

OBSERVATIONS ON THE FINE STRUCTURE OF THE
MACRONUCLEUS OF TOKOPHYA INFUSIONUM

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PLATES 104 TO 107

(Received for publication, July 7, 1955)

INTRODUCTION

As emphasized in a number of previous publications, the protozoan *Tokophrya infusionum* provides unusual opportunities for studying phenomena associated with aging in a single celled organism (1-5). The reason is that the cell retains its identity throughout reproduction (accomplished by endogenous budding) and survives for from several days to 2 or 3 weeks. During the course of some studies on morphological changes accompanying this aging process, the macronucleus has been examined and a few observations made on its fine structure that seem of sufficient interest to report at this time. The interest resides chiefly in the demonstration of morphological order in certain types of chromatin granules and apparently related structures.

It should perhaps be noted at the outset that the macronucleus of *Tokophrya* constitutes only part of the nuclear apparatus in Suctoria, the rest consisting of one or more micronuclei. This nuclear dimorphism is characteristic for the entire group of Ciliophora to which the Suctoria belong. Both types of nuclei have a common micronuclear origin from the synkaryon, formed during conjugation. It is therefore reasonable to assume that at the outset both types are genetically and cytologically similar (6). During development however, the two nuclei diverge, and as a result two distinct bodies are formed, differing not only in their dimensions but also in structure and behavior. The micronucleus diminishes in size, divides by mitosis during budding and contributes to the formation of a new synkaryon during conjugation. The macronuclear anlage, on the other hand, increases greatly in size, acquires dense Feulgen-positive chromatin granules, and develops into a conspicuous body in the organism. It divides amitotically during budding and disintegrates during conjugation. On the basis of these striking differences, the classical concept assigned genetic functions to the micronucleus and somatic or metabolic functions to the apparently polyploid macronucleus.¹

Several important observations made over the last few years have, however, provided reason to question this functional segregation. Quantitative

* Supported by a grant from the National Heart Institute H-1350 (C2).

¹ Sonneborn considers that the macronucleus of *Paramecium aurelia* is composed of many subnuclei (26).

cytochemical investigations made by Moses (7) have shown, for example, that both nuclei in *Paramecium caudatum* are chemically similar since the ratios of their DNA, RNA, and protein contents are the same (1:2:20). This was somewhat anticipated in view of the common origin of both nuclei. Then also, evidence was found that amiconucleated races and species of ciliates are able to divide and some even to conjugate (8-10). Furthermore Sonneborn in his work on *Paramecium aurelia* demonstrated that the phenotype is controlled exclusively by the macronuclear genes (26). Finally, observations made on organisms regenerating after microsurgery show that in many cases parts containing only the macronucleus are able to regenerate, whereas parts with only the micronucleus disintegrate (30). All these observations suggest that the macronucleus is essential for the continued existence of the organism. It is most unusual in always containing large numbers of chromatin granules having the staining properties (and other characteristics noted below) of metaphase chromosomes.

Materials and Methods

Tokophrya infusionum used for these studies has been kept in culture since 1948 and is one of several strains derived from ponds in the vicinity of Laurelton, New York.² Bacteria-free stock cultures were maintained in screw-capped tubes in a 0.05 per cent yeast medium (11). The food organism, axenic *Tetrahymena pyriformis* (12) grown in a proteose-peptone agar medium, was introduced into the cultures every 2nd day. Subculturing was performed on an average of once a week. The organisms reproduced asexually, *i.e.* by *endogenous* budding, and in no instance has conjugation been noted.

For thin sectioning and electron microscopy, the sessile organisms were scraped from the walls of the culture tube and concentrated in the bottom of the tube by a light centrifugation. They were thereafter fixed in 1 per cent OsO₄ at a pH 8.5 (13) for 30 minutes, dehydrated with alcohol, and embedded in *n*-butyl methacrylate. The centrifugation was repeated before each change of fluid. Sections about 50 m μ thick were cut (14) from the resulting plastic blocks and examined with either an RCA, EMU-2c or Philips EM-100 microscope.

For light microscopy, unsectioned and paraffin-sectioned material was used. Whole mounts of *Tokophrya* were prepared from small numbers cultured in hanging drops on coverglasses in Maximow slides, according to the methods used in tissue culture. Every 2nd day food was introduced into such microcultures. When a suitable number of organisms had developed they were fixed in vapors of 2 per cent OsO₄, or in Carnoy's fluid (3 parts absolute alcohol + 1 part glacial acetic acid). Thicker sections for light microscopy were cut from paraffin-embedded material which had been fixed either with 1 per cent OsO₄ at pH 8.5 or with Carnoy's fluid. This fixed material was subsequently stained with either Feulgen's fuchsin-sulphurous acid reagent or aceto-carmine, following Geitler's aceto-carmine procedure (15).

