Nuclear reorganization without cell division in *Paraclevelandia simplex* (Family Clevelandellidae[1]), an endocommensal ciliate of the wood-feeding roach, *Panesthia*.

By

George W. Kidder

(Arnold Biological Laboratory of Brown University, Providence, Rhode Island).

With plate 3.

In a recent paper (KIDDER, 1937) I have described the protozoan fauna occurring in the hind gut of the wood feeding roach, *Panesthia*. Of the nine species of heterotrichous ciliates described, one, *Paraclevelandia simplex*, exhibits a regular and peculiar reorganization process just prior to and during encystment. This process must be called endomixis according to the definition of the term as given by WOODRUFF and ERDMANN (1914) and is accomplished with the minimum of nuclear divisions. In addition to the replacement of the old macronucleus a number of other very interesting phenomena occur which, while clear cut and easily demonstrable in this species, nevertheless are difficult of analysis when compared to the behavior of ciliate nuclei in general. It is the purpose of this paper to record the cytological events that transpire during the encystment of *Para-*

[1]) Due to the fact that the generic name *Clevelandia* is preoccupied (*Clevelandia* EIGENMANN and EIGENMANN, 1888) it is necessary to substitute the generic name *Clevelandella* nom. nov. for *Clevelandia* KIDDER, 1937. In accordance with the International Rules of Zoological Nomenclature the family containing the type genus, *Clevelandella* (*Clevelandia*) must be changed to Clevelandellidae nom. nov. for Clevelandiidae KIDDER, 1937.

clevelandia simplex from the roach *Panesthia javanica* from Mt. Maquilling, Luzon, P. I.

The material for this study was obtained from the same source as previously described (KIDDER, 1937). It has been necessary to examine a great number of roaches in order that all the stages might be obtained. While *P. simplex* was present in the hind gut of fifty percent of the roaches examined a small percentage were found to be in reorganization stages at any one time. Therefore, smear preparations were prepared and each slide carefully studied with this problem in mind. Late stages could be detected easily but the earlier stages were less abundant and much less apparent.

Many techniques were employed but it was found that the most useful results were obtained with the HEIDENHAIN haematoxylin method differentiated in picric acid (KIDDER, 1934) following BOUIN fixation, and the FEULGEN nucleal reaction following warm SCHAUDINN'S fluid. All of the figures were taken from material prepared in one of these ways.

During its motile state *P. simplex* possesses the usual ciliate nuclear complex consisting of an elongate macronucleus and a small compact micronucleus. The macronucleus is enclosed in a peculiar membrane, the karyophore, which connects with the anterior pellicle. The chromatin of the macronucleus is in a finely divided granular state showing only occasional differentiation into larger granules. The gullet is distinct and lined with the characteristic conspicuous membranelles. The ectoplasm is very thick and hyaline possessing irregularities of a vaccuolar nature, through which the peripheral cilia protrude. Motion is relatively slow.

The first evidence of encystment is to the observed in the changes that take place in the nuclear apparatus. The micronucleus enlarges and the chromatin loses some of its staining capacity. It becomes elongate in the direction of the long axis of the body and numerous thread-like chromosomes are formed (Pl. 3 Fig. 1). The number of these threads could not be determined as they are in a very compact state at all times. After further nuclear elongation the chromosomes divide forming two distinct anaphase plates (Pl. 3 Fig. 2). These plates move apart and the nuclear membrane pinches in between them (Pl. 3 Fig. 3). There is never the drawn out condition, however, which is so characteristic of the majority of ciliate nuclear divisions. Instead the pinching in is abrupt and the two daughter nuclei always lie quite close together.

During this activity on the part of the micronucleus the macronucleus is undergoing profound changes which have a great deal of bearing on the subsequent reorganization process. At the mid-region of the macronucleus a slight constriction appears as if the macronucleus were preparing to divide. The chromatin on the two sides of this constriction gradually takes on distinctly different appearances. The half which is directed toward the posterior end of the organism becomes roughly granular and has the appearance of the usual ciliate macronucleus during the early phases of degeneration. Occasionally large spheres of chromatin appear in this region surrounded by clear vesicles. These spheres are irregular in their appearance, however, and ultimately fade from view. The anterior half of the macronucleus retains its finely granular constitution but there invariably appear large spheres of material, staining deeply with the haematoxylin but reacting negatively to the FEULGEN reagent (Pl. 3 Fig. 1). These spheres are surrounded by clear vesicles and have the same general appearance as the X-granules of the macronucleus of *Uroleptus mobilis* and *U. halseyi* as described by CALKINS (1919, 1930). I have been unable to determine whether or not they are cast out as units into the cytoplasm, but I do not believe that is the case. Rather it appears that they are eventually dissolved within the anterior half of the macronucleus where they originated. It is of course possible that the dissolved substances do diffuse into the cytoplasm through the nuclear membrane.

