Macronuclear Regeneration in *Epistylis articulata*

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With one plate (fig. 5)

SUMMARY

The reorganization of the macronucleus from the vegetative micronucleus is described in *Epistylis articulata* (Ciliophora, Peritricha). Individuals which have accidentally been deprived of a macronucleus during binary fission develop a new one from the micronucleus. In such amacronucleate animals, the micronucleus divides into two, one of them growing into a macronucleus, the other remaining as the micronucleus. The phenomenon throws new light on the relative importance of the two parts of the ciliate nuclear apparatus and brings into greater emphasis the undifferentiated nature of the micronucleus. It also establishes the possibility of *de novo* synthesis of DNA in the developing macronuclear rudiment.

INTRODUCTION

THE possession of two nuclei—the macronucleus and the micronucleus—is characteristic of all euciliates. Associated with this separation of the nuclear apparatus into two components, there is also a differentiation in their size, mode of division, and function. The necessity of either or both these nuclei for the normal life of the organism has been repeatedly stressed, but occasional instances where one or the other is absent have been reported, always with more or less conspicuous effects on the organism and its life cycle. For instance, Diller (1940) reported the occurrence of individuals of *Paramecium caudatum* without a macronucleus but regarded the condition as probably due to centrifugation, senility, or changes in nutritional and environmental conditions. In all cases, he thought, such animals died early. Sonneborn (1947) studied, from his stocks of *Paramecium aurelia*, individuals without one or the other nucleus; and while he discovered that those without a micronucleus could survive for considerable periods, animals without a macronucleus met with early death. On these findings he developed the view that while the macronucleus was essential to the vegetative life of the organism and could not be replaced when lost, the organism could do without a micronucleus for a considerably long period.

While making a study of *Epistylis articulata* it was found that amacronucleate individuals turned up in large numbers in certain colonies with such frequency as to merit a closer examination of their origin as well as their fate. Several hundred such individuals were found in our stocks and a minute study revealed certain interesting features.

Quarterly Journal Microscopical Science, Vol. 94, part 2, pp.185–192, June 1953.

Material and Methods

Colonies of *Epistylis articulata* (From.) were obtained from sewage waters about five miles from Bangalore. Among the dominant organisms met with when raw sewage is aerated are peritrichous ciliates of the genera *Vorticella*, *Epistylis*, *Carchesium*, and *Zoothamnium*. This fact was made use of in obtaining large supplies of material which was brought to the laboratory for further examination and treatment. The material was available abundantly throughout the year.

The material was fixed in hot Schaudinn's, Bouin's, and Carnoy's fluids and was stained in haematoxylin, Feulgen, toluidine blue, and Unna's methyl green pyronin mixture. All the diagrams and photographs illustrating this paper are based on Feulgen preparations.

Observations

Amacronucleate individuals of *Epistylis articulata* occur among the normal members of the colony scattered at random and without any special arrangement or distribution. They occur at all levels of the colony. They can be easily recognized by their smaller size and by the absence of the conspicuous macronucleus, which, in this species, is cylindrical and bent, occupying a considerable amount of space in the cytosome.

The amacronucleate condition results from a slight upset of the process of binary fission. In *E. articulata*, binary fission normally starts with the mitotic division of the micronucleus. Long after the daughter micronuclei have separated and moved apart, the macronucleus stretches across the animal at right angles to the cleavage furrow, which, beginning at the peristome, passes towards the stalk. Eventually the macronucleus is cut into two approximately equal parts which are shared by the two daughter organisms.

In the production of the amacronucleate animal, the micronucleus divides in the usual manner, but the macronucleus, instead of being cut into two parts, remains entirely within one of the daughter cells. The amacronucleate individual is much smaller, while the one with the macronucleus is apparently not different from the normal animals of the colony, except perhaps for the visibly larger macronucleus (fig. 1, A, B, and C).

The amacronucleate animal has an interesting future. For a considerable time it remains quiescent and inactive: it is doubtless unable to function as a normal animal. It seems incapable of going through even the one or two fissions that *Paramecium* appears able to achieve (Sonneborn, 1947). The duration of such inactive existence can be easily estimated, in a branching colony like that of *Epistylis*, by a study of the sister individual which has the macronucleus. This latter, it was observed, proceeds with its normal existence, feeding, and reproducing by binary fission. Three or four generations of individuals are produced by the sister animal with the macronucleus while the amacronucleate one has remained, during this period, a small and quiescent organism. It is doubtful if it feeds. It certainly does not seem to grow. It does not divide.

