaginalis*-infected partners simultaneously, Kučera (1950) and Baumeister and Hollinger (1941) were able to prevent recurrences of symptoms in the men. Allen and Butler (1946) were able to cure some of their most resistant cases of trichomonas vaginitis only after the husbands had received adequate treatment for their *T. vaginalis* infections. I have described a case in which relapses of the wife's vaginitis corresponded with recrudescences of *T. vaginalis* urethritis in the husband (Whittington, 1951b). The frequent association of trichomonal and Neisserian infections in both men and women (King, Mascal, and Price,

1936; Liston and Liston, 1939; Liston and Lees, 1940; Allison, 1943) suggests that trichomoniasis, like gonorrhoea, may be transmitted sexually. Observations that *T. vaginalis* infections are more common in prostitutes (Greenblatt, 1945; Breindl—unpublished data cited by Jirovec, Breindl, Kučera and Šebek, 1942) also indicate transmission *in coitu*.

The impression that *T. vaginalis* infestations are more common in women of child-bearing age, that they are rarely demonstrated in post-menopausal women or in children, and are absent from virgins, have been cited as evidence in favour of the sexual transmission of the parasite. It should be borne in mind, however, that the women examined for *T. vaginalis* in the latter three groups are much fewer in numbers than those in the first group, and on this account it would not be right to lay too much stress on this piece of evidence.

There are, however, certain facts that do not support the hypothesis of venereal transmission. Schmid and Kamniker (1926) examined pre- and post-coital vaginal secretions from patients, but failed to find evidence of fresh infection, or of increase in the number of trichomonads already present, in any of the women after sexual intercourse. The authors gave no information about the length of time before and after coitus at which the specimens were taken. If sexual intercourse were the usual method of acquiring the disease, one would expect to find *T. vaginalis* in a larger number of the male contacts of women with trichomoniasis than is in fact the case. The flagellates could only be demonstrated in three out of fifteen such men attending the Whitechapel Clinic and in five out of nine husbands of gynaecological patients, although three series of tests were taken from each of these men, in most instances after the urine had been held all night. Consideration must also be given to the denial of sexual intercourse by patients whose word seems trustworthy. In such cases, alternative explanations for infections must be sought.

Non-Venereal Transmission.—It is possible that trichomonads may find their way into the vagina on inanimate objects like contaminated douche nozzles, or specula or rubber gloves used during vaginal examination.

Toilet seats have been incriminated as a means of infection of women with the parasite. In fact, McCullough (1953) went as far as to say that "The main cause of *T. vaginalis* infection is the ordinary lavatory seat", and claimed that the use of gap toilet seats would reduce the prevalence of the disease by 80 per cent. In the present investigation, an attempt was made to assess the validity of this

statement. *T. vaginalis* is notoriously susceptible to drying, and it was first of all necessary to find out how long the flagellates would live on lavatory seats. Vaginal material containing trichomonads was smeared over an area approximately 0.5×3.0 cm. on the front upper surfaces of three seats in a water closet temporarily kept for experimental purposes in the Whitechapel Clinic. Three types of seat were used: one of bakelite, one of varnished wood, and one of unpolished absorbent wood. At intervals, a little of the secretion was removed from the seats by swabs moistened with saline and placed in tubes of the liver culture medium. The cultures were incubated and examined in the usual way for trichomonads. Table IX shows the survival time of *T. vaginalis* in vaginal exudate on the three different types of seat.

TABLE IX
TIME OF SURVIVAL (MIN.) OF *T. VAGINALIS* ON
LAVATORY SEATS

Experiment	Type of Seat			Control	Room Temperature (°C.)
	Bakelite	Polished Wood	Absorbent Wood		
1	45	45	20	+	+15
2	30	45	30	+	+12.5
3	45	45	30	+	+11.2
4	<10	<10	<10	+	+10.5

The parasite will remain viable for 45 minutes on bakelite and polished wooden seats, and for 30 minutes on an unpolished seat with an absorbent surface.

