

# ELECTRON MICROSCOPY OF MITOSIS IN A RADIOSENSITIVE GIANT AMOEBEA

E. W. DANIELS, Ph.D., and L. E. ROTH, Ph.D.

From the Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois, and the Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa

## ABSTRACT

Various aspects of the ultrastructure of the dividing nuclei in the large radiosensitive amoeba *Pelomyxa illinoisensis* are demonstrated. Evidence of nuclear envelope breakdown is presented, and membrane fragments are traced throughout metaphase to envelope reconstruction in anaphase and telophase. Annuli in the nuclear envelope and its fragments are shown throughout mitosis. During metaphase and anaphase some 15 to 20 mitochondria are aligned at each end of the spindle, and are called polar mitochondria. The radioresistant amoebae *Pelomyxa carolinensis* and *Amoeba proteus* do not have polar mitochondria, and *Pelomyxa illinoisensis* is unique in this regard. The shape of the *P. illinoisensis* interphase nucleoli differs from that in the two radioresistant species, and certain aspects of nucleolar dissolution in the prophase vary. Helical coils in the interphase nucleoplasm are similar to those in the radioresistant amoebae. A "blister" phase in the flatly shaped telophase nuclei of *P. illinoisensis* is described which is interpreted to be the result of a rapid nuclear expansion leading to the formation of the normal spherical interphase nuclei.

## INTRODUCTION

The amoeba *Pelomyxa illinoisensis* is about ten times more sensitive to ionizing radiation than *Pelomyxa carolinensis* (5-8, 30), and some twenty times more sensitive than the uninucleated *Amoeba proteus* (6, 19, 30) and *Amoeba dubia* (10). Our interest in the relative radiosensitivity of *P. illinoisensis* prompted a study of ultrastructural details of the nucleus during mitosis for comparison with nuclei in *P. carolinensis* (25) and *A. proteus* (26).

A second objective of this work has been to extend our study of the spindle filaments and their relationship to the chromosomes, nucleoli, and the breakdown of the nuclear envelope. In addition, we have investigated cytological unknowns peculiar to *P. illinoisensis*: the "polar bodies," minute particles (0.5 to 2.0  $\mu$ ) seen at the ends of

the spindle filaments during metaphase and anaphase (8, 17, 18), and the reported visibility of chromosomes during interphase (18).

## MATERIALS AND METHODS

The multinucleated amoeba, *Pelomyxa illinoisensis* (13, 14), was used. The culture medium was glass-distilled water at pH 6.5 to 7.0 kept at 21° to 24°C, the former being preferable. Food organisms were *Paramecium caudatum* and *Chilomonas paramecium*. About 100 healthy amoebae in different stages of mitosis and interphase were prepared and examined in the electron microscope, the division stages being selected on the basis of external structure (18).

With the exception of the amoebae represented by Fig. 1, which were fixed in acrolein and then treated with osmium tetroxide, the isolated organisms were placed for 1 hour in buffered 1 per cent osmium

tetroxide at pH 7.3 to 8.0 with calcium chloride (0.002 M) as the divalent cation (25). Some fixed amoebae were washed in saline to remove excess  $\text{OsO}_4$ , dehydrated in ethanol, cleared in propylene oxide, and separately embedded in Epon resin (15), while others were embedded in methacrylate by a method previously described (25). Sections were cut at about 100 m $\mu$  on a Forter-Blum microtome with glass knives and observed with an RCA-EMU 3E electron microscope operated at 100 kv.

For light microscopy, interphase and mitotic amoebae were fixed for about 1½ hours in AFA (ethanol 70 per cent, 20 parts, formalin 40 per cent, 2 parts, glacial acetic acid, 1 part), dehydrated in a graded ethanol series, and cleared in xylene. Paraffin-embedded blocks were cut into 10  $\mu$  sections and mounted on glass slides. They were later cleared of paraffin, treated with Feulgen reagent, counterstained with fast green, and examined with and without phase optics at magnifications of 400 to 1100.

## OBSERVATIONS

### Light Microscopy

Interphase nucleoli are Feulgen-negative while the remaining nucleoplasm is weakly Feulgen-positive. Within the central portion of a nucleus are 10 to 25 small (*ca.* 0.5  $\mu$ ), dense, Feulgen-positive areas some of which have a clear halo around them. These areas are similar to those described by McClellan (18) and referred to by him as interphase chromosomes. Although they are Feulgen-positive, indicating the presence of DNA, they are larger and fewer in number than the metaphase chromosomes. These dense areas might result from chromatin condensation during fixation when fixatives other than osmium tetroxide or 10 per cent formalin are used (18). Thus, we are of the opinion that the word chromosome should not be used to describe these granules.

