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Mitosis in the Flagellate *Trypanoplasma borreli* (Kinetoplastidea: Bodonida)

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Summary

The mitosis in both uninucleate and giant multinucleate *T. borreli* culture forms was studied using transmission electron microscopy. The interphase nucleus is of the vesicular type with one prominent nucleolus and dense peripheral chromatin. Nucleosome-like fibres, occasionally with scattered nodules on them of 21–23 nm in diameter, are found in the karyoplasm. The onset of mitosis is signalled by the decondensation of the peripheral chromatin. No individual chromosomes are visible during the whole division. In the course of mitosis the nucleus elongates, the nucleolus becomes fragmented, the nuclear envelope remains intact, and the kinetochore-like plaques and microtubules appear. At metaphase, the spindle microtubules are connected with fibrillar discs that adhere to the inner envelope membrane near nuclear poles. At anaphase, three main bundles of about 40 microtubules each can be found in the spindle. In giant cells the mitotic figures often lie inside a single outer membrane of the envelope, but at different angles to each other. We infer that mitosis in *T. borreli* involves a combination of a primitive membraneous mechanism of chromosome separation with a more advanced mode of chromosome transmission including spindle microtubules and associated structures. This type of nuclear division can be designated as intranuclear closed orthomitosis without discernible individual chromosomes.

Key words: Kinetoplastida; *Trypanoplasma borreli*; Mitosis; Multinucleate cells.

Abbreviations

C = chromatin
ER = endoplasmic reticulum
F = flagellum
FB = fibrillar block
FD = fibrous disc
K = kinetoplast
KH = kinetochore-like dense plaque
MT = spindle microtubules
N = nucleus
NE = nuclear envelope
NU = nucleolus
OM = outer membrane of the nuclear envelope
P = nuclear pore
R = ribosomes

Introduction

Heretofore many aspects of mitosis in the kinetoplastid flagellates remain uncertain in spite of intensive ultrastructural studies, particularly of parasites belonging to the genera *Trypanosoma* and *Leishmania* [2, 3, 5, 7, 11–13, 20, 21, 26, 28, 29, 33, 35, 38, 39, 42–44]. Only little evidence has been provided on the mitosis of lower trypanosomatids [27, 30–34], and practically nothing is known about the fine structural aspects of nuclear division in bodonids. At the same time, knowledge on the mitosis pattern in bodonids would contribute significantly to the understanding of nuclear organization in the kinetoplastids as a whole.

The aim of this report is to describe, by electron microscopy, the course of mitosis in a parasitic bodonid, *Trypanoplasma borreli*. The general ultrastructure of the flagellate was previously described by several authors [4, 14, 16, 19, 24]. However, none give a detailed description of nuclear division. We also draw attention to mitosis in artificial multinucleate culture forms of *T. borreli*, known to occur under the impact of suboptimal conditions in in vitro growth.

Material and Methods

The T-Pg strain of *Trypanoplasma borreli* Laveran et Mesnil, 1901 was used. This strain was isolated from its leech vector *Piscicola geometra* by Hajdú and Matskási [9]. Parasites were maintained in biphasic blood-agar medium SNB-9 [36] supplemented with vitamins (choline chloride 1 mg, L-inosi-

tol 2 mg, folic acid 1 mg, nicotinamide 1 mg, D-calcium pantothenate 1 mg, pyridoxal-HCl 1 mg, riboflavin 0.1 mg, thiamine-HCl 1 mg per 1 l [18]).

The nuclei and their division were studied in trypanoplasms harvested from the 20th–23rd passages from late logarithmic phase culture. Flagellates were collected by gentle centrifugation, and without washing were fixed with 1.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) at 0 °C for 45 min. Samples were then rinsed three times in 0.1 M buffer containing 5% saccharose and post-fixed in 2% osmium tetroxide for 1 h (0 °C). After dehydration in ethanol and propylene oxide series, the parasites were embedded in an epon-araldite mixture. Thin sections were stained with uranyl acetate and lead citrate and examined in a JEM 100 B or a JEM 100 C transmission electron microscope.

Results

Interphasic nucleus

In culture, *Trypanoplasma borreli* has one nucleus ~2 µm in diameter situated in the anterior part of the cell in the vicinity of the kinetoplast (Figs. 1A, 2). The nucleus has a double-membrane envelope with a broad perinuclear space up to 90 nm in width (Figs. 2, 4). The outer nuclear membrane facing the cytoplasm is covered with ribosomes and frequently is continuous with the endoplasmic reticulum. The nuclear pore complexes have an inner diameter of ~40 nm and an outer diameter of ~70 nm (Figs. 2, 3). In electron micrographs of nuclear cross-sections, 3–4 pores are rarely visible per section but a tangential section represented in Fig. 3 suggests up to 40 pores μm^{-2}.

The nucleus typically contains a single roundish nucleolus in the nucleoplasm (Figs, 2. 5). However, far from being a stable organelle, the nucleolus may exhibit fluctuations in shape, volume and ultrastructural organization of its fibrillo-granular material. The condensed chromatin is located mostly in the peripheral area of the nucleus, just beneath the inner membrane of the envelope (Figs. 2, 4). Moreover, a few dense fibrillar blocks about 0.15 µm in diameter can be frequently found between the nucleolus and the envelope. Seven of these blocks are demonstrated in Fig. 2. Threads about 10 nm thick, occasionally with scattered nodules of 21–23 nm in diameter, can be observed in nucleoplasm (Fig. 4, arrows).