OBSERVATIONS

The macronucleus of *Tokophrya infusionum* measures 5 to 20 μ in diameter and the small micronucleus about 1 to 2 μ in diameter. During reproduction (*endogenous* budding) a half of the micronucleus and only a small part of the polyploid macronucleus pass to the offspring. The macronucleus is spherical in the young but oblong to irregular (loboid) in the older organism. In the light microscope it appears to be made up of many (50 to 300) "chromatin"

² The cultures were obtained through the courtesy of Dr. Daniel M. Lilly.

bodies, averaging half a micron in diameter, suspended in a homogeneous matrix. These are evenly dispersed except along a core or central portion of the macronucleus where they are arranged in rows lateral to long, granule-free regions of achromatic material (Fig. 1).

The electron microscope image of a thin section through the macronucleus shows it to contain a number of round, very dense and solid bodies embedded in a fairly homogeneous matrix of much lower density (Fig. 2). It is clear from their number, size, shape, and distribution that the electron-dense elements represent sections through the highly refractile bodies of the light microscope image. They vary greatly in diameter partly because the segment in a thin section (0.05μ thick) may include far less than the diameter of the granule. The larger ones (equatorial segments) are generally regular in outline and measure about 0.5μ .

The matrix material of the macronucleus is considerably less dense than the contained granules and is fairly homogeneous. The long streaks of achromatic material, visible in the living cell (Fig. 1), appear as large granule-free areas in the micrograph shown in Fig. 2. The material contained within them is not greatly different in appearance from the matrix in other parts of the macronucleus. Where, however, the plane of section more nearly coincides with the long axis of these regions, the material constituting them appears finely fibrous.

It is evident from the electron micrographs that the macronucleus is surrounded by a dense line, representing the nuclear membrane. At higher magnifications this is found to be double and to be penetrated by numerous pores, similar to those found in other protozoan and metazoan cell nuclei (16-18).

The chromatin bodies, even at fairly high magnifications, show no striking evidence of order in their structure. They appear instead to have a dense, spongy character, homogeneous throughout (Fig. 3). It is probable, on the basis of structure preserved elsewhere by identical preparation procedures, that the sponge-like structure closely approximates the native distribution of component elements in these bodies, but this assumption requires further investigation. In the structure, or lack of it, shown they resemble very closely the appearance of metaphase chromosomes of higher forms (19). They likewise show no peripheral differentiation that can be taken as representing a membrane.

As noted above, the macronucleus of the living cell shows dense granules (Fig. 1) similar in size, number, and distribution to those in the electron micrographs (Fig. 2). Fixed preparations stained with aceto-carmine (Fig. 6) or Feulgen leuco-fuchsin (after acid hydrolysis) also show the same elements (Figs. 4 and 5). It is evident from the latter that the granules contain the DNA characteristic of chromosomes, a conclusion arrived at earlier by Jiroveč (20).

While these granules represent the dominant component of the macronu-

cleus, they are not the sole element encountered. In older cells, especially, it is not uncommon to find granules with central cavities (Fig. 4). These are easily recognized in preparations stained either by Feulgen's method (Fig. 4) or with aceto-carmin (Fig. 6) and have been reported by other investigators (21, 22). In their occurrence in older cells they coincide with an unusual behavior of the macronucleus termed hemixis (23) in which the macronucleus undergoes repeated divisions without accompanying fissions of the cell (Fig. 5). The phenomenon is thought to accompany some spacial reorganization of the macronucleus resulting probably in a revitalization (as in autogamy (24)) of organisms in old or aging clones (25). Beside the hollow forms or ring-like granules, as they appear in certain focal planes, it is common for Feulgen-stained preparations of these older organisms to show other unusual and irregularly shaped masses of Feulgen-positive material (Fig. 5).

The same unusual forms of Feulgen-positive material may be identified by their shapes in electron micrographs of sections of comparable organisms. The hollow granules appear in thin sections as ring-shaped profiles of material having a density not unlike that of the homogeneous granules (Fig. 7). They are usually larger than the solid granules (evident also in the light microscope image) and differ also in their fine structure. In micrographs of adequate resolution it is occasionally possible to see a fairly high degree of order. This appears, in cross-section, as a close packing of lighter spots in a matrix of denser material. Thus the structure resembles most closely a honeycomb. The lighter areas measure about 220 Å in diameter and the entire structural unit, wall and enclosed less dense material, about 350 Å. The order is not perfect, however, since in the majority of instances examined some irregularities are apparent.

In other sections through granules of comparable size, also possessing a less dense or hollow center, the structural order evident has appeared as a series of parallel lines, more or less evenly spaced (Fig. 8). The greatest distance between them measures about 280 Å. Such images have been taken to represent longitudinal sections of the structure which appears as a honeycomb in cross-section.