The next step was to find out if women with vaginal trichomoniasis do, in fact, leave any material containing the parasites on the lavatory seat after they have used it. Patients with heavy *T. vaginalis* infections were therefore asked to pass water in a water closet fitted with a varnished wooden ovoid seat without a gap. An electric buzzer, wired to sound out of the patient's hearing, was fitted under the seat to indicate whether or not the patient sat on it. Thirty patients unwittingly took part in this experiment (Table X). Seventeen of them sat on the seat, and six of the seventeen left urine on the top of the inner front edge of it. Trichomonads were cultured from two of these six specimens. The remaining thirteen women did not sit on the toilet seat, but five of them left urine on it, and two of these five specimens gave cultures positive for *T. vaginalis*.

These results show that women with *T. vaginalis* vaginitis may leave infective material on a toilet seat after they have used it. Whether other women can

TABLE X
CONTAMINATION BY *T. VAGINALIS* OF LAVATORY SEATS

Total No. of Patients		Sat on Seat	
		Yes	No
30		17	13
Seat Dry after Use		11	8
Material left on Seat after Use	<i>T.v.</i> positive	2	2
	<i>T.v.</i> negative	4	3

acquire trichomoniasis in this way is a question still to be answered.

There is no reliable evidence to support suggestions regarding other sources of infection such as contaminated bath and swimming-bath water, contaminated food or water, transference of intestinal trichomonads to the ano-rectal region and so to the vagina, or animal hosts acting as reservoirs of infection.

Discussion

Accurate and practicable methods of detecting *Trichomonas vaginalis* in secretions are essential both for effective diagnosis and for the assessment of the results of treatment of trichomonal infestations. The laboratory technique which has been used in this investigation, which combines the examination of fresh smears from secretions with cultures in a good medium, has proved both simple and efficient in demonstrating the parasites in specimens from patients, and can be recommended for routine use in hospital laboratories. The method usually relied upon for proving the presence of trichomonads in secretions, the examination of fresh smears, though a quick and easy one, has proved less accurate than the culture of samples in a reliable medium.

Diagnosis presents a problem when there is an inevitable time-lag of many hours between taking and examining the specimens. Experiments with Stuart's transport medium have shown that trichomonads not only survive in this medium for from 1 to 3 days, but that the medium provides a convenient method of sending through the post samples to be examined for the organism.

Trichomonads have been isolated from the urine of women by many observers, and it is clear that the urinary tract provides a place where the flagellates may survive out of reach of the medicaments used in local treatment of the vagina, and from which they may subsequently return to their habitual site when conditions are favourable. It is important to consider whether the relapses, which are so common after treatment for trichomoniasis, are due to this

cause. The pattern of events in the group of women studied in this investigation certainly lends support to this view. Parasites were isolated from the vaginal secretion and urine. Local treatment of the vagina resulted in disappearance of symptoms and failure to detect the parasites in the vagina, although they could still be demonstrated in the urine; subsequently, infection of the vagina occurred within days or weeks of the cessation of vaginal therapy. No matter how effectively the vagina is treated, if the parasites are not eradicated from the urinary tract as well, re-infection of the vagina with trichomonads from this site is possible. Unfortunately, with out-patients there is always another uncertain factor, namely the possibility of re-infection by sexual intercourse. An attempt to clear the parasite from the urinary tract in a few cases by urethro-vesical irrigations with mercury oxycyanide solution proved disappointing. It may be that success in this matter awaits the elaboration of a drug which is trichomonocidal when given by mouth.

T. vaginalis infection does not appear to be confined to any particular part of the lower urinary tract. In special tests designed to determine the sites of infestation, the flagellates were recovered from the urethra, from material expressed from Skene's glands, and from samples of urine obtained with a catheter. The frequency with which the organism was found in the secretions from Skene's glands is a point for particular emphasis.

