CYTOPLASMIC MICROTUBULES IN DIFFERENT ANIMAL CELLS

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ABSTRACT

In avian, murine, and human cells fixed with glutaraldehyde, cytoplasmic microtubules 180 to 250 A in diameter and of undetermined length were found. These cytoplasmic microtubules are similar to those described by Ledbetter and Porter in plant cells after the same glutaraldehyde fixation. The cytoplasmic microtubules in animal cells are connected with the satellites of the centrioles and are similar to the mitotic spindle fibers. Their protein nature and their possible role in maintaining the shape of the cells are discussed. Their presence in all examined animal cells as well as in plant cells favors the hypothesis that they are a permanent component of the cytoplasm.

INTRODUCTION

The search for new fixatives allowing cytochemistry in electron microscopy has led to the introduction of glutaraldehyde fixative (33). This has been found to be good not only for cytochemistry but also for routine electron microscopy, revealing structures not seen after osmium tetroxide fixation alone. Microtubules scattered through the cytoplasm have been observed in glutaraldehyde-fixed cells of different types, in the course of our electron microscopic cytochemical study of avian tumors (11–13). The same cytoplasmic microtubules were also found in normal and neoplastic tissues of murine and human origin.

These microtubules are similar to those described in plant cells after glutaraldehyde fixation by Ledbetter and Porter (22). More recently Porter reported similar findings in the cytoplasm of liver cells (28).

This report will describe in animal cells the relations found between these microtubules and the satellites of the centrioles, and the similarity between these microtubules and the mitotic spindle fibers.

MATERIALS AND METHODS

The avian material included tumors which had been induced by the avian myeloblastosis virus;[1] *i.e.*, myeloblastic leukemia maintained *in vivo* and *in vitro*, kidney tumors, liver tumors, and ovarian tumors. Corresponding normal tissues and some Rous sarcomas were also examined. The mouse material included normal mammary gland and mammary tumors, cells of the ascites form of the Moloney leukemia (27) maintained *in vivo*, and the cells of the Rauscher leukemia (29) cultured *in vitro*. Some human tumors and human leukemic cell preparations were also examined after glutaraldehyde fixation.

The cells in suspension (ascites form or tissue cultures) were fixed with 2.5 per cent glutaraldehyde solution at pH 7.4 (33) for 30 minutes. The solid tissues were fixed with 5 per cent glutaraldehyde solution for 2 to 5 hours. All materials were then postfixed in osmium tetroxide and embedded in Epon 812 (16) or Epon-Araldite mixture (26).

Ultrathin sections were cut with Dupont diamond knives on Porter-Blum and LKB microtomes, double

[1] From the laboratory of Dr. J. W. Beard, Duke University Medical Center, Durham, North Carolina.

stained with uranyl acetate (38) and with lead (21, 30), then examined with a Siemens Elmiskop I using 60 kv accelerating voltage and a 50 μ objective aperture.

RESULTS

General Aspect

It appears that after double fixation (glutaraldehyde-osmium tetroxide) (33) of cells more components are preserved than after osmium tetroxide fixation alone. The ground substance of the cytoplasm between the ribosomes is filled with a fine fibrillar network (see Figs. 1, 4, and 6).

The mitochondria have an appearanace similar to that obtained after chrome-osmium tetroxide fixation (7). The mitochondrial matrix appears as a dense fibrillar network (Figs. 1, 4, and 6), probably resulting from the coagulation of proteins. However, the density of the mitochondrial matrix decreases the apparent contrast of the cristae. The chromatin, which is easily stained with uranyl and lead ions after aldehyde fixation, appears to be condensed at the periphery of the nucleus and around the nucleolus (Fig. 1). The visualization of the cytoplasmic microtubules after glutaraldehyde fixation seems to be one of the main differences between this fixation and fixation with osmium tetroxide alone.

Cytoplasmic Microtubules

Figs. 1 to 3, and 7 to 11 show straight, apparently rigid microtubules in the cytoplasm of glutaraldehyde-fixed cells. These formations are commonly found in the "central area" of the cytoplasm (Golgi zone and centriole) (Fig. 1). They are also present, but to a lesser extent, in the periphery of the cytoplasm (arrows, Figs. 1 and 2). These microtubules often seem to radiate from the area of the centriole toward other areas of the cytoplasm, including the nuclear envelope (Fig. 1). However, no definite connections could be found with either nuclear pores or with any cytoplasmic organelles.