Soon, however, the micronucleus of the amacronucleate animal divides by mitosis (fig. 2). We have not been able to detect any difference between

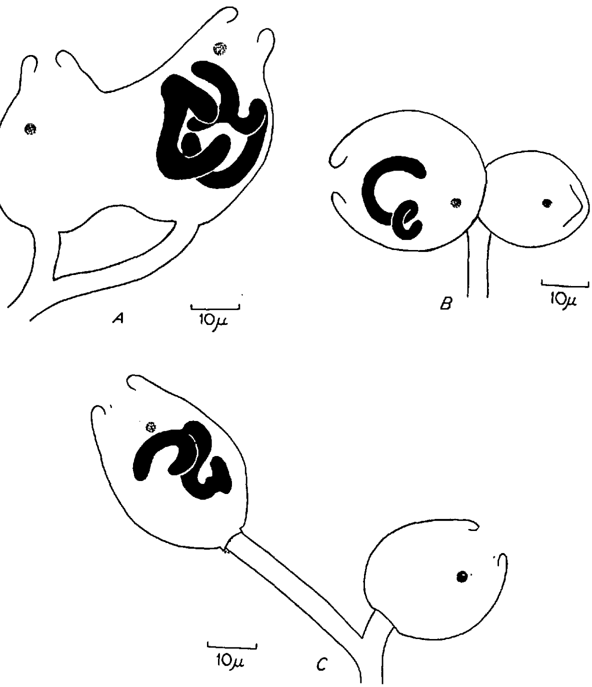

FIG. 1. Formation of amacronucleate animal. An upset in binary fission results in the formation of two individuals, one of which lacks the macronucleus. A, fission is in progress. The micronucleus has divided but the macronucleus is included in one of the animals. B, later stage. Fission complete. The amacronucleate animal is visibly smaller. C, later stage. The difference in the length of the stalk of the two animals is clear.

mitosis of the micronucleus in a normal and an amacronucleate animal; probably there is none. The two daughter nuclei are at first similar in size but soon one of them enlarges and becomes the macronucleus. Even in its initial stages of enlargement, the macronuclear rudiment or primordium shows a striking difference in structure and we have been able to trace the entire process of its differentiation. The micronuclear rudiment, like the micronucleus of the vegetative animal, remains a uniform lightly stained body.

One of the characteristics of the micronucleus is that it shows a smooth homogeneous appearance in preparations made in a variety of ways: haematoxylin, borax carmine, Feulgen, and methyl green. The macronuclear rudiment, on the other hand, shows in its earliest stages of differentiation, coarse deeply staining blocks of chromatin (fig. 3, A). As the rudiment enlarges further it becomes fainter.

The further history of the macronuclear anlage is of interest. It continues to increase in size, and at one time occupies a considerable space in the cell, pushing the micronucleus to a side (fig. 3, B and C). At this stage it remains a faintly staining body. Later, it gradually shrinks in size, and with this shrinkage is associated an unmistakable acquisition of basiphilia, until the macronucleus assumes the final condition characteristic of the vegetative macronucleus. At first spherical or oval, it soon becomes elongated and cylindrical and assumes its definitive form (figs. 4, A and B, and 5).

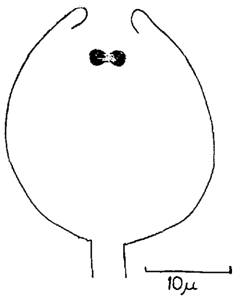

FIG. 2. Amacronucleate animal. Division of the micronucleus is in progress.

With the full development of the macronucleus and the completion of the nuclear equipment, the animal behaves like a normal organism and becomes indistinguishable from it.

DISCUSSION

The regeneration of the macronucleus from the micronucleus of a vegetative individual has never before been reported in any ciliate. That after conjugation or autogamy, the synkaryon possesses this property is well known. That this is shared by the vegetative micronucleus is clear from the above observations.

The nearest parallel we have to this phenomenon is in endomixis. This process was first described by Woodruff and Erdmann (1914) in *Paramecium aurelia*, but it has been noticed in a number of other ciliates too (see Woodruff, 1941, for a review of the subject). Recently some doubt has been expressed on the occurrence of endomixis, especially in *P. aurelia*. Sonneborn (1947) has never met with it in his stocks. The cytological evidence is also not conclusive. However, the resemblance between endomixis as described by Woodruff and the vegetative regeneration of the macronucleus described in this paper is striking. In both cases it is the micronucleus (or one of its division products) of a vegetative organism that produces the macronucleus. There is no fusion of nuclei involved, either in single animals as in autogamy, or in pairing animals as in conjugation.