The finding of *T. vaginalis* in 15.3 per cent. of a series of male patients with non-specific urethritis indicates that men are not infrequent sufferers from trichomoniasis, and that the parasite may be the causative organism in a larger number of genital infections than is generally conceded. The finding of the parasite in the male consorts of women suffering from trichomonal vaginitis shows the desirability of examining the male partner for trichomonads and, if necessary, of treating him.

There is still much speculation about the mechanism by which trichomoniasis is acquired. Venereologists tend to regard sexual intercourse as the means of transmission and there is a considerable amount of clinical evidence in favour of this theory. The ease with which the flagellates die outside the human body, unless protected from drying and extremes of temperature, adds weight to this view. Gynaecologists and general practitioners, on the other hand, are perhaps less aware of the inaccuracy of histories relating to extra-marital sexual intercourse. It is natural that they should wish to avoid the stigma attached to the term "venereal disease" and to seek an explanation of infection by means other than venereal.

It must be admitted that the evidence for non-venereal transmission is very slender. Objects contaminated with secretions containing trichomonads have been suggested as sources of infection, but no actual proof has been advanced that trichomoniasis is commonly acquired in this way. My own experiments with toilet seats only show that infective material is sometimes left on the seat after a woman with vaginal trichomoniasis has used it, and do not, of course, indicate that infection by this method actually occurs.

Summary

(1) A satisfactory technique for the laboratory diagnosis of *Trichomonas vaginalis* infections is described, in which samples of the secretions from patients are examined by fresh smears and cultured in a satisfactory medium. There was agreement between fresh smears and cultures in 1,556 (91.3 per cent.) of the 1,704 samples examined. In 1.9 per cent. of the samples, smears were positive for the parasite while cultures were negative, and in 6.8 per cent. of the samples the reverse was the case. The finding of the trichomonads in the cultures but not in the fresh smears from 23.5 per cent. of the 492 samples positive for the parasite shows the value of the cultural method.

(2) Stuart's transport medium has proved an effective means whereby specimens to be examined for trichomonads can be retained for 24 hours and more and can be sent through the post.

(3) The incidence of vaginal trichomoniasis in 400 gynaecological patients was 12.8 per cent., and in the same number of venereal disease patients, 21.3 per cent.

(4) Trichomonads were demonstrated in the urine of 25 of 32 women with vaginal trichomoniasis. In fourteen cases, local treatment of the vagina appeared to eradicate the flagellates from the vagina but failed to clear them from the urinary tract. Thirteen of these fourteen patients subsequently relapsed with recurrence of symptoms and the parasites were again isolated from the vagina. This evidence supports the view that re-infection from the urinary tract is one cause of relapse.

(5) In special tests carried out on fifteen women to determine the site of *T. vaginalis* infection in the urinary tract, the parasites were recovered from the urethra in two patients, from Skene's glands in seven, and from catheter specimens of urine in three. The frequency of involvement of Skene's glands was unexpected and potentially important.

(6) Trichomonads were found in the urethral secretions or urine in 15.3 per cent. of 326 men with non-specific urethritis, in five out of thirty men after

treatment for gonococcal infections, and in eight out of 24 male contacts of women with vaginal trichomoniasis. No parasites were found in a control group of eleven men with non-venereal diseases or in eleven men with no symptoms of any kind.

(7) It was possible to examine the female consorts of 26 men suffering from trichomonal infection and of 92 men without evidence of such infection. Vaginal trichomoniasis was found in 21 of the former and fifty of the latter.

(8) The incidence of trichomoniasis was 12.5 per cent. in 321 white men, and 28.4 per cent. in 81 coloured men.

(9) None of the 63 men with *T. vaginalis* infections had severe symptoms related to the uro-genital tract; twelve of the 63 were symptom-free.

(10) Observations on nineteen men who received no treatment for trichomoniasis indicated that the disease is self-limiting in some cases.

(11) Trichomonads in vaginal material remained alive for up to 45 minutes on the polished surfaces of lavatory seats. Four out of thirty patients with vaginal trichomoniasis left infective material on a toilet seat after they had used it.

