The Fine Structure of Walker 256 Carcinoma Cells

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Summary. Several ultrastructural features of Walker 256 rat tumor cells which have not been observed before are revealed. Nuclear bodies either singlely or in pairs were found in some cells. Cytofilaments were observed in the cytoplasm and cytoplasmic projections in a few cells. Desmosomes which are usually regarded as a feature of differentiated cells were also observed.

The Walker 256 carcinosarcoma was discovered by GEORGE WALKER[1] and the ultrastructural features of these cells were first described by FISHER and FISHER in 1961[2]. Their electron micrographs, however, were not clear when compared with present day standards. BUCK[3] described the fine structure of these tumors in mitosis. To the best of the author's knowledge, these are the only two publications dealing with the fine structure of this particular tumor. In our studies of the metastatic processes of Walker 256 rat tumor[4–7], the fine structure of these cells was investigated and several features which were not described in the above-mentioned papers were discovered.

Materials and methods. The Walker 256 tumor was used in this study. The tumor has been carried by repeated intramuscular transplantation in the thighs of male Sprague-Dawley rats. Tumor slices, about 1 mm³ each, were fixed in 2.5% glutaraldehyde for 2 h in 0.25 *M* sucrose buffered with 0.1 *M* cacodylate to pH 7.2. The tissues were washed overnight with buffer and post-fixed with 1% osmium tetroxide in cacodylate buffer for 1 h. After dehydration, the tissues were stained en bloc with a staining mixture as recommended by KUSHIDA and FUJITA[8] for 2 h at room temperature. They were then embedded in Spurr (Polyscience Inc. Rydal, Pa.). The thin sections were stained with 6% uranyl acetate and REYNOLD's lead citrate[9] and examined with EM 200.

Results. The Walker 256 tumor cells were found to be either round or ovoid in shape. The nuclei were large, averaging about 8.5μm. The nuclear membrane frequently exhibited an irregular margin and occasional invaginations. The nuclear chromatin was finely distributed around the periphery in the form of a thin layer which was interrupted at intervals (Figure 1). The nucleoli were always prominent and measured about 3 μm. Mitochondria were moderate in number and round to ovoid in shape. They

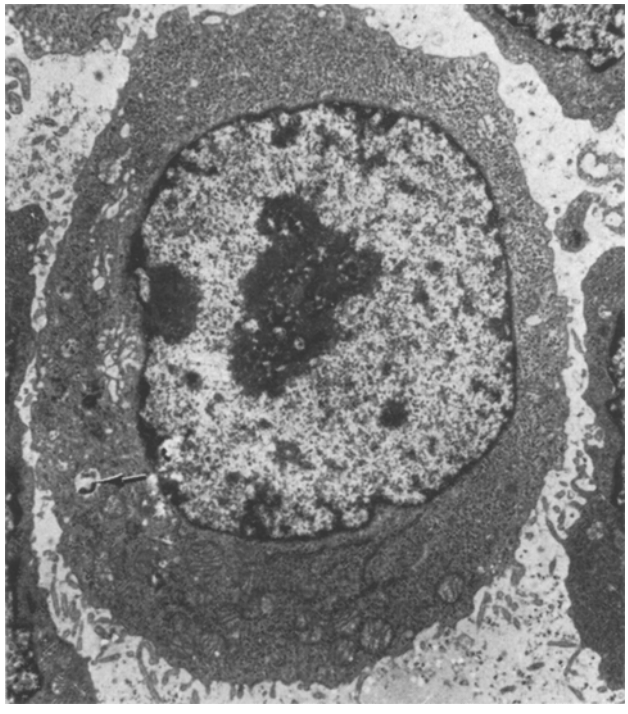

Fig. 1. A Walker 256 tumor cell from a solid intramuscular graft. This cell contains an irregular nucleus and relatively few cytoplasmic organelles. The nuclear chromatin is finely distributed around the periphery. Arrow indicates a phagosome. × 8,500.

