On the Ultrastructure of the Walker 256 Carcinosarcoma*

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SUMMARY

The morphology of the principal cells in Walker 256 carcinosarcoma in rats was studied with the electron microscope. The chromatin structure was composed of fibers 40–80 A in diameter, which were also present in very small amounts in the nucleoli. Particles in nucleoli, nuclei and cytoplasm, presumably containing ribonucleoprotein, seem to be composed of a dense peripheral wall that stained with heavy metals during fixation and dehydration procedures and a central light core. In the cytoplasm of several cells, ribonucleoprotein inclusions were found which were apparently formed in the lumen of endoplasmic reticulum by invagination and sequestration of small portions of cytoplasm containing ribosomes. Irregularly distributed fine fibers of low electron density were also present in the cytoplasm.

The Walker 256 carcinosarcoma of rats has been the subject of many biochemical and microscopic investigations in experimental cancer research. Although ultrastructural studies on this tumor have been made by Mercer and Easty (11), by Fisher and Fisher (6) and by Pool (15), the need for electron-microscopic controls on cell fractions obtained for biochemical studies in this laboratory resulted in the present study of the general ultrastructure of the principal cells of this tumor. In the course of these studies the “ribonucleoprotein particles” in nucleoli, nuclei, and in cytoplasm have been shown to be composed of a dense peripheral layer and a light central core. In addition to the particles that presumably contain ribonucleoproteins, some chromatin-like fibers were seen in nucleoli. The substructure of the nucleolonemas of nucleoli appeared to consist of a network of short tubules and fibers enmeshing small vesicles and granules. In the cytoplasm, inclusions were found that were formed by invagination and sequestration of cytoplasm in the lumen of the endoplasmic reticulum. These cytoplasmic inclusions correspond to the basophilic inclusions seen by light microscopy.

MATERIALS AND METHODS

Small fragments of Walker tumor were fixed for 2–3 hours in 2 per cent OsO₄ in distilled water (3, 13) and in 2 per cent OsO₄ at pH 7.2–9 (18). Some of the samples were “postfixed” overnight at 4°C in 10 per cent neutral formalin (pH 7.8) with saturated calcium carbonate (9). The absolute ethanol used in the dehydration procedure was diluted with 1 per cent uranylacetate to a final concentration of 0.5, 0.3, and 0.1 per cent of uranylacetate for staining and prevention of damage by polymerization during embedding (5, 20). The dehydrated specimens were embedded in Epon (10) or Maraglas (7). Sections were cut with a Porter-Blum ultramicrotome and were studied with an RCA 3F electron microscope. The examination of a series of high-magnification pictures, both over- and underfocused, eliminated the possibility of producing focusing artifacts (12). For controls by light microscopy, smears of the Walker tumor were treated with Giemsa’s stain for general orientation, by the Feulgen reaction for staining of chromatin (1) and with toluidine blue for staining of nucleoli and basophilic structures (2, 16).

RESULTS

Nuclei.—The nuclei were round, oval or kidney-shaped, and frequently invaginations of the nuclear membrane were noted that contained small portions of cytoplasm (Fig. 1). The chromatin which was composed of thin fibers, with a diameter of 40–80 A (Fig. 2), was homogeneous and finely dispersed in some nuclei. In other nuclei chromatin was distributed along the nuclear membrane, and
a few chromatin clusters were noted in the central part of the nucleus (Fig. 1).

The nucleoli were composed of nucleolonemas (Figs. 1, 2, and 3), between which light spaces were seen. In a number of nucleoli the spaces between nucleolonemas were extremely large and appeared to be the vacuoles seen by light microscopy. Nucleolonemas were composed of structures of 90–160 A in diameter (Fig. 3) containing fibers, granules, small vesicles, and short tubules. The walls of the small vesicles and short tubules were densely stained with osmium and uranylacetate during fixation and dehydration. The central parts of these particles had the same degree of electron density as the background. The thickness of the walls of the small vesicles and short tubules was 25–40 A. Some granules and short fibers appeared to be sections of walls of small vesicles and short tubules. Inside the nucleoli a few fibers were found that were similar to the chromatin fibers outside the nucleolus (Figs. 2, 4). The thickness of these fibers was the same as the thickness of chromatin fibers, that is, 40–80 A. Around the nucleoli a layer of “nucleolus-associated chromatin” was usually present (Figs. 1, 2) and sometimes also chromatin clusters.

