

# Abnormal forms of *Trichomonas vaginalis*

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**SUMMARY** Abnormal forms of *Trichomonas vaginalis* have been demonstrated by both conventional and scanning electron microscopy after inoculation of media with clinical material from cases of trichomonal vaginitis. Twenty-six cases of vaginitis have been studied; 10 of them showed the abnormal forms of trichomonads after growth in a modification of the medium described by Bushby and Copp (1955), while 16 showed only normal forms.

## Introduction

Abnormal forms of *Trichomonas vaginalis*, with variation in size and shape, have previously been reported. Marchand (1894) described a large parasite with three tufts of flagella and four nuclei, while other workers have also reported variations, particularly in the size of the parasite (Powell, 1936; Trussell, 1947; Winston, 1974). Some of these findings suggest that the shape varies according to the composition of the growth medium.

Recently, while investigating the effect of various additions to our standard culture medium (Squires and McFadzean, 1962), in order to increase its growth potential, we observed parasites with an abnormal morphology after subculture of the vaginal secretion. These abnormal trichomonads are described in this paper.

## Materials and methods

Twenty-six female patients were studied. A diagnosis of trichomonal vaginitis was made after fresh vaginal discharge had been examined by darkground microscopy. Once a positive diagnosis was made, a sample of the vaginal discharge was inoculated into 20 ml of the medium described by Bushby and Copp (1955), modified by Squires and McFadzean (1962). The vaginal discharge was obtained by using a disposable vaginal pipette. The inoculated medium was placed in the incubator without delay and incubated at a temperature of 35-37°C for 24 hours. At this time, growth was visible in all 26 cultures as a whitish deposit at the bottom of the culture tubes.

Incubation was continued for 72 hours, at which time the growth was examined by darkground microscopy and the appearance and behaviour of the parasites were recorded. The growth was re-inoculated into fresh medium and incubated as before, again the growth was examined on the third day. This re-inoculation process was repeated 10 times for a total observation period of one month.

The growth from the cultures of one patient, which showed a large number of bizarre forms, was also studied under a scanning electron microscope. The specimens were prepared by fixing two to three drops of the centrifuged (at 500 rev/min for five minutes) culture deposit in 10 ml of ice-cooled glutaraldehyde for one hour; this was then again gently centrifuged and the deposit washed in cacodylate solution. After being centrifuged again as above, the deposit was passed through a series of acetone washes (25%, 50%, 75%, and 100%), and allowed to remain in each concentration for 30 minutes. A drop of the suspension from the final acetone wash was then placed on a coverslip under a drop of 100% acetone. The coverslip was then cemented with a current-conducting glue and coated with gold before being examined by the scanning electron microscope.

## Results

Of the 26 cases studied, 10 gave cultures of abnormal *T. vaginalis* and 16 showed only normal forms. None of the 26 patients had shown abnormal forms in the fresh vaginal discharge examined before culture. The abnormal forms started to appear in the first or second passage, and were then seen persistently in the subsequent subcultures, but in

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varying numbers and always intermixed with normal forms. The abnormal parasites appeared under the light microscope to be large and irregular in shape, with multiple nuclei, multiple flagellae, and amoeboid movement (Fig. 1). Large, rounded, non-motile or sluggishly motile forms with a hyaline appearance were also seen, but these were obviously dead (encysted) or dying organisms. Under the scanning electron microscope the following morphology was noted: abnormal forms were larger than organisms of normally accepted size and had irregular surfaces with spiky projections (Fig. 2), or

had multiple stumps and fragmented undulating membrane (Fig. 3). It is possible that some of these stumps were flagellae which had been damaged in the course of preparing the microscopical material; or it is more likely that they were the pseudopodia by which the parasite ingests bacteria or other particulate matter. Figure 4 shows a normal *T. vaginalis* as seen under the scanning electron microscope.

