CYTOLOGICAL INVESTIGATIONS OF COLPODA CUCULLUS

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Inasmuch as the various species of *Colpoda* have interested many previous investigators, especially with regard to factors of encystment and excystment, it was thought worth while to make a careful study of the best known species, *Colpoda cucullus* Muller. So many interesting and hitherto unreported phenomena have been observed that it was thought advisable to present only the cytological results in this first report. Subsequent reports will deal with the many and interesting observations on other phases of the problem which are at hand and these will be supplemented by further and more complete data.

The life histories of various species of *Colpoda* have been investigated from time to time starting with the work of Stein in 1854. He reported the encystment of *Colpoda cucullus*, the subsequent division into two, four, eight and even sixteen smaller individuals and their ultimate escape from the ruptured cyst. Rhumbler (1888) made an extended study of the process of encystment and division in *Colpoda cucullus* and *C. steini* and distinguished between the division cysts, "Theilungscyste," and those cysts within which division does not occur, the permanent cysts, "Dauercyste." He describes in some detail the appearance and activity of freshly encysted and dividing forms, noting that the cilia are retained throughout the division process but are lost during their stay in the permanent cysts. He observed quite accurately the gradual loss of the food inclusions, during permanent cyst formation, although his interpretation of the method of this loss is open to serious question.

Wenyon (1926) has given a rather diagrammatic account of the division of *Colpoda steini*, agreeing with the accounts of Stein and Rhumbler but adding some details of the nuclei. Wenyon's observations were made on fixed and stained material while those of Stein and Rhumbler were mostly obtained from the living material.

Very recently Penn (1937) has reported the occurrence of binary and quadruple division without encystment in a strain of *Colpoda cucullus*. Encystment before division occurred in his race only when the cultures were old or when the cultures became crowded. He was

able to induce the formation of cyst walls by placing "healthy individuals" in infusions containing gelatinous masses of bacteria. In this report are given descriptions of the nuclear phenomena which take place during the divisional process, but details are lacking.

There has been no cytological report of the nuclei of the "permanent" or resistant cysts published to date, as far as we are aware, and the reports of the nuclei during division have all been fragmentary. It is not difficult to see why resistant cysts have eluded observation in the past when one considers the fact that most nuclear investigation has been done after the employment of the various hæmatoxylin stains. As pointed out by Goodey (1913) the cyst walls ("ectocyst and endocyst") stain intensely with both Heidenhain's and Delafield's hæmatoxylins. We have found it impossible to study the contents of the resistant cysts after treatment with these standard stains and no doubt previous workers have experienced like difficulty. It is with the aid of the nucleal reaction developed by Feulgen and Rossenbeck (1924) that the details of the nuclear complex of resistant cysts have been rendered observable.

Hæmatoxylin stains do not react on the division cyst wall as they do on the resistant cyst walls, mainly because of the comparative delicacy of the former. It is possible to obtain a rough idea of what happens to the nuclei during division by employing these stains. But because of the densely packed food inclusions in the cytoplasm during this period fine details are obscured. After the Feulgen reaction, however, the history of the nuclear components may be readily followed. It has been found that this history is a rather surprising one and one that may shed considerable light on the rôle of the macronuclear chromatin in ciliate metabolism. Therefore, because we have observed with considerable exactitude the nuclear phenomena both during division and during the stay within the resistant cyst, and because we feel that these observations will contribute to our understanding of related phenomena among ciliates in general, we offer the following cytological report as the first one of a comprehensive nature on this common organism, *Colpoda cucullus*.

Material and Methods

Colpoda cucullus is a very common form and may be collected in a great variety of places. Our original material was obtained from dry hay taken from the banks of a brackish stream near Stuart, Florida. The hay was placed in spring water from which enormous numbers of the ciliates were later collected and transferred to small dishes. From these dishes a number of motile specimens were selected with a micropipette and placed in individual isolation culture dishes in a drop of

twenty-four-hour-old diluted hay tea. After a number of encystments and segregations had taken place one motile ciliate was selected and all of the others discarded. All glassware was then scrupulously cleaned and boiled for a long period of time to remove any danger of contamination. This obvious precaution was to insure the presence of only one species with which to work. All of our material, therefore, has descended from a single organism.

The standard culture medium used in this work, while not the only one successfully used, has given consistent results and the isolation lines grown in it have exhibited a surprising vitality. It consists of nothing more than ordinary hay tea, used after twenty-four hours at room temperatures. Into this medium single motile ciliates were placed and invariably at the end of twenty-four hours three to four divisions had occurred. All that was necessary to obtain resistant cysts was to allow multiplication to proceed for forty-eight hours or longer without adding fresh medium. Under those conditions the division rate decreased until finally all of the ciliates secreted the heavy wall characteristic of the resistance phase, accompanied by the other phenomena described below. Because of the high fission rate, the hardiness of the species and the predictable response to certain environmental conditions there was always an abundance of material in every phase of the life history under investigation.

For the preparation of permanent slides for cytological study we employed special "recovery dishes"[1] for the collection of material designed by one of us (C. L. C.). Small circular flat-bottomed Pyrex dishes with straight sides, measuring 23 mm. × 12 mm. (inside measurements), were prepared. These dishes will accommodate ⅞ inch circular cover glasses, allowing them to rest on the bottom with very little free space about the edges. Into one of the dishes was placed a cover glass, in some cases thinly coated with fresh egg albumin. The dish was then filled with culture medium and inoculated with a single motile *Colpoda*. By frequent microscopic examinations the stages desired could be accurately noted, the cover glass taken out and placed in the fixing fluid. In this way we were able to recover hundreds of the different stages on each cover glass. Another advantage this method has is that the organisms do not tend to pile up but each adheres to the glass more or less separately. There is a minimum of debris which makes for clarity of the final preparations. The success of these "recovery" dishes caused us to give up entirely our earlier methods of concentrating by centrifuging and of selecting individuals under the dissecting microscope.

[1] These dishes, known as the Claff Recovery Dishes, are being put on the market by Clay-Adams Company, Inc., New York City.

As mentioned in the beginning, our clear preparations resulted from the use of the Feulgen nucleal reaction. We made many preparations with the various hæmatoxylins and carmines, and while these preparations were entirely satisfactory for the motile forms and proved very useful, they were mediocre for divisional phases and entirely useless for resistant cysts. We found that certain modifications of the standard Feulgen procedure were advantageous. After much experimentation it was found that the following times gave excellent results and we highly recommend them for future work on *Colpoda cucullus:* acid hydrolysis—15 minutes at 60° C.; fuchsin sulphurous solution—4 to 5 hours; sodium bisulphite-hydrochloric acid wash—15 minutes (at least three changes). It was found that the increased time of washing had a decided tendency to clear the cytoplasm of any trace of free fuchsin and rendered the preparations beautifully clear.

Many types of fixing fluids were used but it was found that wherever cyst walls were present the more penetrating varieties gave, as would be expected, the best results. Therefore most of our material was fixed in Schaudinn's with 5 per cent acetic, corrosive sublimate in 95 per cent alcohol with 5 per cent acetic acid added, or the Gilson-Carnoy mixture. The latter fixing fluid gave the best results on the resistant cysts.

