Routine cytological examination of vaginal and cervical smears is now an established method of early detection of cancer of the uterine cervix, and the need for screening as many women at risk as possible has been repeatedly stressed in recent leading articles \(\text{British Medical Journal}, 1962, 1963, 1964, 1965; \text{Lancet}, 1963)\.

\textit{Trichomonas vaginalis} may be identified in smears stained by the Papanicolaou method \(\text{Papanicolaou and Traut, 1943)}\). It also produces profound changes in the general smear pattern; the changes were described and discussed at length by Koss and Wolinska \(\text{Koss and Wolinska, 1959}\) and are regarded as so characteristic that the presence of trichomonads may be suspected before the organisms are actually identified \(\text{Wachtel, 1964}\). \text{Way (1963) states that the vaginal smear frequently shows the presence of T. vaginalis when other methods fail and, furthermore, trichomoniasis may be diagnosed from the characteristic cell changes in the absence of the organisms and clinical signs.}\n
At the Clinic for Venereal Diseases of St. Luke’s Hospital in Bradford, we started to collect specimens for cytological examination in the second half of January, 1964, with two aims in view:

1. To contribute to the routine screening of women for cervical cancer;
2. To evaluate the Papanicolaou-stained smear as a diagnostic method of identification of \textit{T. vaginalis} by comparison with the usual methods of wet smear and culture.

A similar comparison of the three techniques for the diagnosis of vaginal trichomoniasis was made in the USA by \text{Kean and Day, 1954}\). They compared the efficiency of the Papanicolaou-stained smear with the hanging-drop technique and a culture method. The culture medium used by them was the Simplified Tripti-case Serum described by \text{Kupferberg, Johnson, and Sprince, 1948} modified by the addition of penicillin and streptomycin \(\text{Williams, 1950}\). In their experience the culture method was superior to the hanging-drop and Papanicolaou smears. In a total of 500 gynaecological patients, a single examination gave positive results in 80 \(16\) per cent.) of the patients were positive in 28 \(9\)-3 per cent.) of 300 of these patients. In no instance was a case positive by the hanging-drop or Papanicolaou smear and negative by culture.

\textbf{Material and Methods}

At first it was arranged with the Department of Cytology that all women over 30 years of age and a limited number of younger women should be screened for both cancer and trichomonias. Later women aged 24 or less were tested for trichomonas only while the age limit of those screened for cancer was reduced to 25, so that of a total of 269 women examined up to the end of March, 1965, only 136 were screened for cervical cancer and 254 were included in the trichomonas study \(\text{fifteen were excluded, because they were not examined by all three methods on the same visit; of these, seventeen were tested twice, making a total of 271 tests. Urethral scrapings from 116 of the women and specimens of urethral discharge or scrapings, and of fluid expressed by prostatic massage from 35 male contacts of women with a trichomonal infection, were also examined by the three parallel methods.}\n
The ages of the 269 female patients ranged from 16 to 55 years and the majority \(\text{154}\) were under 25 years; 225 were born in the United Kingdom, fourteen came from other European countries, twenty were West Indian Negroes, and ten were Asian.

The specimens were collected at the patients’ first visit or at a follow-up attendance. A non-lubricated bivalve speculum was inserted into the vagina and specimens from the posterior fornix and the cervix were collected with an Ayre’s spatula. Where screening for cancer was antici-
pated, separate vaginal and cervical smears were made using the rounded end of the spatula for collecting material from the vaginal pool and the shaped end for scraping the cervix, whereas for testing for trichomomas only one cervico-vaginal smear was prepared. From other sites the specimens were collected with a wire-loop. When the smears were made the slides were immediately immersed in the fixing jar filled with equal amounts of absolute alcohol and methylated ether and were sent to the Department of Cytology for staining and interpretation. A characteristic trichomoniasis cytological pattern was not considered sufficient for diagnosis, and only smears showing trichomonads with a preserved nucleus were accepted as positive.

