PRELIMINARY NOTE

THE ELECTRON MICROSCOPY OF DIVIDING CELLS

by

GEORGE ROZSA AND RALPH W. G. WYCKOFF

Laboratory of Physical Biology, Experimental Biology and Medicine Institute, National Institutes of Health, Bethesda, Maryland (U.S.A.)

Problems of two sorts have impeded electron microscopic studies of the macromolecular structure of cells. One of these is concerned with finding ways to prepare cells with a minimum of alteration to the fine structure of their protoplasm, the other has dealt with the search for ways of getting these preparations of the extreme thinness required for electron microscopy. The evolution of adequate methods for thin sectioning has met in large measure the second of these, but there will for a long time be need of improved methods of specimen preparation.

A knowledge of the macromolecular structure of the chromosomes and of changes in the fine structure of cellular protoplasm during cell division is one of the most important things to be gained through the application of these expanding electron microscopic techniques. We have been carrying out such studies and record here some preliminary results obtained during investigation of cell division in the root tips of the onion, *Allium cepa*. This work has already given much information about both the fine structure of these cells and their chromosomes and the effects of different preparatory procedures on the appearance of these structures. For observation, the excised root tips were immersed in various fixatives for different lengths of time and, after dehydration, were embedded in methacrylate. They were then thinly sectioned according to the methods of NEUMANN, BORYSKO, AND SWERDLOW[1]. The unusually great scattering power of the chromosomes for electrons has made it necessary to use only the thinnest sections. These have been put on formvar-coated grids, the methacrylate has been removed with amyl acetate, and the finished preparations shadowed lightly with palladium for observation under an RCA type EMU electron microscope equipped with a suitable objective aperture. For work at low initial magnifications down to *c.* 900 × a special objective polepiece has been introduced.

When viewed at such a low magnification, these sections have shown the pattern of cells in different mitotic stages familiar to those who have examined this plant material with the optical microscope. As was to be expected, structural details were strongly influenced by the type of fixation employed. In order to use the optically familiar structures as a point of reference for further work, we first examined a series of preparations fixed with FLEMMING's solution and several of its modifications. These fixatives, containing osmic, chromic, and acetic acids in various proportions, have

[1] S. B. NEUMANN, E. BORYSKO, AND M. SWERDLOW, *Science*, 110 (1949) 66.

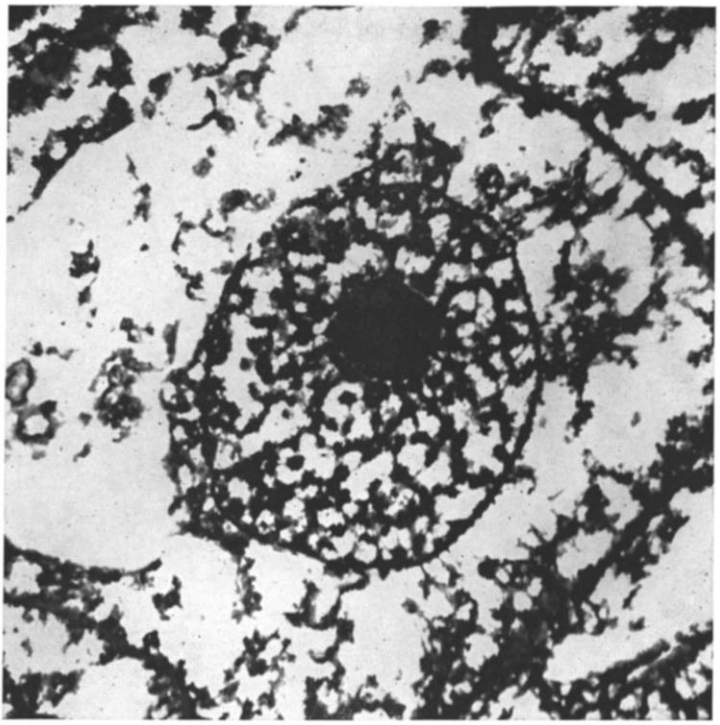

Fig. 1. A resting cell in a sectioned onion root tip fixed with FLEMMING's solution. The nucleus occupies the center of the field, parts of the cell wall run diagonal to the corners of the photograph. Strands of torn cytoplasm are distributed about the extra-nuclear space. Magnification = 5,500 ×

commonly been considered especially good for study of the cell's chromatic and achromatic apparatus. Examples of cells fixed in this way are shown in Figs 1 and 2. Though these photographs reveal familiar structures, the clarity of vision that results from the high resolution of the electron microscope demonstrates that the cells have been severely damaged. The cytoplasm of the resting cell of Fig. 1 appears as a coarse, badly torn net attached in part to the cell wall and the nuclear membrane. The chromatin material of the nucleus consists of dark, irregular bodies connected by a few fine threads; they show a definite tendency to accumulate along the inner side of the nuclear membrane and about the nucleolus which is large and very dense. The nuclear membrane in these preparations seems to be thick and well-defined.