---

### Abbreviations for Figures

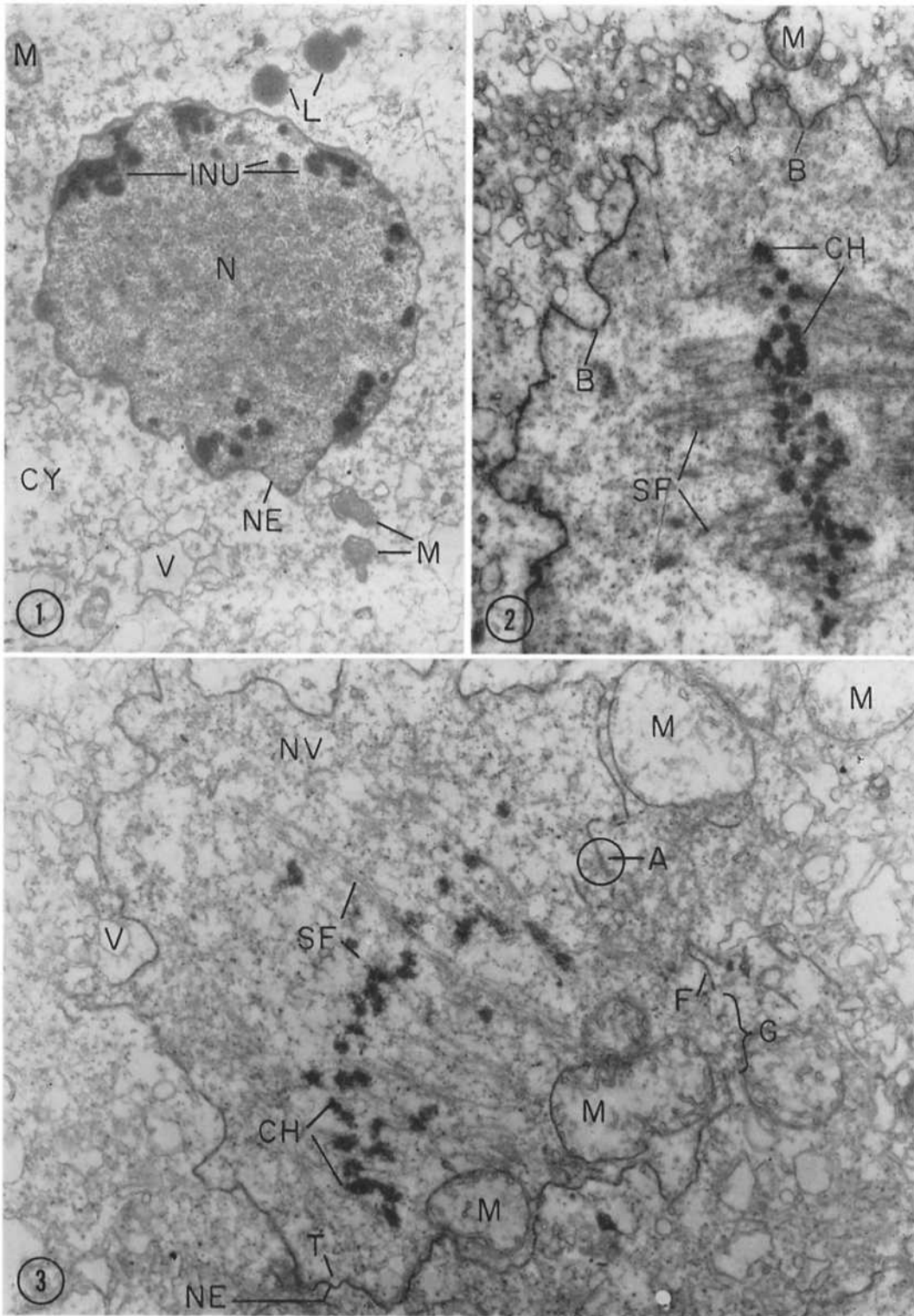
<i>A</i> ,	annuli	<i>L</i> ,	lipid globule
<i>AZ</i> ,	a zone or area of dense nucleoplasm. This is essentially the nuclear content observed in early telophase.	<i>LNS</i> ,	large nuclear spheroids
<i>B</i> ,	break in the nuclear envelope.	<i>M</i> ,	mitochondrion
<i>BZ</i> ,	a zone or area of low electron opacity. It is an area which has filled rapidly in late telophase.	<i>N</i> ,	nucleus
<i>CH</i> ,	chromosomes	<i>NE</i> ,	nuclear envelope
<i>CY</i> ,	cytoplasm	<i>NV</i> ,	nuclear vesicle (to the upper right of <i>NV</i> in Fig. 3)
<i>E</i> ,	end of chromosome plate	<i>PM</i> ,	polar mitochondrion
<i>F</i> ,	fragment of the nuclear envelope	<i>R</i> ,	40 m $\mu$ electron opaque particles
<i>G</i> ,	gap in the nuclear envelope	<i>SF</i> ,	spindle filaments
<i>GO</i> ,	Golgi body	<i>T</i> ,	thin inner layer at the nuclear envelope separating the nucleoplasm from the nuclear envelope
<i>IZ</i> ,	fibers in the interzone	<i>V</i> ,	cytoplasmic vesicle
<i>INU</i> ,	interphase nucleolus	<i>YNU</i> ,	young nucleoli at late telophase and early interphase
<i>K</i> ,	kinetochore region of chromosome		

Note: Amoebae represented by Figs. 2 and 5 were embedded in methacrylate. All others were embedded in Epon 812.