Cultures showed a very high proportion of abnormal forms in the vaginal discharge of a 16-year-old girl, so these were studied under the scanning micro-

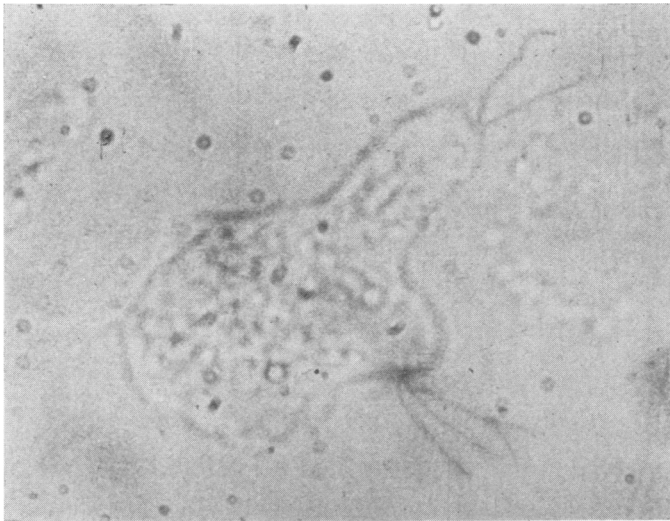


Fig. 1 *Abnormal T. vaginalis* showing multiple nuclei and flagellae. Normal microscopy.

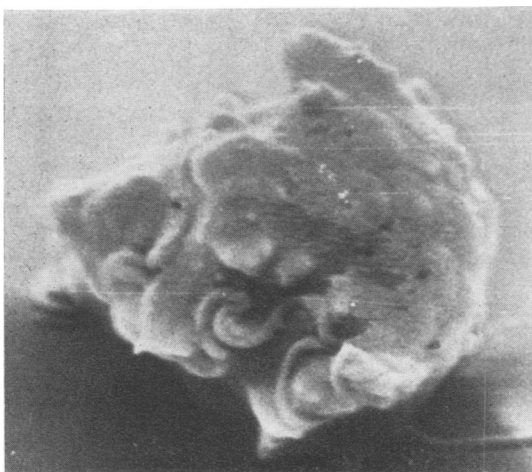


Fig. 2 *Abnormal T. vaginalis* showing spiky projections. Scanning electron microscope.

scope. *T. vaginalis* infection had been diagnosed on seven occasions during a 30-month period. She had had a gonococcal infection on eight occasions and other sexually transmitted infections separately or in association with *T. vaginalis*.

Of the 10 patients who showed abnormal forms of the parasite, three were pregnant at the time of examination and already had children, another three were not pregnant but had previously had children, and four of them were multipara. The group which showed only normal forms were all nulliparous.

There were patients with and without abnormal trichomonads who had gonorrhoea, candidosis, genital herpes, and warts in association with trichomoniasis. However, it is an interesting observation that the history of the group with the abnormal forms shows they had sexual intercourse more often and with more partners than those with only normal forms.

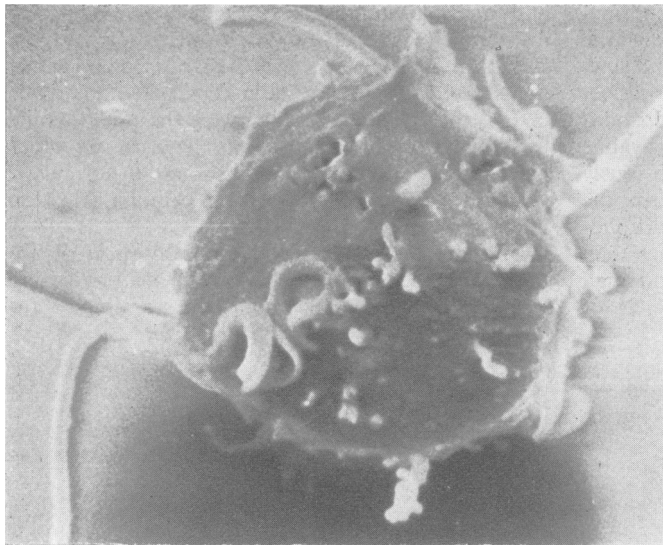


Fig. 3 *Abnormal T. vaginalis with multiple stumps and fragmented undulating membrane. Scanning electron microscope.*