The other structures of exceptional interest encountered in preparations from older cells are less sharply defined than the hollow granules. Nonetheless they seem to be related because of their similar fine structure. The components referred to are wispy condensations of dense material which sweep irregularly through the achromatic matrix of the macronucleus. They are like clouds of denser material, sometimes extending over several microns of the plane of section (Figs. 8 and 9). Structural order is frequently present in such masses, especially in the more central parts. This is to say that the ordered regions may grade into unordered peripheral material of lesser density existing, however, as part of the same wispy mass.

In longitudinal section the fine structure of these condensations usually

appears as an array of evenly spaced (230 Å) lines (Fig. 10). The lines are not sharp or distinct like the edge-on images of membranes but appear somewhat fuzzy and irregular. In cross-section they have the honeycomb configuration (Fig. 11) encountered in cross-sections of the hollow chromatin granules. The resolutions available have not defined the fine structure of any of the walls of the honeycomb, so it is impossible to say whether each is double and so whether the structure represents a close packing of tiny cylinders. The impression is, however, that there is no such separation. As in the case of the longitudinal images, a slight condensation of unorganized material seems to surround each strand of ordered material. Images besides these encountered in the micrographs in Figs. 10 and 11 are readily interpreted as representing oblique sections through these wispy structures.

DISCUSSION

The foregoing is an account of some limited observations on components of the macronucleus of *Tokophrya infusionum*. Many questions regarding the relationship of the structures observed to one another and to the life and reproductive cycle of the organism remain to be answered. For the moment the chief point of interest is the demonstration of clear cut order in certain components of the macronucleus. The elements showing this order seem moreover to be related to the Feulgen-positive chromatin bodies of the nucleus. It thus appears, that at least during limited phases of formation or breakdown, the DNA-containing elements of the nucleus possess a high degree of ordered structure at the macromolecular level, order which can be preserved by OsO₄ fixation and made available for electron microscopy. The basis for these conclusions requires some discussion.

As mentioned earlier, chromatin bodies, commonly found in the macronuclei of Protozoa, have been described as Feulgen-positive (20) and considered to represent chromosome equivalents (27-29). There is no excuse for regarding these in *Tokophrya* as being atypical. They likewise stain intensely by Feulgen's method and, in their fine structure, resemble metaphase chromosomes of other organisms (19). It seems entirely reasonable therefore to consider them as belonging to the general class of chromatin granules encountered elsewhere and most especially in Protozoa.

The point of interest in the above observations resides not so much in the dense granules as in the nuclear components apparently related to them which show a well defined structural order. What then is the evidence of relationship? It appears in the first place that they all stain similarly by Feulgen's test. The hollow bodies are more generally found in older organisms and differ from the solid granules in size and in possession of an ordered structure. It is not evident, however, from observations made thus far whether the hollow units represent a special form of chromatin granule or simply a precursor or derivative of it. A close relationship between the wispy material

and the hollow forms is perhaps better founded in that, besides staining similarly, they show the same honeycomb-like structure.

Such static similarities give unfortunately very slight clues to the sequences if any, through which one component is transformed to the other. Do the chromatin bodies undergo some form of breakdown or degradation in the older organisms, first to a vacuolated but finely ordered structure and thence by further swelling and degradation to the relatively ill defined but well ordered strands? In his claim that the vacuolated chromatin granules are pathological, Collin (21) supports this view.

The reverse sequence seems, however, more likely. In this the wisps of ordered material might originate or "polymerize" from condensations of nucleoprotein in the matrix of the nucleus by a process perhaps not unlike that which goes on in early prophase in metazoan nuclei. These in turn could be supposed to become more discretely defined as hollow bodies (vacuolated form of chromatin body) and thereafter give rise perhaps by budding to the typical chromatin granules. These phenomena, especially characteristic of cells in hemixis, may provide extra chromatin bodies for the precocious division of the macronucleus which takes place at this time.

With so little knowledge of the significance of these organized components to draw on, it is not possible to interpret the meaning of their fine structure. It is clearly evident that they consist essentially of a dense material arranged as a continuum or matrix around long cylinders of less dense material so that, as mentioned above, the whole structure resembles a honeycomb in the electron microscope image. As far as is known, this is the first time a structure of exactly this nature has come to the attention in electron microscopy. Likewise, it represents the first demonstration of this degree of order in components of the nucleus. The staining properties of these various elements lead one to believe that the material of greater density consists in part of desoxyribose nucleic acid but the reason for the arrangement is obscure at this time.

One of the more significant aspects of these findings evident now is the demonstration that OsO_4 can preserve order in chromatin-rich structures. It has seemed heretofore that a reasonably faithful fixation of the nucleus and its contents was being achieved, but there was room for doubt. Micrographs of chromosomes with their homogeneous granular or finely filamentous appearance, and the absence of visible organization where some had been expected, provided the excuse for the doubt. With the findings presented here, however, it is now reasonable to attach greater significance to the image of the chromatin bodies here and in other material.