Fig. 2 is typical of cells in anaphase seen after this type of fixation. The protoplasm of this cell is the same kind of torn network visible in Fig. 1. There is, however, a well defined spindle and the newly formed middle lamella is apparent as the cell-plate. The chromosomes seem shrunken; their naturally high opacity has been considerably enhanced by the osmium they have bound. In suitably thin preparations, however, the internal structure of these chromosomes has been photographed at initial magnifications up to 10,000 × and such photographs show that aside from the fragments of protoplasm adhering to or embedded in their peripheries, they do not present a definite fine structure at any order of magnitude visible in the electron microscope.

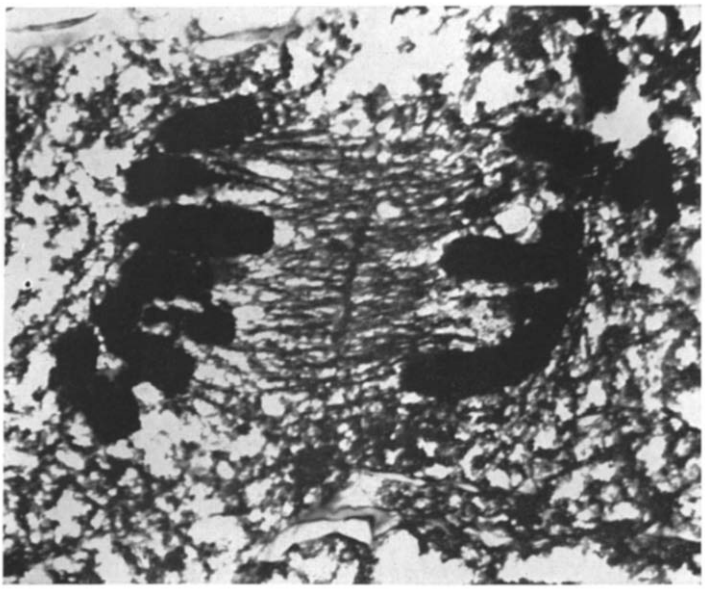

Fig. 2. An onion root tip cell in anaphase showing a well-developed spindle and equatorial plate FLEMMING's fixative. Magnification = 4,500 ×

An analysis has been made of the action of the components of the FLEMMING solution. This made it clear that the acetic acid was the most destructive, both in the speed and the extent of the damage it caused. The chromic acid had similar effects but acted more slowly. Osmic acid proved to be the least important component of this class of fixative largely because of its slow diffusion rate; its chief contribution therefore was a non-specific enhancement of the already high opacity of the chromosomes. The destructive action of acetic acid is especially important because of its presence in frequently used fixatives such as acetic-alcohol mixtures and in stains which act also as fixatives, like acetic-carmine and orcein.

Reduction in the acidity of any of the fixatives we have tried has resulted in fewer aggregates and less destruction. Figs. 3 and 4 are reproduced, for comparison with Figs 1 and 2, to illustrate the effect of substituting a neutral for an acid fixative. For these preparations neutral 4% formalin was employed, but indistinguishable results were obtained when neutral chromate also was present. Fig 3, like Fig. 1, shows a resting cell but there are striking differences in the texture of both nuclei and cytoplasm. The cytoplasm of Fig. 3 is not distorted and broken up but appears as a fine, uniform network in which there are a few vacuoles and many mitochondria. These "mitochondria" are areas of somewhat greater electron scattering, perhaps due mainly to their finer texture; though sharply delineated from the surrounding cytoplasm, they have no obvious membrane. Most of the mitochondria appear to be elongated objects though they usually are in more or less oblique section. The karyolymph fills the nucleus and has a uniform texture that is definitely different from that of the cytoplasm. It has been surprising that in all pictures of these better fixed preparations there has been