Our Feulgen prepared material was very often counterstained with either fast green in 95 per cent alcohol, methylene blue in 70 per cent alcohol, or the acid component of the Borrel mixture (Calkins, 1930). The last-named stain was modified as to balance from the original, containing $\frac{1}{3}$ indigo carmine to $\frac{2}{3}$ picric acid. Counter stains reacted well in the cytoplasm of trophic and divisional forms, penetrating the thin cyst membrane of the latter with ease. The resistant cyst membranes appear to be entirely impermeable to fast green, methylene blue and the indigo carmine component of the Borrel mixture. The picric acid of the Borrel stain penetrates very readily, however, staining the cytoplasm an intense yellow. After this counterstain striking preparations are obtained if the material has been taken from a culture in which trophic forms, divisional cysts and newly formed resistant cysts are present. The cytoplasm of the trophic forms and the divisional cysts is a brilliant green while the cytoplasm of the resistant cysts is yellow.

It should be mentioned that numerous preparations employing the silver nitrate method of Klein were made on the trophic forms as an aid to our positive determination of the species at the beginning of the investigation. The extreme variability of the size and form of these ciliates is so great that specific identification becomes difficult without

careful study of the ciliary pattern. The question of variation of the ciliary pattern we hope to present in another report.

It has been found possible to check very accurately the times when the various nuclear changes occur during each phase by starting with resistant cysts, inducing them to excyst and fixing material at frequent, timed intervals. In carrying out these timed observations a large number of "recovery dishes" were prepared at one time and their contained organisms fixed in order, first noting the condition of each in the living state. Due to this procedure we have a complete series of slides demonstrating the nuclear activity from excystment, through several reproductive divisions and through a second resistant encystment. As a result of these observations we are certain that the sequence of events to be described is the normal one and occurs in a regular fashion in this ciliate. All the cytological details observed from the timed material have been repeatedly checked from mass cultures.

The Life Cycle—General

Conjugation has never been observed by us in our strain of *Colpoda cucullus* and as far as we have been able to determine it has never been reported in the literature. Enriques (1908) mentions conjugation as having been noted by him in *Colpoda steini* but not in *C. cucullus*. Therefore we will use the term "life cycle" to denote the sequence of events taking place from one resistant cyst stage back to another.

Colpoda cucullus appears to possess the ability to accommodate itself to a wide variety of environmental conditions. The great factor in this extreme accommodation seems to be its ability to secrete, in a minimum of time, protective or semiprotective cyst membranes. Under normal conditions all reorganizational phases accompanying reproduction and resistant encystment are carried out when the organism is enclosed in some type of membrane or membranes. Feeding, only occurring in the trophic stage, is accomplished in a very efficient manner, for within a few minutes after resistant excystment the cytoplasm will be found to be literally packed with spherical food vacuoles.

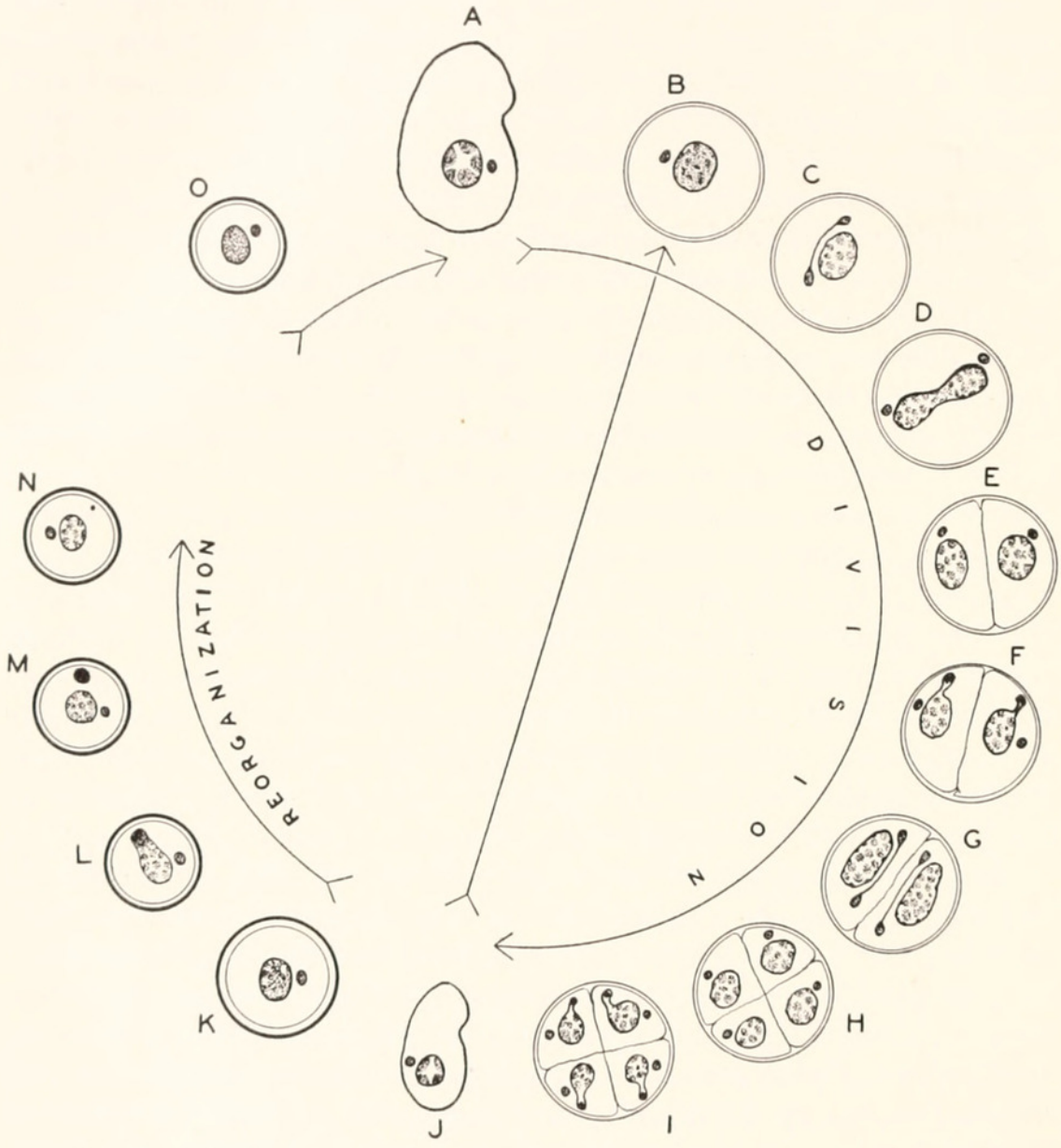
When placed in fresh culture medium the resistant cyst undergoes certain changes very rapidly. These changes lead to a rupturing of the heavy outer cyst wall (ectocyst of Goodey, 1913) and finally to liberating the swimming ciliate from the thin inner wall (endocyst of Goodey). One of us (C.L.C.) has been investigating the mechanism of excystment under normal and experimental conditions and will report these findings at an early date.

