FURTHER OBSERVATIONS ON THE FINE STRUCTURE OF THE MACRONUCLEUS IN TOKOPHRYA INFUSIONUM

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Plate 143

Among Protozoa, the group Ciliophora (except Opalinidae) is peculiar in having a nuclear apparatus composed of a macronucleus and a micronucleus. It has been assumed that this nuclear dimorphism provides for a functional separation, the micronucleus serving for genetic and the macronucleus for metabolic functions only. Results of several observations and experiments during the last years indicate, however, that the macronucleus may perform both functions (1–4) and that its absence may prevent conjugation (5).

The macronucleus in *Tokophrya infusionum*, as described in previous papers (6–8), is composed of numerous dense Feulgen-positive chromatin bodies suspended in a fairly homogeneous matrix and surrounded by a double perforated membrane. The chromatin bodies are large enough (0.5 μ in diameter) to be seen clearly in the light microscope and therefore easy to identify in electron microscopy (9). At high resolutions they comprise a dense spongework of very thin filaments resembling closely electron micrographs of metaphase chromosomes in metazoan cells (10). This striking similarity together with their Feulgen-positive reaction strongly suggest that the chromatin bodies represent chromosomes. Similar views have been already expressed by other investigators (11, 12).

It was noted earlier (8) that the usually spherical chromatin bodies may change considerably during the life span of *Tokophrya*, and most significantly in older individuals. The changes seem to coincide with the occurrence of hemixis (13), a phenomenon in which the macronucleus undergoes division or fractionation not accompanied by cytoplasmic fission. At this time among chromatin bodies of the usual spherical shape and size, some were observed to be much larger and to contain a central cavity. Electron micrographs of the dense, Feulgen-positive, hollow bodies revealed in cross-sections a fine honeycomb structure and in longitudinal sections an array of parallel lines, about 120 A thick. The spacing of the lines was about 230 A. In more recent studies the same kind of defined structure was found in organisms which had been overfed (14–17).

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Materials and Methods

For these experiments cultures of young *Tokophrya* were fed living ciliates (*Tetrahymena*) 2 to 3 times daily for a period of 4 days and thereafter collected and prepared for electron microscopy in the same way as described in the previous paper (8). They were fixed in 1 per cent OsO_4, buffered at a pH 8.5 (18) for 30 minutes, dehydrated with alcohol, embedded in *n*-butyl methacrylate and left for 24 hours in the incubator at 45°C. The material embedded in plastic was used for light and electron microscopy. Sections about 50 mμ thick were cut (19) and examined with the RCA model EMU-2C microscope. For light microscope examination, sections about 2 μ thick were cut and after the removal of the methacrylate stained with the Feulgen technique.

OBSERVATIONS AND COMMENTS

Sections cut through macronuclei of overfed organisms and stained with Feulgen show that some of the chromatin bodies are very large and contain an internal cavity (Fig. 1), resembling the hollow granules found in the macronuclei of older organisms. In electron micrographs of thin sections such bodies show in cross-section the same ordered structure as described for the hollow granules (8), *i.e.*, a close packing of less dense spots in a dense matrix. The structure resembles a honeycomb or a fine lattice composed of light areas surrounded by dark walls (Fig. 4). The walls measure about 120 A in thickness, the light spots about 230 A in diameter; the dimensions are the same as found in the macronuclei of older organisms (8).

It was noted further that the chromatin material appears frequently in the form of large irregular masses, with no evidence of separate chromatin bodies. Fig. 2 represents a light microscope picture of a section through such a macronucleus stained with Feulgen. The macronucleus seems to be changed almost beyond recognition. Instead of separate bodies as in Fig. 1, the chromatin material appears as a mass most probably formed by fusion of the chromatin granules. The same kind of macronuclei in which the chromatin material is not differentiated into separate bodies may be easily identified in electron micrographs of thin sections from the same block. One such is depicted in Fig. 3. The predominance of the very well organized honeycomb structure in this mass is striking, and there are only a few spots in which the sponge-like structure characteristic of the regular chromatin body may be seen. Both structures are contiguous in the same mass. The thickness of the dense walls in the honeycomb structure is about 120 A, the same as found in the older organisms. The dimension of the light areas surrounded by the walls changes from place to place, but this may be due to slight changes in the angle of sectioning or compression during sectioning.

In sections of some macronuclei it is possible to see the honeycomb structure side by side with the parallelly aligned structure (Fig. 5). This provides clear evidence that both images (the honeycomb and parallel lines) belong to the same structure seen at different planes of section. Oblique sections produce all

expected deviations between the regular honeycomb and the array of parallel lines.