FIGURE 1 Interphase nucleus fixed in acrolein followed by  $\text{OsO}_4$ .  $\times 4,000$ .

FIGURE 2 Later stage in prometaphase showing alignment of chromosomes, outward radiation of spindle filaments, and early complete breaks in the membrane of the nuclear envelope.  $\times 7,000$ .

FIGURE 3 Prometaphase to metaphase transformation with large gaps in the nuclear envelope. Mitochondria have been admitted into the nucleoplasmic area, and many electron-opaque, 40 m $\mu$  particles are seen both within and without the nucleus. Spindle filaments are relatively longer.  $\times 9,000$ .



In late telophase a "blister" phase occurs during the transformation of the flatly shaped nucleus into the nearly spherical, early interphase nucleus. McClellan (18) observed a blister in the central portion of the telophase plate. We also observed this in our Feulgen sections and in addition found a number of telophase nuclei each of which had a blister at each end of the plate, but none in the central area.

### Electron Microscopy

#### INTERPHASE

The nucleoli in a mature interphase nucleus of *P. illinoisensis* (Fig. 1) are larger and more irregular than those of early interphase, the latter appearing as electron-opaque areas (ca. 0.3 to 0.5  $\mu$ ) near the nuclear envelope (Fig. 10, *YNU*). The interphase and early prophase nucleoplasm contains helices similar to those first described by Pappas, in *P. carolinensis* and *A. proteus* (20-22), to be in areas which react positively to the Feulgen reagent.

#### PROPHASE AND PROMETAPHASE

**NUCLEOLI:** The irregularly shaped nucleoli of interphase, ranging in size from 0.3 to 2  $\mu$ , become rounded in prophase and gradually disappear in late prophase and early prometaphase. They do not migrate centrally as in *P. carolinensis* (12, 17, 18). One to a few large, spheroidal (ca. 2  $\mu$ ) nucleoli are occasionally seen after the others have disappeared, but these also fade by the time the spindle is fairly well organized. They resemble electron-opaque bodies in late telophase (Fig. 10, *LNS*). Dense particles in the size range of 10 to 15  $m\mu$  can be seen in the prophase nucleoli. Both chromosomes and fading nucleoli may be present simultaneously for a short time.

**NUCLEAR ENVELOPE:** Bleb formation takes place and the shape of the entire nucleus is particularly irregular. In one amoeba we observed infoldings of the nuclear envelope which formed 2- $\mu$  pockets and apparent vesicles containing cytoplasm.

Just before the breakdown of the nuclear envelope, minute vesicular spaces (ca. 100  $m\mu$ ) may form along the inner surface of the envelope (Fig. 3, *T*). Complete breaks then develop and widen to form gaps allowing larger cytoplasmic components to enter (Figs. 2 and 3, *B* and *G*). The nuclear envelope then continues to fragment (Fig. 4).

**CHROMOSOMES AND SPINDLE FILAMENTS:** As in *P. carolinensis*, the 100 or more chromosomes appear in late prophase and prometaphase as electron-opaque spheroids about 0.3  $\mu$  in diameter (Figs. 2, 3, and 4). Dense particles in the 10 to 20  $m\mu$  range are seen within them. The spindle filaments can first be seen at the chromosomes (Fig. 2, *SF*) and they gradually extend farther toward the nuclear envelope (Figs. 3 and 4, *SF*).

**NUCLEAR ENVELOPE ANNULI:** Annuli remain in the fragments of the nuclear envelope throughout mitosis (Figs. 3, 4, 6, and 9, *A*). In a group of new daughter *P. illinoisensis* amoebae, the annuli averaged 70  $m\mu$  in diameter and 170  $m\mu$  center-to-center, which dimensions are within the range of those of annuli in other types of cells (24).