The newly excysted ciliate is devoid of food vacuoles but very rapidly engulfs great quantities of bacteria and bacterial debris. This

food is invariably formed into spherical compact masses and distributed at random throughout the cytoplasm. One average sized ciliate (70 μ) may have as many as two hundred such food vacuoles (Plate I, Figs. 1 and 2). Growth ensues, the ultimate size appearing to be dependent on the excellence of the cultural conditions but independent of the ability to reproduce. Within a few hours after excystment the trophic form begins to round up, without, however, losing the motility of the cilia. The cytostome becomes indistinct and a thin membrane is secreted outside the still moving cilia. This is the "Theilungscyste" of Rhumbler (1888) and undoubtedly corresponds to the "pseudocyst" of *Tillina canalifera* recently described by Turner (1937). In the vast majority of cases quadruple division occurs within this cyst resulting in the liberation of four small daughter ciliates. The cilia may be continually observed throughout the process. Occasionally binary division occurs resulting in the liberation of but two daughter ciliates in which case they are proportionately larger in size. We have never observed divisions resulting in more than four daughters although Stein (1854) has reported as many as sixteen daughter ciliates escaping from a division cyst. The report of Penn (1937), in which he maintains the normal condition in *Colpoda cucullus* to be quadruple division without encystment, was not confirmed in our study. Binary and quadruple division without encystments were encountered only occasionally among the great number of encysted forms irrespective of the bacterial condition of the medium. It is possible that lack of encystment is a peculiarity of the strain studied by Penn.

If no additions are made to the culture medium for a period of forty-eight hours the rate of division cyst formation decreases and stops and the ciliates become very small and less active. They round up very quickly and secrete a heavy, wrinkled cyst wall about themselves. This results in the resistant cyst which is able to withstand desiccation. The formation of resistant cysts may be induced by simply drawing off a part of the medium and replacing it with old medium from a culture in which resistant cyst formation has taken place. In the literature are numerous accounts of the necessity for drying before resistant cyst formation occurs (Barker and Taylor, 1931; Bodine, 1923; etc.). We have found that resistant cysts form readily even in an abundance of fluid, and may be kept for long periods without recourse to evaporation. As pointed out by Penn (1937), if conditions inducing resistant cyst formation occur rapidly enough (evaporation, according to Penn), some of the division cysts become resistant cysts by the simple expedient of forming a heavy wall about the outside of the division cyst wall. Thus resistant cysts may contain one, two

or occasionally four cells, all motor organelles dedifferentiating until nothing can be seen but the nuclear apparatus and the granular cytoplasm.



TEXT FIG. 1. Diagrammatic representation of the "life cycle" of *Colpoda cucullus* illustrating the sequence of events during reproduction and resistant cyst formation. *A–J*, normal reproductive activity repeated (*J* to *B*) under favorable cultural conditions. *K–O*, resistant cyst with space between *N* and *O* representing the lapse of an indefinite amount of time while the arrow from *K* to *N* represents a short space of time during which macronuclear reorganization and chromatin elimination takes place. Arrow from *O* to *A* represents the return of favorable conditions for excystment.

A young resistant cyst possesses many food inclusions but these are rapidly absorbed, leaving characteristic refringent inclusions in their stead. Within a few hours even the refringent bodies have disappeared

and the cytoplasm becomes compact and evenly granular, containing the nuclear apparatus. The compactness of the cytoplasm is brought about by the actual shrinkage in the size of the organism even after the cyst wall has been laid down. Resistant cysts are always very much smaller than all but the smallest of the trophic forms or division cysts.

The above account agrees essentially with the previously published descriptions of the "cycle" of *Colpoda cucullus* and will serve as a basis for the details of the nuclear activity described below.

Text figure 1 illustrates the normal course of events during the "life cycle" of *Colpoda cucullus* which, with the accompanying legend, will serve to clarify the above description.

The Nuclei of Colpoda Cucullus

Enriques (1908) gives as a specific characteristic of *Colpoda cucullus* the possession of a macronucleus with a lobed karyosome "cariosoma lobato." He was able to observe this after staining with carmine. From his figures it may be assumed that, except for the karyosome, the macronucleus is devoid of stainable material (chromatin). Wenyon (1926) figures the macronucleus of *Colpoda cucullus* with irregular karyosome-like bodies toward the center. He does not state what stain was employed but it would be safe to assume the use of a hæmatoxylin. That both of these observations were due to the type of stain used is demonstrated by a comparative study of organisms stained in borax carmine, Heidenhain's hæmatoxylin and after the Feulgen reaction. In the first two cases the stainable material seems to be concentrated toward the center of the macronucleus surrounded by a faintly stained, irregular periphery. But after the Feulgen reaction the picture is reversed. The chromatin is seen as irregular plaques surrounding and extending into the colorless nucleoplasm (Plate I, Fig. 1). After any of the counter stains used with the Feulgen reaction the nucleoplasm is sharply contrasted to the chromatin and is seen to have the form described as the karyosome by previous workers. Penn (1937) illustrates this general condition in his figures from Feulgen prepared material. We wish to emphasize the chromatin configuration of the macronucleus of the trophic form, therefore, as being in the form of irregular plaques around the periphery and surrounding the non-chromatin nucleoplasm of the center, because of the prevalent use of this character in classification (see the description of Kahl, 1931).

A single micronucleus is always present in the trophic stage. It lies near the macronucleus but may be on any side of it. The chromatin is quite compact and in fixed preparations is usually seen to have

shrunken away from the nuclear membrane. The micronucleus is quite commonly elongated, flattened on one surface, and slightly pointed at the ends. We have never found more than the single micronucleus in the trophic forms. Penn (1937) mentions the occasional occurrence of two or four micronuclei in his strain. We believe that it is possible that he interpreted the balls of extrusion chromatin from the macronucleus as micronuclei inasmuch as he completely overlooked these interesting bodies (see our Plate I, Fig. 23).

In our material the micronucleus behaves in an orthodox fashion during the divisional activities. After the division cyst membrane is laid down it enlarges and assumes a spherical shape. The chromatin becomes slightly less compact and is seen to be finely granular (Plate I, Fig. 3). It then becomes distinctly striated and the whole nucleus elongates and enlarges. The striations within the chromatin become more marked and finally irregular threads may be observed all oriented with the long axis of the nucleus (Plate I, Fig. 4). Contraction of the chromatin threads results in the formation of the metaphase plate (Plate I, Figs. 5, 6, 9). There appear to be a great many chromosomes and we were never able to make even an approximate count, as a glance at the figures will reveal. The anaphase is formed by a separation of the chromosomes of the metaphase plate into two groups; the nature of this separation has not been determined. The two daughter chromosome groups move to opposite ends of the fully formed spindle leaving between them definite clear fibers which remain into the elongated telophase (Plate I, Figs. 7 and 8). The chromatin becomes compact again in the daughter telophase groups and these move farther apart (Plate I, Figs. 10 and 19), retaining for some time the connecting strand formed from the nuclear membrane. Finally this strand breaks and the two micronuclei round up and take their positions at opposite ends of the now elongated macronucleus (Plate I, Figs. 11 and 12). This sequence is repeated prior to every cell division without any appreciable variation.