SUMMARY

The macronucleus in *Tokophrya infusioformis* is composed of numerous Feulgen-positive chromatin bodies (about 0.5μ in diameter) which appear in

thin sections as a dense spongework, homogeneous throughout. The same appearance characterizes metaphase chromosomes of higher forms.

Some chromatin bodies of the macronucleus were found to possess a highly organized structure in certain old organisms. This structure appears in cross-sections as a honeycomb and in longitudinal sections as parallel lines about 120 Å in diameter evenly spaced (about 230 Å).

As far as is known this is the first time a regular structure has been found in bodies of chromosomal character at the dimensional level presently explored by electron microscopy. The demonstration that OsO₄ can preserve order in chromatin material is another significant aspect of these findings.

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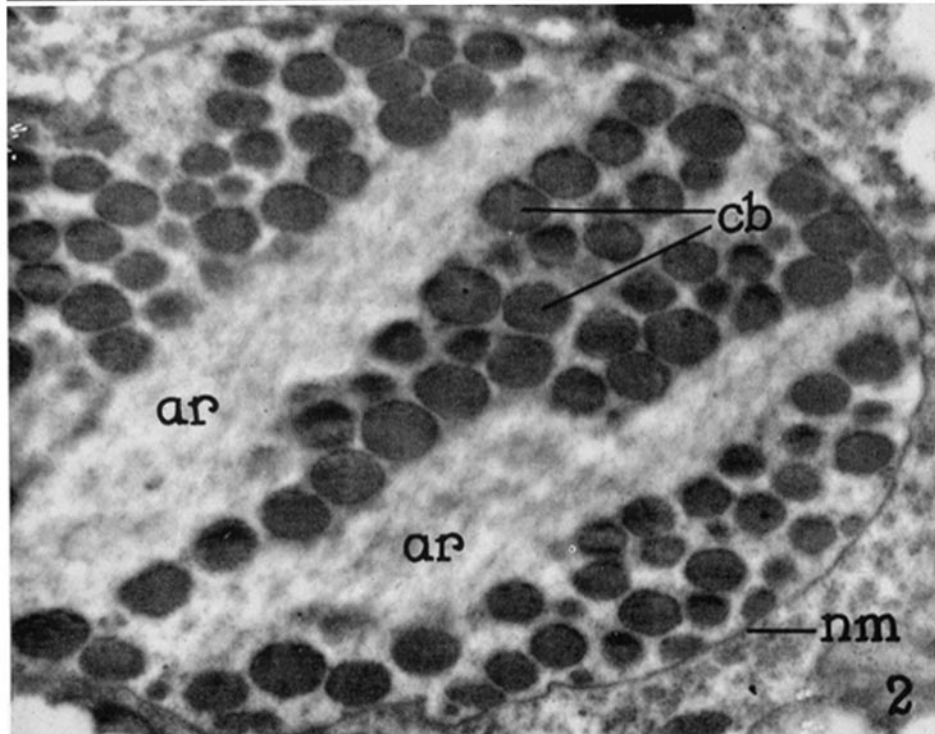
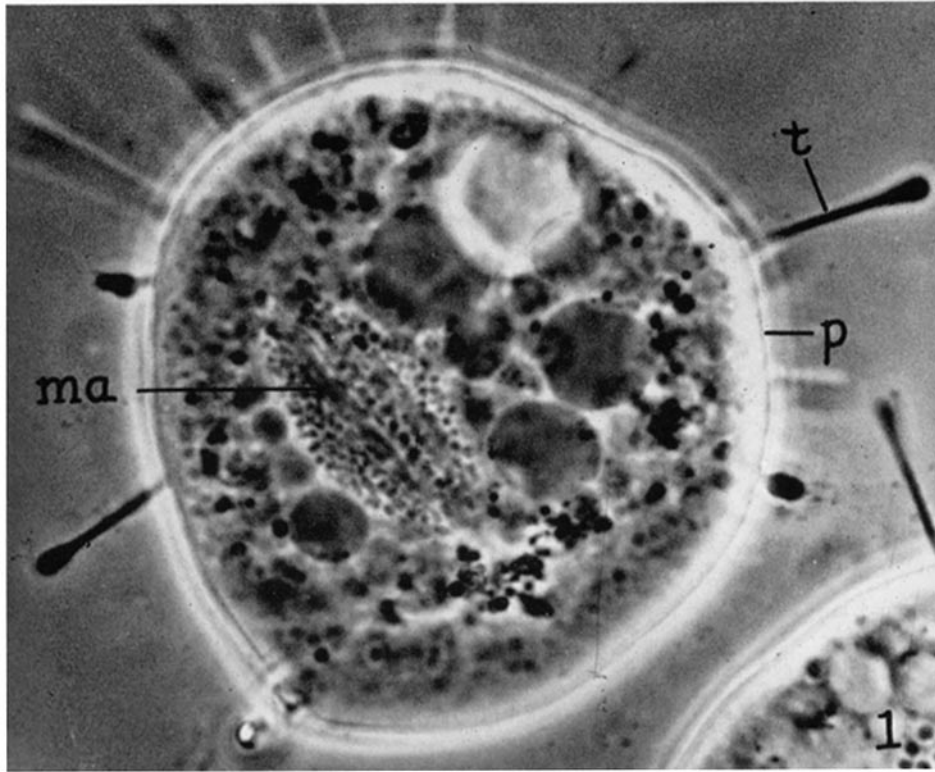
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EXPLANATION OF PLATES