#### METAPHASE

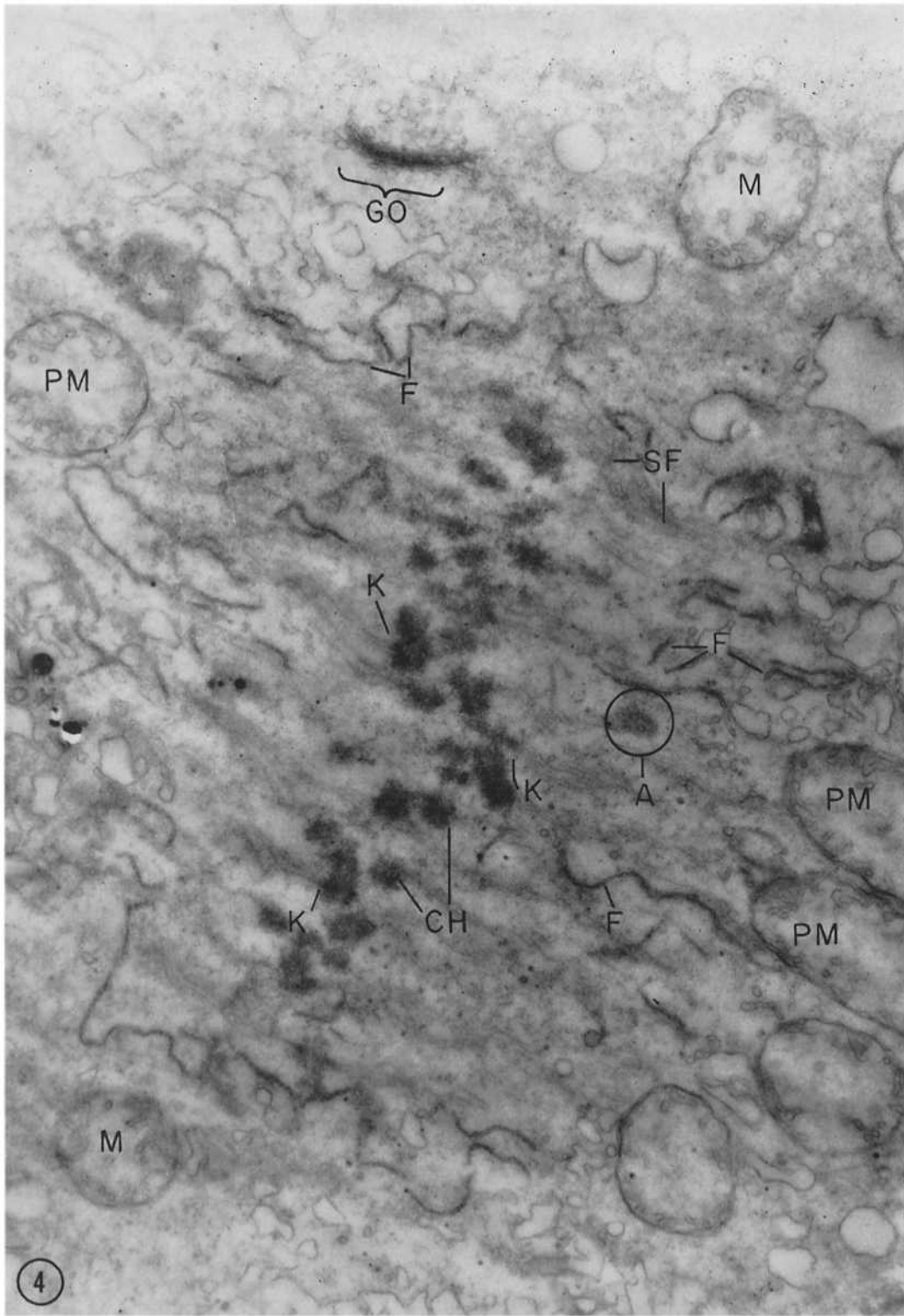
**MITOCHONDRIA:** At metaphase, the mitochondria accumulate in an orderly array primarily on opposing sides of the metaphase plates (Fig. 5). In a given section of a mitotic figure usually about 12 to 15 polar mitochondria are located at each of the two ends of the spindle distal to the metaphase chromosomes. They remain in this position until the end of anaphase. We were not able to see any distinct connections between the spindle filaments and the mitochondria. However, multiple spindle filaments are attached to single chromosomes at kinetochore-like junctions (Fig. 4, *K*). The filaments in all stages were 15  $m\mu$  in diameter with a dense cortex, as seen in other electron microscope studies of the mitotic apparatus (11, 25).