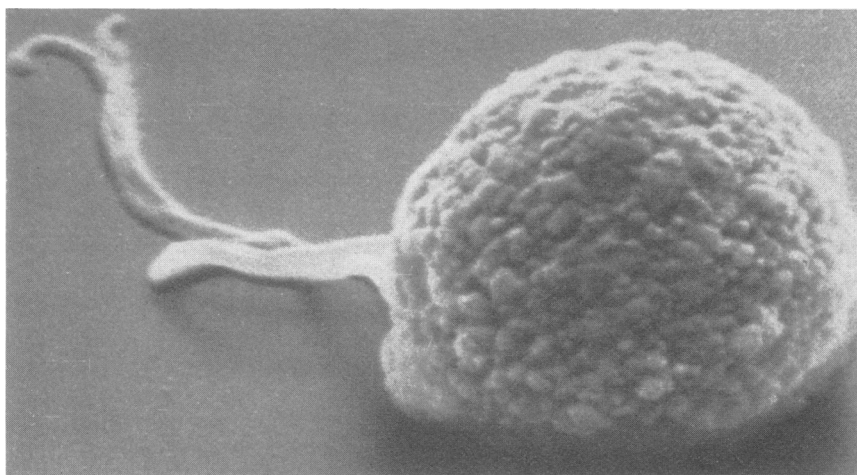


Fig. 4 *Normal T. vaginalis. Scanning electron microscope.*

The abnormal forms were tested for their sensitivity to metronidazole and were found to be as sensitive as the normal forms.

#### Discussion

Abnormal forms of *T. vaginalis*, with variation in size and shape have previously been described. Wenrich (1930) reported trichomonads varying in size between 7 and 23  $\mu\text{m}$ , Whittington (1957) observed some as large as 100  $\mu\text{m}$ , and Winston (1974) reported on giant forms measuring between 30 and 50  $\mu\text{m}$  in diameter and appearing in liquid

medium 12 days after incubation. Multinucleated and multiflagellated forms were also observed. There are also reports of variation in shape depending on the environment, some authors claiming that the shape of the organism varies according to the composition of the growth medium (Künstler, 1883). It has also been suggested that the morphology of the parasite varies according to the external environment, but the smaller the organism the more regular is the shape. Honigberg and King (1964), using phase contrast microscopy, showed ellipsoidal, ovoid, and spherical forms; they also suggested that pseudopodium-like protrusions could be seen.

In addition to the process of reproduction, usually accepted as being one of binary fission (Donné, 1836), Marchand (1894) reported multiple fission forms and described a large trichomonad with three tufts of flagellar apparatus and four nuclei. These observations have also been supported by other workers. Powell (1936) described an organism with two nuclei and four sets of flagella, Trussell (1947) noticed in cultures of trichomonads some with eight nuclei, while Winston (1974) reported giant forms which contained a dozen or more nuclei with multiple blepharoplasts each giving rise to three or four flagellae. The abnormal forms seen in our observations showed a larger size than normal, irregular in shape, and with multiple nuclei. Furthermore, they were seen only after subculture in liquid medium. Winston (1974) was of the opinion that, in a favourable environment, the organisms reproduce rapidly, assuming a small size, and these cause the inflammation and symptoms of the disease, whereas when the environment is unfavourable the parasite reproduces slowly, becomes larger, and lives as a saprophyte. Kupferberg (1940) showed that at an unfavourable pH, the rate of multiplication decreased, and large trichomonads appeared in the cultures.

The significance of these abnormal forms is still to be assessed. Are they in any way related to the long-standing use of chemotherapeutic agents? (some were described before the use of drugs); or are they forms capable of multiple fission in response to the adverse environment in which they were situated—a prolonged disease state? We feel that the relapse of patients from whom these abnormal forms are isolated should now be investigated, and that the influence of the constituents of the culture medium on their development should also be studied.

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