It is within the chromatin of the macronucleus that events occur that offer an interesting problem in ciliate cytology. Before the division cyst membrane is laid down the chromatin loses its plaque-like configuration and becomes flocculent, being roughly dispersed throughout the nucleoplasm. It is still slightly granular but the granules are exceedingly minute (Plate I, Fig. 2). By the time the division cyst membrane is formed the chromatin of the macronucleus has begun to take on a definite configuration (Plate I, Fig. 3), that of granular aggregates suspended in the clear nucleoplasm (Plate I, Figs. 9, 10 and 11). This configuration is retained until the daughter organisms are

ready to emerge from the cyst, in most cases through the two divisions. The chromatin aggregates stain uniformly and rather intensely but because of their scattered condition the macronucleus as a whole appears as a loosely knit body, lying among the numerous food inclusions. By the time the micronucleus has reached its telophase stage the macronucleus has begun to elongate. This elongation marks the future division plane of the cell, being always at right angles to it. Further elongation stretches the macronuclear membrane until a typical constriction appears separating the chromatin into two daughter halves (Plate I, Fig. 12). The two daughter halves of the macronucleus quickly separate and round up and the fission plane forms, dividing the cell into two equal daughter cells, each containing a single micronucleus and macronucleus and numerous food inclusions (Plate I, Fig. 13).

Shortly after cell division there becomes differentiated simultaneously within each daughter macronucleus an irregular, granular mass of chromatin. This mass stains much more intensely than the general macronuclear aggregates and is first seen lying close to the nuclear membrane. It is rapidly separated from the chromatin aggregates and is pushed out from the surface of the macronucleus being surrounded by a pocket formed from the macronuclear membrane. This activity is nearly or exactly synchronous in each daughter macronucleus (Plate I, Fig. 14). This deeply staining chromatin mass is the "extrusion chromatin" or "residual chromatin" and is ultimately broken away from the macronucleus and cast into the cytoplasm (Plate I, Figs. 15 and 17). Within the cytoplasm it becomes compacted into an intensely staining ball which rapidly diminishes in size until it disappears from view. It is usual that complete absorption of the extrusion chromatin is accomplished before the start of the second fission.

The second division is initiated, as in the first, by the activity of the micronucleus. All stages appear to be the same as in the preceding division (Plate I, Figs. 18 and 19) with the result that four daughter ciliates are formed within the original division cyst membrane. Occasionally the cyst wall becomes soft and irregular and liberates the two daughters before the second division. Figure 17 represents a case of binary fission just before the liberation of the daughter cells. The extrusion chromatin has not been absorbed yet, and may not be until after the daughters become free-swimming organisms.

Following the second cell division each macronucleus again undergoes a reorganizational process whereby more extrusion chromatin is formed and cast out into the cytoplasm (Plate I, Figs. 20 and 21).

After this process has taken place the four daughter ciliates become more active, the cyst wall becomes softer and more irregular and finally ruptures. In the majority of cases enough time elapses during the freeing process for the extrusion chromatin to become absorbed but sometimes the cyst membrane ruptures early and the young daughter organisms each carry with them the remains of the residual ball (Plate I, Fig. 23). This residual ball might easily be mistaken for a supernumerary micronucleus, as we feel sure has been the case in the report of Penn (1937) on the occurrence of more than one micronucleus.

When, as occasionally happens, as mentioned above, binary or quadruple division takes place without the formation of a definite cyst membrane there occurs exactly the same nuclear activity as found in the normal division cyst. Chromatin extrusion follows each division in as regular and predictable a fashion as that just described. Figure 22 represents the end result of a quadruple division without cyst formation. These daughter ciliates are completely reorganized and all trace of the residual chromatin has disappeared. We have been able to find all stages representing the above process of chromatin elimination in these divisions but because of the duplication of the conditions found during the normal process and because division without cyst formation was the exception in our material we felt that illustrations would be redundant.

The above process of the alternation of a free-swimming, feeding organism with reproduction within the division cyst is repeated every eight hours, on the average, either in isolation or mass cultures so long as fresh medium is provided. Whether or not there will be found a waning vitality over extended periods of time we cannot tell at present. Experiments testing this point are being carried out and will be reported at a later date.

When conditions within the culture change due to the accumulation of waste products resistant cysts are formed. This process is accomplished in a very short period of time and involves the laying down of a thick, relatively impermeable wall, the absorption of the food bodies and the concentration of the cytoplasm. No activity on the part of the micronucleus is observed but the macronucleus proceeds to the most profound reorganization as yet reported for any holotrichous ciliate.

Resistant cyst formation in a trophic organism proceeds with a diminution in size and a rounding up of the ciliate. As the heavy cyst wall is secreted the cilia disappear and the cyst becomes firmly attached to the substrate. The nuclear apparatus is much the same in appearance as in the trophic stage except for the fact that both

nuclei become smaller and slightly more dense (Plate II, Fig. 24). The food inclusions are rapidly absorbed and the cytoplasm then contains numerous minute refringent bodies (Plate II, Figs. 25 and 26). After the disappearance of the food balls but before the disappearance of the refringent bodies the macronucleus begins to elongate and to differentiate into two distinct regions. One end becomes more intensely staining than the other as if the chromatin was becoming com-

Explanation of Plates

All figures are of *Colpoda cucullus*, and with the exception of Figs. 4–8 the magnification is × 1,000. Figs. 4–8 represent a magnification of × 2,000. All figures are from preparations treated with the Feulgen reagent. Fixatives used were Schaudinn's fluid with 5 per cent acetic acid for the preparations illustrated on Plate I and Gilson-Carnoy fluid for the illustrations on Plate II. The cilia, which are present in all stages represented on Plate I, have been omitted from the illustrations. Asterisks (*) represent residual chromatin.

Plate I

Explanation of Figures

Fig. 1. Trophic ciliate showing typical nuclear apparatus, food inclusions, contractile vacuole and cytoplasmic vacuoles. Note the plaque-like arrangement of the macronuclear chromatin.

Fig. 2. Ciliate about to undergo encystment prior to reproduction. The macronuclear chromatin has become dispersed and flocculent.

Fig. 3. Early division cyst. Micronucleus enlarged and the macronuclear chromatin beginning to collect in aggregates.

Figs. 4–8. Representative micronuclei during mitosis.

Fig. 9. Micronucleus in metaphase and macronuclear chromatin in the form of irregular aggregates.

Fig. 10. Later stage. Macronucleus elongating.

Fig. 11. Daughter micronuclei at the poles of the elongated macronucleus.

Fig. 12. Constriction of the macronucleus.

Fig. 13. Plasmotomy completed and the two daughter macronuclei rounded up. No indication of chromatin differentiation for elimination as yet.

Fig. 14. Budding off of extrusion chromatin (*).

Fig. 15. Slightly later stage.

Fig. 16. About the same condition as the previous stage and a timed preparation. This cyst represents the first one formed after emerging from the resistant cyst. Note small size.