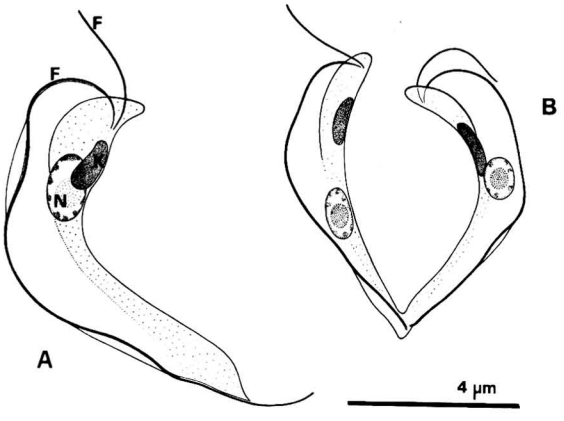
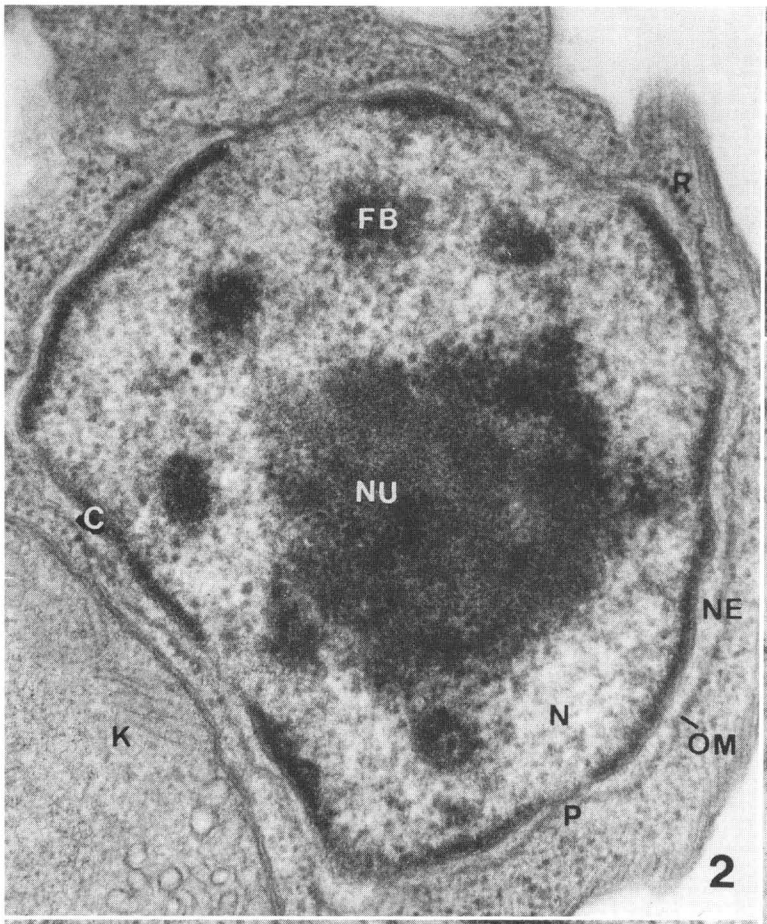


Fig. 1. Schematic drawing of *in vitro* cultivated *Trypanoplasma borreli*. **A.** Non-dividing form, F = flagellum, K = kinetoplast, N = nucleus. **B.** Final stage of cytokinesis. Scale bar = 4 µm.

Giant multinucleate cells

In cell cultures among normal uninucleate *T. borreli*, giant multinucleate flagellates are regularly observed (Figs. 5, 6). The nuclear morphology of some of them is typical for *T. borreli* with peripheral chromatin and a single prominent nucleolus (Fig. 5). The other nuclei differ from cell to cell with regard to the presence or absence of condensed chromatin, assembled nucleolus, or nodular threads (Fig. 6). Apparently, all nuclei in giant cells are interconnected by membranes of rough endoplasmic reticulum (Fig. 5). Moreover, in some cases these nuclei are surrounded by two separate inner membranes and by a single common outer membrane of the envelope (Fig. 6). Thus, the nuclei of the giant cells acquire a partly joint perinuclear space. Another intranuclear feature of the giant cells are tightly coiled membraneous complexes (Fig. 7). Such concentric complexes may occupy a large part of the nucleus. Similar membraneous complexes are also found free in the cytoplasm or in close association with the nuclear envelope (Fig. 7). Sometimes the piles of goffered membranes may occur in the perinuclear space (Fig. 7).

