Reproduction in *Colpoda cucullus.*

By

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With 3 figures in the text.

The reproduction of *Colpoda cucullus* MULLER was first studied by STEIN (1854). This process in all instances, according to STEIN, is preceded by encystment. Within its cyst the animalcule divides variably into either two, four, eight, or as many as sixteen, spore-like bodies, which ultimately escape through the rupture of the wall of their prison-house into a form which, while smaller, is in all respects identical with that of the parent animalcule.

In a study of different types of cysts of *Colpoda*, RHUMBLER (1888) distinguished three types of cysts, namely, "division-cysts", "sporocysts" and "permanent cysts". His drawings indicate that "division-cysts" are those in which the individual undergoes binary fission, that "sporocysts" are those in which the individual undergoes multiple division and that "permanent cysts" are those in which the individual does not divide but remains inactive. He did not say whether or not the animal may divide without being encysted.

Subsequent investigators, GOODEY (1913), BODINE (1923), WENYON (1926), ADOLPH (1929) and BARKER and TAYLOR (1931), who worked on *Colpoda cucullus*, accepted the fore-going account without any reservation. BARKER and TAYLOR maintain:

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"Encystment in *Colpoda* is of two distinct types. Preceding division, this ciliate always encysts. Within the cyst it divides once, twice, or three times, and then immediately excysts to produce two, four (most commonly), or eight free swimming organisms.

"The other type of encystment results in the formation of 'permanent cysts' RHUMBLER. These are commonly single or double cysts of the same general appearance as the division cysts. They do not excyst in the medium in which they are formed unless given a definite stimulus."

During a study of vacuole-contraction of *Colpoda cucullus*, the present investigator was struck by the fact that, in the race of *Colpoda cucullus* kept in this laboratory, the process of reproduction deviates greatly from what has been described by former workers. The present paper is a presentation of the results of an investigation of the process of reproduction of this race of *Colpoda*.

Material and methods.

The race of *Colpoda cucullus* used in this investigation is identical with that described by MULLER. In the present race there is a single micro-nucleus which MULLER made no reference to in his race.

At the beginning of this investigation, ten clones were isolated from a mass culture to see if there were any variations. When no variation of any significance had been found, only a single clone was maintained for investigation.

The ciliates were grown in the cavities of depression slides. As medium, a 0.5 % hay infusion was prepared by boiling timothy hay in tap water for eight minutes. The infusion, after being cooled, was transferred into glass jars and inoculated with bacteria from an old hay infusion. The jars were covered and left for two days in order to allow bacteria to grow. Six drops of clear solution from this 2-day old infusion were transferred by means of a small pipette into each cavity of a number of depression slides, and then a single individual of *Colpoda* is added into each cavity. These culture slides were placed in Petri dishes with moist filter paper and were supported on glass rods in order to avoid any adhesion on the slide of water from the wet filter paper.

Under these conditions, the ciliates divided two or three times a day. After two days, there were more than one hundred healthy and uniform individuals descended from a single parent.

Observations on living material were made under simple and compound binocular microscopes. Cytological slides were prepared by using SCHAUDINN'S sublimate alcohol as fixtative and FEULGEN'S reaction to bring out chromatin configurations.

Reproduction and encystment.

Under normal conditions, this race of *Colpoda cucullus* reproduces invariably by quadruple division without being preceded by encystment. Binary fission with or without preliminary encystment occurs only in old cultures when food is getting scarce and the medium is getting toxic. In mass cultures, viz. thousands of individuals in a large amount of infusion, binary fission and octuple division occasionally occur. Now, the questions arise: Why should the individuals in fresh cultures reproduce invariably by quadruple division while some of those in old cultures and in mass cultures reproduce by binary fission or occasionally by octuple division? Why should there be a variation in the process, normally without being preceded by encystment and sometimes with previous encystment?

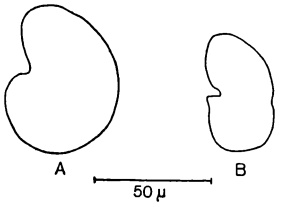

Fig. 1. Comparison of the shape and size of individuals: A, A large healthy individual which reproduces by quadruple division; B, A small starving individual which reproduces by binary fission. × 310.

The fact that those individuals which undergo binary fission are usually smaller individuals containing only a few food vacuoles (cf. Fig. 1) points to the possibility that binary fission is due to the reduction of material present in the body, which is in turn due to the scarcity of food. In order to test this point, the following experiments were performed.