But the parallel ends there. In endomixis there are two complications which are absent in the macronuclear regeneration described in this paper.

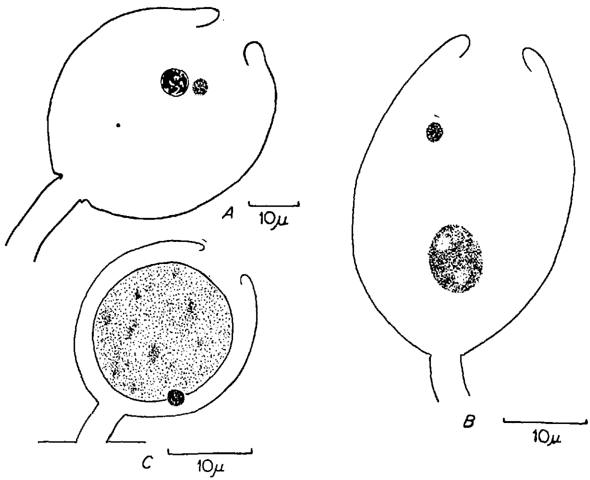

FIG. 3. Differentiation of the macronucleus. A, an early stage, showing the macronuclear rudiment with deeply staining chromatin. B, later stage in differentiation. The enlarging pale macronuclear rudiment is clear. C, stage showing maximum enlargement of the macronuclear rudiment. The micronucleus lies to one side.

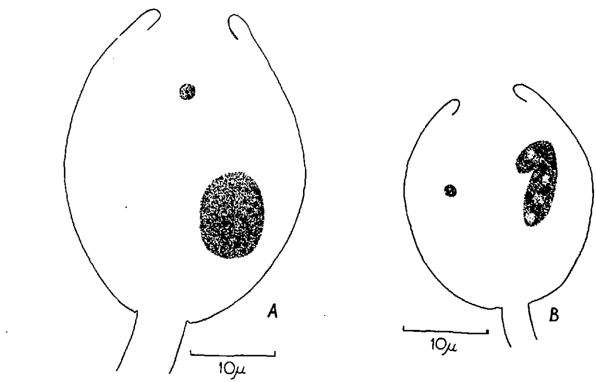

FIG. 4. A, differentiation of the macronucleus. The rudiment has visibly shrunk in size and stains deeply. B, an early stage in the acquisition of the definitive form of the macronucleus.

First, the micronucleus in endomixis passes through divisions which recall the progamic divisions in conjugation and autogamy. Secondly, the animal has, in endomixis, a macronucleus, which, however, breaks up and is absorbed in the cytoplasm in much the same way as in conjugation and autogamy.

Doubts have been expressed, on both cytological and genetical grounds, of the occurrence of endomixis in ciliates. These refer particularly to the two divisions of the micronucleus immediately before differentiation. Their occurrence in conjugation and autogamy is of significance in that they are meiotic, reducing the diploidy of the micronucleus of the vegetative animal to haploidy, which is restored to diploidy by syngamy. In the absence of syngamy, the divisions could not be meiotic and it is difficult to imagine the necessity for these divisions in endomixis. It seems likely, as Diller (1936) remarks, that Woodruff and Erdmann were mixing up stages of autogamy and hemixis and have lumped them together under endomixis. On this analysis, the behaviour of the micronucleus described in this paper seems simple and straightforward. The single division of the micronucleus must be a mere mitotic division and both the rudiments are diploid. The growth of one into a macronucleus is a subsequent phenomenon not affecting the essential nature of the ploidy of the nucleus.