The abundance and frequency with which the ordered structure appears in the macronuclei of overfed organisms permit its clearer and better three-dimensional reconstruction. It is assumed that it might be composed of units having the form of very fine, long, closely packed cylinders or hexagons, each being surrounded by a dense wall with a less dense core inside. Such a structure would appear in cross-sections as a honeycomb and in longitudinal sections as parallel lines, representing the walls of the cylinders, or hexagons. In the structure described above one would expect to see separate walls for each unit. It must, however, be pointed out that so far no clear evidence was found for separate walls. It might be that the tightness of the neighboring walls is responsible for this. In this case thinner sections and higher resolution could be helpful.

Since the regular structure is so far the first demonstration of such a degree of order in a component of the nucleus, it seems worth while to investigate its nature and meaning. For this purpose, the finding of the close relationship between overfeeding and the appearance of the organized structure in the macronucleus should prove of considerable value, for it permits one to obtain this configuration at will and in striking amounts.

SUMMARY

In a previous paper (8) an organized structure was described in the macronuclei of certain old organisms of *Tokophrya infusionum*. It was found that the same honeycomb structure appears in great abundance in the macronuclei of overfed organisms. This permitted a better three-dimensional reconstruction of the described structure.

Since the defined structure may be experimentally induced, it offers an opportunity for further more detailed studies as to its nature and meaning.

BIBLIOGRAPHY

1. Schwartz, V., Konjugation micronukleoloser *Paramecien*, *Naturwissenschaften*, 1939, **27**, 724.
2. Chen, T. T., Conjugation of *Paramecium bursaria* between animals with very different chromosome numbers and between animals with and without micronuclei, *Proc. Nat. Acad. Sc.*, 1940, **26**, 243.
3. Balamuth, W., Regeneration in Protozoa: A problem of morphogenesis, *Quart. Rev. Biol.*, 1940, **15**, 290.
4. Sonneborn, T. M., Recent advances in the genetics of *Paramecium* and *Euplotes*, *Advances Genet.*, 1947, **1**, 263.
5. Sonneborn, T. M., Macronuclear Control of the Initiation of Meiosis and Conjugation in *Paramecium aurelia*, *J. Protozool.*, 1955, **2**, 12.
6. Rudzinska, M. A., and Porter, K. R., The fine structure of *Tokophrya infusionum*

with emphasis on the feeding mechanism, *Tr. New York Acad. Sc.*, 1954, **16,** 408.

7. Rudzinska, M. A., and Porter, K. R., The structure and behavior of the macronucleus in *Tokophrya infusionum* as revealed by light and electron microscopy, *J. Protozool.*, 1955, **2,** 5.
8. Rudzinska, M. A., and Porter, K. R., Observations on the fine structure of the macronucleus of *Tokophrya infusionum*, *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 421.
9. Rudzinska, M. A., The use of OsO_4 as fixative for Feulgen-stained preparations, *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 472.
10. Porter, K. R., Changes in the cell fine structure accompanying mitosis, *Proc. VIII Internat. Cong. Cell Biol.*, Leiden, September, 1954.
11. Grell, K. G., Die Struktur des Makronucleus von Tokophrya, *Arch Protistenk.*, 1953, **98,** 466.
12. Kimball, R. F., The structure of the macronucleus of *Paramecium aurelia*, *Proc. Nat. Acad. Sc.*, 1953, **39,** 345.
13. Diller, W. F., Nuclear reorganization processes in *Paramecium aurelia* with descriptions of autogamy and "hemixis," *J. Morphol.*, 1936, **59,** 11.
14. Rudzinska, M. A., The influence of the amount of food on the reproduction rate and longevity of a suctorian (*Tokophrya infusionum*), *Science*, 1951, **113,** 10.
15. Rudzinska, M. A., Overfeeding and life span in *Tokophrya infusionum*, *J. Gerontol.*, 1952, **7,** 544.
16. Rudzinska, M. A., Giant individuals and vigor of populations in *Tokophrya infusionum*, *Ann. New York Acad. Sc.*, 1953, **56,** 1087.
17. Rudzinska, M. A., Differences between young and old organisms in *Tokophrya infusionum*, *J. Gerontol.*, 1955, **10,** 469.
18. Palade, G. E., A study of fixation for electron microscopy, International Conference on Electron Microscopy, 1954, London, *Proc. Roy. Micr. Soc.*, in press.
19. Porter, K. R., and Blum, J., A study of microtomy for electron microscopy, *Anat. Rec.*, 1953, **117,** 685.