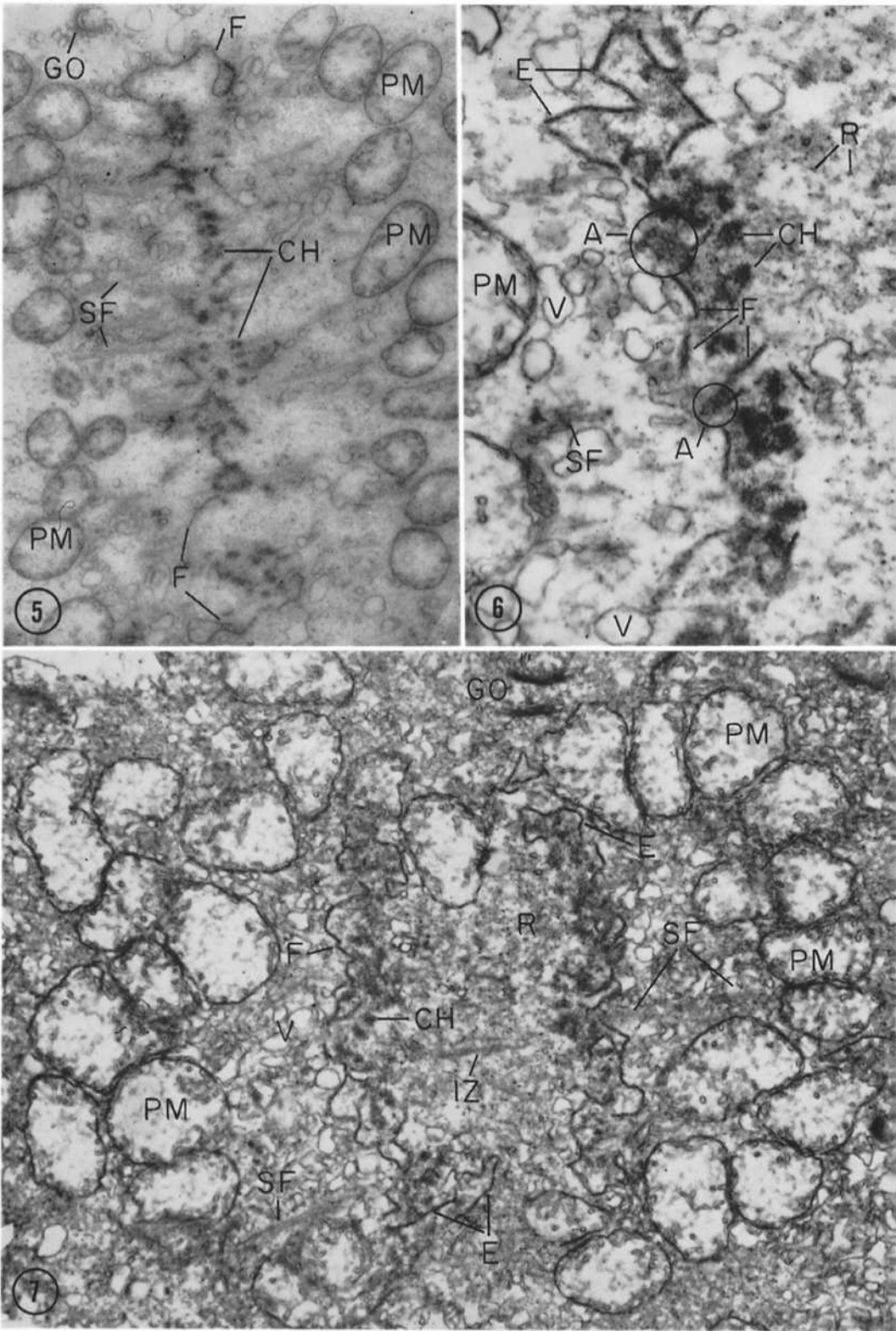
#### ANAPHASE

**NUCLEAR ENVELOPE FRAGMENTS:** At metaphase, the fragments of the nuclear envelope

---

FIGURE 4 Metaphase demonstrating fragments of nuclear envelope, many of which show annuli either in side or front views.  $\times 20,000$ .





are seen between and parallel to the filaments (Figs. 4 and 5, *F* and *SF*), and they are carried through anaphase close to the chromosomes (Figs. 6 and 7, *A*, *E*, *F*, and *CH*). While there is some variation in the sizes of fragments, most of them measure about 1  $\mu$  or less. One envelope fragment at metaphase measured 400 by 700  $m\mu$  and contained 16 annuli.

As the paired chromosomes separate at anaphase, fragments of the old nuclear envelope are located almost exclusively on the poleward side of the two chromosome plates and are lacking in the interzone (Fig. 7, *F*). After the plates have separated a distance of about 5  $\mu$ , their ends are usually capped with a more or less continuous membrane (Figs. 6 and 7, *E*), and slightly later the chromosome plate is again enclosed by a continuous envelope (Fig. 8, *N*).

**FINE PARTICLES:** Electron-opaque, 40- $m\mu$  particles can be seen randomly distributed in the cytoplasm (Figs. 3, 6, 7, and 8, *R*). Similar particles are also found in mature interphase and prophase nuclei. In early anaphase they often appear concentrated in the interzonal space (Fig. 7, *R*), and later at telophase are excluded from the nucleus. Small vesicles (ca. 0.5  $\mu$ ) often occur in the space between the polar mitochondria and the chromosomes (Figs. 6 and 7, *V*). These vesicles and the 40- $m\mu$  particles appear randomly distributed as the chromosome plates move farther apart.

**SPINDLE FILAMENTS:** During anaphase, spindle filaments are found in the interzonal region (Fig. 7, *IZ*) as well as in the space between the chromosome plate and its polar mitochondria (Fig. 7, *SF*). One micrograph of late anaphase, not shown here, demonstrates several filament bundles lying lengthwise in the interzone between chromosome plates. Filament bundles lying in the cytoplasm, similar to those of *P. carolinensis* at

telophase (25), were found in *P. illinoisensis* at the anaphase-telophase transformation period. The individual spindle filaments in the bundles have a dense periphery with a less dense interior and are 15  $m\mu$  in diameter.

