Mitosis and Polyploidy in Nuclei of *Opalina ranarum*

The problem of nuclei and mitosis in *Opalina* has been studied by many authors. The most detailed data were given by CHEN[1-3] for species of the genus *Zelleriella*.

This author has established that: (1) there is evidence of acentriolar mitotic divisions; (2) nuclei of trophonts are diploid; (3) there is clear and visible attachment between chromosomes and nucleoles. In other Opalinata, however, not all details of mitotic divisions were fully described because of the relatively small size of their nuclei and chromosomes, and poor staining due to the low content of DNA (CHEN[1]).

In the present paper the Feulgen method was used. Some of the slides were subsequently dried and stained with toluidin blue. Double staining gives an excellent contrast of chromosomes. RNA was stained by UNNA's method. Some slides treated with ribonuclease were used as a control.

Results. The observation showed that normal mitosis occurs in trophonts of *Opalina ranarum* and *Cepedea dimidiata*. An asynchrony of nuclear divisions was found in individuals of both species. The prophasic, metaphasic, anaphasic and telophasic stages were clearly distinguishable. In contrast to DEVIDE's results[4], the arrangement of chromosomes in equatorial region during metaphase was observed in this work. The exact number of chromosomes was not established because of their small size. In the best pictures, about 18 chromosomes could be distinguished in *O. ranarum*, but, because of their relatively small size, their bivalent character and position of kinetochores during metaphase were not seen.

On the other hand, there are clear-cut differences between individual chromosomes in size and shape during the late prophase and metaphase. In 2 populations of trophonts of *O. ranarum* from *Rana temporaria*, large nuclei were clearly visible. They often occur together with nuclei of normal size in the same trophonts.

These populations thus included: (1) trophonts with only large nuclei, (2) trophonts with both large and normal nuclei and (3) trophonts with only normal nuclei. Large nuclei in resting stage were often elongated and somewhat irregular in shape. In the case of large nuclei, their number per individual was usually smaller than in the case of normal nuclei. It was evident that these large nuclei divide mitotically and all phases of mitosis were observed (see Figures).

These large nuclei have a higher number of chromosomes than normal ones. Both the size and shape of chromosomes in all nuclei is the same and depends only on the stage of mitosis. In Figure 1 are shown 2 adjacent nuclei of the same individual; a normal one (a) and a large one (b). The polyploid character of nucleus (b) is visible. About 60 chromosomes can be distinguished, but the actual number of chromosomes may differ from this estimation. Three different nuclei during metaphase are shown in Figures 2, 3 and 4 (Figures 3 and 4 show nuclei presumably during prometaphase). The nucleus in Figure 2 is normal, the 2 others have different levels of polyploidy because the distances between chromosomes are similar in these nuclei, which differ only in size. The degree of polyploidy, however, has not been established. Figures 5 and 6 give a comparison of normal and large nuclei during anaphase.

In normal nuclei a few nucleoles lie close under the nuclear envelope (NOIROT-THIMOTHÉE[5,6], SUKHANOVA[7]). On the other hand, in a large nucleus very large nucleoles of different size are present (Figure 7). Associations of nucleoles and chromosomes were not observed. CHEN[2]