After the spheres have appeared and dissolved, the anterior half of the macronucleus begins to round up and the constriction between it and the posterior half becomes more pronounced (Pl. 3 Fig. 2). The anterior half eventually assumes a more or less spherical form and becomes separated from the posterior half. In this condition the rounded half has the appearance of a smoothly granular reorganized nucleus (Pl. 3 Fig. 3).

By the time the anterior half of the macronucleus has rounded up the two daughter micronuclei have become immensely swollen with the chromatin in the form of an eccentrically located mass in a clear vesicle. Both micronuclei appear identical at this time, but gradually one begins to grow smaller and usually migrates a short distance from the macronuclear sphere. The other increases slightly and remains in juxtaposition with the macronuclear sphere.

From this period a set of amazing phenomena take place. The larger of the two daughter micronuclei sinks into the macronuclear

sphere and the chromatin of the sphere becomes displaced, forming a shell of considerable thickness around the micronuclear vesicle (Pl. 3 Fig. 4). For some time the vesicular micronuclear product remains intact (Pl. 3 Fig. 5) but eventually the macronuclear chromatin invades it forming a compact chromatin mass.

During this process the posterior half of the old macronucleus begins to degenerate and the smooth outline of the membrane is lost. It appears that this disintegrating chromatin passes into the reservoir in the anterior end. The contents of the reservoir react very positively to the FEULGEN reagent during this period, the reaction becoming weaker as the reorganization process proceeds. As far as I have been able to observe directly there is no passage of stainable material through the pellicle to the outside, but the structure of the reservoir indicates that such a passage may occur. In any event it appears that the reservoir receives all the disintegrating macronuclear chromatin. This is supported both by the staining capacity of the reservoir contents at this period and by the morphological continuity of the reservoir and the macronuclear half. Complete removal of the chromatin of the posterior macronuclear half to the reservoir and the disappearance of the old karyophore is completed by the time the macronuclear anlagen has reached its maximum growth.

The growth of the macronuclear anlagen is apparently quite rapid. As the swelling takes place the chromatin, of macronuclear and micronuclear origin, spins out into twisted and extremely elongated threads. In the early growth stages it is possible to differentiate between the two types of chromatin by the relatively large size of the strand formed from macronuclear chromatin and the thin, beaded thread formed from the micronuclear chromatin (Pl. 3 Fig. 6). It is difficult to tell whether or not this is a single thread or a number of threads. There appears to be a growth of the chromatin as the anlagen enlarges until eventually the anlagen is in the form of a huge sphere occupying the greater portion of the central part of the cell. At its maximum size the thread is rather thick and there is no longer any distinction between micro- and macronuclear chromatins (Pl. 3 Fig. 7). The anlagen appears as a large "ball of yarn" and is in every way similar to the macronuclear anlagen of *Nyctotherus cordiformis* after conjugation as described by STEIN (1868), SCHNEIDER (1886) and recently by WICHTERMAN (1937). This is the stage most frequently encountered when one observes the fully formed cysts. It seems to be the longest stage of any in the process.

Finally the thread-like appearence of the macronuclear anlagen becomes indistinct, the chromatin breaking up into granules (Pl. 3 Fig. 8). The staining capacity in slight at first but as contraction of the anlagen progresses it becomes increasingly basophylic. It is during this contraction stage of the macronuclear anlagen that the first evidence of excystment appears. In the blunt end of the cyst the beginning of the new cytostome is visible. This enlarges and eventually membranelles grow out from its inner border.

The macronuclear anlagen continues to contract in the direction at right angles to the long axis of the cell until it finally becomes rod-shaped. During this contraction it moves from the center of the cell toward the narrow (anterior) end and to one side (Pl. 3 Fig. 9). The micronucleus also moves forward and comes to lie near the macronucleus at about its mid-region. As the nuclei are completing their migration peripheral cilia make their appearance. There is no rupture of the cyst wall, rather a slight decrease in its thickness. The cilia seem to grow out through the heavy pellicle from the basal bodies along the ever present body striations. The peripheral cilia increase in size and number as the cytostomal membranelles are differentiating and soon the ciliate becomes active. The karyophore seems to be the last structure to be differentiated in the cell. By the time all traces of the endomictic process, as judged by the nuclei, are past, the karyophore appears. I was unable to observe its formation, however, as at this stage it is difficult to differentiate between ordinary trophic organisms and post-endomictic ones.