Fig. 17. Probably a case of binary fission with the two daughter ciliates about to leave the wrinkled cyst membrane. Note extrusion chromatin (*).

Fig. 18. Prophase of the second division. The extrusion chromatin has been absorbed in the cytoplasm.

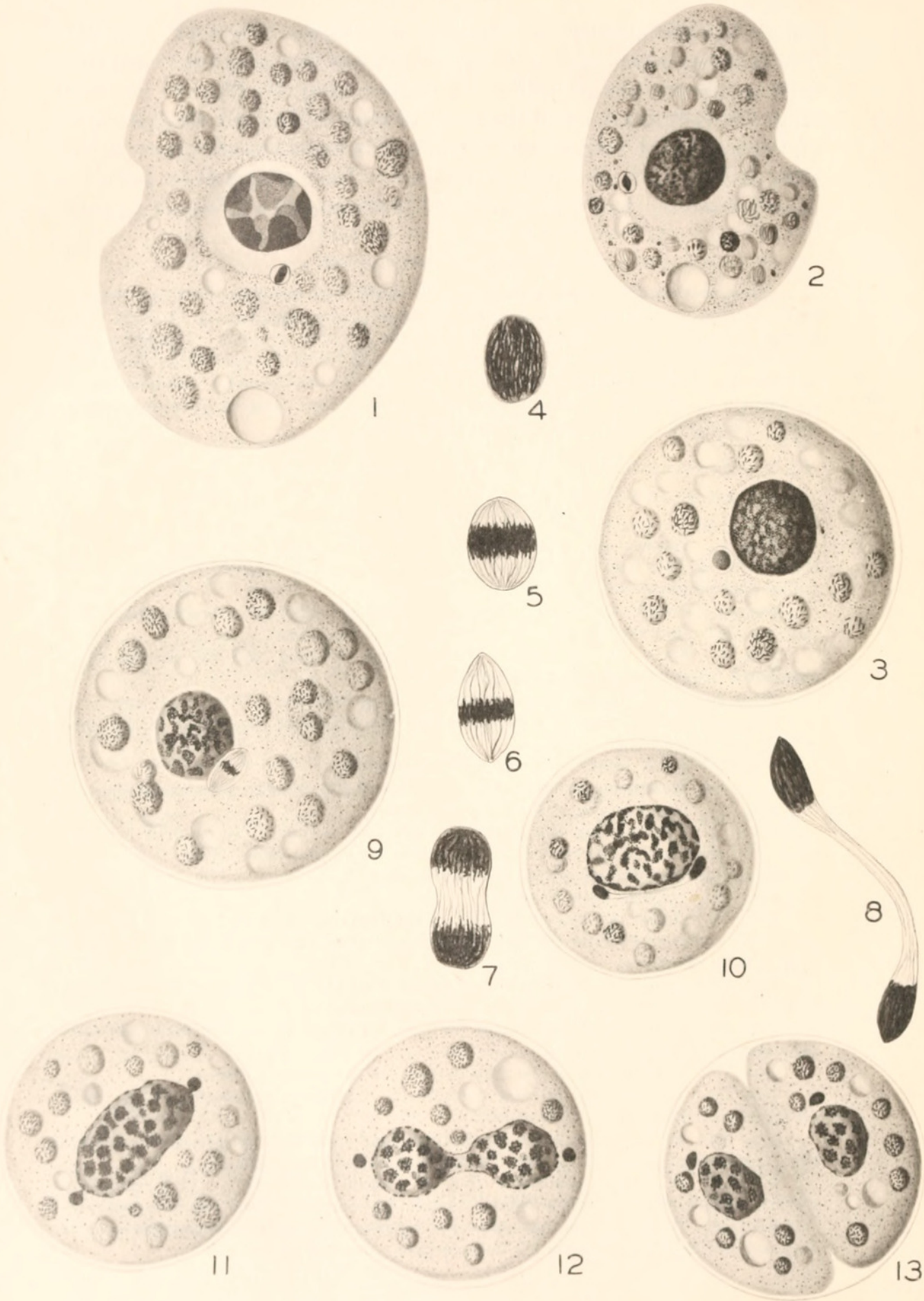
Fig. 19. Constriction of the macronuclei for the second division.

Fig. 20. Second cell division completed. Extrusion chromatin being given off from each daughter macronucleus.

Fig. 21. Four small ciliates about to emerge from the division cyst. Within the cytoplasm of each will be seen the residual ball of chromatin (*).

Fig. 22. A case of quadruple division without encystment. This represents the last stage with the daughter ciliates completely reorganized and about to separate.

Fig. 23. A free-swimming ciliate just after emerging from the division cyst. The residual ball of chromatin (*) has not been absorbed as yet.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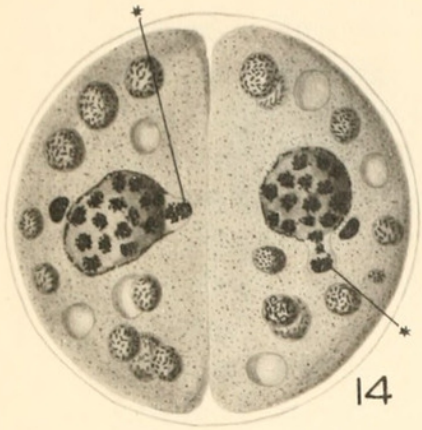

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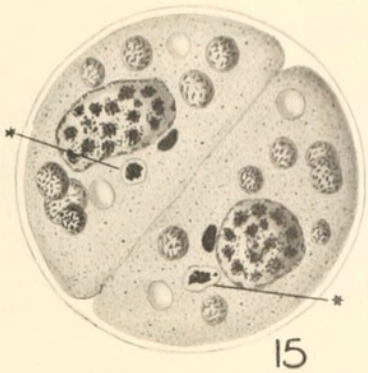

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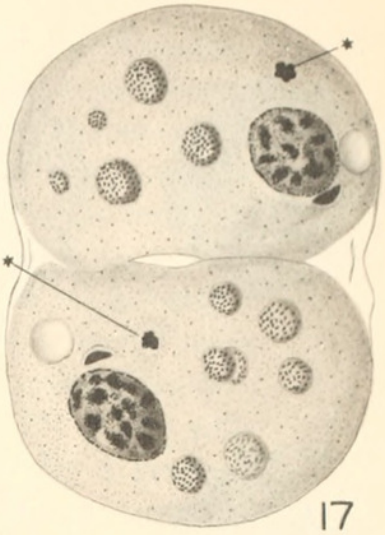

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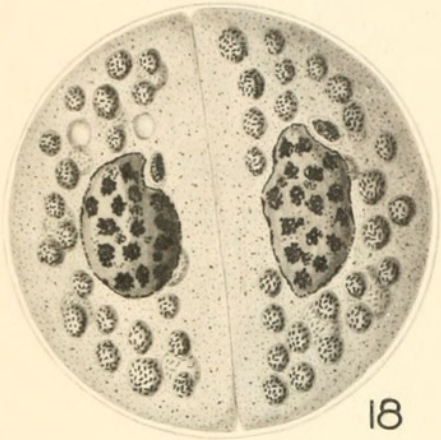