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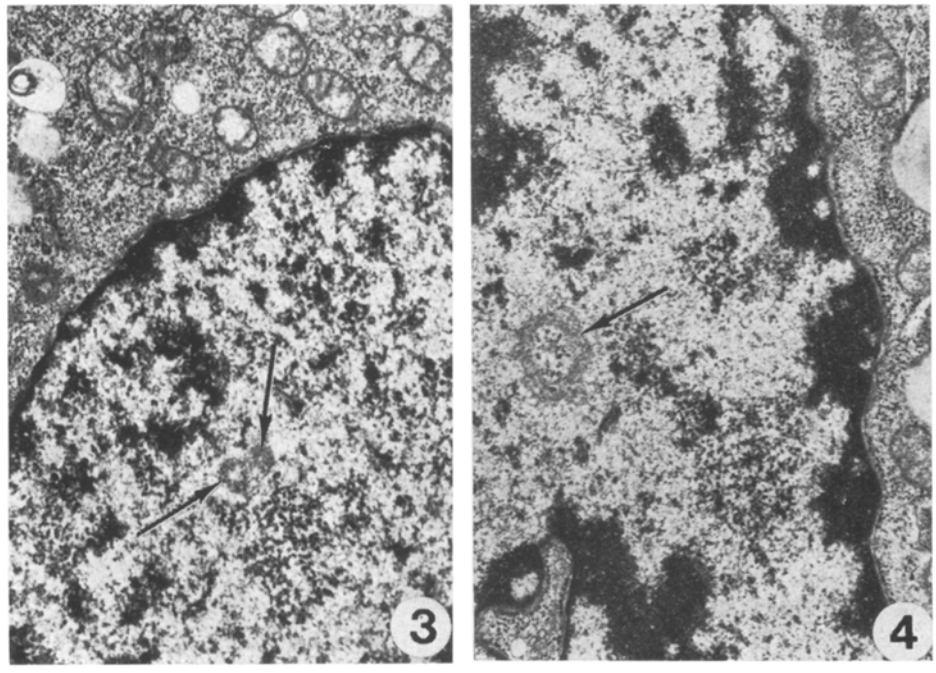
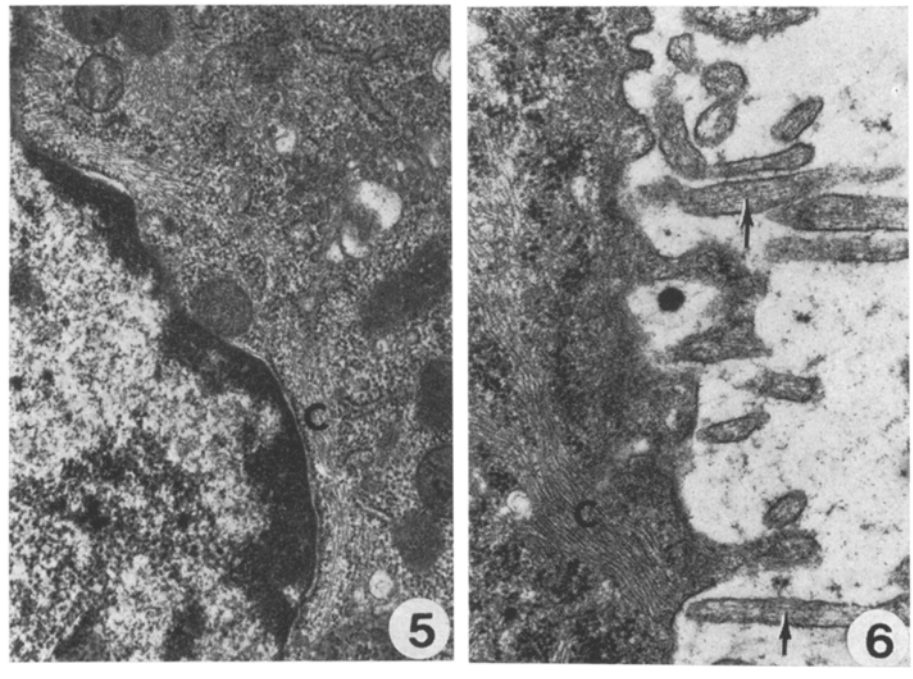
Fig. 2. Part of a Walker 256 tumor cell showing tubular structures (arrow) in the cytoplasm. × 40,000.

contained only a few cristae (Figures 1 and 3). The endoplasmic reticulum comprised scanty tubules with ribosomes attached to them. Large numbers of free and rosette types of polyribosomes were distributed in the cytoplasm and were aggregated in some areas. Golgi apparatus was always prominent. Small vesicles and phagosomes were often present in the cytoplasm (Figure 1). Tubular structures were observed in some tumor cells (Figure 2). Nuclear bodies either singlelyl or in pairs were observed in some cells (Figures 3 and 4). These were spherical structures ranging from 0.8 to 1.8 μm in diameter. A few cells contained cytofilaments which were either located perinuclearly (Figure 3) or at the periphery (Figure 4). A few cytofilaments were also present in cytoplasmic projections (Figure 6). Desmosomes were present between some adjacent cell membranes (Figure 7).

Discussion. The cytoplasm of Walker 256 tumor cells contains numerous free and rosette types of polyribosomes suggesting active protein synthesis in these cells. The fine distribution of nuclear chromatin around the nuclear membranes indicates that the nucleus is active in metabolism.



