A note on the cultivation of *Tricercomitus termopsidis* and its method of cyst formation [1].

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(With 1 figure in the text.)

In the course of work on the cultivation of the intestinal protozoa of the termite, *Termopsis angusticollis*, cultures were obtained of *Tricercomitus termopsidis* KIRBY, 1930, one of the ordinarily non-xylophagous flagellates.

The organism has been grown on a solid medium and in several liquid media, all having the following salt base [2]:

Distilled water	1000 ccm
NaCl	1.169 g
$Na_3C_6H_5O_7 \cdot 2H_2O$ (citrate)	2.943 g
$NaHCO_3$	0.840 g
$NaH_2PO_4 \cdot H_2O$	0.690 g
KCl	0.745 g
$CaCl_2$	0.111 g

The liquid media contained in addition 0.1 % dextrose, or 0.1 % xylose, or 0.2 % LÖFFLER's dehydrated blood serum, and were

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[2]) The elaboration of this and other media for the cultivation of several intestinal flagellates of termites will be described in a separate paper.

sterilized by filtration through a Berkefeld filter, while the solid medium contained 0.1 % dextrose plus 0.01 % peptone plus 1.5 % agar, and was autoclaved 15 minutes at 17 pounds pressure. The solid medium, in the form of slants, is most suitable for the maintenance of stock cultures, and on it 3 strains of *Tricercomitus* have been cultured for over 2 years.

Primary cultures of *Tricercomitus* can be obtained in the following way. A termite is sterilized externally by immersion in 1—1000 mercuric chloride solution for at least 10 minutes. It is then dipped in 95 % alcohol and finally in sterile water. The gut is removed with sterile instruments and is placed on a slant or a plate of the solid medium. About 3 to 5 days later a good growth of *Tricercomitus*, accompanied by bacteria derived from the termite's gut, is present. Subculture is effected by picking up some of the material on the end of a platinum needle and streaking it on the surface of a fresh slant or plate. Within 2 days after subculture, a very heavy growth of the flagellates is present along the streak. It is thus possible to subculture every other day, but this is by no means necessary. At room temperatures, subculture every 2 or 3 weeks is often enough, while at a temperature of about 5° C cultures are still viable after storage for 6 months. On slants the growth is even, filiform, smooth, glistening and almost colorless. In liquid media a thin surface pellicle is formed.

The 2-year-old culture strains are accompanied only by a small Gram-negative bacillus, of which it has been impossible to rid them. Bacteria-free "cysts" (concerning which more will be said shortly) of the protozoa were obtained by the following methods. "Cyst" suspensions were treated for 2 hours with 10 % potassium bichromate, then washed in sterile medium and finally deposited on a plate of solid medium. In the other method, 0.5 ccm of a "cyst" suspension was inoculated into the peritoneum of a white mouse and, after lengths of time varying from 2 to 24 hours, a little of the peritoneal fluid was aspirated and deposited on a plate of solid medium. In both cases, after 3 or 4 days of incubation, "cysts" were seen with no surrounding bacterial growth, but such "cysts" never "excysted", while others on the same plate, treated in the same way but not freed of bacteria, did.

The morphology of the cultural *Tricercomitus* has been studied both in the living state and in smears fixed in SCHAUDINN's fluid with acetic acid and stained with HEIDENHAIN's iron-alum haematoxylin. In 2-day-old cultures on solid medium, practically all the

organisms are long motile forms (Fig. 1, *1*) similar to those described by KIRBY (1930) in post-molt termites. Although many of the nuclei show the chromatin-block structure figured by KIRBY as typical, others show a central granule with radial filaments and peripheral chromatin (Fig. 1, *2—3*). Comparatively few division stages (Fig. 1, *4—7*) may be found, in spite of the rapid multiplication taking place in a 2-day-old culture. After the third day, the number of motile forms rapidly decreases, and spherical, highly refractile bodies appear in increasing numbers. Microscopic examination of a 4 or 5 day growth along a streak on a plate shows that the round bodies predominate at the center of the growth while the motile forms predominate at the periphery. In liquid media the same progression of forms occurs, but the cycle is longer and more variable. The round bodies have a single nucleus of the same structure as that of the motile forms, are difficult to destain, and show blocks of heavily stained material around the periphery (Fig. 1, *8*). In round bodies stained with iodine, 1 or 2 small dark brown "glycogen" particles are visible, as well as the nucleus with its central granule (Fig. 1, *9*).

