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THE CULTIVATION OF TRICHOMONAS OF THE HUMAN MOUTH (TETRATRICHOMONAS HOMINIS).

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Plates 34 to 37.

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Few attempts have been made to cultivate the parasitic flagellates of man. The first report on the cultivation of the trichomonas was published by Lynch (1) in 1915.

Lynch found numerous trichomonades in the vagina and gums of a negress who was suffering from catarrhal vaginitis and acute gingivitis. He placed the material in neutral and acid bouillon and kept it alive for 3 days at 30°C. Then, as the organisms began to be affected by bacterial growth he transferred them to new media and was thus able to carry the culture through several generations.

The experiment described in this communication was carried out at first without the knowledge of Lynch's report and differs considerably from his.

Material.—The tartar of the mouth which has long been known to harbor trichomonades was used. In most instances they are few in number.[1]

Culture Media.—A number of different media were employed. Acid bouillon, neutral bouillon, Ringer's solution, ascitic fluid, ascitic bouillon (equal parts), ascitic fluid-isotonic salt solution (equal parts), ascitic fluid-Ringer's solution (equal parts), bouillon-Ringer's solution, sheep serum water, sheep serum water-Ringer's solution (equal parts), rabbit serum water, nutrient agar, ascitic agar, ascitic Ringer's agar, sheep serum-glucose agar. Fresh tissue was placed in both the fluid and solid media.

Most of the media were unsuitable; only a few gave relatively good results; the mixture of ascitic fluid and Ringer's solution in equal

[1] We wish to express here our indebtedness to Dr. M. I. Schamberg for his courtesy in providing us with some of the materials used for the present study.

portions and in its natural alkalinity gave entire satisfaction. Samples of ascitic fluid from different sources were used and gave equally good results. The bouillon employed by Lynch was not so suitable as our ascitic Ringer's solution, the chief reason probably being that it enables the contaminating bacteria to multiply so rapidly and profusely that the trichomonades no longer find it a suitable medium. Undiluted ascitic fluid was also found to be too rich in protein material, although in this respect it is more suitable than bouillon.

Culture.—When the culture medium is inoculated with a small amount of dental tartar, the trichomonades are so few that it takes some time to find one. But by placing the inoculated tubes at a temperature of 37°C.[2] and then transferring a small quantity of the culture into new medium every 24 hours for 4 or 5 days in succession, the number of the organisms gradually increases and no difficulty was experienced in finding quantities of agglomerating flagellates as well as free individuals in every field. The organisms group together in clusters of varying size around the masses of bacteria (Fig. 1). At lower temperatures (23–27°C.) the growth of bacteria as well as of protozoa proceeds much more slowly than at higher temperatures, and the transfer may be made every 48 hours.

The method of transfer is simple. As the trichomonas grows at the bottom of the tube with bacterial sediments, it is necessary to suck up this precipitate with a capillary pipette and transfer it to new tubes. In both sucking up and transferring precaution should be taken not to stir the culture too much. In this way strains were carried for over twenty generations, and the loss of the strain was usually due to an accident or negligence in the transfer.

A brief note on the *Entamœba gingivalis* Gross (*Entamœba buccalis* Steinberg), which is almost always encountered in this medium, may be made here. In this medium the entameba can easily be kept alive over 24 hours, and with a little precaution sometimes over 48 hours; that is, by keeping the medium always at about 37°C. and by having a sufficient amount of culture fluid. If the alkaline reaction of the medium is reduced by adding drops of a 1 per cent solution of acetic acid to almost the neutral point indicated by litmus paper,

[2] Lynch could not get cultures at 37°C.

the rapidity of the growth of the trichomonas is somewhat reduced, whereas that of the entamebæ is accelerated. By transferring the sediment into a new, already heated medium once every 24 hours, the amebæ can be kept alive over a week; in one instance they were kept alive as long as 10 days. Judging from the fact that only a small amount of the sediment is carried from one culture tube into two, three, or more new tubes each time, the amebæ remain alive and multiply, though not so strikingly as in the case of the trichomonades.

Morphological and Biological Properties of the Culture Trichomonas.

The body of the trichomonas is uniformly pear-shaped; but being highly flexible it can take any shape according to circumstances, especially at the time when it passes through the narrow spaces in the bacterial masses, as observed in a fresh preparation. The protrusion of any definite pseudopodium as drawn by Kuczynski (2) in the case of *Trichomonas augusta* has not been seen in our culture, but a prolongation of the protoplasm near the posterior end (Fig. 2) was often observed. This is often used as an anchor, while the main part has a lively oscillating movement (von Prowazek's *haftpseudopodialer Fortsatz*). This prolongation of the protoplasm, however, has nothing to do with a real pseudopodium as we understand it in the case of the ameba, and there is no evidence that it is used for obtaining food. On the other hand, the presence of a cytostome (peristome) is probable, though we could not discern it clearly. In one instance a long chain of bacilli was gradually taken in through a point near the beginning of the flagella. After it became embedded in the protoplasm the chain broke into two parts, one of which was soon cast off from the body through the same point by which it had first entered, while the other seemed to be digested.