A single small individual containing a few food vacuoles was isolated from a mass culture into the cavity of each of 20 depression slides with 6 drops of a fresh 1 % hay infusion without bacteria. A single large individual containing many food vacuoles was isolated into the cavity of each of another 20 slides with the same medium. The slides were placed in moist PETRI dishes. Observations on the mode of division were made and recorded. A similar experiment with BARKER and TAYLOR'S medium (1931) in place of hay infusion was performed on individuals taken from another mass culture. The results of these two experiments are presented in tables 1 and 2.

Table 1.
Mode of reproduction in relation to size of individuals. (In hay infusion.)

Reproduction and encystment	Large individuals	Small individuals
Quadruple division	20	4
Binary fission	0	11
Encysted	0	5

Table 2.
Mode of reproduction in relation to size of individuals. (In Barker and Taylor's medium.)

Reproduction and encystment	Large individuals	Small individuals
Quadruple division	18	2
Binary fission	?	9
Encysted	1	9

The foregoing tables indicate that whether an individual undergoes binary fission or quadruple division depends on the size of the individual, which, in turn, is a function of the amount of food.

The occasional occurrence of octuple division in mass cultures, is difficult to explain. Octuplets, when isolated, gave rise only to individuals which undergo quadruple division. Experiments designed to produce octuplets experimently did not yield positive results.

Reproduction preceded by encystment occurs in cultures more than three days old and also in mass cultures. The process in the formation of cyst membrane is similar to that of permanent cyst. Prior to cell division, the ciliate gradually rounds up and secretes a membrane around itself. Then the animal inside the cyst undergoes either quadruple division or binary fission (cf. Fig. 2). The entire process from the formation of cyst membrane until the escape of daughter individuals from the cyst takes from five to seven hours. The details of this process are the same as described by Stein.

The fact that, in fresh cultures, reproduction is never preceded by encystment seems to indicate that the formation of cyst membrane prior to division is correlated with the aging of the culture as a result of crowding of individuals and accumulation of excreta.

Multiplication with preliminary encystment can be experimentally produced by transferring healthy individuals into an infusion containing an extremely dense population of bacteria — so dense that the infusion appears grayish white to the naked eye and that, under the microscope, the bacteria are seen to form gelatinous

masses. In a former paper (PENN, 1935), it has been demonstrated that the excreta of bacteria facilitate encystment in Pleurotricha. All these findings lead to the conclusion that excretory products of the animal itself and that of bacteria serve as a stimulus in the secretion of the cyst membrane in the Protozoa.

"Permanent cysts" are formed by small starving individuals. Individuals in these cysts undergo dedifferenciation. All the organelles, except the nuclear apparatus and the contractile vacuole, gradually disappear, and, as a result, a homogeneous mass of protoplasm is formed. The contractile vacuole persists so long as the cyst is kept in solution. The rate of contraction of the contractile vacuole gradually slows down with the increasing in age of the cyst until the vacuole disappears altogether when the culture solution is evaporated.

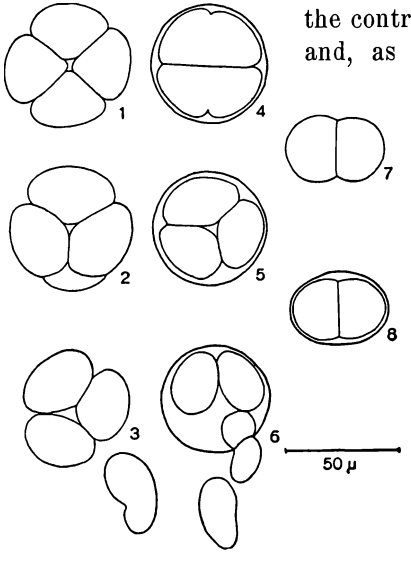

Fig. 2. Camera Lucida sketches from living specimens: 1—3, quadruple division without preliminary encystment; 4—6, quadruple division preceded by encystment; 7, binary fission without preliminary encystment; 8, binary fission preceded by encystment. × 310.

Individuals in such "permanent cysts" are never found to divide and they never excyst unless the cysts are transferred from old cultures into fresh infusion.

"Dividing cysts" may become "permanent cysts" when cultures are allowed to evaporate. The process of division may be arrested at different stages while the permanent dividing cysts are being formed. Thus cysts containing dividing individuals at various stages are found. These cysts survive drying just like single permanent cysts. When such cysts are placed in fresh infusion, they require a much longer time to excyst than the single permanent cysts.

Chromatin configurations during quadruple division.

Multiple division as a method of reproduction is of common occurrence among the Protozoa, especially among the flagellates. In all cases, the process of division takes place inside some sort of

membrane — a cyst, a spore case, or other structures of similar nature. Among the ciliates, multiple division has been reported to occur only in two cases, in Colopoda and in Otostoma. In both cases, the process of division has been reported to be preceded by encystment. Therefore, the present case of multiple division as a normal method of reproduction and not preceded by encystment is entirely a new phenomenon.