The over-all diameter of the tubules varies from 180 A to 250 A. Their length is undetermined but can be traced for several microns in some sections (Figs. 1, 7, and 10). They possess a 40 to 60 A thick wall, the constitution of which seems to be more fibrillar than membranous (Fig. 5). Their inside diameter measures around 100 to 120 A. In cross-section (Figs. 4, 5, and 8) no internal density can be seen, but in longitudinal section the inside of the cylinder is denser than the cytoplasmic background, probably due to the fact that the entire thickness of a microtubule can be seen in one ultrathin section.

Relations with the Centriole

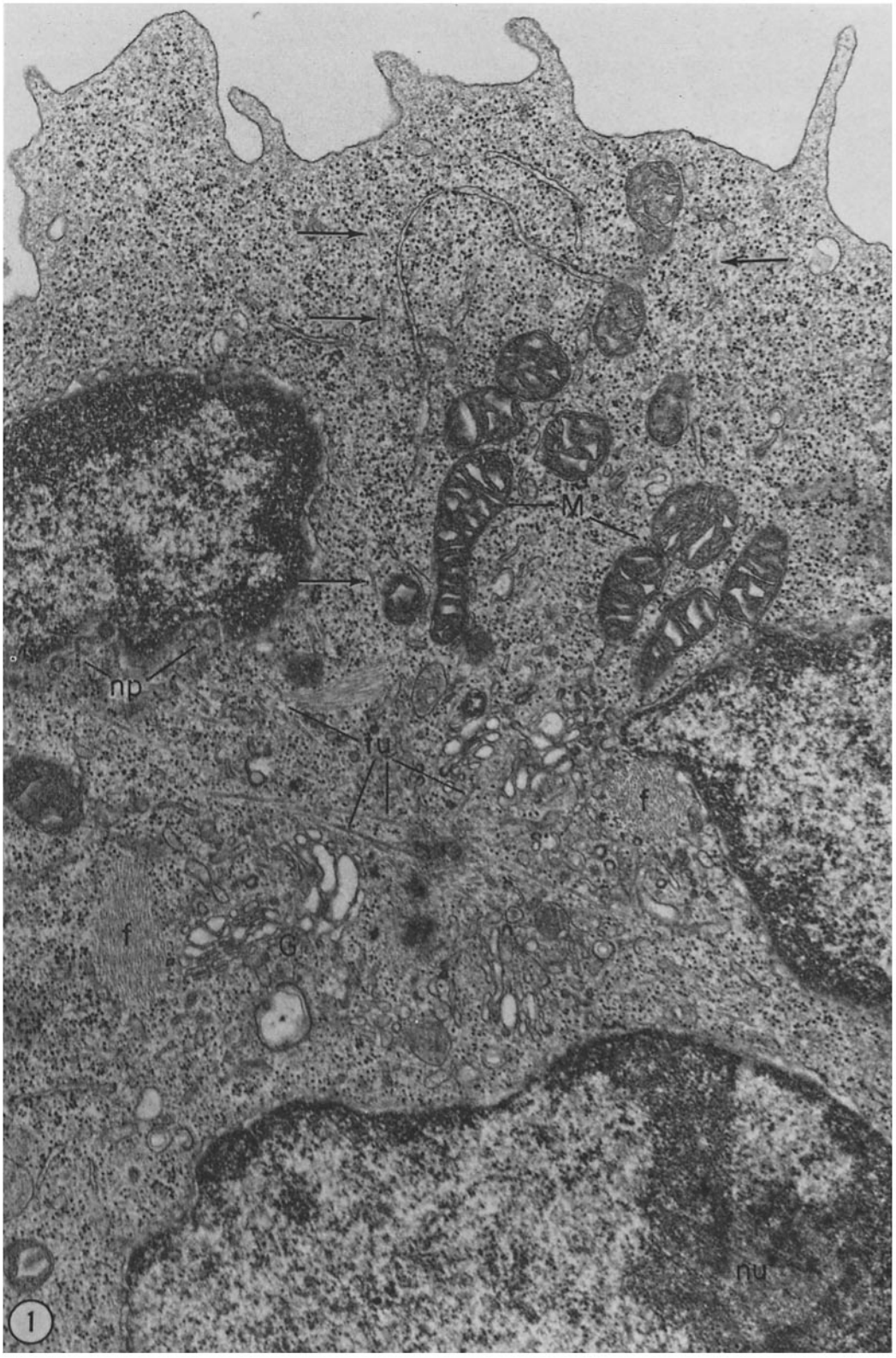
A close association between the microtubules and the centrioles was frequently observed (Figs. 1, 7, 8, 10, and 11). The microtubules were never in contact with the centriolar cylinder itself, but were attached to the dense paracentriolar formations (see Figs. 7 to 11), which have been termed "massules," "satellites," or "procentrioles" by Bessis and Breton-Gorius (4), Bernhard and de Harven (3), and Gall (17), respectively. These different terms appear to refer to similar formations since the tubular elements described by Gall in the procentrioles (see Fig. 23 in reference 17) are also suggested in the present material (see arrows in Figs. 8 and 12), although these structures have been somewhat overstained. Fig. 7 shows numerous satellites or procentrioles to which microtubules are attached.