The interchromatinic areas were light spaces between chromatin structures (Fig. 1). In relatively thicker sections a continuity was observed between the interchromatinic areas. In interchromatinic areas some dark particles were present. At higher magnification some of these dark particles seem to have a dense peripheral wall stained with metals during fixation and dehydration. The central parts of these particles had the same degree of electron density as the background. The thickness of the walls of the small vesicles and short tubules was 25–40 A. Some granules and short fibers appeared to be sections of walls of small vesicles and short tubules. Inside the nucleoli a few fibers were found that were similar to the chromatin fibers outside the nucleolus (Figs. 2, 4). The thickness of these fibers was the same as the thickness of chromatin fibers, that is, 40–80 A. Around the nucleoli a layer of “nucleolus-associated chromatin” was usually present (Figs. 1, 2) and sometimes also chromatin clusters.

The nuclear membrane was composed of two dense layers, and sometimes cistern-like dilatations were found between these two layers. In the last stages of mitosis, the formation of the nuclear membrane was associated with the endoplasmic reticulum (Fig. 6).

Cytoplasm.—The size of the cytoplasmic layer around nuclei and the number of different cytoplasmic structures were very variable in ultrathin sections. The cell surface was smooth, but had occasional protrusions that were short pseudopod-like structures or long, thin microvilli (11). The degree of contact of tumor cells varied since intercellular spaces of varying sizes were present between the cells.

Most mitochondria were round or ellipsoid, but abnormally shaped mitochondria were also found, such as the T-shaped mitochondrion in Figure 7. In ultrathin sections in one block, abnormally shaped mitochondria were found in 6 of 89 cell sections and in 2 of 84 cell sections in another block. In mitochondria, a few granules ranging in size from 240–255 A were present. In some mitochondria the spaces between cristae were greater, and in others cristae could not be observed. Golgi bodies (Fig. 8) were usually well developed and frequently were hypertrophic.

In all cells there was variation in the sizes of vesicles and tubules of the endoplasmic reticulum. The extensive vacuolization in some cells seemed to be caused by dilatation of endoplasmic reticulum (Fig. 9). Palade granules ranging from 120–145 A in diameter were present in the cytoplasm, free or associated with endoplasmic reticulum.1

In a few cells vacuole-like dilatations of the endoplasmic reticulum were present and contained cytoplasmic saclike inclusions with a great number of ribosome-like particles (Fig. 10). These ribonucleoprotein inclusions were apparently formed by the invagination of cytoplasm (4, 18) with ribosomes in the lumen of the endoplasmic reticulum (Fig. 11). The diameter of the ribosomes in cells that formed inclusions appeared to be larger (160–200 A) than the diameter of the ribosomes in the other tumor cells (120–145 A). In smears stained with toluidine blue these inclusions were basophilic. The variability of appearance of these inclusions was evident from the fact that in ultrathin sections, 10 inclusions were found in 85 cell sections of one block and in none of 85 cell sections in another block.

The multivesicular bodies present in many cells of Walker tumor generally had two forms: large, light bodies (Fig. 19) and small, dense bodies (Fig. 19). They were composed of outer membranes surrounding small vesicles or of bodies 400–470 A in diameter. In a few light multivesicular bodies double membranes were found that were similar to the double membrane of mitochondrial cristae (Fig. 14). Vesicles similar to those present in multivesicular bodies were also found free in the cytoplasm of some cells (Fig. 15).

Lamellar bodies were found that were composed of many concentric membranes (Figs. 16, 17). Some of these bodies seem to originate on or near Golgi membranes. In several lamellar bodies small particles were present. In the cytoplasm, very fine filaments 80–100 A in diameter, were also found (Fig. 18). They were distributed very irregularly in the cells, and their electron density was lower.

1 With higher magnification some Palade granules appeared to be composed of darker peripheral wall, 25–35 A thick, and a central light area (Figs. 10, 11). It is possible such structures may represent partial uncoiling of molecules of high axial ratios.
than that of other membranous structures. The composition of the fine fibers irregularly distributed in the cytoplasm is not known, but similar fibers were found in other tumors (19) and also in nontumor cells (14). Lipoid particles (Fig. 1) had irregular forms, and they were present in only a few cells and in very small number.

DISCUSSION

In general, the description of the ultrastructure of the cells of Walker tumor is similar to the descriptions in earlier studies (6, 11, 15). With higher magnifications it was found that particles in nucleoli, nuclei, and cytoplasm that presumably contain ribonucleoprotein, have similar substructures. They seem to be composed of a dense peripheral wall, stained with heavy metals during fixation and dehydration procedures, and of a light, unstained center. The possibility of the existence of a substructure of ribonucleoprotein particles was noted by de Petris et al. (14) in their description of Palade granules in phagocytes and by Hay and Revel (8) in their description of interchromatinic granules in nuclei of cells from regenerating salamander limb.