The process of quadruple division of normal individuals in fresh cultures is initiated by gradual rounding up of the cell, resembling the initiating step in encystment; but no cyst is formed. The absence of a cyst wall during division is manifested by careful direct observation on living material and on prepared slides under high magnification with different illuminations, and also by dissecting with glass needles. After the process of division has been completed, the daughter individuals separate and move away in any direction. There is left at the spot a small amount of gelatinous substance secreted by the dividing animal for attachment, but no cyst wall is left behind.

Now, two more problems arise. Is this quadruple division really quadruple, or is it just a close succession of two consecutive binary divisions? How do the macronucleus and the micronucleus behave during such quadruple division? In order to solve these problems, a cytological study is necessary.

Permanent slides were prepared by using SCHAUDINN'S sublimate alcohol with a little acetic acid as fixative and FEULGEN'S reaction to bring out chromatin configurations. Figure 3 represents the behavior of both the macro- and the micro-nuclei.

In the resting stage, the individual contains typically a macronucleus and a micronucleus. The macronucleus appears more or less round in shape and consists of loosely arranged chromatin with a varied number of dense chromatin clumps. The micronucleus is a small, compact, olive-shaped body, situated near one side of the macronucleus where it is usually slightly indented.

When the animal is rounding up, the micronucleus migrates away from the macronucleus, enlarges, and then divides into two. At the same time, some of the chromatin material of the macronucleus organizes into 7 (sometimes 8) chromatin clumps attached to the periphery of the nucleus. Later on, these clumps disappear and the macronucleus becomes granular, enlarges and then elongates and divides into two. At the time when the macronucleus is elongated, the two micronuclei have migrated to situate at the opposite

poles of the elongated macronucleus. Some of the chromatin material of the two macronuclei is organized into chromatin clumps, usually 7 in number. These clumps disappear shortly after and the two macronuclei again become granular. At this time or shortly before, the two micronuclei divide to form four. Then the two granular macronuclei elongate and eventually divide into four.

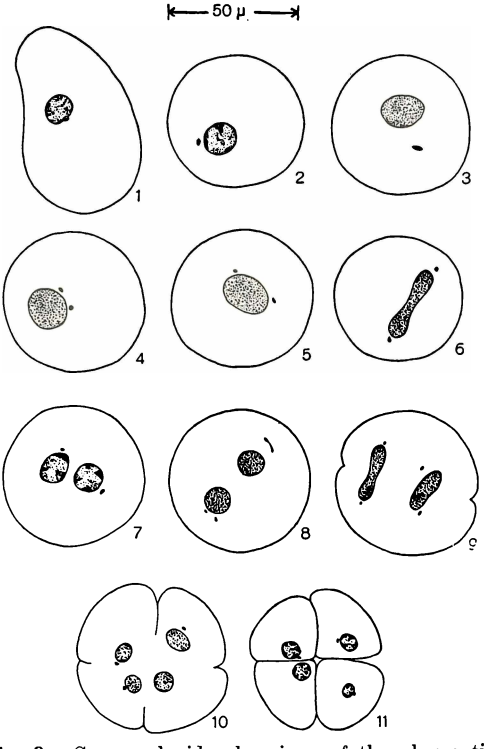

Fig. 3. Camera lucida drawings of the chromatin configurations during quadruple division without being preceded by encystment. × 340.

The above account is the normal sequence of chromatin configurations. This sequence may vary in some cases. Thus, on some slides there are found individual containing 2 or 4 micronuclei with the macronucleus showing yet no sign of change, and also individuals with a pair of micronuclei at the opposite ends of the single elongated macronucleus.

The first cytoplasmic division begins when the two macronuclei are becoming granular or when they are elongating. Before the first cytoplasmic division is completed, the second division sets in. As a result, four daughter cells are formed simultaneously.

Observations on living material show that, in very many cases, the second cytoplasmic division does not begin until the first has been entirely completed. In such cases, a 2-cell stage is very distinct. These two cells never separate but remain together and wait for the second division.

WENYON (1926) studied the cytological changes during multiple division of *Colpoda steini*. A comparison of his figures and Fig. 3 given above reveals the existance of a great deal of difference between *Colpoda cucullus* and *C. steini* in the behavior of the nuclear apparatus.

Summary.

1. *Colpoda cucullus* reproduces normally by quadruple division without being preceded by encystment.

2. Binary fission occurs only in old cultures as a result of scarcity of food.

3. Quadruple division with preliminary encystment occurs occasionally in old cultures and also in mass cultures. Secretion of the cyst membrance can be induced by placing healthy individuals in infusion containing gelatinous masses of bacteria.

4. The chromatin configurations during quadruple division have been carefully investigated.

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