Specimens for culture were collected on charcoal-impregnated swabs and sent in bijou bottles filled with Stuart’s transport medium to the Bradford Public Health Laboratory where, usually within 24 hours, the growth medium was inoculated. The medium was made up as follows:

- Oxoid Hartley’s digest broth . . . . 1 litre
- Dehydrated liver (Oxoid), . . . . . . 5·5 g.
- Dextrose . . . . . . . . . . . . . . . 2·5 g.
- Cysteine hydrochloride . . . . . . . 1·3 g.

Adjust pH to 6·0 with N/1 NaOH; heat in autoclave at 10 lb. for 30 min., and then add:

- Ascorbic acid . . . . . . . . . . . . . 2·0 g.
- Inactivated horse or sheep serum . . . . . 100 ml.
- Chloramphenicol succinate . . . . enough to give a final concentration of 100 μg per ml.

Distribute in universal bottles and store at 4°C.; warm to 37°C. before inoculation.

Bottles with the inoculated medium were incubated at 36°C. and examined after 3 and 5 days. The majority of the positive results were obtained after 3 days.

Wet smears were examined immediately by direct illumination at the clinic.

**Results**

**Identification of T. Vaginalis**

The comparative results of the three parallel methods are shown in Tables I, II, and III.

In the cervico-vaginal specimens (Table I), *T. vaginalis* was identified by the Papanicolaou-stained smear in 83 (77·5 per cent.) of the 107 positive cases, as compared with 51 (47·6 per cent.) positive wet smears and 92 (86·1 per cent.) positive cultures. There was a high proportion of agreement between the Papanicolaou smear and the two other methods (75·7 per cent.) and in eight cases (7·4 per cent.) this was the only method by which the organism was detected. In sixteen of the 24 cases in which the Papanicolaou smear was negative and the wet smear or culture positive, the general smear pattern was characteristic of trichomoniasis, though the organisms could not be found.

### Table I

**TRICHOMONAS IN THE VAGINA**

**ANALYSIS OF 107 POSITIVE RESULTS IN 271 CASES EXAMINED BY THREE PARALLEL METHODS**

<table>
<thead>
<tr>
<th>Method of Detection</th>
<th>Wet Smear</th>
<th>Papanicolaou-</th>
<th>Culture</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Three</td>
<td>38</td>
<td>35·5</td>
<td>38</td>
<td>35·5</td>
</tr>
<tr>
<td>Two</td>
<td>6</td>
<td>5·6</td>
<td>31</td>
<td>29·0</td>
</tr>
<tr>
<td>One</td>
<td>1</td>
<td>0·9</td>
<td>8</td>
<td>7·4</td>
</tr>
<tr>
<td>Totals</td>
<td>51</td>
<td>47·6</td>
<td>83</td>
<td>77·5</td>
</tr>
</tbody>
</table>

### Table II

**TRICHOMONAS IN THE FEMALE URETHRA**

**ANALYSIS OF 27 POSITIVE RESULTS IN 116 CASES EXAMINED BY THREE PARALLEL METHODS**

<table>
<thead>
<tr>
<th>Method of Detection</th>
<th>Wet Smear</th>
<th>Papanicolaou-</th>
<th>Culture</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Three</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Two</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>One</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Totals</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

### Table III

**TRICHOMONAS IN THE URETHRA AND PROSTATE**

**ANALYSIS OF 8 DETECTED CASES IN 35 MALES EXAMINED BY THREE PARALLEL METHODS**

<table>
<thead>
<tr>
<th>Clinical Diagnosis</th>
<th>No. of Patients</th>
<th>Positive Results by All Three Methods</th>
<th>Wet Smear and Culture</th>
<th>Culture only</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U</td>
<td>P</td>
<td>U</td>
<td>P</td>
<td>U</td>
</tr>
<tr>
<td>Asymptomatic Contacts</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Non-Gonorrhoeal Urethritis</td>
<td>12</td>
<td>1*</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Gonorrhoea</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Prostatitis</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1*</td>
</tr>
<tr>
<td>Totals</td>
<td>35</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

* In the Papanicolaou-stained smears Trichomonas was suspected, but not definitely identified.
† In two cases the organism was cultured from both sites.

In the female urethra (Table II), there were 27 positive results, of which 21 were obtained by culture, four by wet smear and culture, one by wet smear alone, and only one by Papanicolaou-stained
smear and culture. In two of the cases detected by culture the infection was confined to the urethra and this was confirmed by a second culture.