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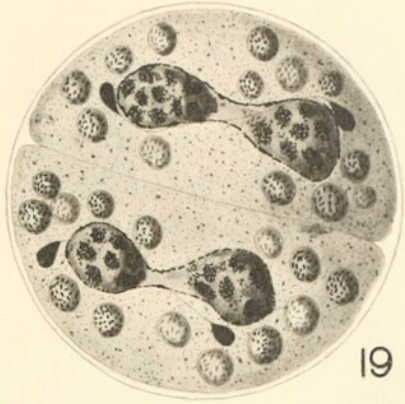

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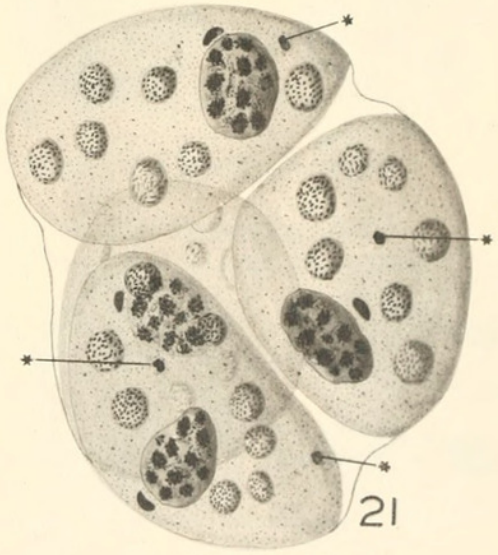

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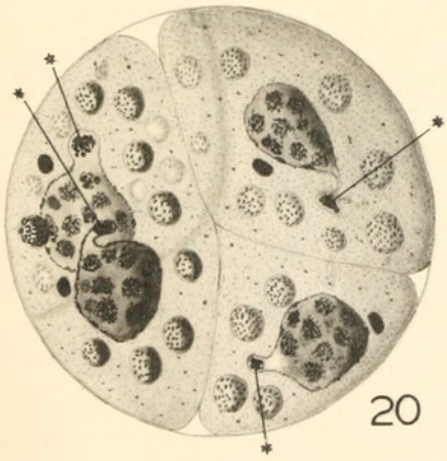

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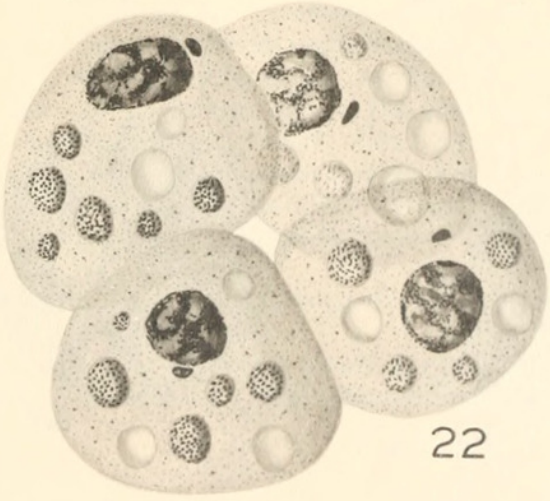

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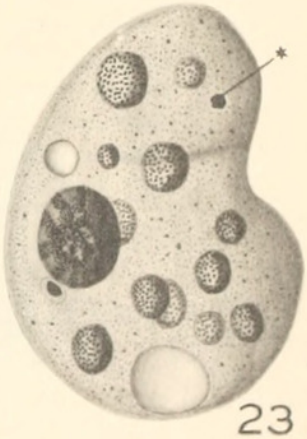

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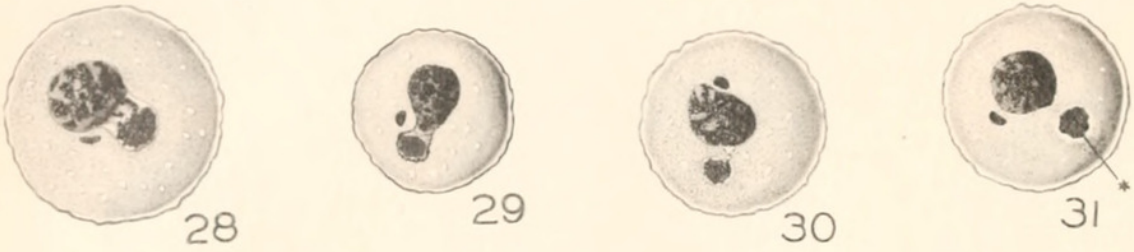
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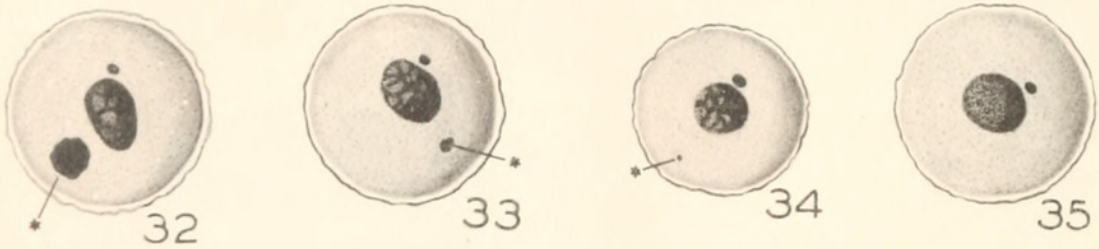
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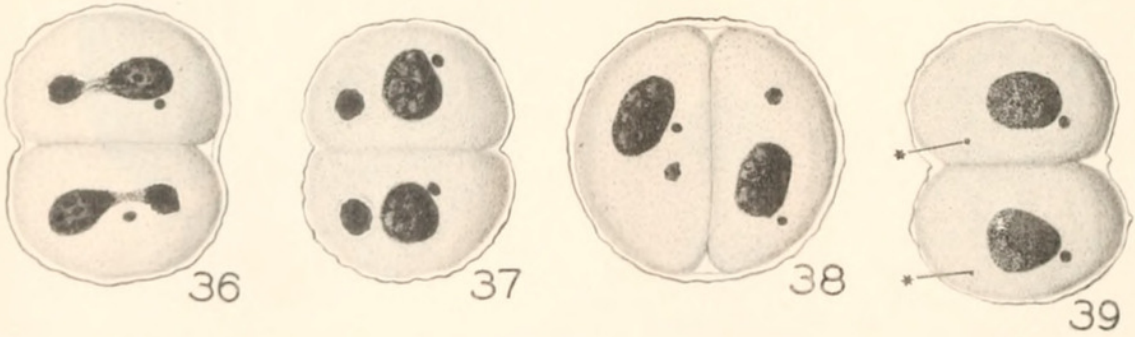
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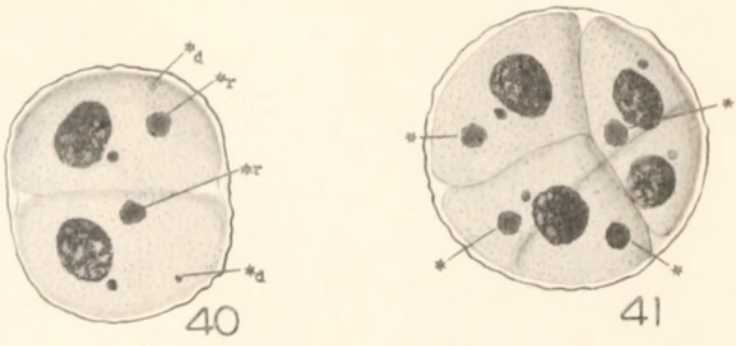
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pacted. This compacted region varies in size from one-third to one-half the whole nucleus (Plate II, Fig. 27). Very rapidly the compacted area buds off from the rest of the macronucleus leaving its chromatin in an irregular granular condition (Plate II, Fig. 28). The compact bud moves farther away from the nucleus until the connection between the two severs and the deeply-staining chromatin rounds up in the cytoplasm (Plate II, Figs. 29, 30 and 31). The amount of chromatin extruded varies considerably in the different cysts. In extreme cases it forms a ball as large as the remaining reorganized macronucleus but usually it is somewhat smaller (Plate II, Figs. 31 and 32). The ultimate resorption of the cast-out chromatin takes place by a gradual diminution in size but no apparent diminution in staining capacity (Plate II, Figs. 33 and 34). Within a few hours after encystment the extruded chromatin has disappeared and there is no further activity within the cyst until excystment. The refringent bodies have gradually disappeared during this process of nuclear reorganization and the resting cyst then possesses a very clear, slightly granular cytoplasm in which the micro- and macronucleus are embedded (Plate II, Fig. 35).