Discussion.

The foregoing account of endomixis during encystment in the ciliate *Paraclevelandia simplex* is unique in a number of respects. In the first place it is the first case on record, as far as I am aware, where renewal of the macronucleus is accomplished by incorporating part of the old macronuclear chromatin. This sort of phenomenon has been reported as occurring during exconjugant reorganization in *Boveria labialis* (Ikeda and Ozaki, 1918) and in *Euplotes patella* (Turner, 1930). In these cases there was very little evidence that the old macronuclear chromatin was reorganized before incorporation. In *Paraclevelandia simplex*, on the other hand, there seems to be complete transformation from the loosely granular condition of the old macronucleus to the finely divided state of a new nucleus. In many ways this transformation suggests the condition

found in the division macronuclei of a number of the hypotrichous ciliates after the passage of the "reorganization bands", particularly in *Euplotes* (GRIFFIN, 1910; YOCOM, 1918; TURNER, 1930), *Aspidisca*, *Diophrys* and *Stylonychia* (SUMMERS, 1934). But in these forms there is no disintegration of macronuclear parts and no fusion of micro- and macronuclear chromatin. In a number of holotrichous ciliates it has been shown that during the divisional and post-divisional reorganization of the macronucleus parts are separated off to disintegrate in the cytoplasm. The list of forms exhibiting this phenomenon is rather long and is summarized elsewhere (KIDDER and CLAFF, 1938).

The disintegration of approximately half of the old macronucleus following the reorganization process, as it ocours in *Paraclevelandia simplex*, has its counterpart in the activity of the macronucleus of the young resistant cyst of *Colpoda cucullus* (KIDDER and CLAFF, 1938). In this ciliate the macronucleus reorganizes and eliminates extremely large quantities of its chromatin into the cytoplasm. The micronucleus takes no active part in this reconstruction process, however.

In *Urostyla grandis* TITTLER (1935) has reported the occurence of a process of endomixis taking place in pre-cystic forms. The old macronuclear complex is completely disorganized and resorbed while new macronuclear anlagen form from some of the products of micronuclear mitoses. This process resembles, in a single organism, the conditions found during conjugation.

It is evident that there are different mechanisms employed among the ciliates for reorganizing their macronuclei and also that there are different grades of reorganization and reconstitution of this cell element. Following conjugation it appears to be usual that the old macronuclear complex is completely disorganized and resorbed, but in at least two cases even here some parts may be utilized in the new complex (*Boveria labialis* IKEDA and OZAKI, 1918 and *Euplotes patella*, TURNER, 1930). Without conjugation macronuclear reorganization, with visible changes occuring, may take place without the aid of micronuclear activity as has been demonstrated in *Colpoda cucullus* (KIDDER and CLAFF, 1938); or, as in the case reported in this study, through the amalgamation of both macronuclear and micronuclear chromatin. Or, as in *Urostyla grandis* (TITTLER, 1935) and *Paramecium* (WOODRUFF and ERDMANN, 1914; DILLER, 1936), a complete reconstitution of a new macronucleus from micronuclear (amphinuclear in *Paramecium*, according to DILLER, 1936) derivitives.

What factors, apparently inherent in the species, initiate micronuclear activity during macronuclear reorganization in some cases and suppress it in others are entirely unknown. The end result, as far as we can judge by observation, no matter what variation of the mechanism is employed, is the formation of a new macronucleus devoid of the accumulations of the waste products of previous cell activity.

In *Paraclevelandia simplex* as in the majority of forms, there appears to be dedifferentiation of the motor organelles during encystment. It appears, then, that the period of encystment in this organism functions as a time when renewal of all the vital cell elements, at least those that can be observed, takes place. It has not been ascertained what relation this period bears to the environmental conditions. It appears that encystment and excystment can and do take place side by side within the limited confines of the gut of the host.

It should be noted that dedifferentiation of motor organelles during encystment has been reported without any apparent nuclear reorganization. In *Didinium nasutum*, according to BEERS (1935), the macronucleus and the micronucleus remain inactive throughout this period. BEERS fails to confirm the earlier assertions of CALKINS (1915, 1916) that macronuclear reorganization takes place within the cyst. There appears to be a possibility that the reorganization process mentioned by CALKINS takes place very rapidly in the newly encysted organism, as it does in *Colpoda cucullus* (KIDDER and CLAFF, 1938). BEERS fails to give an analysis of the period between the start of encystment and what he terms the "immature cyst", which is about 12 hours old. It would be very desirable to determine the reaction of the "endoplasmic spheres", considered food material by BEERS, to the FEULGEN reagent, in as much as they increase in number during the encysted stages.