Figs. 2–6. Fine structure of nuclei in normal and giant cells of *Trypanoplasma borreli*. **2.** The interphase nucleus (N), C = chromatin, FB = fibrillar block, K = kinetoplast, NE = nuclear envelope, NU = nucleolus, OM = outer membrane of the nuclear envelope, P = nuclear pore, R = ribosomes, ×60 000. **3.** Nuclear pores (P) on tangential sections, ×56 000. **4.** Nucleosomal filaments with supranucleosomal granules (arrows), C = chromatin, NE = nuclear envelope, ×62 000. **5.** Nuclei (N) in the giant cell which are interconnected by the rough surface membranes of endoplasmic reticulum (ER), C = chromatin, NU = nucleolus, ×42 000. **6.** Apposed nuclei (N) in a giant cell with a partly joint perinuclear space (arrowhead), OM = outer membrane of the nuclear envelope, ×52 000.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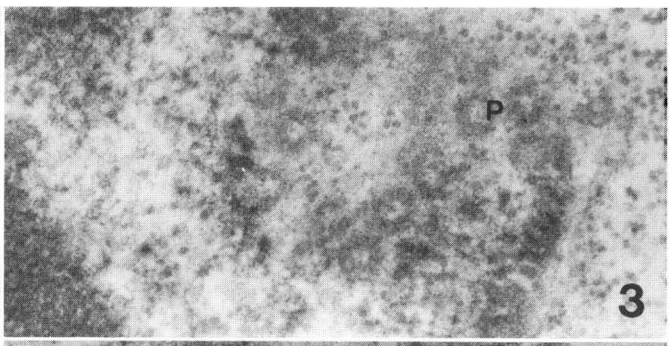
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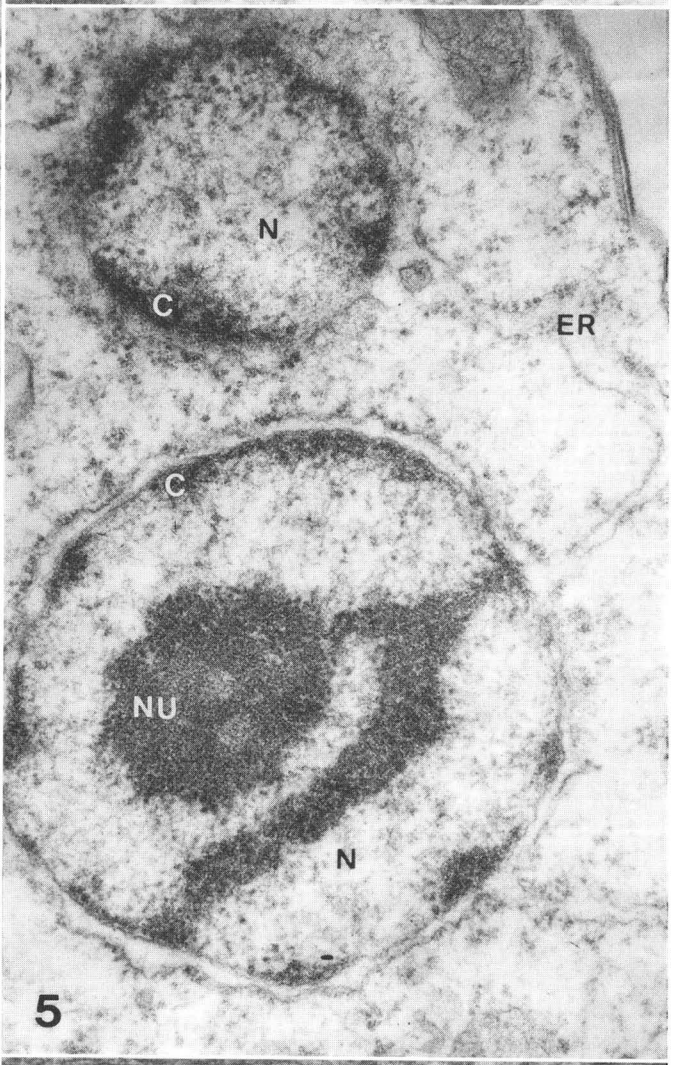
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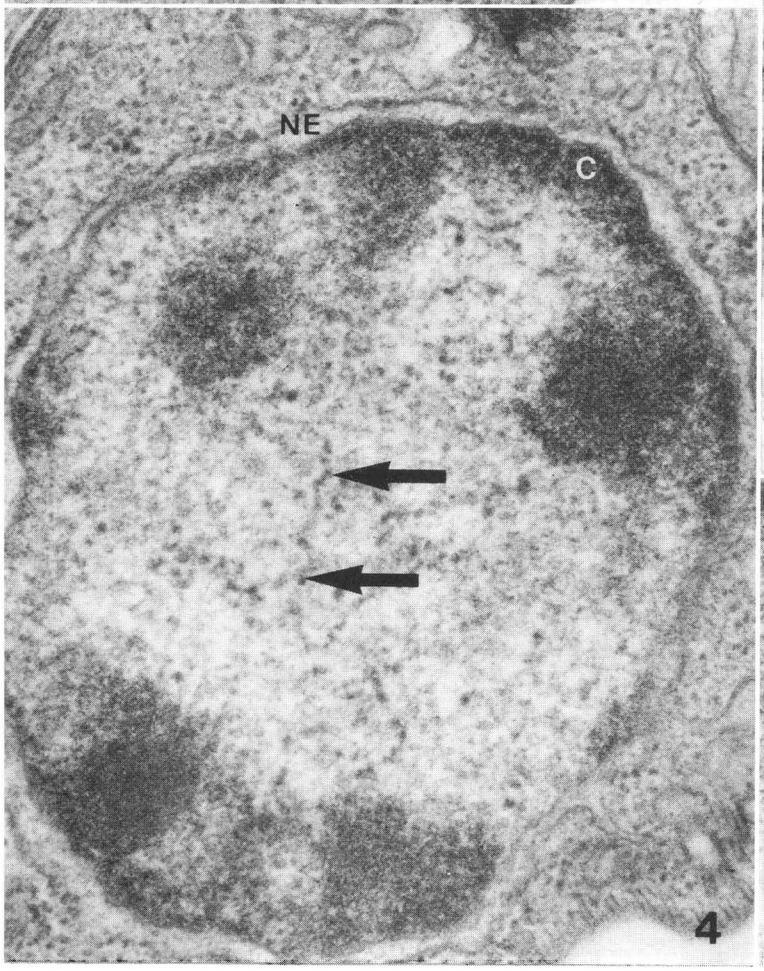
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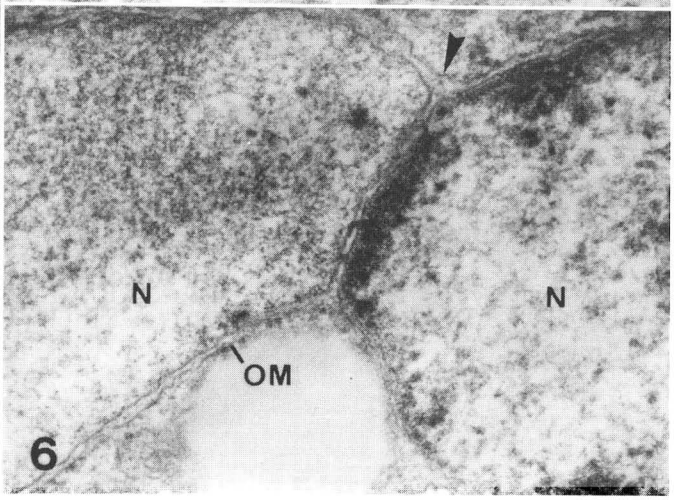
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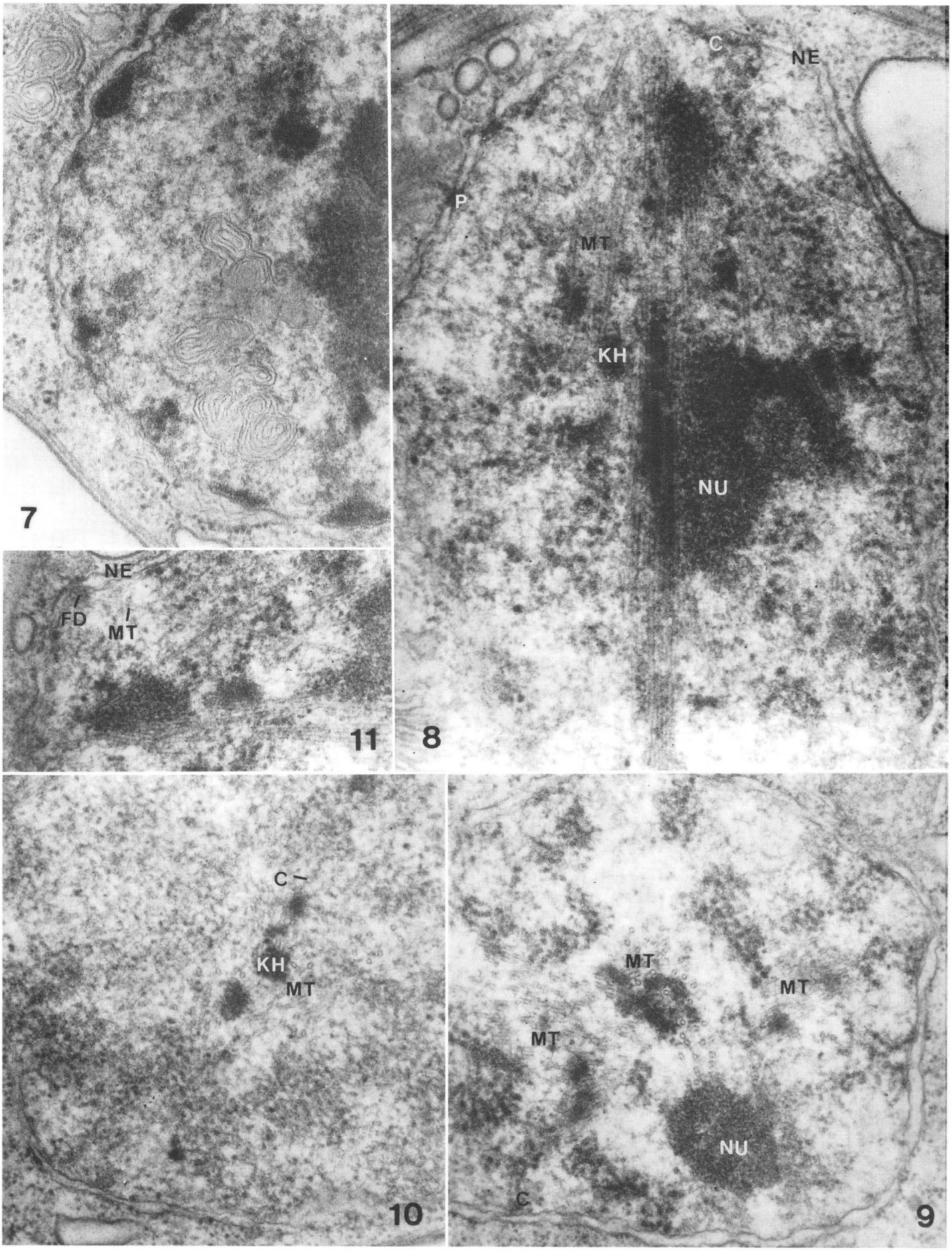
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Mitosis