The other matter of the breaking up of the macronucleus characteristic of conjugation, autogamy, and endomixis raises a problem of even greater interest—the origin of the desoxyribose nucleic acid (DNA) of the developing macronuclear anlage. It is clear from a number of earlier studies (Seshachar, 1947, 1950; Seshachar and Dass, 1951; Seshachar and Srinath, 1946 and 1947) that the ciliate macronucleus has a large amount of DNA in it. The synthesis of DNA in the developing macronucleus after conjugation has formed a subject of considerable interest, and more than once it has been suggested that the DNA of the new macronucleus must in some manner come from the old and degenerating macronucleus and its break-down products (Seshachar, 1947; Baker, 1948; Glass, 1949). There has been some indirect evidence for this transfer in the form of a gradual degeneration and disappearance of the fragments of the old macronucleus as the new macronucleus is acquiring its DNA. In *Vorticella convallaria* (Seshachar and Dass, 1951) there is a gradual reduction in the number of fragments, associated with the acquisition of DNA by the rudiments. In *Epistylis articulata* (Seshachar and Dass, in press) the fragments of the old macronucleus, which in the early stages are uniform, brilliantly

FIG. 5 (plate). Photomicrographs showing the formation of amacronucleate animal and the differentiation of the macronucleus in it.

A, formation of amacronucleate animal.

B, typical amacronucleate animal.

C, early stage in the differentiation of the macronucleus. The difference between the two rudiments is clear.

D, macronuclear rudiment in the process of enlargement.

E, macronuclear primordium at maximum enlargement. The micronucleus lies to one side.

F, macronuclear primordium has shrunk in size and stains deeply. Compare with D.

Feulgen-positive bodies, gradually develop Feulgen-negative spaces within them, until each fragment finally becomes a Feulgen-negative body. On the basis of a specific staining technique like that of Unna-Pappenheim (pyronin methyl green) we have reasons to believe that this involves a fundamental change in the nucleic acids of the fragments the DNA being gradually replaced by RNA (ribose nucleic acid). Then again, there is abundant evidence of a more general nature, in a number of ciliates, where, concomitantly with the development of the new macronucleus, there is a gradual dwindling and disappearance of the old. Around these observations has developed the idea that the synthesis of DNA in the new macronucleus must in some manner be connected with the disintegration and disappearance of the old, and a causal connexion must exist between the two, however undemonstrable cytologically it might be. It seems, however, not necessary to believe that a *de novo* synthesis of DNA in the new macronucleus is impossible. Actually, considerable *de novo* synthesis of DNA has been demonstrated by photometric methods in the developing macronucleus (after conjugation) of *Chilodonella* (Seshachar, 1950).

The observations recorded in this paper seem to put the entire problem of the *de novo* synthesis of DNA in the macronucleus beyond reasonable doubt. There is no macronucleus or its fragments to cloud the issue.

We also see another interesting resemblance between the present instance of macronuclear regeneration in vegetative individuals of *Epistylis articulata* and the development of the macronuclear rudiment in *Chilodonella uncinatus* after conjugation, described by one of the authors (Seshachar, 1950). In both cases, the initial stage of macronuclear differentiation consists in an enlargement of the rudiment to considerable dimensions, without acquisition of DNA at a corresponding rate; so it appears as a large faintly staining body, occupying much of the cell space (figs. 3, C and 5, E). Later it shrinks in size, meanwhile acquiring DNA and the final form of the macronucleus. The initial process involving a conspicuous enlargement of the rudiment to great dimensions, occurring as it does in two very different circumstances (one after conjugation and the other in vegetative reorganization), is one of the most striking phenomena in the nuclear reorganization of ciliates, whose precise significance is not clear, but whose interest is not, on that account, any the less.

Then there is the final question of the relative importance of the two parts of the ciliate nuclear apparatus to the life and life-history of the animal. Sonneborn (1947) believes that in *Paramecium*, an individual without a macronucleus is unable to survive long, while it is capable, deprived of the micronucleus, to live for a considerable length of time. In the light of the observations set forth in this paper, it seems necessary to modify this concept, at least in so far as it relates to peritrichous ciliates. The lost or missing macronucleus *can* be regenerated by the vegetative micronucleus, outside the conventional processes of reproduction and reorganization—conjugation, autogamy and endomixis. This, however, does not take away any of the importance attached to the role of the macronucleus in vegetative life; rather it adds to it.

But it also emphasizes the role of the definitive micronucleus, not the synkaryon alone, in the development of the macronucleus. The macronucleus has been accepted for a long time as a highly organized body irreversible in its differentiation, while the micronucleus is comparatively undifferentiated and capable of producing both nuclei. We had been led to believe that only the synkaryon was in this protean condition; it now seems necessary to regard the formed micronucleus also as sharing this character.

It is a pleasure to thank the National Institute of Sciences of India for the award of the I.C.I. (India) Research Fellowship to one of us (C. M. S. D.).

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