Summary.

1. *Paraclevelandia simplex*, an endocommensal heterotrichous ciliate of *Panesthia*, undergoes encystment and excystment within the gut of the host.

2. Prior to and during this process dedifferentiation of the motor organelles and nuclear reorganization occur.

3. The nuclear reorganization is a type of endomixis.

4. The micronucleus undergoes one mitotic division.

5. The macronucleus undergoes a reconstitution resulting in the breaking down and elimination, apparently through the reservoir, of approximately one half of its chromatin.

6. The remaining half rounds up and becomes incorporated with one daughter micronucleus to form the macronuclear anlagen.

7. The macronuclear anlagen then swells and the chromatin assumes the form of twisted threads. The anlagen appears as a "ball of yarn", characteristic of the macronuclear anlage in the exconjugants of *Nyctotherus*.

8. By contraction and loss of the identity of the chromatin threads the new rod-shaped macronucleus results.

9. Motility of the ciliate is resumed after the peripheral cilia grow out through the thinned cyst wall. The membranelles form in the developing cytostome during this time.

Literature cited.

BEERS, C. D. (1935): Structural changes during encystment and excystment in the ciliate Didinium nasutum. Arch. Protistenkde **84**, 133.

CALKINS, G. N. (1915): Didinium nasutum. I. The life history. J. of exper. Zool. **19**, 225.

— (1916): General biology of the protozoan life cycle. Amer. Naturalist **50**, 257.

— (1919): Uroleptus mobilis ENGELM. I. History of the nuclei during division and conjugation. J. of exper. Zool. **27**, 293.

— (1930): Uroleptus halseyi CALKINS. II. The origin and fate of the macronuclear chromatin. Arch. Protistenkde **69**, 151.

DILLER, W. F. (1936): Nuclear reorganization processes in Paramecium aurelia, with descriptions of autogamy and "hemixis". J. Morph. a. Physiol. **59**, 11.

EIGENMANN, C. H. and R. S. EIGENMANN (1888): A list of the American species of Gobiidae and Callionymidae, with notes on the specimens contained in the Museum of Comparative Zoology, at Cambridge, Massachusetts. Proc. Calif. Acad. (2) **1**, 76.

GRIFFIN, L. E. (1910): Euplotes worcesteri sp. nov. II. Division. Philippine J. Sci. **5**, 315.

IKEDA, I. and Y. OZAKI (1918): Notes on a new Boveria species, Boveria labialis, n. sp. J. Coll. Sci., Imp. Univ. Tokyo **23**, 287.

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.

— (1937): The intestinal protozoa of the wood-feeding roach Panesthia. Parasitology **29**, 163.

KIDDER, G. W. and C. L. CLAFF (1938): Cytological investigations of Colpoda cucullus. Biol. Bull. **74**, 178.

Summers, F. M. (1935): The division and reorganization of the macronucleus of Aspidisca lynceus Müller, Diophrys appendiculata Stein and Stylonychia pustulata Ehrbg. Arch. Protistenkde. **85**, 173.

Tittler, I. A. (1935): Division, encystment and endomixis in Urostyla grandis with an account of an amicronucleate race. La Cellule **44**, 189.

Turner, J. P. (1930): Division and conjugation in Euplotes patella Ehrbg. with special reference to the nuclear phenomena. Univ. California Publ. Zool. **33**, 193.

Woodruff, L. L. and R. Erdmann (1914): A normal periodic reorganization process without cell fusion in Paramecium. J. of exper. Zool. **17**, 425.

Yocom, H. B. (1918): The neuromotor apparatus of Euplotes patella. Univ. California Publ. Zool. **18**, 337.

Explanation of plate.

Plate 3.

All figures are of *Paraclevelandia simplex* during endomixis. Magnification × 1000.

Figures 5 and 8 were drawn from material fixed in Schaudinn's fluid with 5 per cent acetic acid added and treated with the Feulgen reagent. The remaining figures were drawn from material fixed in Bouin's fluid, stained with Heidenhain's haematoxylin and differentiated in aqueous picric acid.

Fig. 1. Pre-cystic form. Macronucleus differentiating into degenerating posterior, and reorganizing anterior chromatin. Micronucleus in prophase. Peripheral cilia present but not shown.