In the group of 35 male contacts of the infected women (Table III), specimens were collected from the urethra and prostate in all cases with the exception of four patients with acute gonorrhoea from whom only specimens of urethral discharge were taken. In eight patients T. vaginalis was cultured from six urethral and four prostatic specimens; in one of the urethral and two of the prostatic specimens the organism was found in the wet smear also, and in the two prostatic specimens positive by wet smear and culture the organism was suspected but not definitely identified in the Papanicolaou-stained smears.

These results indicate that the Papanicolaou-stained smear is a reliable method of identification of T. vaginalis in the vagina and cervix. It compares favourably with the wet smear which, however, remains the simplest method of immediate diagnosis. The culture method is the most efficient, but the diagnosis is delayed. The combination of the three methods gives the highest yield of positive results.

The Papanicolaou smear is of little value in the detection of T. vaginalis in specimens from the female urethra or from the male urethra and prostate.

Screening for Cervical Cancer

Table IV shows the age and race distribution of the 136 women who were screened for cervical cancer.

Of the European patients, 107 were born in the United Kingdom and ten came from Eire or the continent of Europe. Of the six Asians, four were from Pakistan and one from India, and one was Chinese. 69 were married, 29 were divorced or separated from their husbands, three were widowed, and 35 were unmarried. All the Asians were married.

Nineteen women were childless, fifteen had one child, and 102 were multiparous; the highest parity was in a woman aged 35 who had had ten children and four miscarriages. Ten women were pregnant at the time of examination, but only four of them were married. As regards the clinical diagnosis, 101 had various sexually-transmitted diseases, often in combination, and 68 were known to be very promiscuous and had a history of multiple previous incidents of venereal disease; 35 were consorts of infected men, but were found to be free from infection and did not require treatment.

In this very small series of women screened, six cases of malignancy were detected; the details of these cases are shown in Table V.

In the case in which the result of cone biopsy is shown as inconclusive, definitely malignant cells were found in the smear, but the histological examination of the biopsy material was performed in another town; however, this patient remains under the supervision of a gynaecologist.

### Table IV

<table>
<thead>
<tr>
<th>Age Group (yrs)</th>
<th>Racial Group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>European</td>
<td>West Indian</td>
</tr>
<tr>
<td>16-20</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>21-25</td>
<td>28</td>
<td>1</td>
</tr>
<tr>
<td>26-30</td>
<td>33</td>
<td>4</td>
</tr>
<tr>
<td>31-35</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>36-40</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>41-45</td>
<td>6</td>
<td>—</td>
</tr>
<tr>
<td>Over 45</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>117</td>
<td>13</td>
</tr>
</tbody>
</table>

### Table V

<table>
<thead>
<tr>
<th>Time of Detection</th>
<th>Age of Patient (yrs)</th>
<th>Origin</th>
<th>Marital Status</th>
<th>Parity</th>
<th>Clinical Diagnosis</th>
<th>Result of Cone Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>March, 1964</td>
<td>37</td>
<td>UK Born</td>
<td>Married</td>
<td>II</td>
<td>Trichomonas Vaginitis</td>
<td>Early invasive Cancer</td>
</tr>
<tr>
<td>April, 1964</td>
<td>25</td>
<td>UK Born</td>
<td>Separated</td>
<td>II</td>
<td>Gonorrhoea</td>
<td>Invasive Cancer</td>
</tr>
<tr>
<td>May, 1964</td>
<td>35</td>
<td>West Indian</td>
<td>Single</td>
<td>II</td>
<td>Gonorrhoea 34 wks Pregnancy</td>
<td>Invasive Cancer</td>
</tr>
<tr>
<td>November, 1964</td>
<td>31</td>
<td>UK Born</td>
<td>Separated</td>
<td>V</td>
<td>Vaginal Candidiatis</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>December, 1964</td>
<td>33</td>
<td>West Indian</td>
<td>Single</td>
<td>VI</td>
<td>Gonorrhoea Urethral Trichomoniasis</td>
<td>Carcinoma in situ</td>
</tr>
<tr>
<td>January, 1965</td>
<td>39</td>
<td>UK Born</td>
<td>Separated</td>
<td>I</td>
<td>Gonorrhoea</td>
<td>Carcinoma in situ</td>
</tr>
</tbody>
</table>
**Discussion**

"Cervical cancer occurs most frequently in women of low socio-economic status living in the city, who marry early, whose marriages are frequently broken, and whose lives are unhappy. It is prevalent in groups in which these factors are prominent, such as immigrants and prostitutes". This is how Graham, Sotto, and Paloucek (1962) conclude their discussion of the aetiology of carcinoma of the cervix. Five of our six cancer patients belong to these categories.