The size of the culture trichomonas varies within wide ranges, but it usually measures 10 to 15 μ in length and 4 to 8 μ in width (Fig. 11). Sometimes particularly large forms are encountered, about 25 μ long and 12 μ wide (Figs. 12 and 13). Flagella at the anterior end are uniformly four in number, radiating out from a basal granule situated just in front of the nucleus. They are unequal in length, in most instances

two of them being a little longer than the other two. The length of the flagella averages 14 to 16 μ. An undulating membrane rising from a blepharoplast, which lies close to, but differs from the basal granules, is distinctly seen (Fig. 8). The distal end of the limiting line of the membrane usually lies within the body, while its terminal end sometimes projects beyond the margin as a short, free flagellum (Fig. 3).

The axostyle usually seems to take its origin at the distal end of the spindle-shaped nucleus, but sometimes its course may be traced through the nucleus as far up as the basal granule, or very close to it. In its distal course the axostyle runs downward through the body and projects beyond the margin at the posterior end of the organism, forming a free tail 5 μ or less in length. The nucleus is single, oval, more or less elongated in shape, has a distinct karyosome, and measures 1 to 1.5 μ by 2 to 3 μ.

The protoplasm shows no differentiation of ectoplasm and endoplasm, and no contractile vacuoles or chromidial apparatus could be observed other than fine granulation with scattered, coarse particles. In some specimens the protoplasm is filled with ingested bacteria. In preparations stained with Giemsa's solution two or more characteristic purple rings are found, not unlike the forms described by some authors as a stage of so called cyst formation (Fig. 6).

The common method of reproduction seems to be a binary, longitudinal fission (Figs. 14, 15, 16, 17, and 18); multiple fission (or rather budding) may also occur in the culture. Figs. 2 to 10 show a series of successive stages of division, which is complete within from 30 minutes to 1 hour in the hanging drop. The number of daughter cells of multiple fission is not great (4, 6, 8, etc.); they do not seem to separate from each other at the same time, but usually bud off one after another in succession, so that the general impression differs considerably from the usual mode of schizogony as in other protozoa (Figs. 19 and 20). The division of blepharoplast is of the mitotic type (Fig. 19), while that of the nucleus itself is promitotic (Fig. 21). In cultures no form has been observed which may be interpreted as a cyst.

DISCUSSION AND SUMMARY.

Trichomonades from the mouth were studied by Steinberg[3] who proposed to group them into three distinct types; namely, *Trichomonas elongata*, *Trichomonas caudata*, and *Trichomonas flagellata*. Doflein (3) regards them as probably identical with *Trichomonas hominis*. Opinions differ as to whether or not *Trichomonas vaginalis* Donné and *Trichomonas hominis* Grassi are the same species. Lynch, for instance, believes that they are the same species, while von Prowazek (4), Bensen (5), and others (6, 7) insist that they are different types. Bensen's view seems to be well supported by the difference alleged to be found between the mode of encystment in the two trichomonades, were it not for the fact that our knowledge about the so called cyst of trichomonades is still obscure. According to Alexeieff (8) many of the so called cysts were evidently blastomyces contained in the cell body of the trichomonas. An autogamy alleged to take place in cysts as described by Bohne and von Prowazek (9) has not been confirmed by Dobell (10). And Wenyon (11) contends that it has never been found possible to produce any development of these cysts outside the body on the warm stage as can be done with the cysts of *Entamœba coli*. Therefore, it is still premature to take the process of encystment into consideration as far as the classification of trichomonas is concerned. On the other hand, Rodenwaldt (12) seems to think that there are many species of trichomonas in the human intestines, and Wenyon has described a new trichomonas from the human intestines (*Macrostoma mesnili* Wenyon).

Further cultural studies in the morphology and biology of these organisms must be carried out in order to solve these problems.

In the light of modern investigations there are five subgenera to be included under the genus *Trichomonas* Donné. They are as follows:

(1) *Protrichomonas* Alexeieff, with three anterior flagella, without an undulating membrane.

(2) *Trichomastix* Bütschli, with three anterior flagella and a trailing flagellum (*Schleppgeissel*) without an undulating membrane.

[3] Steinberg, quoted by Doflein, F., in Lehrbuch der Protozoenkunde, Jena, 3rd edition, 1911, 492.

(3) *Trichomonas* Donné, with three anterior flagella and an undulating membrane.

(4) *Macrostoma* Alexeieff, Amend, Wenyon (11), with three anterior flagella and an undulating membrane wedged in a deep groove (peristome).