I am glad to have the opportunity of thanking the following members of the London Hospital staff for their assistance: Mr. Victor Lack, Senior Consultant to the Gynaecological Department; Mr. A. J. King, Dr. C. S. Nicol, and Dr. Eva Gallagher of the Whitechapel Clinic; Dr. I. N. Orpwood Price, Director of the Venereal Diseases Reference Laboratory, by whose ready co-operation this work was made possible; and Professor Clifford Wilson for helpful criticism and advice. I am also indebted to Dr. G. L. M. McElligott, of the Special Clinic, St. Mary's Hospital, Paddington, for the vaginal specimens in Stuart's medium sent by post.

REFERENCES

- Allen, E., and Baum, H. C. (1943). *Amer. J. Obstet. Gynec.*, **45**, 246.
 — and Butler, S. (1946). *Ibid.*, **51**, 387.
 Allison, G. G. (1943). *Sth. med. J. (Bgham, Ala.)*, **36**, 821.
 Baumeister, C., and Hollinger, N. (1941). *Arch. Surg. (Chicago)*, **43**, 433.
 Coutts, W. E., and Silva-Inzunza, E. (1954). *British Journal of Venereal Diseases*, **30**, 43.
 —, Vargas-Salazar, R., Silva-Inzunza, E., Olmedo, R., Turteltaub, R., and Saavedra, J. (1955). *Brit. med. J.*, **2**, 885.
 Debiasi, E. (1939). *Ann. Ostet. Gynec.*, **61**, 281.
 Draper, J. W. (1955). *Int. Rec. Med.*, **168**, 563.
 Drummond, A. C. (1936). *Amer. J. Surg.*, **31**, 98.
 Durel, P., Roiron-Ratner, V., Siboulet, A., and Sorel, C. (1954). *British Journal of Venereal Diseases*, **30**, 69.
 Feinberg, J. G., and Whittington, M. J. (1957). In the press.
 Feo, L. G. (1944). *Amer. J. trop. Med.*, **24**, 195.
 Fowler, W. (1953). *British Journal of Venereal Diseases*, **29**, 166.
 Greenblatt, R. B. (1945). *Western J. Surg.*, **53**, 135.
 Jackson, M. H., Malleon, J., Stallworthy, J., and Walker, K. (1948). "Problems of Fertility in General Practice", p. 46. Hamish Hamilton, London.

- Jira, J., Rössler, J., and Švejcár, J. (1955). *Čas Lék. čes.*, **94**, 1233.
- Jirovec, O., Breindl, V., Kucera, K., and Šebek, V. (1942). *Zbl. Bakt., I. Abt. Orig.*, **148**, 338.
- Kean, B. H. (1955). *Amer. J. Obstet. Gynec.*, **70**, 397.
- King, A. J., Mascall, W. N., and Price, I. N. O. (1936). *Lancet*, **2**, 18.
- Kukera, K. (1950). *Čas Lék. čes.*, **89**, 508.
- Lanceley, F. (1953). *British Journal of Venereal Diseases*, **29**, 213.
- (1954). *Ibid.*, **30**, 163.
- and McEntegart, M. G. (1953). *Lancet*, **1**, 668.
- Liston, W. G., and Lees, R. (1940). *British Journal of Venereal Diseases*, **16**, 34.
- and Liston, W. A. (1939). *J. Obst. Gynaec. Brit. Emp.*, **46**, 474.
- McCullagh, W. McK. H. (1953). *Lancet*, **1**, 698.
- Moffett, M., Young, J. L., and Stuart, R. D. (1948). *Brit. med. J.*, **2**, 421.
- Pattysen, R. A. (1937). *N.Y. St. J. Med.*, **37**, 41.
- Peter, R., and Jirovec, O. (1950). *Praxis*, **39**, 357.
- Peterson, P. (1938). *Amer. J. Obstet. Gynec.*, **35**, 1004.
- Roth, R. B. (1944). *Vener. Dis. Inform.*, **25**, 163.
- Schmid, A. L., and Kamniker H. (1926). *Arch. Gynäk.*, **127**, 362.
- Sorel, C. (1954). *Presse méd.*, **62**, 602.
- Trussell, R. E. (1947). "Trichomonas vaginalis and Trichomoniasis", p. 103. Thomas, Illinois.
- Whittington, M. J. (1951a). *J. Obst. Gynaec. Brit. Emp.*, **58**, 399.
- (1951b). *Ibid.*, **58**, 614.
- Young, R. L. (1949). *Rocky Mtn med. J.*, **46**, 928.