The microtubules appear to radiate in different directions from the satellites (Fig. 9). In some cases small parts of such microtubules can be observed near the satellites of material fixed with osmium tetroxide alone (Fig. 12).

Connections between satellites and centriole were described as "bridges" by Bessis and Breton-

All micrographs are of tissues fixed with glutaraldehyde and postfixed with osmium tetroxide except Fig. 12 which is of tissue fixed only with osmium tetroxide.

FIGURE 1 Survey view of a leukemic ascites cell (Moloney). In the central part of the cytoplasm are seen microtubules (*tu*) radiating from centriolar formations in all directions. Many of these microtubules are near the nuclear membrane on the left, but no connections can be seen with the well delineated nuclear pores (*np*). Segments of microtubules can also be seen in the periphery of the cell (arrows). The mitochondria (*M*) have a dense matrix. Fibrillar structures (*f*) are similar to those described by de Petris *et al.* (14). *G*, Golgi area; *nu*, nucleolus. × 27,000.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Gorius (4). In some instances (Figs. 11 and 12) these bridges seem double, joining each satellite to two neighboring triple fibers of the centriole. The tubular nature of these bridges (4) is possible (Fig. 12) but not clearly seen.

Aspect During Mitosis

The spindle fibers of the mitotic apparatus after osmium tetroxide fixation have been described as fibrils (19) or tubules (32) with a diameter of 150 A, and in some cases as tubules 200 A in diameter (10, 20). After glutaraldehyde fixation (Figs. 13 and 14) they clearly appear as tubules (see also reference 22) with a diameter varying between 180 A and 220 A. When seen in cross-section the spindle fibers appear identical with the cytoplasmic microtubules of the cell in interphase (compare Fig. 13 with Figs. 4 and 5).

DISCUSSION

Cytoplasmic microtubules have been visualized in animal cells after glutaraldehyde fixation. These microtubules are very similar to those reported in plant cells by Ledbetter and Porter (22) and in hepatic cells by Porter (28) after glutaraldehyde fixation. Slautterback (34), using osmium tetroxide fixation, has also reported the presence of cytoplasmic microtubules in *Hydra*.

Comparison with Other Known Structures

Certain specialized structures seen after fixation with osmium tetroxide are somewhat similar to the microtubules observed after glutaraldehyde fixation. These structures include the tubules associated with myofibrillogenesis (2), the tubular fibers of the marginal band of the nucleated red cell (15), the cytostomal fibers in *Trypanosoma mega* (35), the cytoplasmic tubules arising from some parabasal bodies (18), the tail fibrils of spermatogenic cells (5), and perhaps the tubules in spermatozoids of fern described by Manton (24), or the striations found in shadowed preparations of *Trypanosoma Cruzi* (25). However, at variance with such particular formations, the microtubules described herein have been found in all animal cells examined after glutaraldehyde fixation.

The similarity between these microtubules and the spindle fibers of the mitotic apparatus suggests that they represent the same structures, for the spindle fibers are also tubular (10, 20, 32). The apparent differences in diameter can be accounted for by the differences in technique utilized by the various authors. The persistence of spindle structures during interphase has been suggested by Lettré and Lettré (23). The observations reported here confirm their hypothesis, although definite connections between the microtubules and the nuclear pores were not found.

Nature and Origin of the Microtubules

The protein nature of these microtubules is very probable, since "the dialdehydes are excellent cross-linking agents that react rapidly, especially with active hydrogen, amino, and imino groups in protein. . . This cross-linking property results in the *in situ* insolubilization of many proteins and gives what can be regarded as a relatively undistorted fixation of cellular structures, . . . especially