(5) *Tetratrichomonas* Parisi (13), with four anterior flagella and an undulating membrane.

As far as our culture trichomonas from the human mouth is concerned, it has been shown that it is not strictly a trichomonas and that it should be classed under the subgenus *Tetratrichomonas*.

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EXPLANATION OF PLATES.

All the specimens were obtained from cultures of *Tetratrichomonas hominis*.

PLATE 34.

FIG. 1. Dark-field illumination of a colony of *Tetratrichomonas hominis* in culture. × 1,000.

PLATE 35.

Air-dried, fixed in methyl alcohol, and stained with Giemsa's solution. Drawn with camera lucida, oc. 4, obj. $\frac{1}{12}$, oil immersion. Tube 160.

FIG. 2. Typical shape.

FIGS. 3 to 9. Different shapes.

FIG. 10. A phase of binary fission.

PLATE 36.

Moist, fixed in Schaudinn's sublimate alcohol with a few drops of acetic acid, and stained with Heidenhain's iron and hematoxylin.

FIG. 11. Small type.

FIGS. 12 and 13. Large types.

FIGS. 14 and 15. Preliminary phases of binary fission.

FIG. 16. Beginning of binary, longitudinal fission of the protoplasm.

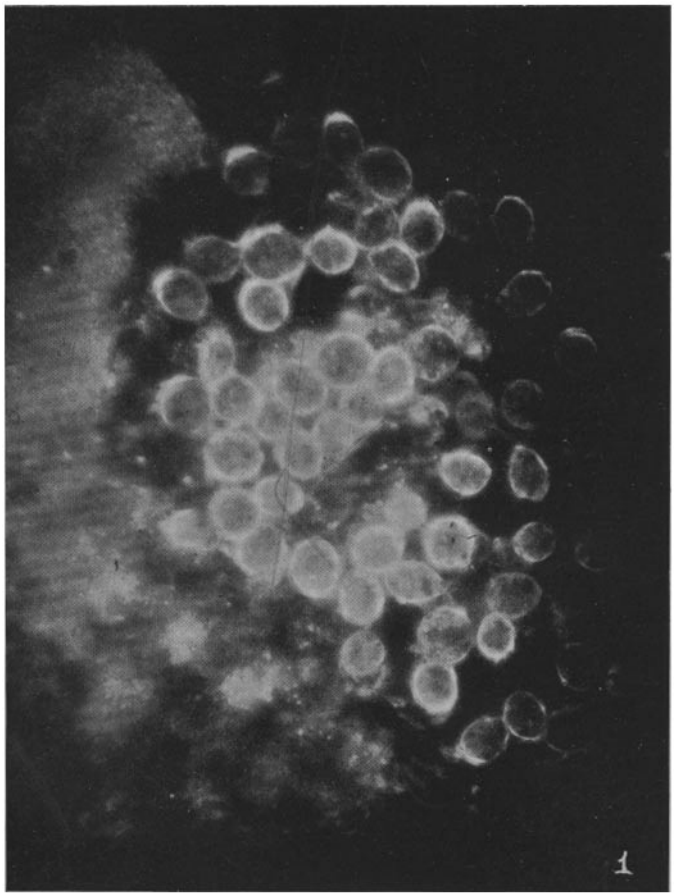
FIGS. 17 and 18. Final phases.

PLATE 37.

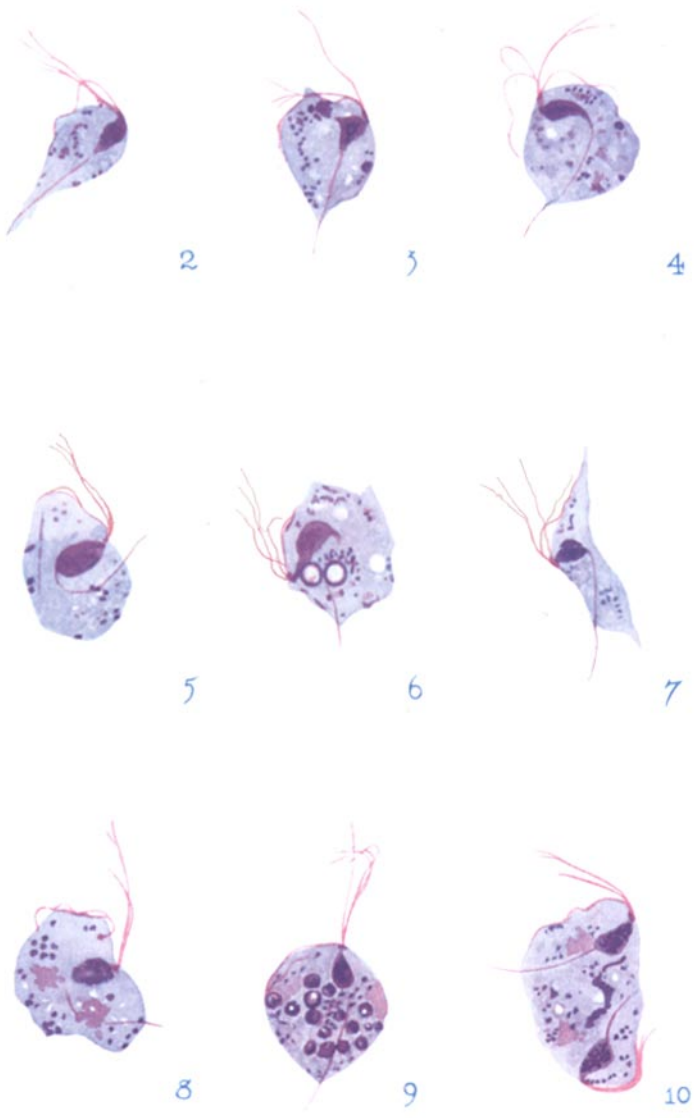
FIG. 19. A phase of multiple fission, showing six daughter nuclei and a mitotic spindle of a basal granule.