Fig. 2. Later stage. Micronucleus in anaphase. Further differentiation of macronucleus. Cilia as in Fig. 1.

Fig. 3. Telophase of micronucleus. Note the smoothly granular anterior half of the macronucleus.

Fig. 4. Fusion of daughter micronucleus with reorganized portion of macronucleus. Posterior portion of macronucleus shrinking away from the old nuclear membrane. Enlarged condition of the daughter micronuclei quite characteristic.

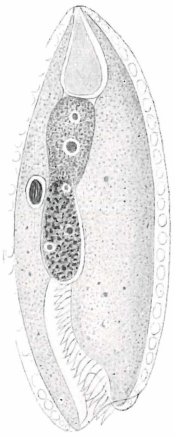
Fig. 5. Later stage. Posterior half of old macronucleus smaller and more irregular. Contents of the reservoir react positively to the Feulgen reagent. Peripheral cilia have disappeared and membranelles are being resorbed.

Fig. 6. Complete encystment. Large macronuclear anlagen containing the chromatin threads. Note the fine and the course threads. Micronucleus contracted.

Fig. 7. Later stage. This is the most common stage of the process encountered.

Fig. 8. Dissolution of the chromatin threads. The beginning of a new cytostome can be seen.

Fig. 9. Nuclear apparatus reorganized. Peripheral cilia have reappeared (not figured) and the new membranelles are present. The karyophore has not yet been re-formed.



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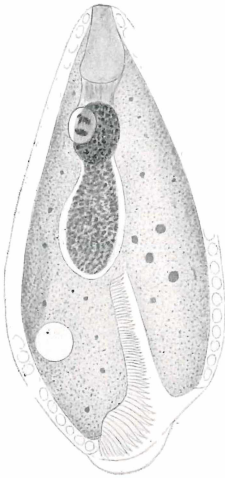

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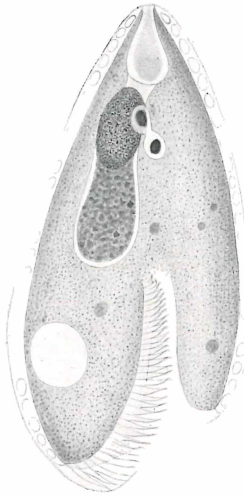

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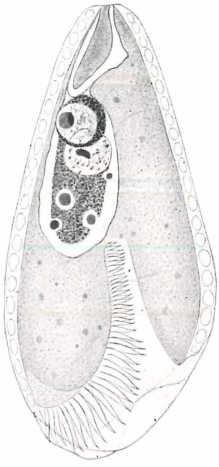

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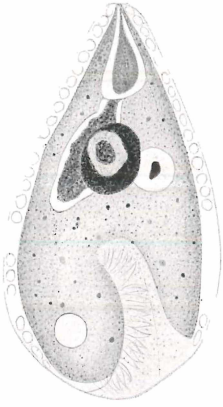

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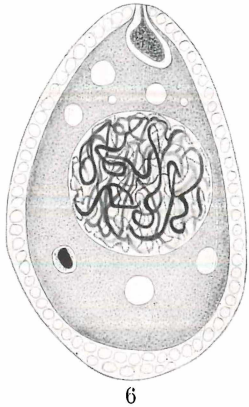

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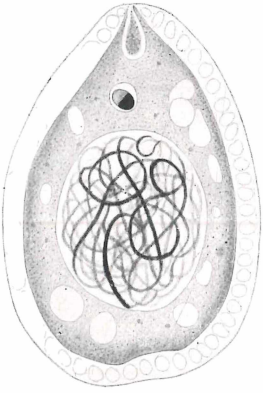

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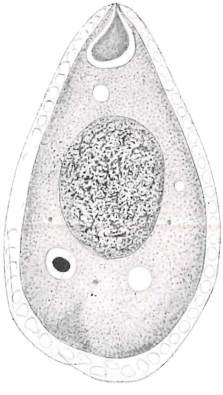

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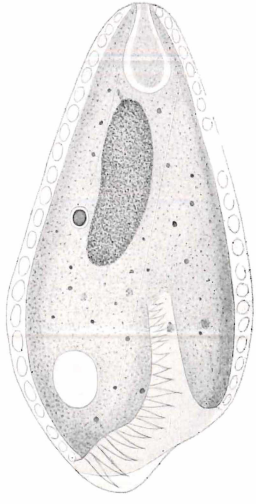

9

Kidder.

VERLAG VON GUSTAV FISCHER IN JENA.