#### TELOPHASE AND YOUNG INTERPHASE NUCLEI

In early telophase, the nucleus is discoid, the nuclear envelope is continuous, and the chromosomes are no longer seen (Figs. 8 and 9, *N*). The bilister phase in late telophase, shown in Fig. 10, is characterized by an area of denser nucleoplasm on one side (Fig. 10, *AZ*) and an area of watery, very light nucleoplasm in the remainder of the nucleus (Fig. 10, *BZ*). The nuclear envelope appears to have ballooned out from one side of the flattened disc stage of Figs. 8 and 9, leaving the early or denser telophase nucleoplasm essentially in place (Fig. 10).

In late telophase, one to a few relatively large (ca. 1 to 2  $\mu$ ) electron-opaque structures (Fig. 10, *LNS*), similar in appearance to the large nucleoli of late prophase, are present in the nucleoplasm. They disappear soon after the stage shown in Fig. 10 and are not seen in the young interphase nuclei which have significantly smaller nucleoli (ca. 0.3 to 0.5  $\mu$ ) that are also more numerous (Fig. 10, *YNU*). As the young nucleus matures, its nucleoli enlarge and become more angular and irregular in shape, thus assuming the mature interphase characteristics (Fig. 1, *INU*). Meanwhile, the entire nucleus becomes spherical and enlarges as it prepares for the next mitosis.

#### DISCUSSION

**RADIOSENSITIVITY:** There are many similarities and few differences in the mitotic events of the radiosensitive *P. illinoisensis* and the radio-resistant *A. proteus* and *P. carolinensis* amoebae. A

---

FIGURE 5 Metaphase showing polar mitochondria. Relatively long segments of the membrane of the old nuclear envelope are located at the ends of the chromosome plate.  $\times 5,500$ .

FIGURE 6 View of an early anaphase chromosome plate showing details of nuclear envelope fragments.  $\times 14,000$ .

FIGURE 7 Early anaphase. Nuclear envelope fragments are seen mostly around the poleward surface of the plates and at their ends. Electron-opaque, 40  $m\mu$  particles can be seen between the plates. Polar mitochondria are still in place.  $\times 7,000$ .

striking feature of mitosis in *P. illinoisensis*, that is completely absent in the radioresistant amoebae, is the polar aggregation of mitochondria at metaphase and anaphase. The polar mitochondria cannot be distinguished structurally from other mitochondria in this species (9) and show no evidence of specialization.

Other distinctive features characterize the mitotic events of *P. illinoisensis*: during prophase the

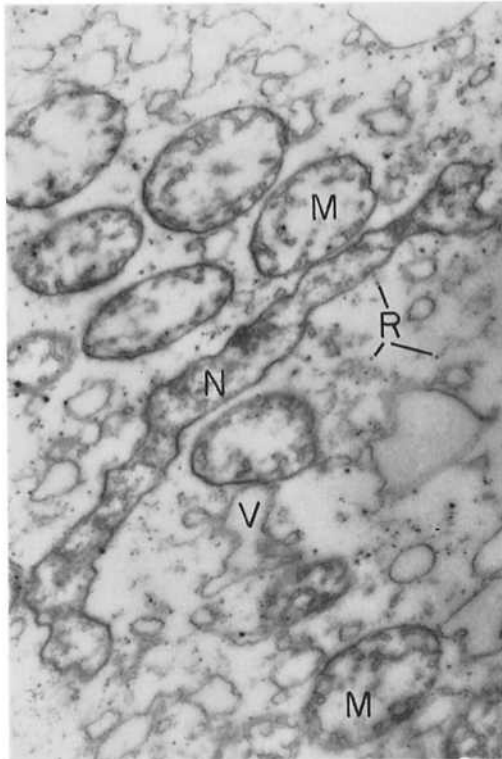


FIGURE 8 Cross-section of early (disc-shaped) telophase nucleus. The electron-opaque, 40  $m\mu$  particles have been excluded from the nucleus.  $\times 11,000$ .

nucleoli do not migrate centrally, and in telophase the nuclei are characterized by a large blister phase not seen in the radioresistant organisms. The intermitotic time of *P. illinoisensis* (8) is about twice that of either *P. carolinensis* or *A. proteus*.

**FILAMENTS OF THE MITOTIC APPARATUS:** The mitotic apparatus of the giant amoebae deserves careful study because an unusual degree of spindle elongation occurs. Short (27) and Berkeley (4) reported that the pole-to-pole distance in *P. carolinensis*, about 10  $\mu$  at metaphase, is in-

creased to about 60  $\mu$  by the end of anaphase. Kudo (12) showed data of the same kind from the same species, the increase being four-fold. These investigators found little or no change in the chromosome-to-pole distance when metaphase and anaphase figures were compared. Similar observations have also been made on *P. illinoisensis* (8, 18).