FIG. 20. Final stage.

FIG. 21. Promitotic division of the nucleus.



(Ohira and Noguchi: Trichomonas of the Human Mouth.)



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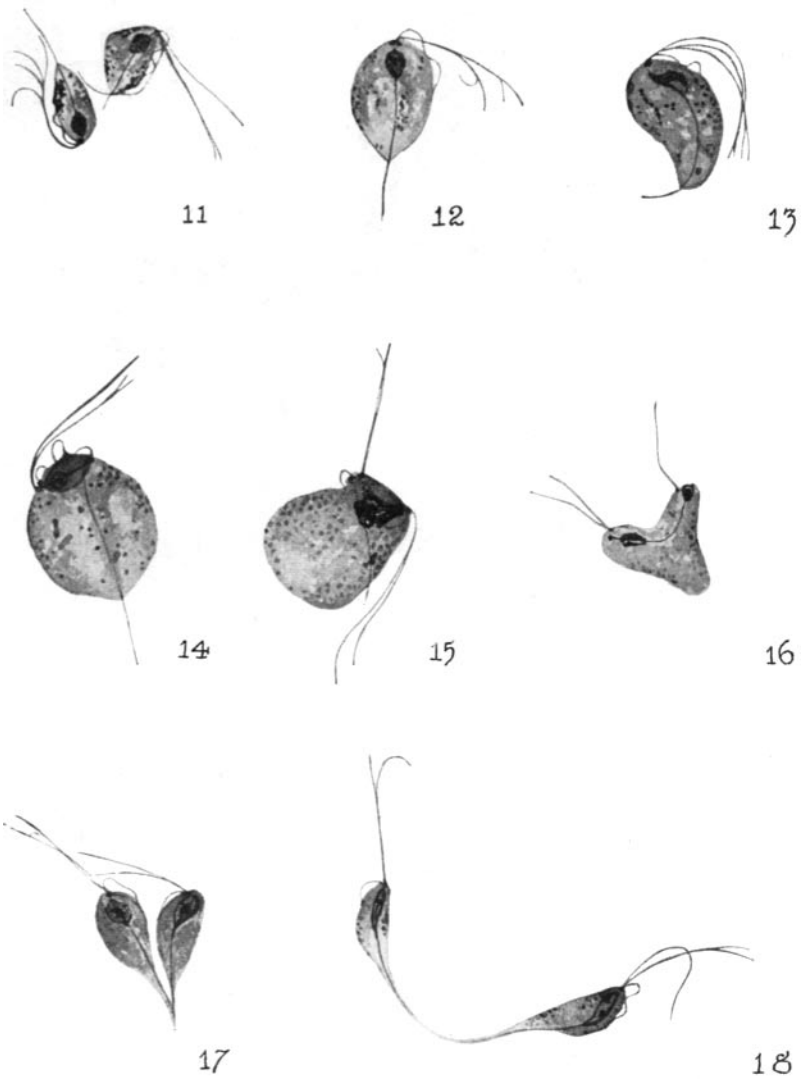
(Ohira and Noguchi: Trichomonas of the Human Mouth.)



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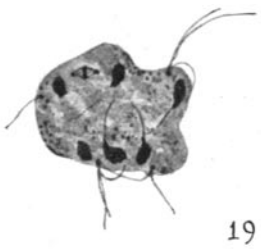

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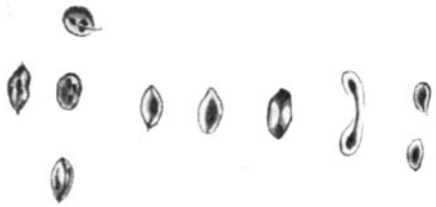

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