PLATE 104

FIG. 1. Photomicrograph of phase contrast image of living *Tokophrya*. The limits of the cell are marked by the pellicle (*p*). Tentacles (*t*) are evident where they fall within the focal plane. The macronucleus (*ma*) is centrally located and oblong in shape. Its limits are not sharply defined in this image. It contains a large number of refractile granules (chromatin bodies) and a longitudinal organization of these runs from end to end of the nucleus. Long agranular areas separate the rows of granules. Magnification 1960.

FIG. 2. Electron micrograph of thin section through macronucleus of a fairly young organism. The round, dense elements (*cb*) in the micrograph represent sections through chromatin bodies. The less dense matrix around them is, in the plane of section, without evidence of order. It is continuous with the wide agranular regions (*ar*), which seem in most instances to run through the mid-region of the macronucleus. The dense line limiting the macronucleus represents the nuclear membrane (*nm*). Magnification 12,700.



(Rudzinska and Porter: Fine structure of macronucleus)

PLATE 105

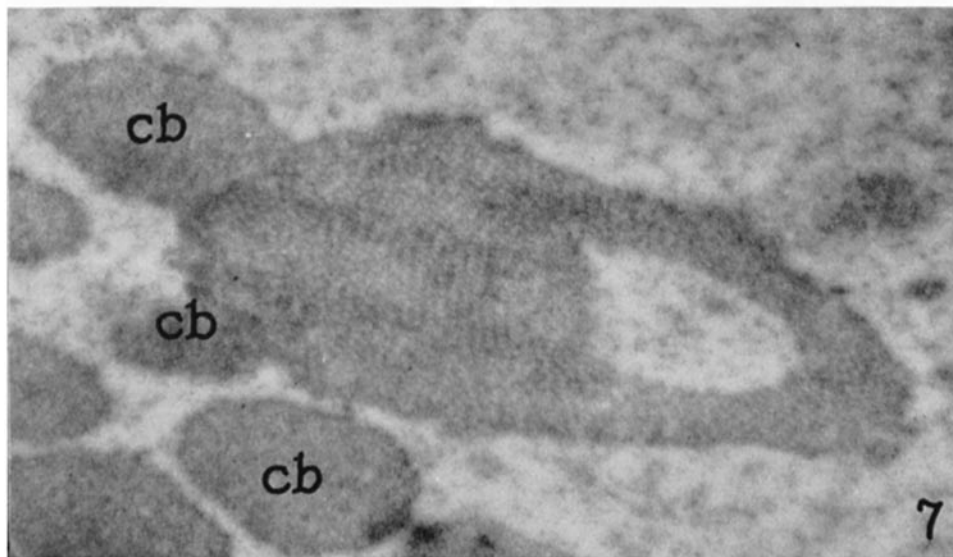
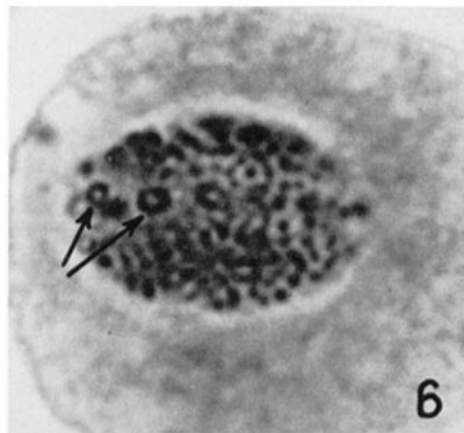
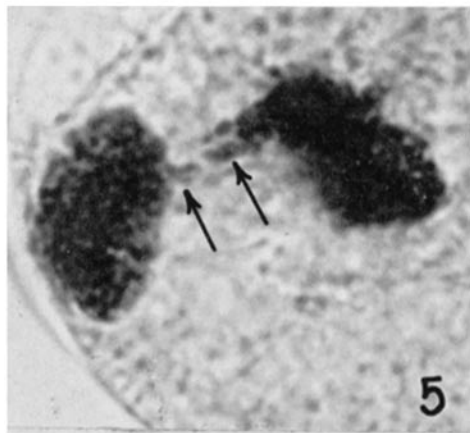
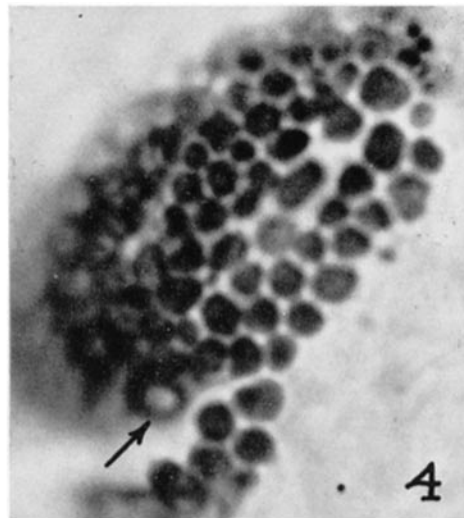
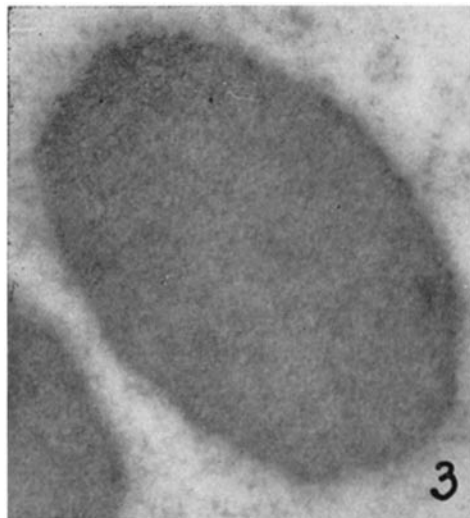
FIG. 3. Micrograph of single chromatin body. The material constituting it is finely granular to finely fibrous and shows no evidence of order in its disposition. Whether the spongy character of the fine structure results from fixation or faithfully represents the native structure is difficult to decide at this time. There is no indication in the image of a limiting membrane or any differentiation at the surface. Magnification 65,700.