Our present study on *P. illinoisensis* shows that the structure of filaments, except for this elongation, is not altered during mitosis. The increase in pole-to-pole distance is apparently accomplished by anaphase growth of the spindle, probably by the addition of protein to the filaments in such a way that the same molecular architecture exists throughout the anaphase as in the metaphase. Mazia (16, Page 297) discusses the anaphase spindle from the viewpoint that its growth is the assemblage of preformed molecular constituents. That little or no synthesis is taking place during anaphase has been stated before (16, page 139; 29) and is substantiated by Plesner (23) in recent studies on *Tetrahymena*. Therefore, in view of the electron microscopic evidence in this species and *P. carolinensis* that the filament structure is constant, we can suggest that the interzonal filaments are lengthened by the addition and orientation of previously synthesized molecules of the surrounding mixoplasm (28). As a result of this, the chromosomes are pushed apart.

That an influx of cytoplasm takes place very soon after the rupture of the envelope is shown in this study by the observation of mitochondria and smaller cytoplasmic elements inside the nucleus (Figs. 2 and 3). This mixture correlates closely with the formation of filaments, and it is our hypothesis that such mixing is essential for the formation of the mitotic apparatus. We visualize the lengthening of the interzonal fibers as a continuation of this process. The concentration of electron-opaque, 40- $m\mu$  particles in the interzonal space (Fig. 7) in early anaphase might play a role in the lengthening of the adjacent filaments (24).

**THE NUCLEAR ENVELOPE DURING THE MITOTIC CYCLE:** One of the most important contributions of this study is the demonstration that fragments of the envelope with their annuli are conserved intact during mitosis and are, judging from our evidence, reused to form the new envelopes at telophase. Annuli are retained throughout the process, giving positive identification to the fragments during the time between nuclear



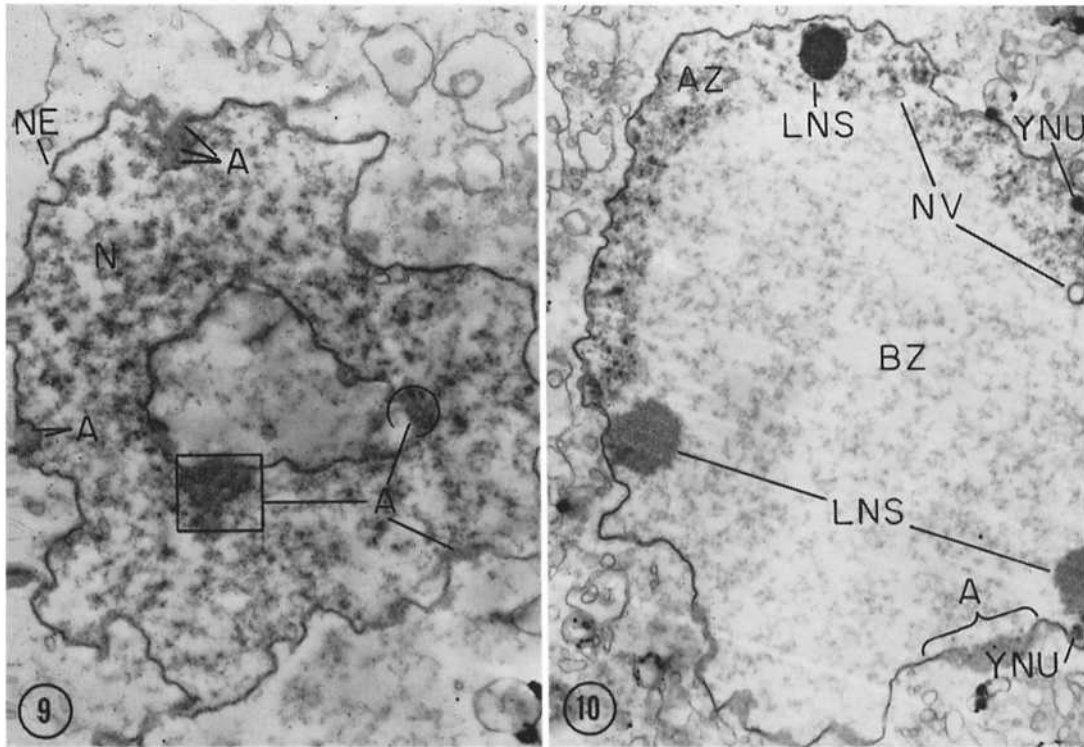


FIGURE 9 Longitudinal section through the telophase nucleus at the beginning of its expansion. Annuli in the membrane of the nuclear envelope are demonstrated.  $\times 9,000$ .

FIGURE 10 "Blister" phase of late telophase during nuclear expansion showing the large, intranuclear, electron-opaque spheres.  $\times 5,000$ .

envelope breakdown and reformation. Thus, these cells carry a unique "label" that allows decisive identification of the origin of the new envelope; consequently, a considerable question remains regarding the hypothesis that the en-

velope re-forms from the endoplasmic reticulum at each mitosis (1-3, 16, 24).

This work was performed under the auspices of the United States Atomic Energy Commission.

Received for publication, March 4, 1963.