We were at first uncertain concerning the nature of these round bodies, since cysts of *Tricercomitus* have never been observed in termites, but it has been possible to show that they are resistant forms of the flagellate. Thus, material from a culture containing many round bodies was spread on fragments of sterile coverslip. These were dropped into dry sterile tubes and at the end of varying lengths of time a slip was removed and dropped into a tube of liquid medium. Such smears, a few minutes after being made, were perfectly dry and all the motile forms on them dead, so that the experiment was a test of the resistance of the round forms to drying. In one case, material dried for 2 days produced a culture, although the round forms usually did not resist more than one day of drying. They have repeatedly withstood an exposure of as great as 2 hours to 10 % potassium bichromate, and are killed in 10 minutes at 60° C but not in 10 minutes at 55° C.

There can thus be no doubt that the round bodies are resistant forms of *Tricercomitus*, for when they are subcultured into fresh medium, after treatment which kills the motile forms, motile organisms appear within a few days. Whether these resistant forms are true cysts depends on our definition of the word "cyst". If we define a cyst as a hard wall secreted around the organism and left behind after excystation, then the resistant forms of *Tricer-*

comitus are not cysts, as will be apparent from the following account of the processes of "excystation" and "encystation".

Material containing many round and a few motile forms was allowed to dry on a coverslip so that the motile ones were killed. A loopful of liquid medium was put on the dry smear and the coverslip set up as a hanging drop preparation. About 3 hours later "excystation" had begun and observations on the process were made in several individuals. The experiment has been repeated a number of times and all organisms observed "excysting" went through the same process. The "cyst", 3 to 4 μ in diameter, highly refractile and resembling a little glass bead, swells during a period of several hours to almost twice its original size. It is still spherical but is no longer highly refractile, and its protoplasm appears about the consistency of that of the motile forms. A slight movement then becomes apparent at one point, previously undistinguishable, on the "cyst", and soon the 3 anterior flagella, united in a bundle and looking like a single flagellum, become visible waving about within the cytoplasm and attached at the place where movement first appeared (Fig. 1, *10*). This flagellar