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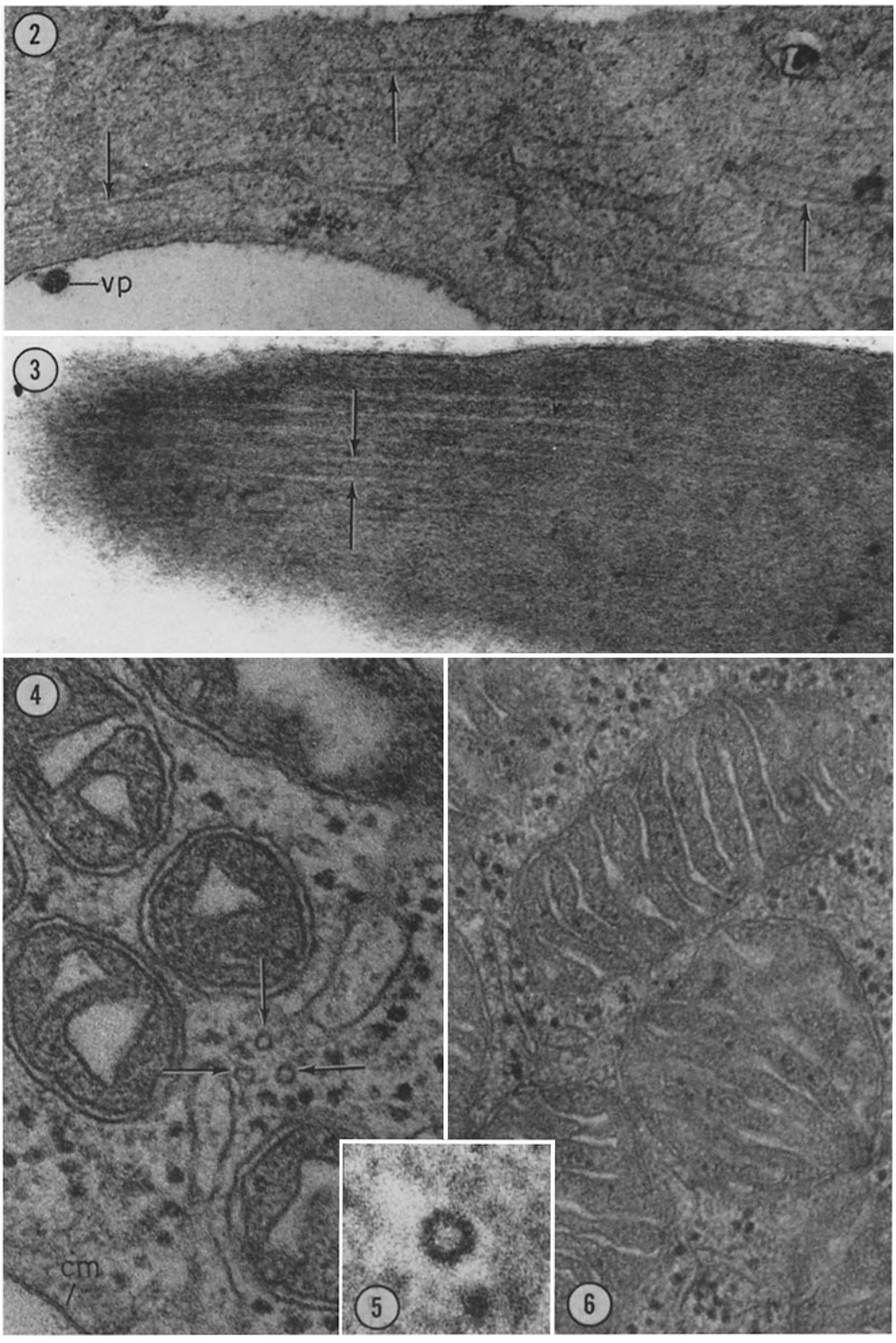
FIGURE 2 Peripheral portion of a spindle-shaped Rous sarcoma cell, showing many cytoplasmic microtubules (arrows) all oriented in the long axis of the cell. *vp*, virus particle. × 42,000.

FIGURE 3 Tangential section of an avian nucleated red cell, showing similar parallel microtubules (arrows). × 92,000.

FIGURE 4 Periphery of the cytoplasm of an ascites tumor cell, showing cross-sections of three microtubules (arrows) and mitochondria with a dense matrix made up of a fibrillar network. The cell membrane (*cm*) appears composed of 3 layers (Robertson's unit membrane, see reference 31). × 122,000.

FIGURE 5 Higher magnification of a cross-section of a cytoplasmic microtubule where no membranes are visible. × 350,000.

FIGURE 6 Mitochondria of an avian leukemic myeloblast, showing a dense matrix with some small granules also visible after chrome-osmium tetroxide fixation. Note the fine fibrillar network of the cytoplasm between the ribosomes. × 86,000.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in the case of glutaraldehyde . . ." (33). This coagulative property of glutaraldehyde might also account for the dense fibrillar network of the mitochondrial matrix and of the cytoplasmic ground substance.

The nature of the mitotic apparatus has been studied in amebae by Roth and Daniels (32) who concluded "that spindle fibrils are composed of polymerized, oriented protein molecules that are in equilibirum with and bathed in non-oriented molecules of the same protein."