Plate II

Fig. 24. Newly-formed resistant cyst characterized by the possession of food spheres.

Fig. 25. Later stage showing the diminution of food bodies and the concomitant appearance of the small, refringent bodies.

Fig. 26. The food bodies have entirely disappeared and the refringent bodies are numerous.

Fig. 27. Early stage in the differentiation of the extrusion chromatin within the macronucleus. The extrusion chromatin stains more intensely than the chromatin which is destined to remain.

Figs. 28–30. The budding off of the ball of extrusion chromatin.

Fig. 31. The connection between the extrusion bud and the macronucleus has broken. Note the globular condition of the large extrusion mass (*).

Fig. 32. A large extrusion mass (*) somewhat later than the preceding stage.

Figs. 33–34. The extrusion chromatin mass (*) diminishes in size. Note also that the refringent bodies within the cytoplasm have disappeared.

Fig. 35. A reorganized resistant cyst with compact clear cytoplasm and a smoothly granular, compact macronucleus. In this condition the resistant cyst will remain until the cultural conditions are altered sufficiently to induce its excystment.

Figs. 36–39. Chromatin elimination from the macronuclei of two-cell resistant cysts duplicating, in each cell, the conditions seen in the single-celled resistant cyst.

Fig. 40. Two-celled resistant cyst in which cyst formation occurred before the extrusion chromatin of the divisional reorganization was completely absorbed. The divisional extrusion chromatin is represented by the small, deeply staining balls (*d) while the resistant cyst extrusion chromatin is represented by the large masses (*r).

Fig. 41. Four-cell resistant stage showing typical extrusion chromatin in each cell. This type of cyst is relatively rare.

Fig. 42. Small, clear, cyst-like structures which appeared in a few old liquid cultures and which are thought to represent degenerate resistant cysts.

We have found a few cases where two buds of waste chromatin have been extruded from the reorganizing macronucleus but these are rare.

When, as often happens, division cysts become resistant cysts there is a complete reorganization within each of the daughter ciliates identical with that of the single-celled cyst described above. Usually there appears to be enough time for the divisional reorganization of the macronucleus to proceed to completion and the subsequent resorption of the extrusion chromatin to take place before resistant cyst macronuclear reorganization sets in (Plate II, Figs. 36, 37, 38, 39 and 41). Rarely are there found resistant cysts where both divisional extrusion chromatin and resistant cyst extrusion chromatin are present (Plate II, Fig. 40). Also resistant cysts containing two cells are much more frequent in occurrence than those containing four cells.

Discussion

In our opinion the actuality of macronuclear reorganization involving the elimination of residual chromatin may possibly be demonstrated universally among the holotrichous ciliates. The definite establishment of the elimination of residual chromatin as a single ball during divisional reorganization has been made in the following forms: *Kidderia* (*Conchophthirius*) *mytili* (Kidder, 1933*a*), *Ancistruma isseli* (Kidder, 1933*b*), *Conchophthirius anodontæ*, *C. curtus*, *C. magna* (Kidder, 1934), *Myxophyllum* (*Conchophthirius*) *steenstrupii* (Rossolimo and Jakimowitsch, 1929), *Allosphærium convexa* (Kidder and Summers, 1935) and *Urocentrum turbo* (Kidder and Diller, 1934). A number of other species undoubtedly fall into this group if we can judge by the published reports (*see Loxocephalus*, Behrend, 1916 and *Eupoterion pernix*, MacLennan and Connell, 1931). Post-divisional chromatin elimination, i.e. the casting into the cytoplasm of residual chromatin from each of the daughter macronuclei after separation, is known to occur in *Ichthyophthirius multifiliis* (Haas, 1933), *Colpidium colpoda*, *C. campylum* and *Glaucoma scintillans* (Kidder and Diller, 1934), *Chilodonella labiata* and *C. faurei* (MacDougall, 1936).

In all the cases cited above the chromatin to be eliminated is differentiated within the macronucleus prior to its division and thus advertises itself. All species, therefore, in which no differentiation into regions occur prior to fission have been described as having a "clean" macronuclear division. As the vast majority of ciliates divide without encystment, and the two daughter cells separate immediately after fission, this phase of their cytology has been neglected. It seems entirely possible to us that if attention were paid to the young daughter

cells after fission the occurrence of macronuclear reorganizational processes would be discovered in a great many if not all species. Another possibility that suggests itself is that the many cases of chromatin-like fragments in the cytoplasm so often reported in ciliate studies may be explained by some process of macronuclear elimination during reorganization. We wish to emphasize the necessity for more thorough and critical cytological work with this problem in mind to determine whether or not *macronuclear reorganization with chromatin elimination will be found to be a universal principle applicable to all holotrichous ciliates.*

As to the exact significance of this regular though complicated process, we are still unable to say. It has been suggested (Kidder, 1933*a*, 1933*b*, 1934; Kidder and Diller, 1934) that the eliminated chromatin represents worn-out material and the process might be a cleaning out of the macronucleus toward a perfect organization. It was further suggested (Kidder and Diller, 1934) that the profound reorganization which occurs at every division of *Colpidium colpoda*, *C. campylum* and *Glaucoma scintillans* might account for the fact that conjugation rarely occurs in these species, the reorganizational process serving to restore the cells to their fundamental condition and thereby decreasing the necessity for conjugation. The above suggestion seems to us to apply as well as any other to *Colpoda cucullus.*

Concerning the drastic reorganization that occurs immediately after the resistant cyst is formed, it seems logical to suppose that this represents the ridding of the macronucleus of materials no longer needed in the state of decreased or suspended metabolism. Materials accumulated in the macronucleus through the very activity of encystment may be detrimental to the resting cell or to the process of excystment, to come at a later date. Unfortunately we have very few records of what goes on within the resistant cysts of the various species of ciliates with which to make comparisons. We know from a few sources (see Tittler, 1935) that a process of endomyxis sometimes accompanies encystment, whereby the old macronucleus is completely discarded and a new one formed from micronuclear material. These cases would seem to represent simply a different method for accomplishing the same general result as occurs in *Colpoda cucullus*, the production of a purified macronucleus.