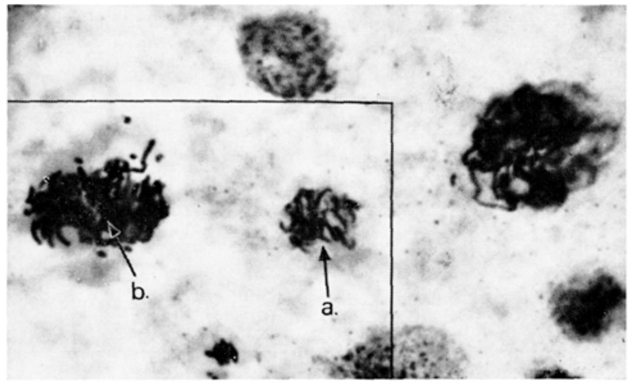

Fig. 1

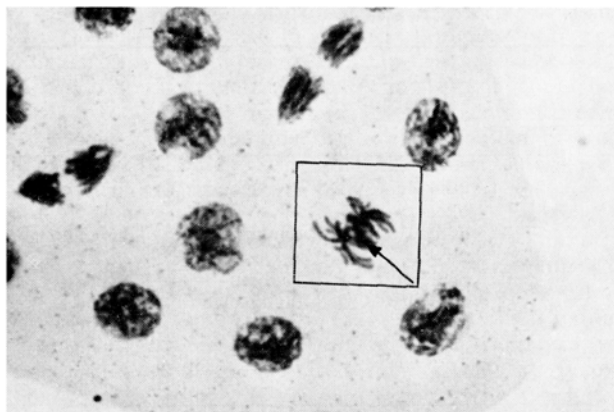

Fig. 2

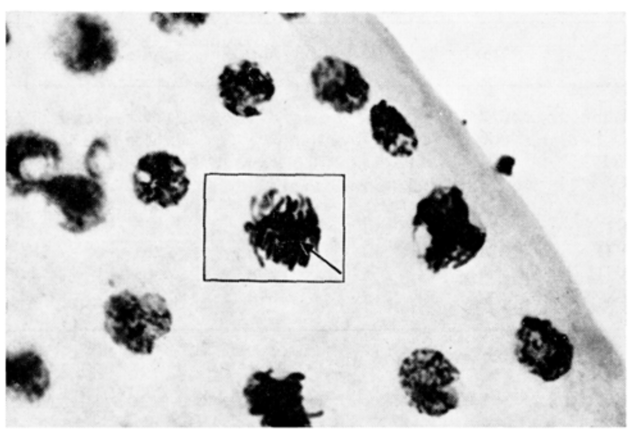

Fig. 3

[1] T. T. CHEN, Proc. natn. Acad. Sci. USA *22*, 594 (1936a).
[2] T. T. CHEN, Proc. natn. Acad. Sci. USA *22*, 602 (1936b).
[3] T. T. CHEN, J. Morph. *83*, 281 (1948).
[4] Z. DEVIDE, Bull. int. Acad. yougos. Sci. Classe Sci. med. *3*, 75 (1951).
[5] C. NOIROT-THIMOTHÉE, Annls Sci. nat., Zool. *12*, 265 (1959).
[6] C. NOIROT-THIMOTHÉE, Protistologica *3*, 301 (1967).
[7] K. M. SUKHANOVA, Citologia *3*, 577 (1961).

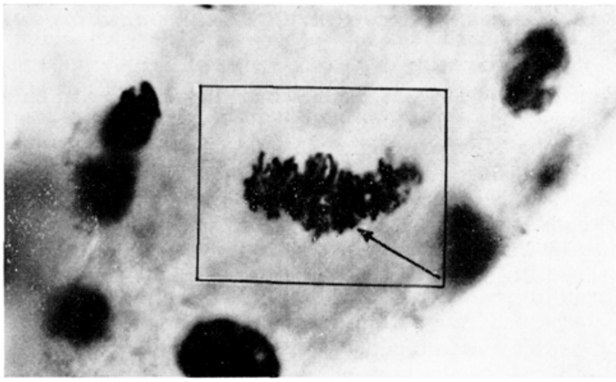

Fig. 4

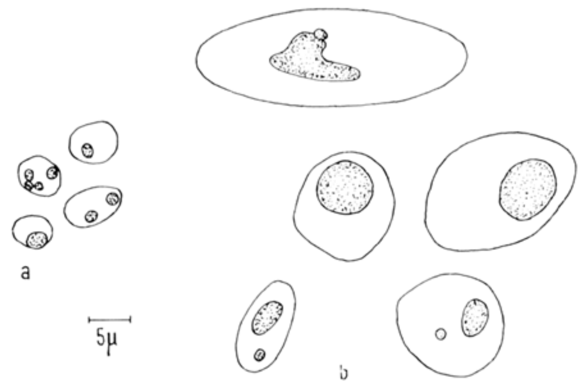

Fig. 7. (a) Nucleoles in normal nuclei; (b) nucleoles in large nuclei.