Concerning the origin of the microtubules, the conclusion of Ledbetter and Porter is that "centrioles are apparently not essential to their development, for they are absent in *Pelomyxa* as in plant cells" (22). On the contrary, Slautterback describes the microtubules arising from the periphery of a very special centriolar formation in *Hydra* (34). In the micrographs of Bernhard and de Harven (3), of Gibbons (18), and of Gall (17), among others, portions of microtubules similar to those described herein can be seen in close relation with the centriolar formations. In the present material the satellites appear to be the site of attachment of the microtubules.[2] However, in hepatic cells where similar microtubules are present (28, 8), centrioles are rarely seen. In cells which have no centrioles, the origin of microtubules might be in structures which represent the points of attachment of the mitotic fibers.

It seems not unreasonable to suggest that the synthesis of the constitutive proteins of the microtubules might take place within the satellites of the centrioles, under the control of the ribonucleic acid present in the centriolar formations (1, 9, 37).

Protein synthesis has been shown to occur in isolated mitotic apparatus (36) and on spindle fibers of cells infected with reovirus (6) where a protein coat comparable with that of the viral capsid appears on the spindle fibers. On the other hand, the diameters of both the microtubules and the centriolar elementary tubules are similar (10), suggesting that the centriolar tubules may be the "model" for the synthesis of the microtubules or spindle fibers.

Role of these Microtubules

It is not known at the present time whether these microtubules are only rudiments or remnants of the mitotic fibers having no role during interphase, or whether they play an active role in determining the shape of the cells, as suggested in plant cells (22). In animal cells, we have found few examples of microtubules ending near or at the cell membrane (see Fig. 3). On the other hand, spherical ascites cells have radiating microtubules (Fig. 1), whereas elongated fibroblasts or flattened red cells have microtubules parallel to the axis of the cell (Figs. 2 and 3). These microtubules are perhaps involved in the movements of the cytoplasmic organelles or in the movements of the entire cell.

The author is very grateful to Dr. A. J. Dalton, National Cancer Institute, for helpful suggestions and comments during this study. The excellent technical assistance of Mr. Ben Elliott, Mrs. Marian Smith, and Mr. Douglas Jones is also very much appreciated.

This work was completed while the author was a Visiting Scientist at the Laboratory of Viral Oncology, National Cancer Institute, National Institutes of Health.

Received for publication, December 27, 1963.

[2] *Note added in proof:* While this paper was in press, confirmation of the attachment of the spindle fibers to the satellites of the centriole was given by J. André and W. Bernhard at the 11th International Congress for Cell Biology, Providencce, Rhode Island, August 30 to September 5, 1964, in their presentation on "The centriole and the centriolar region."

FIGURE 7 Ascites cell, showing longitudinal and cross-section of two centrioles surrounded by many "satellites" (3) or procentrioles, *pc*, (17). Note that microtubules (*tu*) arise from some of them. The microtubules and centriolar tubules have a similar diameter. × 85,000.

FIGURE 8 Ascites cell, showing cross-section of a centriole surrounded by at least two satellites (*pc*) and many cross-sections of microtubules (*tu*). At left, cross-sections of tubular structures are suggested in the upper satellite (double arrows). × 100,000.

FIGURE 9 Part of a centriole area showing, at upper left cytoplasmic microtubules radiating from one locus (arrow) into different directions. To the right are two satellites (*pc*), the lower with possible tubular structures. × 120,000.