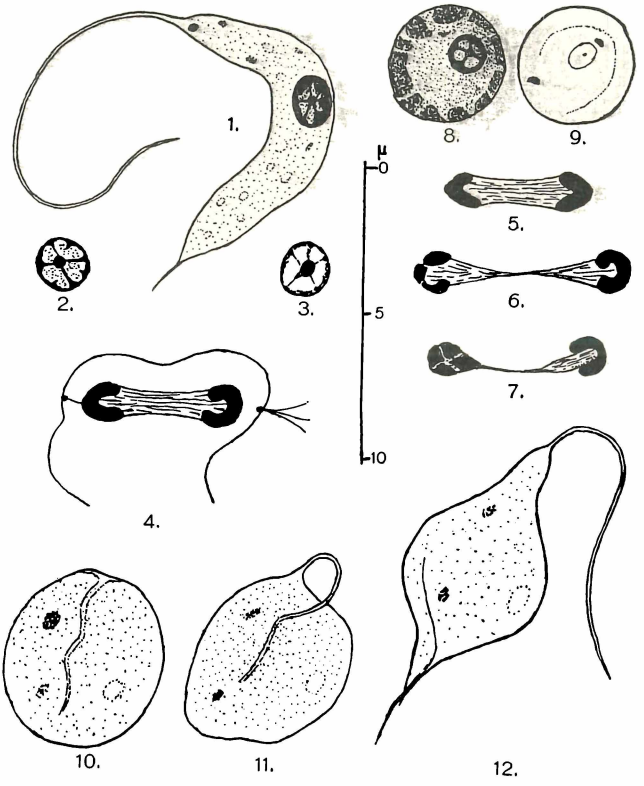

Fig. 1. *1* Motile form. Schaudinn's-Heidenhain's. *2*, *3* Nuclear structure. *4—7* Stages in nuclear division. *8* "Cyst". Schaudinn's-Heidenhain's. *9* "Cyst". In iodine. *10—12* Stages in "excystation".

bundle then begins to be pulled out of the body, the part nearest the point of attachment appearing free of the body first (Fig. 1, *11*). Meanwhile, the shape of the organism has become less regular. After about 10 minutes, the flagellar bundle is completely free and it moves about in a slow uncoördinated manner. The body itself begins to undergo euglenoid movement and elongates at the posterior and anterior ends while the central part is still rounded (Fig. 1, *12*). The organism appears to gradually move itself into shape, while the movements of the flagellar bundle become more rapid and more coördinated. The trailing flagellum becomes visible and, 20 or 30 minutes after the first visible movements, the anterior flagellar bundle acquires its typical rapid corkscrew motion and the flagellate, its body now completely elongated, swims away. There is no shell left behind.

When a hanging drop preparation such as used for the observation of "excystation" is 3 or 4 days old, many of the flagellates have again "encysted" and a few may be seen undergoing "encystation". The body becomes rounded chiefly toward the posterior end, while at first the anterior end remains long and narrow. At this stage the flagellate is still very active. During the course of several hours it becomes more rounded and now appears much like the round forms described by KIRBY in termites. The trailing flagellum shortens, being reduced to a stump within about 20 minutes, and finally becomes invisible. Even before this, the organism loses its ability to swim, and rotates in one place by means of the beat of the anterior flagellar bundle. The flagella composing this bundle slowly shorten until, about 15 minutes after the disappearance of the trailing flagellum, only a pointed stump, still capable of movement, remains. The stump then seems to be inverted into the "cyst" (this could not be clearly seen), and can no longer be distinguished. By this time the flagellate is considerably smaller than it formerly was. Slight irregular movements of the "cyst" continue a few minutes after disappearance of the flagella, and then all movement ceases, although the "cyst" is not as refractile as it later becomes.

"Encystation" thus consists of a rounding up, resorbtion of the flagella, loss of motility, and loss of water as indicated by the decreased size and increased refractility ot the "cysts". "Excystation" is a reversal of the process. This is quite different from the well-known process of excystation in free-living and parasitic amoebae, flagellates, and ciliates, in which the encysted organism shrinks

away from, and then emerges through an opening in the cyst wall. Even in the case of *Tricercomonas intestinalis*, supposedly a close relative of *Tricercomitus*, the encysted form has a definite cyst wall (Thomson and Robertson, 1925). Of all the resistant forms of flagellates so far described, the round forms of *Tricercomitus* appear to be most like the round, aflagellate, resistant forms of certain insect Trypanosomidae. Wenyon's experiments (Wenyon (1926), p. 351) with *Leptomonas ctenocephali* and a flagellate from the human flea showed that forms resistant to dessication were present. The question as to whether these round resistant forms have a cyst wall has not been definitely settled, for no one has observed them during the process of "excystation". Becker (1923) states that the round forms of *Crithidia gerridis* found in the rectum of the water-strider have no cyst wall. Many other workers, however, have reported cyst walls in such forms, but their work is based chiefly on dried films stained with Romanowsky stains. Wenyon (1926, p. 342) has pointed out the fallacies which may arise in this way. Not only may various artifacts be easily mistaken for cysts, but the round forms themselves may be surrounded by a deposit of stain, and such a deposit can "hardly be considered as evidence of the presence of a cyst wall".

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