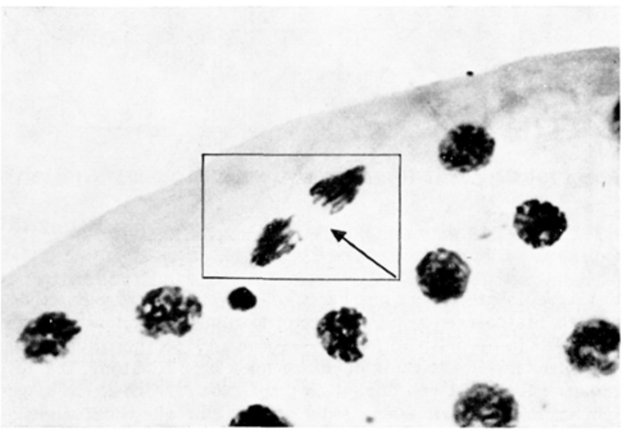

Fig. 5

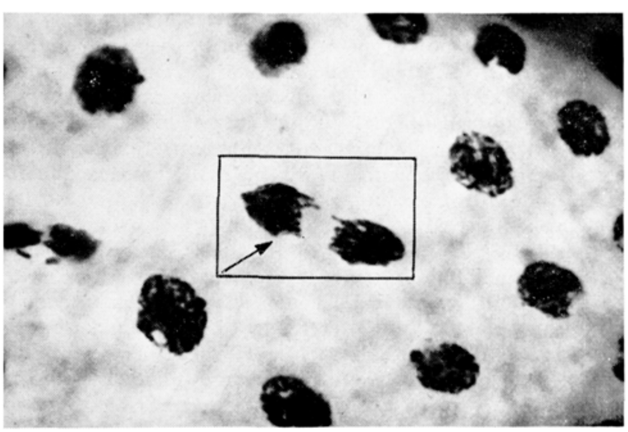

Fig. 6

Figs. 1–6. × 1500.

described very close relations between chromosomes and nucleoles in *Zelleriella*. On the other hand, he observed random fusing of nucleoles which are connected with different chromosomes. Presumably large irregular nucleoles are formed in large nuclei in a similar way. The presence of large nuclei in *Opalina* has been described previously by some authors (DEVIDE[4], NERESHEIMER[8]). DEVIDE[4] believed that such nuclei do not possess a higher number of chromosomes, but only that their distribution is looser, and his drawings suggest that nucleoles are larger in such nuclei.

The fact that polyploid nuclei have been found in trophonts of *Opalinata* may be important for further comparisons of *Opalinata* and *Ciliata*. The populations described are only random. The frequency of such populations has not been established. The mechanism of polyploidization in this case is not known. On the other hand, it is interesting that nuclei of polynuclear *Opalinata* can become polyploid under some circumstances. Especially interesting is the fact that the nuclei in the same trophont may differ as regards their chromosomal content and size like the micronuclei and macronuclei in *Ciliata*.

It means also that the principal difference between these 2 groups of protozoans is not necessarily connected with disability of nuclei of *Opalinata* to polyploidization and dimorphism.

The principal difference between these major protozoan groups is presumably connected with the mechanism of genetical continuity and different regulation of the function of the 2 kinds of nuclei in *Ciliata*.

Such mechanisms are associated with conjugation in *Ciliata* and are absent in *Opalinata*.

Résumé. Dans certaines populations de *Opalina ranarum* nous avons trouvé d'importantes différences dans la grandeur des noyaux. Ces différences tiennent au degré de polyploïdisation. Les grands polyploïdes conservent la faculté de se diviser par mitose.

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19 February 1968.

[8] E. NERESHEIMER, Archs Protistenk. Suppl. *1*, 1 (1907).