That the reorganizational process occurring within the resistant cyst takes the place of the divisional reorganization of the macronucleus is denied by direct observation. In the very first division after emergence from the resistant cyst the normal reorganizational chromatin elimination occurs. This was determined by timed preparations

and Figure 16 illustrates a first division cyst. The small size is usual as the ciliates emerging from the resistant cysts are very small and usually reproduce before full growth is attained, a condition noted in the case of *Tillina magna* by Gregory (1909). The time factor may play an important rôle here, however, as there is the possibility of the aging of the macronucleus during its long period within the resistant cyst, resulting in the necessity for reorganization immediately upon again taking up an active life.

The actuality of a regular and predictable macronuclear reorganization with the elimination of quantities of chromatin during division and within the resistant cyst has been established for *Colpoda cucullus* but a completely satisfactory explanation of its significance awaits further investigation. Experiments are now under way which, it is hoped, will throw some light on this question.

Summary

1. A complete description of the nuclear activity of *Colpoda cucullus* Muller is given for the first time.

2. In our strain the normal method of reproduction takes place within a thin cyst membrane. Usually two divisions result giving rise to four daughter organisms which break out of the cyst and repeat the process. Occasionally binary fission occurs within the cyst. Rarely quadruple division occurs without encystment, as described by Penn (1937).

3. Following each cell division there occurs a reorganizational process within the daughter macronuclei resulting in the elimination of a quantity of residual chromatin. The residual chromatin is cast into the cytoplasm where it is absorbed. Elimination of residual chromatin is regular and synchronous in each cell whether the division has occurred within a cyst or not.

4. When cultural conditions are poor resistant cysts are formed.

5. The resistant cysts are formed by the secretion of a heavy cyst membrane, the absorption of the food inclusions and the concentration of the whole protoplasmic mass.

6. Immediately following the formation of the resistant cyst membrane the macronucleus undergoes a profound reorganization during which a variable, but always a considerable amount of chromatin is budded off and cast into the cytoplasm where it is absorbed. No micronuclear activity occurs at this time.

7. The question of chromatin elimination from the macronuclei of holotrichous ciliates is discussed and the opinion expressed that this phenomenon may represent a universal principle.

LITERATURE CITED

BARKER, H. A., AND C. V. TAYLOR, 1931. A study of the conditions of encystment of Colpoda cucullus. *Physiol. Zoöl.*, **4**: 620.

BEHREND, K., 1916. Zur Conjugation von Loxocephalus. *Arch. f. Protist.*, **37**: 1.

BODINE, J. H., 1923. Excystation of Colpoda cucullus. *Jour. Exper. Zoöl.*, **37**: 115.

CALKINS, G. N., 1930. Uroleptus halseyi Calkins. II. The origin and fate of the macronuclear chromatin. *Arch. f. Protist.*, **69**: 151.

ENRIQUES, P., 1908. Sulla morfologia e sistematica del genere Colpoda. *Arch. Zool. Exper. et Gén.*, **8**: (N. & R.) 1.

FEULGEN, R., AND H. ROSSENBECK, 1924. Mikroscopisch-chemischer Nachweis etc. *Zeitschr. Physiol. Chem.*, **135**: 203.

GOODEY, T., 1913. The excystation of Colpoda cucullus from its resting cysts, and the nature and properties of the cyst membranes. *Proc. Roy. Soc. London*, (B) **86**: 427.

GREGORY, L. H., 1909. Observations on the life history of Tillina magna. *Jour. Exper. Zoöl.*, **6**: 383.

HAAS, G., 1933. Beiträge zur Kenntnis der Cytologie von Ichthyophthirius multifiliis Fouq. *Arch. f. Protist.*, **81**: 88.

KAHL, A., 1931. Die Tierwelt Deutschlands. 21 Teil: Protozoa. Fischer, Jena.

KIDDER, G. W., 1933*a*. Studies on Conchophthirius mytili de Morgan. I. Morphology and division. *Arch. f. Protist.*, **79**: 1.

KIDDER, G. W., 1933*b*. On the genus Ancistruma Strand (Ancistrum Maupas). I. The structure and division of A. mytili Quenn. and A. isseli Kahl. *Biol. Bull.*, **64**: 1.

KIDDER, G. W., 1934. Studies on the ciliates from fresh water mussels. II. The nuclei of Conchophthirius anodontae Stein, C. curtus Engl., and C. magna Kidder, during binary fission. *Biol. Bull.*, **66**: 286.

KIDDER, G. W., AND W. F. DILLER, 1934. Observations on the binary fission of four species of common free-living ciliates, with special reference to the macronuclear chromatin. *Biol. Bull.*, **67**: 201.

KIDDER, G. W., AND F. M. SUMMERS, 1935. Taxonomic and cytological studies on the ciliates associated with the amphipod family Orchestiidæ from the Woods Hole district. *Biol. Bull.*, **68**: 51.

MACDOUGALL, M. S., 1936. Étude cytologique de trois espèces du genre Chilodonella Strand. Morphologie, Conjugaison, Réorganisation. *Bull. Biol. France et Belg.*, **70**: 308.

MACLENNAN, R. F., AND F. H. CONNELL, 1931. The morphology of Eupoterion pernix, gen. nov., sp. nov.: a holotrichous ciliate from the intestine of Acmæa persona Eschscholtz. *Univ. Calif. Publ. Zoöl.*, **36**: 141.

PENN, A. B. K., 1937. Reproduction in Colpoda cucullus. *Arch. f. Protist.*, **88**: 366.

RHUMBLER, L., 1888. Die verschiedenen Cystenbildung und die Entwicklungsgeschichte der holotrichen Infusoriengattung Colpoda. *Zeitschr. f. wissensch. Zool.*, **46**: 549.

ROSSOLIMO, L. L., AND FRAU K. JAKIMOWITSCH, 1929. Die Kernteilung bei Conchophthirius steenstrupii St. *Zool. Anz.*, **84**: 323.

STEIN, F., 1854. Die Infusionsthiere auf ihre Entwicklung untersucht. Leipzig.

TITTLER, I. A., 1935. Division, encystment and endomyxis in Urostyla grandis with an account of an amicronucleate race. *La Cellule*, **44**: 189.

TURNER, J. P., 1937. Studies on the ciliate Tillina canalifera n. sp. *Trans. Am. Microsc. Soc.*, **56**: 447.

WENYON, C. M., 1926. Protozoölogy. New York.