DISCUSSION

The President, Dr. S. M. Laird (*Manchester*), said that infestation with *Trichomonas vaginalis* in both male and female was of great importance to the venereologist. It was necessary to know much more about diagnostic methods and the epidemiology of this infection, and progress in treatment was also essential. For some years, venereologists had felt strongly that research on these problems was much needed and Miss Whittington's excellent paper indicated that a promising start had already been made.

Dr. J. A. Burgess (*Wakefield*) thanked Miss Whittington for her most instructive and interesting paper.

Miss Whittington had stressed that, in the diagnosis of trichomoniasis, superior results were obtained by the use of cultural methods in addition to wet films. He asked the lecturer if she would give details of the culture and transport medium which she used.

In the vast majority of cases, examination of films of vaginal secretion from patients with trichomonas vaginitis showed numerous pus cells; but, in rare cases, wet films showed living flagellates and large numbers of epithelial cells, but no leucocytes. Was there a non-pathogenic strain of *T. vaginalis*?

Dr. R. R. Willcox (*London*), having congratulated Miss Whittington on her painstaking and most useful work, expressed interest in the incidence of

trichomonads in Skene's tubules. In all of the three cases in which they were found in the bladder, they were found in Skene's tubules also, and the possibility that bladder infestation was secondary to infection of these tubules was not unlikely.

It was interesting to note that the lavatory habits of the women with trichomoniasis were approximately divided by half into those who sat and those who did not. Both groups were equally apt to deposit the parasite on the seat. If the disease was commonly acquired in the lavatory, it would be expected that female patients with trichomoniasis would show a higher percentage of sitters than non-sitters and some control figures of the female population at large would be helpful.

Finally Dr. Willcox, in noting the failure of local therapy in cases of urinary trichomoniasis, inquired whether the newly flaunted 2-acetylamino-5-nitrothiazole preparations had been tried. This drug he understood was of great importance in preventing blackhead disease in turkeys and in the saving of millions of birds each year. Blackhead disease was caused by a flagellated protozoon. Two preparations (Tritheon and Trichorad) of 2-acetylamino-5-nitrothiazole were on the British market, advertised as potent orally against trichomoniasis. His own experience of the drug in vaginal trichomoniasis had been most disappointing, and he would be glad to hear of the experience of others of its effects in other sites. Of twelve cases of vaginal trichomoniasis treated with oral Tritheon, ten had been followed, and *T. vaginalis* had been found in all of them without any difficulty after one week. He felt that the drug might have been prematurely applied to human medicine by analogy from veterinary experience but, this time, the wrong kind of turkey has been chosen!

Dr. R. D. Catterall (*London*) described a series of fourteen patients treated with Trichorad, and stated that there had been no therapeutic effect after using one tablet three times a day for 10 days in any of the cases. Furthermore, samples of urine at the completion of treatment showed no anti-trichomonal effect.

He had also treated 23 patients with local Trichomycin pessaries, and a control series with S.V.C. The relapse rate with Trichomycin at the end of one month's observation was twice as high as with S.V.C. A group of patients had been treated with oral Trichomycin in high doses, but no therapeutic effect whatever had been seen after 14 days' treatment in any of the patients, nor did the urine show any anti-trichomonal effect.

Dr. E. Dunlop (*London*) stated that he had had a little experience with oral trichomonicidal agents.