Many factors have been reported to be pre-disposing to or frequently associated with cancer of the cervix. Those of particular interest to the venereologist include syphilis (Belote, 1931; Harding, 1942; Levin, Kress, and Goldstein, 1942; Lombard and Potter, 1950; Morris and Meigs, 1950; Röjel, 1953; Jones, Macdonald, and Breslow, 1958), trichomonal infection (Koss and Wolinska, 1959; Way, 1963), prostitution (Röjel, 1953; Pereyra, 1961), first coitus at an early age, frequent coitus, in particular coitus unprotected by contraceptives, and promiscuity (Wynder, Cornfield, Schroff, and Doraiswami, 1954; Stocks, 1955; Terris and Oalman, 1960; Stern and Dixon, 1961), and poor penile hygiene in uncircumcised sexual partners (Wynder, 1955; Elliott, 1964).

These reports suggest that routine screening for cervical cancer at the clinics for venereal diseases would give a higher than usual proportion of positive cases. Our finding of six cases in 136 women screened confirms this assumption, but the series is too small to be statistically significant. Farrer and Tatham (1962) detected two cases of carcinoma in situ and two of invasive carcinoma in 235 patients tested in a VD clinic during 18 months. Recently Greene, Oppenheim, and Olswang (1964) reported that, of 9,636 patients screened for cervical cancer during 4 years at two venereal diseases clinics in Brooklyn, 306 showed positive cytology; ninety were lost for follow-up, and in 216 a biopsy was performed. The biopsies revealed 125 cases of carcinoma in situ and eight cases of invasive cancer.

Fergusson (1961) reported on 77 girls who were 19 years of age or less when they were discovered to have a positive cancer smear. The majority failed to attend for biopsy, but ten of those who did so were found to have intra-epithelial carcinoma of the cervix. He contended that no age limit should be imposed on cytological screening for cervical cancer: if a girl is old enough to have a vaginal examination she is old enough to have a cervical cytological examination. Of the cases discovered at the two Brooklyn VD clinics, 107 were in the 21 to 35-year age group and seven, one with invasive cancer, were under 20. The youngest of our patients with positive smears was aged 25, but she had an invasive cancer which clinically proved to be Grade III carcinoma with parametrial involvement.

If, as it now seems to be generally accepted, sexual activity is a major contributory pathogenetic factor, women attending the VD clinics are at a greater risk than the rest of the female population and the venereologist has an important part to play in the early detection of cancer of the uterine cervix.

**Summary**

1. The Papanicolaou-stained smear was evaluated as a method of identification of *T. vaginalis* by comparison with the wet smear and culture techniques on 271 specimens from the vagina and cervix, 116 specimens from the female urethra, and 35 from the male urethra and prostate.

   Of a total of 107 positive findings in the cervico-vaginal specimens, *T. vaginalis* was identified by the Papanicolaou smear in 83 cases (77.5 per cent.), as compared with 51 (47.6 per cent.) positive wet smears and 92 (86.1 per cent.) positive cultures. In 81 cases (75.7 per cent.), the Papanicolaou smear was in agreement with either or both of the two other methods, and in eight cases (7.4 per cent.) it was the only method by which the organism was identified.

   In specimens from the female urethra, *T. vaginalis* was identified by the Papanicolaou smear in only one of the 27 cases found to be positive by culture or wet smear. In the male the organism was suspected but not definitely identified in two specimens from the prostate out of a total of six urethral and four prostatic specimens which were positive by culture.

   The Papanicolaou-stained smear is a reliable method of identification of *T. vaginalis* in the vagina and cervix. The combination of the Papanicolaou smear with the two other methods gives the highest yield of positive results. It is of little value, however, in the diagnosis of trichomonal infection in the female or male urethra or in the prostate.