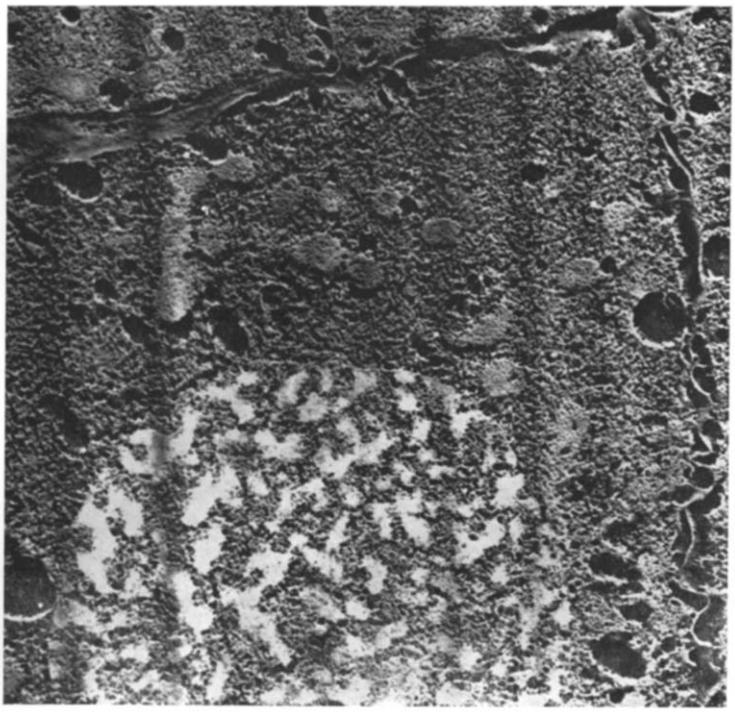

Fig. 3. Part of a resting cell fixed in 4% neutral formalin. Fine-textured mitochondria are scattered through the cytoplasm above the nucleus. The absence of a pronounced nuclear wall is remarkable. The cell boundary runs nearly parallel to the top and right side of the photograph. Magnification = 12,000 ×

no trace of the well-marked nuclear "membrane" seen in such poorly fixed material as that of Fig. 1; instead there is only an abrupt change in protoplasmic texture across the nuclear boundary. The chromatin of the resting nucleus is dense and in the form of small objects of various shapes. Because of the thinness of the sections in which they are best seen, we do not feel justified in deciding yet if these objects are irregular granules often stuck together, or well-defined strands.

Fig. 4 demonstrates the equally different structures that are seen in a dividing cell when a neutral instead of an acid fixative is used. The uniform texture of the protoplasm throughout the cell is especially noteworthy. We have now made several hundred photographs of dividing cells fixed in this way without finding in them the same sort of evidence for a spindle that is so apparent in the acid fixed Fig. 2. These photographs record many steps in the transition from the interphasic nucleus to the prophase, with the concomitant disappearance of the nucleolus. They also have shown under conditions capable of giving resolutions of 50 Å the internal structure of prophase and later stage chromosomes. The chromosomes as thus seen are completely devoid of any particulate internal fine structure. Metaphase chromosomes appear at high magnifications as dense but structurally homogeneous bodies; where structural detail could be recognized apparently within their confines, it could be identified as protoplasmic structure seen through the chromosome. The long thin chromosomes seen in electron

Fig. 4. A formalin-fixed cell in anaphase. The uniform fine texture of the protoplasm enveloping the chromosomes is in striking contrast to that of Fig. 2. Magnification = 5,500 ×

micrographs of prophase should undoubtedly be identified with the chromonema, but we have been unable in photographs of this or later stages to see distinctions between chromonema, matrix, and pellicle. Since the chromosomes have always shown a homogeneous internal structure, nothing has been seen which could be recognized as chromomeres, or as genes. We have obtained equally revealing photographs of the chromosomes in acid-fixed preparations without finding a particulate internal structure within them. These acid-fixed chromosomes have adhering to them clusters of objects of dimensions down to the limit of optical resolution and below, but their superficial character and the fact that they are bits of coagulated cytoplasm are obvious under the electron microscope.

This work has demonstrated that it is now possible to study the internal structure of chromosomes with the electron microscope and to visualize with complete clarity those details associated with chromosomes and cell division that have for years been observed under the far lower resolving powers of the optical microscope. It emphasizes the differences in this detail that arise from different methods of tissue preparation and the need for continued research on methods of fixation that will give a minimal protoplasmic alteration as judged by the high resolution of the electron microscope. We will publish elsewhere in greater detail our results dealing with the fine structure of onion root tip and other chromosomes.

SUMMARY

Electron micrographs have been made of thinly sectioned onion root tip treated with various fixatives. These demonstrate the gross damage done to all fine cell structures by acid fixation and illustrate the appearance of some of these structures, including chromosomes, after non-acid fixation.

RÉSUMÉ

Des micrographies électroniques de coupes minces de pointe de racine d'oignon traitées par des fixateurs différents font apparaître le gros dommage que cause la méthode de fixation acide à toutes les fines structures cellulaires. Elles permettent également de voir certaines structures, dont les chromosomes, après fixation par des agents fixateurs non-acides.

ZUSAMMENFASSUNG

Elektronenmikrogramme von dünnen Schnitten von Zwiebelwurzelspitzen, die mit verschiedenen Fixiermitteln vorbehandelt sind, beweisen welch grosse Schäden die saure Fixiermethode in allen Zellen-feinstrukturen anrichtet. Nach Fixierung mit nicht sauren Fixiermitteln kann man mit Hilfe dieser Elektronenmikrogramme einige andere Strukturen, einschliesslich der Chromosomen, erkennen.

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