FIG. 4. Photomicrograph of Feulgen-stained macronucleus from older organism. The dense bodies are chromatin granules and one at arrow shows definite cavity. Magnification 4100.

FIG. 5. Photomicrograph of Feulgen-stained preparation showing phenomenon of hemixis in *Tokophrya*. Fission of the macronucleus was apparently in progress at the time of fixation. Certain of the elongate bodies (arrows) connecting the two groups of chromatin bodies may be equivalent to organized material of irregular outline pictured in Figs. 8 and 9. Magnification 1730.

FIG. 6. Photograph of phase contrast image of organism stained with acetocarmine following Geitler's method. The dark granules represent chromatin bodies and those at arrows have a cavity inside. Magnification 2940.

FIG. 7. Electron micrograph of section through large chromatin body with central cavity. More usual chromatin bodies in section are indicated at *cb*. The unusual form with central region of lower density is of special interest because of the structural order that it shows. In cross-section, as pictured here, this has the appearance of a fine honeycomb. Small points of less density are surrounded by walls of greater density. The latter material resembles in its density that of the material making up the homogeneous chromatin bodies. The contact between the chromatin bodies at *cb* and the larger organized body is not interpretable at this time. Magnification 33,000.



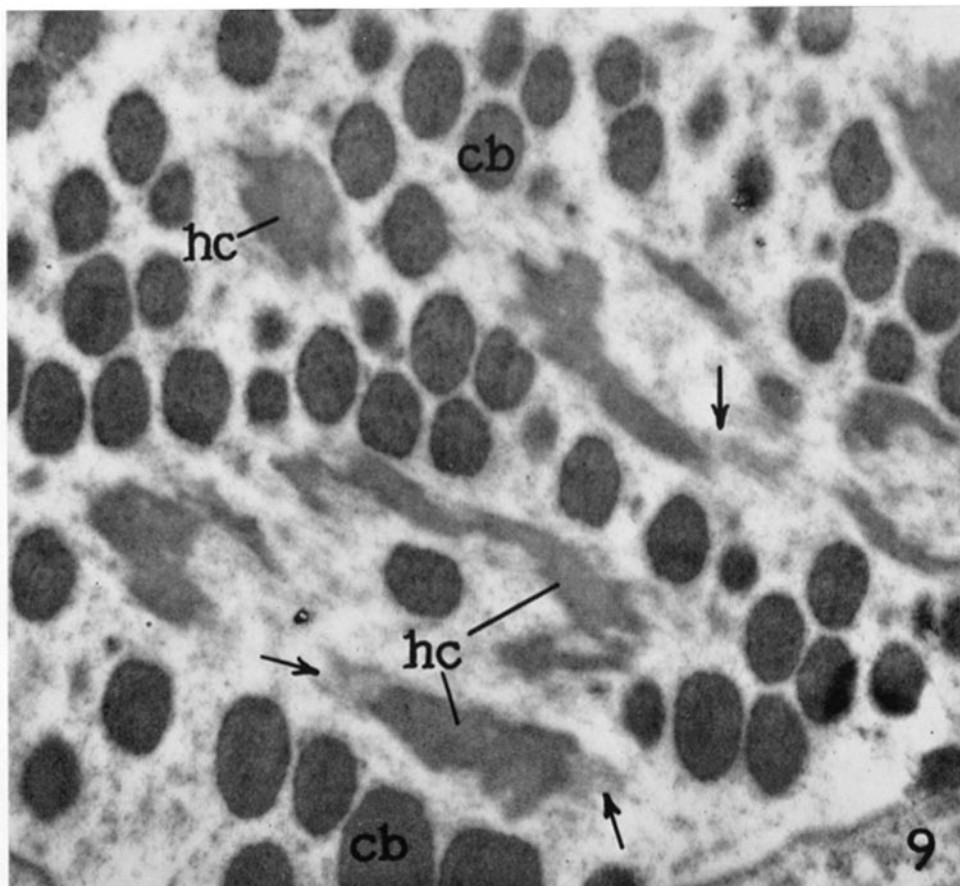
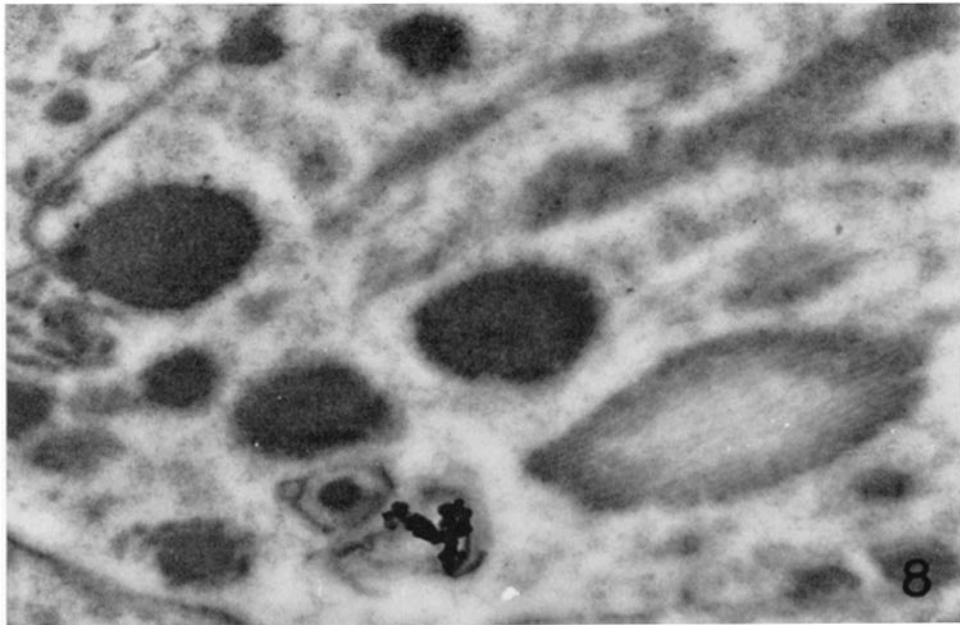
(Rudzinska and Porter: Fine structure of macronucleus)

PLATE 106

FIG. 8. Micrograph of thin section through chromatin body with central cavity, this time showing the longitudinal aspect of the organization evident in the granule in Fig. 7. The fine structure appears as a series of parallel dense lines which are interpreted as representing the walls of the honeycomb. These are not evenly spaced in this instance, presumably because of differences in the relation of the plane of section to the honeycomb organization. Some are 280 A apart, others appear as close as 160 A. It is conceivable that some of this irregularity is a product of compression resulting from sectioning.

The long wispy condensations at the upper right show no clear cut evidence of order in their structure, and in this are similar to material lying peripheral to organized masses in Figs. 9 and 10. Magnification 34,000.

FIG. 9. Low power micrograph of macronucleus from older organism showing distribution of irregular masses (*hc*) of apparently fibrous material suspended in matrix of different character and lower density. In their electron-scattering properties, these irregular masses are not much less effective than the round chromatin bodies. At the margins of these masses, the organized (see Fig. 10) and more dense material shades into an unorganized substance of lesser density (arrows). Magnification 22,500.