The inclusions containing ribonucleoprotein particles in the cytoplasm of the cells of the Walker tumor probably correspond to the inclusions composed of dense material noted by Pool (15). Ribosomes in Walker tumor cells with these inclusions had a greater diameter than ribosomes in other cells of the tumor. Inclusions containing ribonucleoprotein particles were described in pulmonary adenoma in mice by Svoboda (18) and in mouse plasmocytomas by Dalton (4). There the inclusions were also formed by invagination and sequestration of small portions of cytoplasm with Palade granules in the lumen of endoplasmic reticulum.

The differences in the appearance of the chromatin structure in nuclei of Walker tumor in the present study and earlier studies may be due to the use of different fixation procedures. In earlier studies fixation was carried out only with OsO₄, and this procedure did not satisfactorily fix the chromatin structure. In the present study post-fixation with formalin was used. The appearance of chromatin structures after this fixation and staining with uranylacetate in the dehydration procedure was very similar to the appearance of chromatin in smears treated by the Feulgen reaction or with Giemsa stains. The diameter of the chromatin fibers approximates that previously described for such fibers (8). A few chromatin-like fibers were also found in nucleoli and may account for the reports of the presence of deoxyribonucleic acid in nucleoli (2). The Feulgen reaction was essentially negative in nucleoli, and hence the amount of chromatin observed by electron microscopy is apparently below the limit of detectability by current histochemical procedures.

The electron micrographs show a continuity of interchromatinic areas containing dark particles and suggest that these dark particles were part of the nuclear ribonucleoprotein network found by light microscopy (17). The association of this network with nucleoli was noted by light microscopy, and the contact with nucleoli of interchromatinic areas containing dark particles was observed by electron microscopy.

Most of the components observed by electron microscopy of the Walker tumor cells have been found in other cells. However, the abnormally shaped mitochondria and the cytoplasmic inclusions are less commonly found in nontumor cells.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Robert M. O'Neal and Mr. Gordon Adams from the Department of Pathology for their advice and help. The authors also wish to express their appreciation to Mr. Charles Taylor for transplantation of the Walker tumor.

REFERENCES


Fig. 1.—Cell from Walker tumor. NO = nucleolus, Ch = chromatin, I = interchromatic area with dark particles, G = Golgi body, Mi = mitochondria, L = lipide, E = endoplasmic reticulum, X23,500.

On each figure the measured line indicates 1 micron, except where otherwise indicated.
Fig. 2.—Nucleolus and nucleolus-associated chromatin. 
Nm = nucleonema, Ch = chromatin, arrows = chromatin fibers in nucleolus, X78,000.
FIG. 3.—Nucleolus at high magnification. Arrows = tubular and vesicular structures, X113,000.

FIG. 4.—Nucleolus showing a few chromatin-like fibers inside the nucleolar structure and around the periphery, X113,000.
FIG. 5.—Dark particles in interchromatinic area. Note that particles are composed of a dark peripheral part and central light area, X154,000.

FIG. 6.—Forming of nuclear membrane from endoplasmic reticulum. Palade granules are clearly seen along the outer layer and occasionally along the inner layer of the forming membrane. Ch = chromatin, X40,000.
FIG. 7.—Abnormal mitochondrion, \( \times 78,000 \).
FIG. 8.—Golgi body in tumor cell, \( \times 45,300 \).
FIG. 9.—Dilatation of endoplasmic reticulum, ×38,000.

FIG. 10.—Ultrastructure of basophilic inclusions composed of small portions of cytoplasm with ribosomes in the lumen of endoplasmic reticulum, ×47,000.
Fig. 11.—Formation of a basophilic inclusion by invagination of a small portion of cytoplasm in the endoplasmic reticulum, X180,000.

Fig. 12.—MV = light multivesicular body, X76,000.
FIG. 13.—MV = dark multivesicular bodies, ×84,000.
FIG. 14.—Multivesicular body (MV) containing structures with double membranes reminiscent of cristae-arrow, ×62,000.
Fig. 15.—Vesicles (V) in the cytoplasm similar to those of a multivesicular body. Note the presence of a microvillus in the intercellular space (arrow), ×81,000.

Fig. 16.—Multilamellar body containing particles, ×81,000.
Fig. 17.—Multilamellar body, ×115,500.

Fig. 18.—Fine fibers of low electron density in the cytoplasm, ×108,000.
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Cancer Res 1963;23:1600-1603.

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