PLATE

EXPLANATION OF PLATE 143

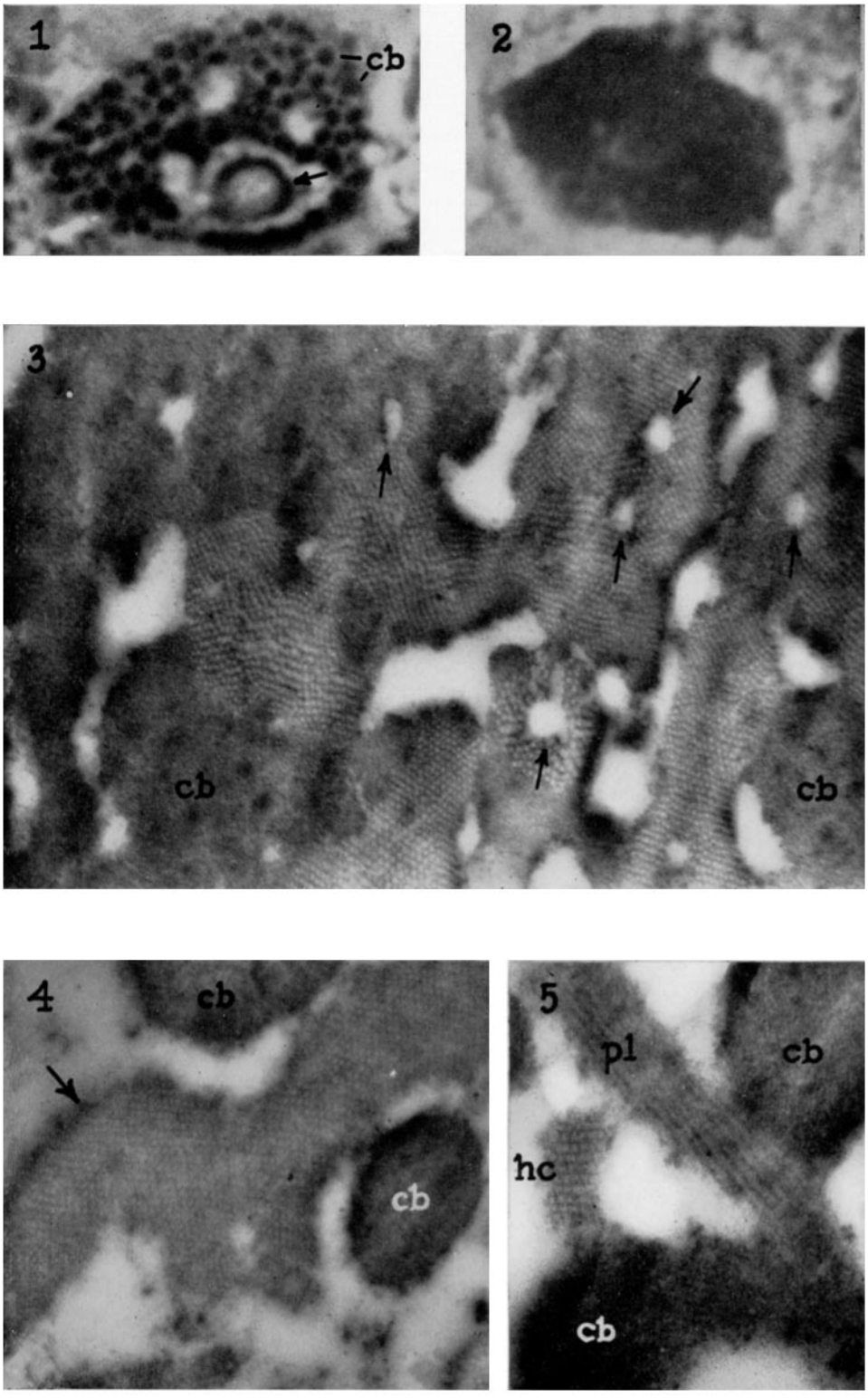
FIG. 1. Photomicrograph of a section 2 μ thick, through the macronucleus of an overfed organism. The microorganisms were fixed in 1 per cent OsO_4 buffered at 8.5 pH and embedded in methacrylate. After sectioning, the methacrylate was removed and the preparation stained with the Feulgen reagent. Among regular chromatin bodies (*cb*) one, at arrow, is enormously large and has a cavity inside. × 3,600.

FIG. 2. Photomicrograph of a 2 μ thick section through the macronucleus of an overfed organism, stained with the Feulgen technique and prepared in the same way as in Fig. 1. The chromatin material is fused to one mass with no evidence of the single, separate chromatin bodies which can be so clearly seen in Fig. 1. × 3,600.

FIG. 3. Electron micrograph of thin section through the macronucleus of an overfed organism from the same block as Fig. 2. The honeycomb structure extends throughout the macronucleus; there are only a few spots where the chromatin material shows the sponge-like and rather homogeneous character of the regular chromatin body (*cb*). In some places (at arrows) there are spaces, which probably represent residual central cavities of the hollow granules. These latter are fused together to form the irregular chromatin mass evident in the electron micrograph. × 34,800.

FIG. 4. Electron micrograph of thin section through a part of the macronucleus of an overfed organism. The very large chromatin body with a cavity inside, at arrow, shows the honeycomb structure, while others (*cb*) regular in shape and size look homogeneous. × 45,240.

FIG. 5. Electron micrograph of part of the macronucleus from an overfed organism showing in the same section parallel lines (*pl*), the honeycomb (*hc*), and the sponge-like structure of the regular chromatin body (*cb*). × 54,000.



(Rudzinska: Macronucleus in *Tokophrya infusionum*)