T. borreli divide by binary fission, which is initiated by the formation of two new anterior flagella [22]. Mitosis takes place, followed by transverse cleavage of the kinetoplast. In *T. borreli* flagellar bases are not found in association with spindle poles. It seems that the basal body cycle has temporal, but not spatial coordination with mitosis. The kinetoplast-flagellar base complex association is also not obvious in *T. borreli*. The division is completed by longitudinal fission of the mother cell into two daughter individuals (Fig. 1B).

For the intranuclear mitosis in trypanosomatids, Solari [28] has suggested four stages: preliminary, equatorial, elongational, and reorganizative. These stages are analagous to prophase, metaphase, anaphase, and telophase of classical mitosis. However, taking into account the specificity of intranuclear mitosis in these flagellates, the former classification is generally accepted. In an attempt to obviate the discrepancies between the trypanosomatid and bodonid mitosis, the terminology used here respects Solari's classification.

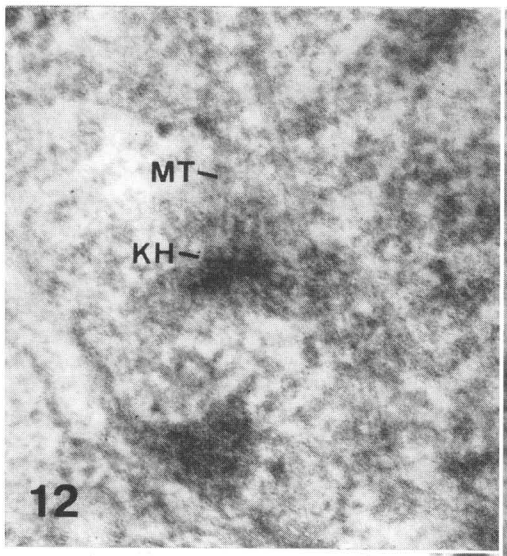
At the preliminary stages of mitosis, peripheral condensed chromatin of *T. borreli* becomes less apparent. At the equatorial stages, the nucleus has an ovoid appearance with slightly flattened poles (Fig. 8). The nuclear envelope remains intact throughout mitosis and with persistent pores and connections with cisternae of the endoplasmic reticulum (Figs. 8, 9, 13, 14). The outer membrane of the envelope bears numerous ribosomes. At this stage the peripheral chromatin disaggregates considerably. Chromatin-like ribbons of 150–190 nm, formed by the loosely packed fibres 10 nm in diameter, can be seen (Figs. 8–10). These ribbons are extended to the central domain of the nucleoplasm to be partly attached to the nuclear envelope. However, no typical discrete chromosomes are seen at any stage of nuclear division. At the equatorial stage, intranuclear microtubules are formed (Figs. 8–10). The two half-spindles of microtubules appear to be distributed in three main bundles of about 40 microtubules each (Figs. 8, 9). At the pole zones, these microtubules converge and seem to interact with the envelope. As seen in Fig. 11, a fibrous disc adheres to the inner membrane of the envelope, giving rise to a single microtubule. This disc may be the attribute of the microtubule organizing centre (MTOC). Individual fibrillar plaque-like structures are positioned inside the bundles of microtubules (Figs. 8, 10, 12). These plaques can send one or two microtubules towards both poles and attach to the chromatin fibers by their lateral margins (Figs. 8, 10, 12). In some profiles the plaques display a trilaminar structure with two outer dense plates and a middle transitional zone (Fig. 12). At the equatorial stage, the nucleolus is highly fragmented, its parts randomly distributed between the intranuclear microtubules (Figs. 8, 9). The plaques are masked by nucleolar fragments, making it difficult to count their actual number.

At a stage that can be classified in the light microscope as the elongational stage, the nucleus extends grossly, becoming first sausage-like and then dumbbell-shaped (Figs. 13, 14). In the sausage-like nucleus, the poles of the former half-spindles seem to become broader and each of their three main bundles join together and/or partly overlap, forming again three bundles, but of extremely long and mostly parallel microtubules (Fig. 13). Sometimes nucleolar fragments can be found inside these main bundles, separating them locally into smaller sub-bundles (Fig. 13). The microtubules in the bundles reveal extensive cross bridges not shown in this paper, especially in the interzonal region. At the extreme ends of the spindle, the microtubules seem to be set against the inner nuclear membrane and appear to push the opposite nuclear poles apart (Fig. 13). Nucleolar components laterally associated with the spindle microtubules are seen to migrate towards the poles. The amount of condensed chromatin attached to the envelope increases and the fibrillar plaques are no longer detected (Fig. 14).

At the reorganizative stage, the daughter nuclei gradually re-establish their interphase profiles (Fig. 15). After breaking down from the polar zones (Fig. 14), spindle microtubules disappear. The nucleolar material aggregates in the centre of the nucleus and the peripheral chromatin layer recondenses (Fig. 15).

Mitosis profiles can be also found in the giant multinucleate *T. borreli* (Fig. 16). It is of interest that in these forms spindles usually lie at different angles to each other and inside the single outer membrane of the nuclear envelope. However, the continuous inner nuclear membranes prevent the adjacent nuclei from direct contacts between their nucleoplasms (Fig. 16). The pe-