2. Of a total of 136 women screened for cervical cancer, six were found to have definitely malignant cells in the smears. Biopsy revealed three cases of invasive cancer and two of carcinoma in situ, while one case was unconfirmed and the patient remains under gynaecological supervision. One of the women with an advanced invasive cancer was only 25 years of age.
Factors which are deemed to contribute to the development of cervical cancer and which are prominent in patients attending clinics for venereal diseases are discussed in the light of published reports, and the important part the venereologist should play in the early detection of cancer of the uterine cervix is emphasized.

The co-operation of Dr R. A. McInroy, Consultant Pathologist, and Mr R. G. Taylor and Mr W. Callaghan, Senior Laboratory Technicians of the Department of Cytology of St Luke’s Hospital, Bradford, and of Dr H. G. Smith, Director of the Bradford Public Health Laboratory, is gratefully acknowledged.

REFERENCES


Examen cytologique routinier pour la détection du cancer cervical et de l’infection à trichomonas dans un dispensaire de maladies vénériennes

RéSUMÉ

(1) On a comparé la valeur diagnostique de différentes méthodes d’identification du *T. vaginalis* (l’examen des frottis colorés par la technique de Papanicolaou, l’examen direct des frottis mouillés, et la culture) sur 271 spécimens vaginaux et cervicaux et 116 spécimens urétraux prélevés chez la femme, et sur 35 spécimens urétraux et prostatiques prélevés chez l’homme. Sur un total de 107 résultats positifs dans les spécimens cervico-vaginaux le *T. vaginalis* fut identifié dans 83 cas (77,5%) par la méthode de Papanicolaou, comparé avec 51 (47,6%) résultats positifs dans l’examen direct des frottis mouillés, et 92 (86,1%) cultures positives. Dans 81 cas, la méthode de Papanicolaou donna les mêmes résultats que l’une des autres méthodes ou les deux ensemble, et dans 8 cas (7,4%) ce fut la seule par laquelle le microbe fut identifié.

Dans les spécimens urétraux féminins on n’identifia le *T. vaginalis* par la méthode de Papanicolaou que dans un seul cas, alors que 27 résultats positifs furent obtenus par les 2 autres méthodes. Chez l’homme on soupçonna l’existence du trichomonas sans l’identifier de façon certaine dans 2 spécimens prostatiques, alors qu’on trouva 6 échantillons urétraux et 4 de la prostate positifs par la culture.

La technique de Papanicolaou offre une méthode sûre d’identification du *Trichomonas* dans le vagin et le cervix. La combinaison de cette dernière avec les deux autres méthodes donne la récolte la plus abondante de résultats positifs. Elle a cependant peu de valeur dans le diagnostic des infections trichomonales de l’urètre mâle ou femelle ou de la prostate.

(2) Des 136 femmes examinées en vue de détecter un cancer cervical, on en trouva six dont les frottis contenaient des cellules définitivement malignes. La biopsie révèle trois cas de cancer envahissant et deux de cancer localisé in situ, tandis qu’un cas restait non confirmé et sous supervision gynécologique. Une des femmes atteintes de cancer extensif avancé était âgée de 25 ans seulement.

On étudie à la lumière des travaux publiés les facteurs considérés comme favorisant le cancer cervical souvent au maximum chez les malades des dispensaires vénériens, et on insiste sur le rôle important du vénéréologue dans la détection du cancer cervical.

THE PRACTITIONER

The symposium published in the November, 1965, issue of *The Practitioner* comprises the following articles:

The Treatment of Syphilis: A. J. KING
The Treatment of Gonorrhoea: C. B. S. SCHOFIELD
Reiter’s Disease: J. A. H. HANCOCK

Trichomoniases: L. WATT
Venereal Diseases and Teenagers: R. D. CATTERALL
Venereal Diseases and Immigrants: R. R. WILLCOX.
Routine exfoliative cytology for cancer and trichomonas detection at a clinic for venereal diseases.

L Z Oller

*Br J Vener Dis* 1965 41: 304-308
doi: 10.1136/sti.41.4.304

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