Figs. 3 and 4. Two Walker 256 tumor growing in solid intramuscular grafts. Arrows indicate nuclear bodies found in some tumor cells. × 18,900.



Figs. 5 and 6. Two Walker tumor cells showing cytofilaments (C) either perinuclearly located (Figure 5) or at the periphery (Figure 6). Cytofilaments are also present in cytoplasmic projections (arrows in Figure 6). × 23,800; × 40,000.

Nuclear bodies were occasionally found within the nuclei of some tumor cells. Similar structures have been found in normal, diseased tissues and in tumors[10–13]. Morphologically, the nuclear bodies observed in this study correspond to Type I and Type II nuclear bodies classified by BOUTEILLE et al.[10]. WEBER et al.[14] believe that the nuclear body is a frequent internuclear inclusion which seems to be related to cellular hyperactivity. This hyperactivity may be physiological, hormonal, drug-induced, viral or tumoral. The appearance of nuclear bodies in Walker tumor cells is likely to be related to hyperactivity.

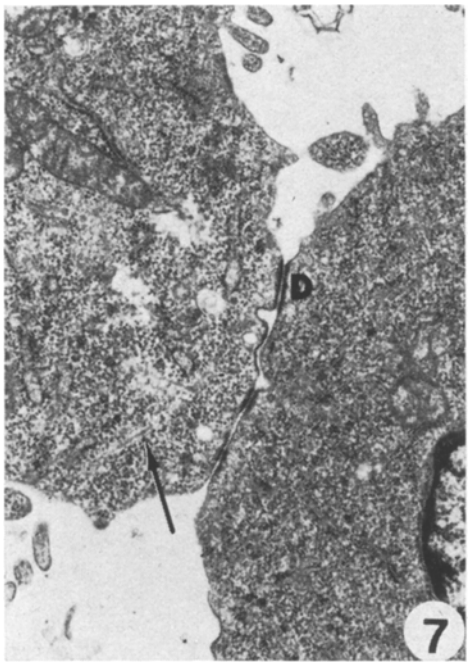


Fig. 7. Walker 256 tumor cells showing desmosomes (D) between the adjacent cell membranes. Arrow indicates tubular structure within the cytoplasm. × 18,900.

Cytofilaments have been observed in many fibrogenic and non-fibrogenic cells as well as epidermal cancer cells[15,16]. In these observations, bundles of cytofilaments were always seen close to the nucleus. However, in the present investigation, cytofilaments were found to be either perinuclearly located or at the periphery. They were also present in cytoplasmic projections. Some authors believe that they play a role in cytoplasmic viscosity or act as a cytoskeleton[15,17]. MALECH and LENTZ[16] suggested that the presence of cytofilaments in malignant cells may be correlated with the motile, invasive properties of these cells. The Walker 256 tumor cells do exhibit ameoboid movement and invasiveness (unpublished results). Thus the author is tempted to agree with MALECH and LENTZ.

The presence of desmosomes is usually regarded as a feature of differentiated cells. They have been observed in several types of tumor cells such as Wilm's tumor[18] and mouse sarcoma cells[19]. The presence of desmosomes in Walker 256 cells has not been reported before. We previously discounted as a Walker 256 cell, any showing desmosomes, but there is clear proof from examining solid Walker 256 tumors that they are formed.

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Pregnancy Following Segmental Isthmic Reversal of the Rabbit Oviduct[1]

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Summary. Microsurgical reversal of a segment of rabbit proximal tubal isthmus has been followed by normal pregnancy in the first two animals to undergo the procedure. Establishment of pregnancy despite radical modification of the oviduct furnishes the opportunity to gain new insights into the mechanisms controlling tubal ovum transport and emphasizes the evolving feasibility and importance of tuboplastic microsurgery both as a research tool and clinical procedure.

Transport of ova through the oviduct into the uterus is a complex, discontinuous process characterized by a pause of varying, species-specific duration at the ampullary-isthmic junction (AIJ) with or without an additional pause at the tubo-uterine junction (TUJ) before final entrance of ova into the uterus[4]. In order to investigate the hypothesis that the tubal isthmus and its junctions constitute the primary physiologic mechanism controlling tubal transport and entrance of ova into the uterus, microsurgical modification of the rabbit oviduct isthmus has been employed. In the course of preliminary investigations, reversal of a segment of proximal tubal isthmus with end-to-end reanastomosis has been followed by pregnancy despite an earlier report that segmental reversal results in infertility[5]. A future report will examine the physiologic consequences of tubal isthmic reversal with particular emphasis upon electrophysiologic, contractile and ciliary activity following completion of this procedure in additional subjects. We describe here in detail the microsurgical technique being used and the resulting pregnancies obtained following our initial isthmic reversal procedures.