Figs. 7–11. Fine structure of nuclei in *Trypanoplasma borreli*. **7.** Membraneous complexes within the karyoplasm, perinuclear space, and free in the cytoplasm, ×56 000. **8.** Longitudinal section through part of the nucleus at the equatorial stage of mitosis, C = chromatin, KH = kinetochore-like dense plaque, MT = spindle microtubules, NE = nuclear envelope, NU = nucleolus, P = nuclear pore, ×55 000. **9, 10.** Cross sections through the nuclei at the equatorial stage of mitosis, C = chromatin, KH = kinetochore-like dense plaque, MT = spindle microtubules, NU = nucleolus, magnifications are ×44 000 and ×48 000, respectively. **11.** Fibrous disc (FD) adheres to the inner membrane of the nuclear envelope (NE) near the spindle pole, MT = spindle microtubules, ×50 000.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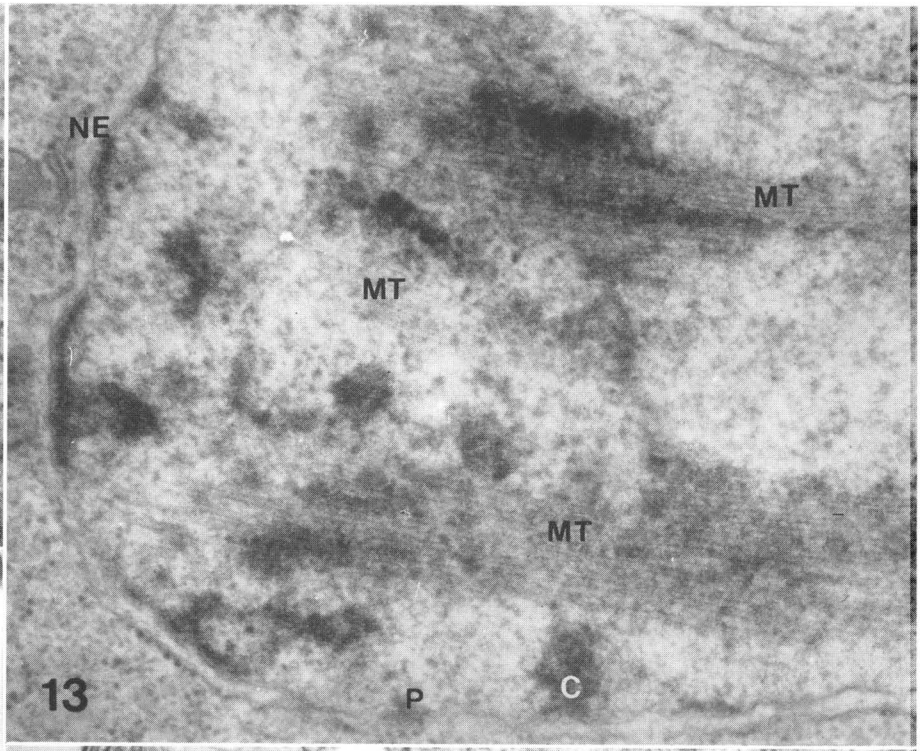
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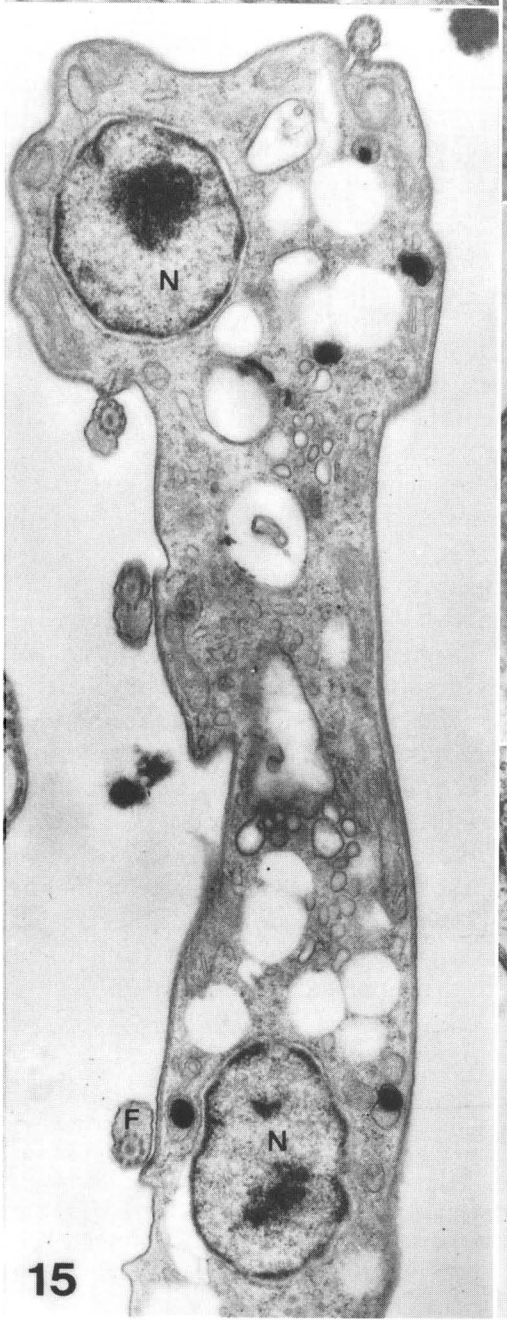
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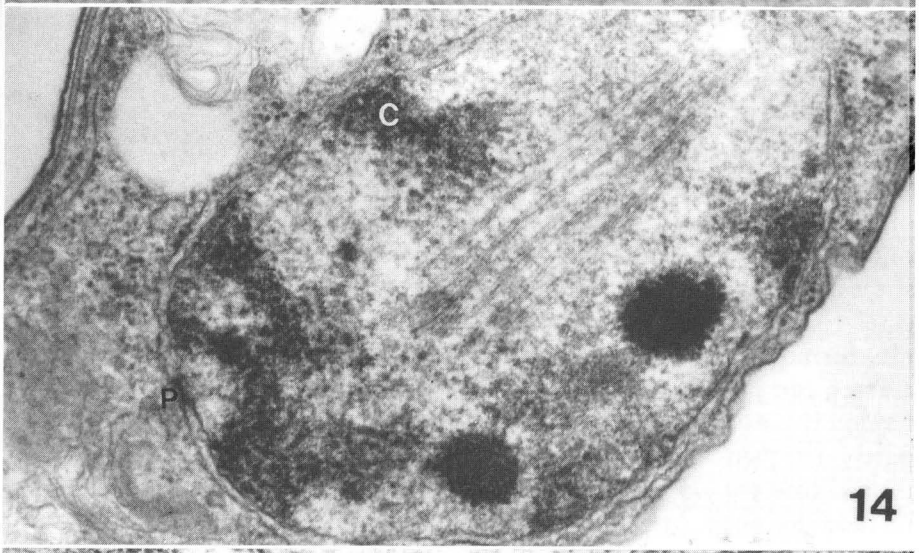
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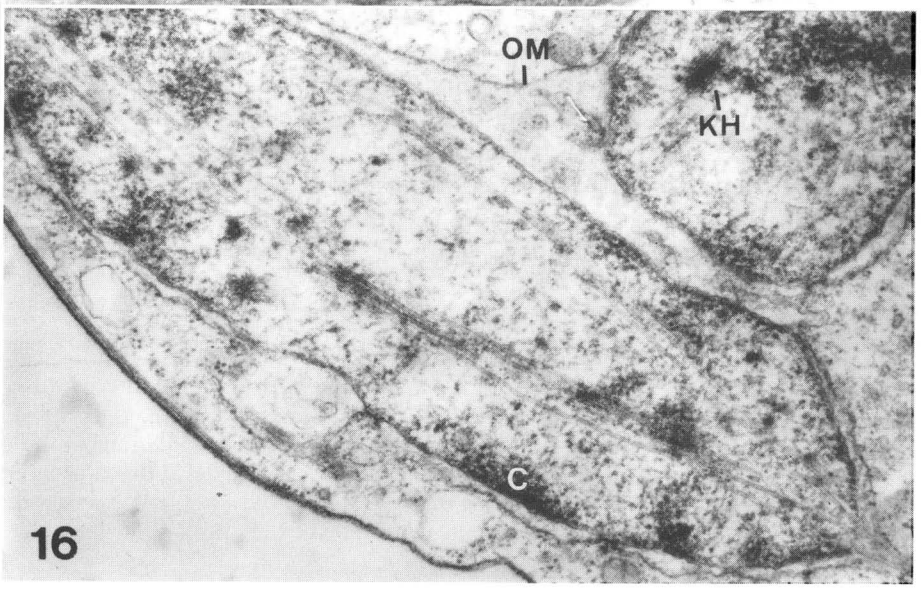
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ripheral chromatin of the giant cells decondenses at the preliminary stage to re-appear at the reorganizative stage of mitosis. The dividing nuclei of the giant cells also display the dense plaques, spindle microtubules and the same cycle of nucleolar reorganization as in the uninucleate flagellates (Fig. 16).