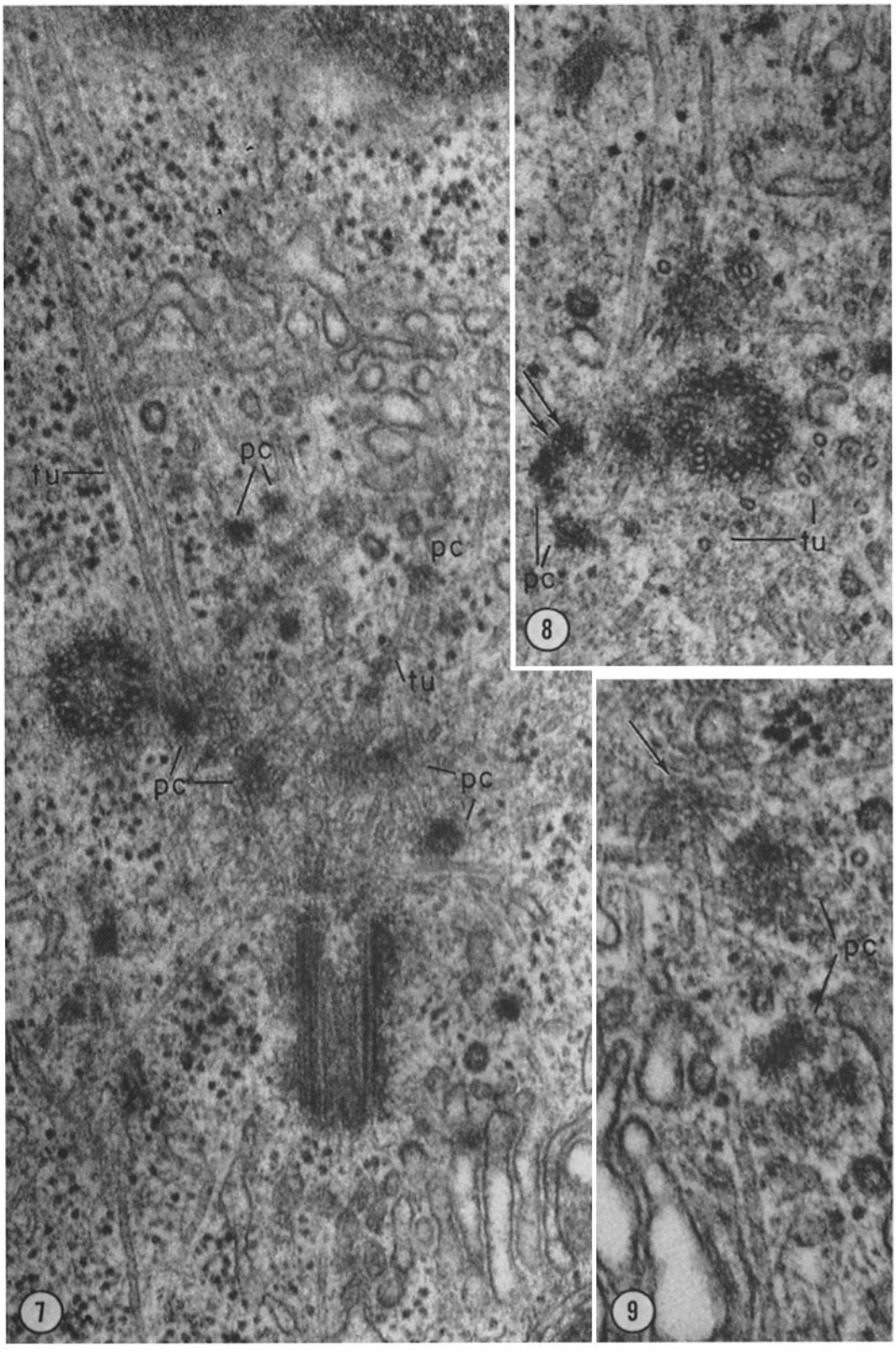
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FIGURE 10 Part of the cytoplasm of an avian myeloblast showing two perpendicular centrioles. Underneath them is a satellite or procentriole (*pc*) from which a long microtubule (*tu*) arises. *G*, Golgi area. × 71,000.

FIGURE 11 Higher magnification of a centriole and satellite joined by a double bridge cut obliquely (arrows). A microtubule (*tu*) originates from the satellite (*pc*) and extends down toward the lower right. × 145,000.

FIGURE 12 Centriole area of an avian thymocyte fixed with osmium tetroxide alone. The 9 triple-tubules of the centriole are clearly visible. Two central formations are visible (suggesting the 9 + 2 fiber pattern of cilia). Outside the centriole, five satellites or procentrioles (*pc*) are present. In some of them, tubular structures can be seen (arrows). Between these satellites and the mother-centriole are double bridges joining each satellite to two neighboring triplets of the centriole. × 125,000.