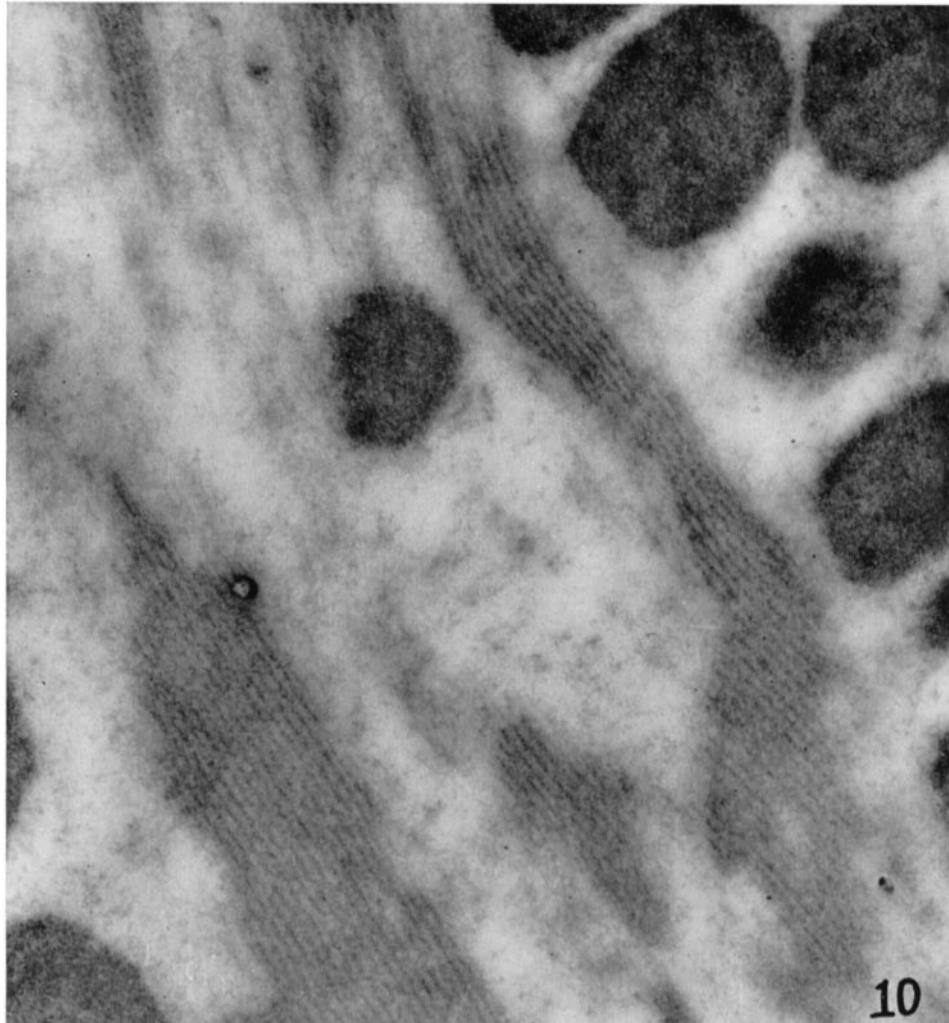


(Rudzinska and Porter: Fine structure of macronucleus)

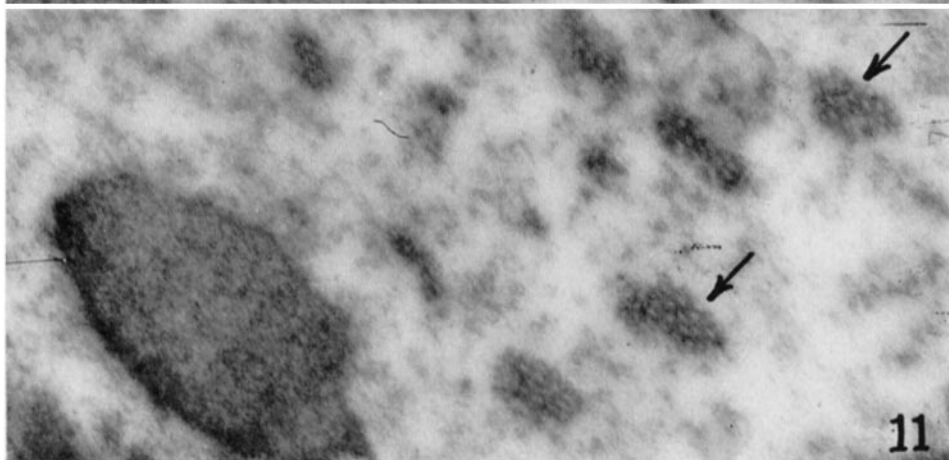
PLATE 107

FIG. 10. Higher magnification of irregular masses shown in Fig. 9. The parallel arrangement of dense lines is clearly shown, and in this instance the lines are evenly spaced at about 230 A. They are more distinct in some places than in others. This is doubtless a reflection of the relation of the plane of section to the honeycomb structure shown in Figs. 7 and 11. It is readily understood that wherever the plane of section passes from the longitudinal axis of the organization, the image of the walls vertical to the section will be altered by the interposition of the walls parallel or nearly parallel to the plane of section. Again attention is directed to condensations of unorganized wispy material lying peripheral to the organized masses. Magnification 55,000.

FIG. 11. Micrograph showing wisps of organized material in cross-section. The honeycomb structure is seen to best advantage at arrows. The lighter spots in the cross-section measure 230 A, the walls separating them, and representing a continuous matrix, vary considerably in thickness but average roughly 120 A. There is a small quantity of fluffy unorganized material around each small mass of honeycomb. Magnification 65,600.



10



11

(Rudzinska and Porter: Fine structure of macronucleus)