Discussion

Our results show that the interphase nucleus of cultured *T. borreli* contains largely peripheral chromatin, 10 nm thick threads, and a single nucleolus. Similar ultrastructure is displayed by non-dividing nuclei in aberrant *T. borreli* gigantic culture forms. According to Raikov [23], all these nuclei can be classified into the vesicular type. Their general fine structure has a strong resemblance to that of other kinetoplastids studied [44].

Of special interest are the 10 nm thick threads with occasional nodules on them. Direct attention to such threads has been paid only in a few trypanosomatid species (e.g. in *Crithidia oncopelti* [25]). However, similar threads may be found in many electron micrographs of the kinetoplastid nuclei published earlier [44]. The diameter of these threads coincides with that of a typical nucleosomal filament [37], though the nodules are considerably larger. So far as the presence of nucleosomes is clearly established in the kinetoplastid nucleus [10], the above mentioned threads seem to correspond to the nucleosomal filaments, whereas the nodules can be identified as the supernucleosomal granules (nucleomeres).

To our knowledge, *T. borreli* appears to be the first, but apparently not the only bodonid to form aberrant gigantic culture forms. On the other hand, in many trypanosomatid species the multinucleate giant cells are rather common both in cultures and appropriate stages of natural life cycles [6, 8, 25]. Little is known about functions of kinetoplastid giant cells. In the *T. borreli* forms observed here giant forms are a response to the culture condition and are thus an artificial phenomenon, though in other cases it has been proposed that the multinucleate trypanosomatids might represent systems for genetic exchange [6, 8, 25]. In *T. borreli* we have seen several types of association between nuclei of the giant cells (Figs. 5, 6). These nuclei are partially connected by cisternae of the endoplasmic reticulum, while others are surrounded by a single outer membrane and by separate inner membranes of the nuclear envelope. Thus, the nuclei acquire a common perinuclear space which seems to facilitate all possible nuclear interactions. Sometimes in multinucleate giants mitotic spindles and associated structures can be found.

It has been demonstrated that the multinucleate giant cells of *T. borreli* posses tightly coiled membraneous complexes within the karyoplasm, perinuclear space and the free cytoplasm. Comparable structures were described in intimate association with the membraneous capsule surrounding the kinetoplast of trypanosomatids [44].

Our electron microscopical investigation of *T. borreli* indicates that the nuclear envelope does not break down throughout nuclear division. At the onset of mitosis the dense peripheral chromatin decondenses but individual chromosomes remain discernible. Nevertheless, even at equatorial stages of mitosis when the chromosome decondensation reached its peak the chromatin-like ribbons appear to be associated with the inner nuclear membrane. At the elongational stage, the association of chromatin masses with the envelope becomes more prominent and at the reorganizative stage practically the whole condensed chromatin appears attached to the inner nuclear membrane. Vickerman and Preston [43] identified the significance of nuclear envelope in distribution of chromosomes between daughter cells of trypanosomatids. From the present study it seems possible to extend this supposition to bodonids as well. In kinetoplastids the mechanism of genome segregation with the help of nuclear envelope is based on the idea that the lateral, but not the pole portions of the inner nuclear membrane contain attachment loci for the discernible chromosomes. In the course of mitosis the actively growing nuclear envelope between the attachment sites of daughter chromosomes move them apart.