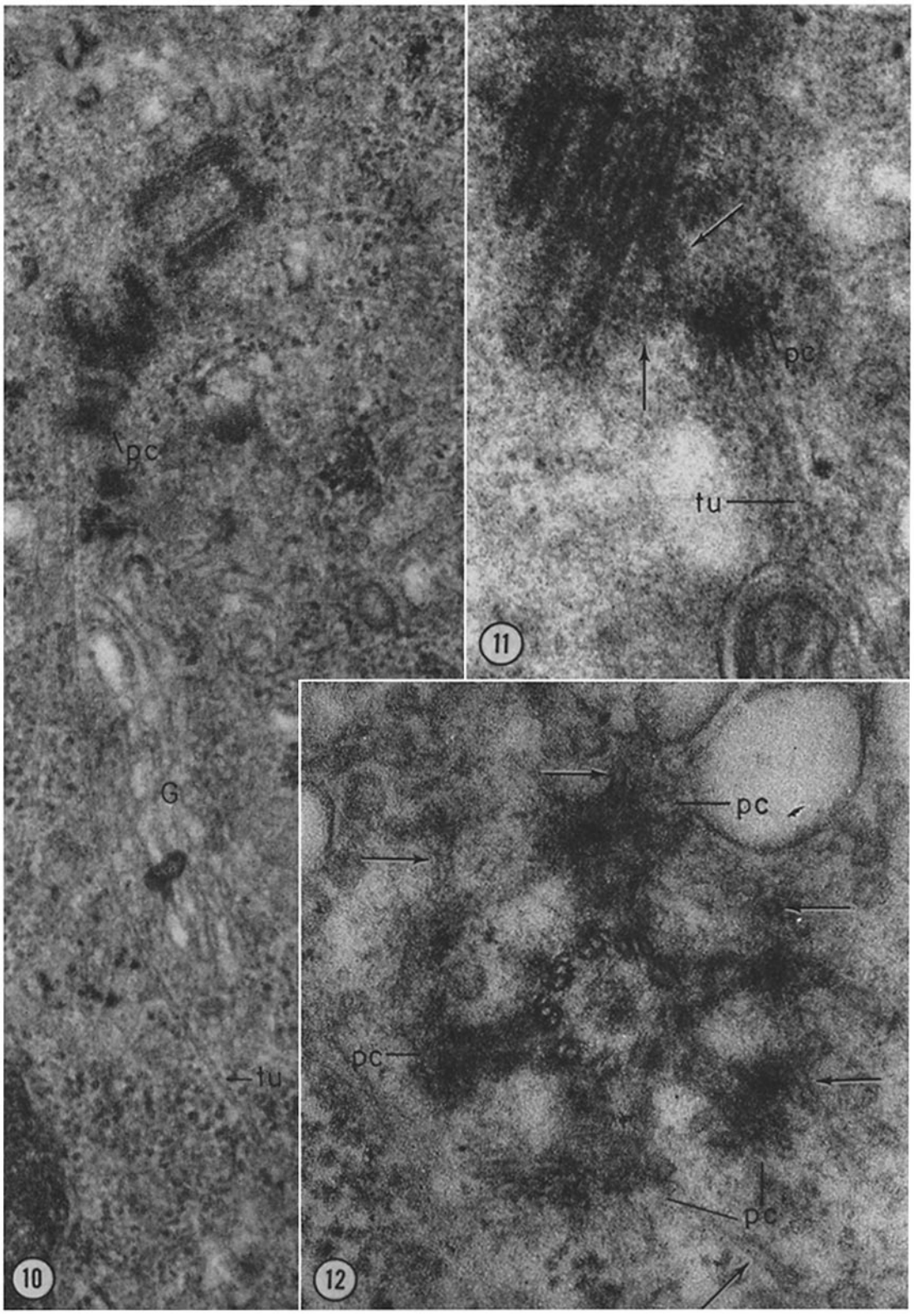
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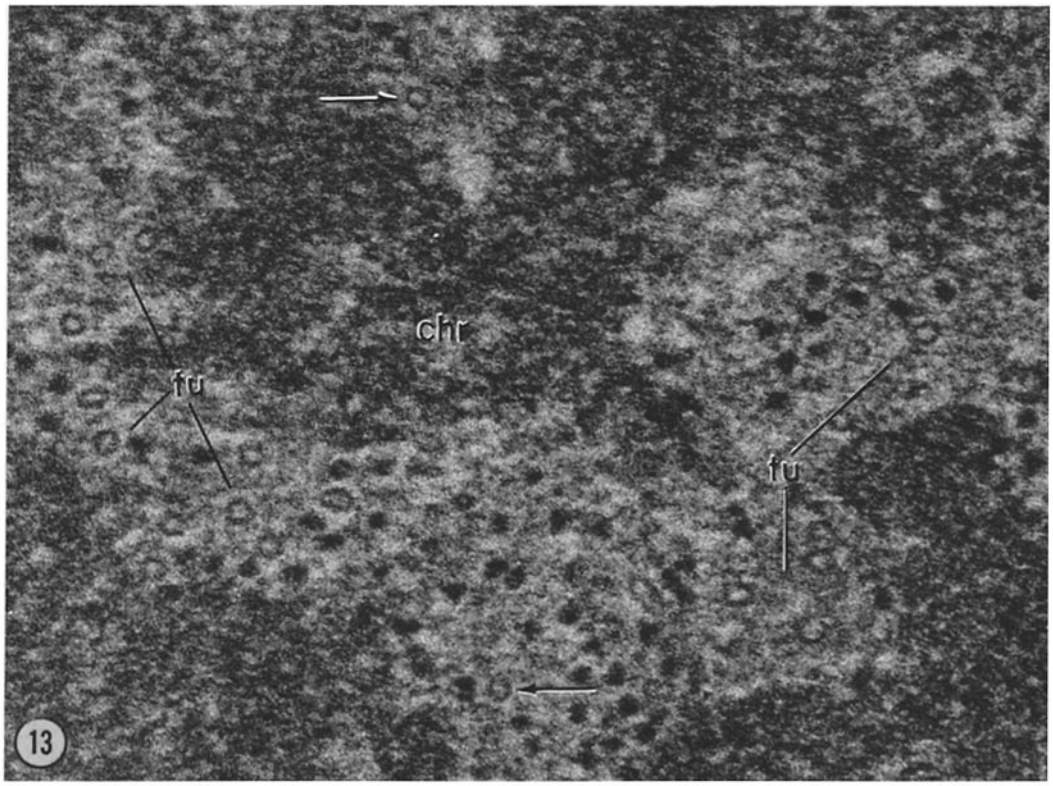
FIGURE 13. Mitosis of ascites cell, showing cross-section of mitotic spindle fibers (*tu*, and arrows). The size and appearance of such fibers (clearly seen as tubules) are similar to those of the cytoplasmic microtubules. Between the ribosomes a fine fibrillar network can be seen. *chr*, chromosome. × 160,000.