#### REFERENCES

1. BARER, R., JOSEPH, S., and MEEK, G. A., The origin of the nuclear membrane, *Exp. Cell Research*, 1959, 18, 179.
2. BARER, R., JOSEPH, S., and MEEK, G. A., The origin and fate of the nuclear membrane in meiosis, *Proc. Roy. Soc. London, Series B.*, 1960, 152, 353.
3. BARER, R., JOSEPH, S., and MEEK, G. A., *Electron Microscopy in Anatomy*, London, Edward Arnold, Ltd., 1961, 160.
4. BERKELEY, E., Spindle development and behavior in the giant amoeba, *Biol. Bull.*, 1948, 94, 169.
5. DANIELS, E. W., Studies on the effect of x-irradiation upon *Pelomyxa carolinensis* with special reference to nuclear division and plasmotomy, *J. Exp. Zool.*, 1951, 117, 189.
6. DANIELS, E. W., Survival and cell division following x-irradiation of three species of amoebae, *U. S. A. F. Radiation Lab. Quart. Prog. Rep.*, 1953, 8, 89.
7. DANIELS, E. W., X-Irradiation of the giant amoeba, *Pelomyxa illinoisensis*. I. Survival and cell division following exposure. Therapeutic effects of whole protoplasm, *J. Exp. Zool.*, 1955, 130, 183.
8. DANIELS, E. W., X-Irradiation of the giant amoeba, *Pelomyxa illinoisensis*. II. Further

- studies on recovery following supralethal exposure, *J. Exp. Zool.*, 1958, **137**, 425.
9. DANIELS, E. W., and ROTH, L. E., X-Irradiation of the giant amoeba, *Pelomyxa illinoensis*. III. Electron microscopy of centrifuged organisms, *Radiation Research*, 1961, **14**, 66.
  10. DANIELS, E. W., unpublished data.
  11. HARRIS, P., Some structural and functional aspects of the mitotic apparatus in sea urchin embryos, *J. Cell Biol.*, 1962, **14**, 475.
  12. KUDO, R. R., *Pelomyxa carolinensis* Wilson. II. Nuclear division and plasmotomy, *J. Morphol.*, 1947, **80**, 93.
  13. KUDO, R. R., A species of *Pelomyxa* from Illinois, *Tr. Am. Micr. Soc.*, 1950, **69**, 368.
  14. KUDO, R. R., Observations on *Pelomyxa illinoensis*, *J. Morphol.*, 1951, **88**, 145.
  15. LUFT, J. H., Improvements in epoxy resin embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 409.
  16. MAZIA, D., in *The Cell*, (J. Brachet and A. E. Mirsky, editors), New York, Academic Press, Inc., 1961, **3**, 77.
  17. MCCLELLAN, J. F., Observations on nuclear divisions in *Pelomyxa illinoensis* Kudo, 1954, Thesis, University of Illinois, Urbana, Illinois.
  18. MCCLELLAN, J. F., Nuclear division in *Pelomyxa illinoensis* Kudo, *J. Protozool.*, 1959, **6**, 322.
  19. ORD, M. J., and DANIELLI, J. F., The site of damage in amoebae exposed to x-rays, *Quart. J. Micr. Sc.*, 1956, **97**, 29.
  20. PAPPAS, G. D., Helical structures in the nucleus of *Amoeba proteus*, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 221.
  21. PAPPAS, G. D., Electron microscope studies on amoebae, *Ann. New York Acad. Sc.*, 1959, **78**, 448.
  22. PAPPAS, G. D., A study of the fine structure of the helices found in the nuclei of free living amebas, Abstracts of the First Annual Meeting of the American Society for Cell Biology, Chicago, 1961, 163.
  23. PLESNER, P., Nucleotide metabolism and ribosomal activity during synchronized cell division, in *Symposia of the International Society for Cell Biology*, Liège, 1962, (R. J. C. Harris, editor), New York, Academic Press, Inc., 1963, 77.
  24. PORTER, K. R., in *The Cell*, (J. Brachet and A. Mirsky, editors), New York, Academic Press, Inc., 1961, **2**, 621.
  25. ROTH, L. E., and DANIELS, E. W., Electron microscopic studies of mitosis in amoebae. II. The giant amoeba, *Pelomyxa carolinensis*, *J. Cell Biol.*, 1962, **12**, 57.
  26. ROTH, L. E., OBETZ, S. W., and DANIELS, E. W., Electron microscopic studies of mitosis in amoebae. I. *Amoeba proteus*, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 207.
  27. SHORT, R. B., Observations on the giant amoeba, *Amoeba carolinensis*, *Biol. Bull.*, 1946, **90**, 8.
  28. WASSERMANN, F., Wachstum und Vermehrung der lebendigen Masse, in *Handbuch der mikroskopischen Anatomie des Menschen*, (W. von Mollendorff, editor), Berlin, Springer Verlag, 1929, **1**, No. 2.
  29. WENT, H. A., Dynamic aspects of mitotic apparatus protein, *Ann. New York Acad. Sc.*, 1960, **90**, 422.
  30. WICHTERMAN, R., and HONEGGER, C. M., Action of x-rays on the two common amebas, *Chaos diffluens* and *Chaos chaos*, *Proc. Penn. Acad. Sc.*, 1958, **32**, 240.