However, in kinetoplastid mitosis the exclusively membraneous mechanism can hardly account for the accurate genome segregation. Theoretically only the small-sized chromosomes have an opportunity for successful transmission by the nuclear envelope. At present the so-called minichromosomes ranging in size from 50 to 150 Kb have been found in several species of the genus *Trypanosoma* (e.g. *T. brucei* and *T. equiperdum* [40]). Minichromosomes are thought to be envolved in antigenic variation of these flagellates [40]. However, the absence of the minichromosomes in the

Figs. 12–16. Mitosis in *Trypanoplasma borreli*. **12.** Kinetochore-like plaque (KH), MT = spindle microtubules, ×98 000. **13.** Portion of the nucleus at the elongational stage, C = chromatin, MT = spindle microtubules, NE = nuclear envelope, P = nuclear pore, ×49 000. **14.** Portion of the dumbbell-shaped nucleus, C = chromatin, P = nuclear pore, ×45 000. **15.** Daughter nuclei (N), F = flagellum, ×26 000. **16.** Mitosis in the giant multinucleate cell, C = chromatin, KH = kinetochore-like dense plaque, OM = outer membrane of the nuclear envelope, =25 000.

genomes of many other species strongly suggests that they are not essential for vital functions in trypanosomatids. Thus, the random distribution of minichromosomes and their accidental loss during mitosis may not be fatal for parasites. Whether the minichromosomes exist in the bodonids is a question for future research.

The pulsed-field electrophoresis technique resolves large linear DNA molecules indicating that the kinetoplastids studied usually contain more than 18 chromosomes in the 250–5700 Kb range [1, 15, 40, 41]. If this is the case, it becomes difficult to explain the physical separation of the large decondensed chromosomes by the membraneous mechanism only. Undoubtedly, the spindle microtubules and associated structures are involved in the mitotic process in these flagellates. The electron dense plaques have been found in spindle microtubules in most studies of nuclear division in trypanosomatids [20, 26–33, 38, 39, 43]. These plaques are constant in number for each species, possess laminar structure and behave as kinetochores of decondensed chromosomes. Moreover, up to 1984 it was generally accepted that the number of chromosomes in kinetoplastids is equivalent to the number of their dense plaques. With this assumption the number of chromosomes in *Trypanosoma cruzi* was taken as 10 [28, 29], 4 in *T. danilewskyi* [20, 26], 3 in *Blastocrithidia triatomae* [31], 4–5 in *B. miridarum* [27], and 6 in several *Leishmania* species [38, 39].

However, as mentioned above, pulsed-field electrophoresis shows that these flagellates have far more discrete chromosome bands than kinetochore-like plaques. From these results it has been suggested that only the large trypanosomatid chromosomes (presumably 1500 Kb or slightly more) possess kinetochore-like plaques [27]. Another possibility, the formation of composite chromosomes, seems less probable to us because of difficulties in separation of long decondensed chromosomes.

In *T. borreli* mitosis the kinetochore-like plaques are found to congregate toward the equatorial plate. Moreover, in some flagellates we found at least 7 dense fibrillar blocks just before the nuclear division (Fig. 2). The ultrastructure of these blocks is very similar to that of *Leishmania adleri* at the appropriate stage of its cell cycle [38]. This data has made them candidates for the prokinetochores, or the chromatin clumps not bound to the nuclear envelope. In the former proposition the number of kinetochore-like plaques in *T. borreli* may be at least 7. Unfortunately, at the equatorial and elongational stages of mitosis, the kinetochore-like plaques are usually difficult to discern from the chromatin elements and scattered nucleolar fragments. Several kinetochore-specific antibodies, including autoantibodies from sera of scleroderma patients, have recently been described [17]. It is reasonable to expect that at least a fraction of these antibody probes will cross-react with the kinetochore-like plaques of kinetoplastids.

With the beginning of equatorial stage the intranuclear microtubules of *T. borreli* form the monaxone-symmetrical bipolar spindle. We show that the microtubules seem to contact the nuclear envelope or the fibrillar disc which lies at the inner side of the nuclear membrane. The presence of fibrillar discs at the spindle poles suggests the existence of intranuclear MTOCs in *T. borreli*. From ultrastructural studies, clear MTOCs are reported from *T. raiae* [43] and *T. equiperdum* [21], but not from most other trypanosomatids.

It is clear from previous observations [20, 26, 27, 30, 31, 38, 39], that depending on the species, the trypanosomatids' spindles usually possess from 10 to 50 microtubules, tending to assemble in a few bundles. In *T. cruzi*, the spindle develops as two half-spindles of about 60 microtubules each. These half-spindles overlap at the equator of the nucleus [28]. In *T. borreli* at the elongational stage, the mitotic spindle contains three main bundles of about 40 microtubules each. These microtubules seem to press the opposite enlarged poles to push them apart.

In summary, mitosis in *T. borreli* can be described as intranuclear closed orthomitosis without condensed individual chromosomes. We infer that the nuclear division in this species displays a combination of a primitive membraneous mechanism of segregation of genetic material with a more evolutionary advanced mode of chromosome separation involving spindle microtubules, MTOCs, and kinetochore-like plaques. Results from this and other studies lead us to conclude that mitosis in trypanosomatids and bodonids uses practically the same basic structures and mechanisms for the nuclear genome segregation. The problem still remains in necessity to recognize the respective roles of the nuclear envelope and the spindle apparatus during mitosis. It would be especially important to map the attachment sites of these structures and the "invisible" chromosomes. This is the problem of the future to be solved by the immunocytochemical approach.

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