FIGURE 14 Mitotic figure of an avian leukemic myeloblast showing, in longitudinal view, spindle fibers as tubules which are very similar to the cytoplasmic microtubules. The dense material present at the cell membrane is a lead phosphate deposit resulting from the incubation of the cell in the Wachstein and Meisel medium (see reference 13). *vp*, myeloblastosis virus particle showing ATPase activity at its envelope; *chr*, chromosomes; *ce*, centriole. × 53,000.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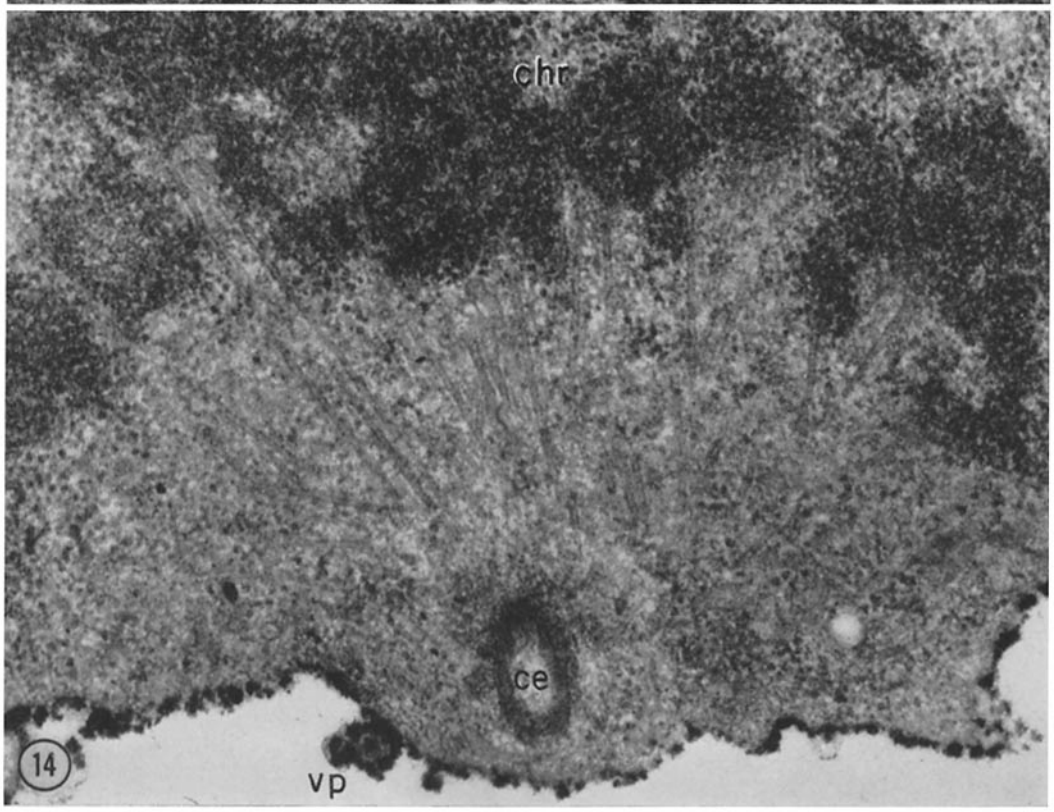
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