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Cytoarchitecture of Ehrlich Ascites Carcinoma Implanted in the Hind Limb of Ascorbic Acid-Supplemented Mice*

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Abstract—*Several morphologic alterations in tissue architecture were observed in solid Ehrlich ascites tumors implanted in the hind limbs of mice drinking distilled water supplemented with* 0.1% *ascorbic acid. Light microscopic observations of non-necrotic tumor sections revealed clusters of cells and occasional regions of denser connective tissue among the tumor cells. Ultrastructural examination revealed tumor cell groupings with peripheral fibroblasts, deposition of cellular product between cells, close association of tumor cells within clusters, and the presence of basement membrane. Tumors from mice maintained on distilled water alone did not exhibit any clustering of tumor cells.*

INTRODUCTION

THERE is accumulating evidence that ascorbic acid plays an important role in maintaining cellular and tissue integrity. As a result, considerable interest has arisen concerning the possible role of vitamin C as a therapeutic agent. Few studies have focused upon the cellular response to ascorbic acid in the non-scorbutic state.

We have investigated this aspect using the solid form of the Ehrlich ascites carcinoma. Previous studies have shown that the growth pattern of the solid Ehrlich tumor is sensitive to environmental factors within the tissue [1–4]. Recent studies have shown that growth of the solid form of the Ehrlich ascites tumor is significantly slower in mice maintained on distilled water supplemented with 0.1% ascorbic acid than growth in mice maintained on distilled water alone [5]. The present work was undertaken to learn if there might be a histological basis for this slower tumor growth.

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MATERIALS AND METHODS

Animals and tumor induction

Two strains of male mice (Swiss mice and CF_1 mice) drank either distilled water or distilled water supplemented with 0.1% ascorbic acid for 1 week prior to and following tumor induction. Mice were housed three to a cage in constant temperature quarters and supplied food and water *ad libitum*. Solid tumors were induced by injecting 1.6–1.8 $\times 10^6$ Ehrlich ascites carcinoma cells (in 0.1 ml saline) i.m. into the mouse hind limb. Tumor tissue was sampled for electron microscopy 13 days after tumor induction. At these times, tumor masses were approximately 1.5–3.4 gm [6]. Tumors from 12 ascorbic acid-supplemented mice (6 Swiss and 6 CF_1) and 12 non-supplemented mice (6 Swiss and 6 CF_1) were sampled for electron microscopic examination.

Electron microscopy

Sections of tumors were minced in Karnovsky's fixture [7] at 4°C for 1.5 hr, rinsed overnight in 0.1 M sodium cacodylate buffer and then fixed 1.5 hr in 2% OsO_4 in 0.1 M cacodylate buffer containing 0.5% $CaCl_2$. Tissues were then rinsed three times in buffer, treated with tannic acid [8], dehydrated and embedded in a low viscosity epoxy

resin [9, 10]. Thick sections (about 1 μm) stained with methylene blue–azure II [11] were examined with a light microscope to select non-necrotic regions of the tumors. Thin sections of appropriate regions were cut with a diamond or glass knife, grid stained with uranyl acetate and lead citrate and examined in a RCA EMU electron microscope.

RESULTS

The solid form of the Ehrlich ascites tumor is well circumscribed and characterized by a high degree of cellularity, only a small amount of connective tissue stroma and poor vascularization [12]. The histologic pattern is undifferentiated (Fig. 1); observation of any definite structural arrangement in the tumor tissue is rare [12]. Figure 2 is a representative micrograph of cell ultrastructure in the solid tumor in a CF_1 mouse drinking distilled water. Nuclei are large and lobulated. Tumor cells in animals maintained on distilled water did not exhibit any clustering or cellular associations. Occasionally, neighboring cells did have membranes closely opposed, but this was observed on the ultrastructural level only for single pairs of cells. Both light and dark staining cells are present in tumor sections.

The most prominent feature of tumors of ascorbic acid-supplemented animals was the formation of cell clusters and occasional regions of denser connective tissue coursing among the cells (Figs. 3 and 4). Densely staining material was present between cells in some of the clusters (Fig. 5). Cells in groupings frequently exhibited close apposition of cell membranes. This was seen between both light and light and light and dark staining cells (Fig. 6). Although marked clusters were not found in all sections of all tumors, they were prominent in 8 out of 12 tumors of the ascorbic acid-supplemented animals examined in this study.

In other tumor regions, cells with extensive microvillar surface projections were observed (Fig. 7). These were often complemented by extensions from neighboring cells although mitotic cells did not seem to exhibit as many processes. Long regions of basement membrane were also present in tumors from ascorbic acid-supplemented mice (Fig. 8).

DISCUSSION

Our observations, although limited to one type of tumor, indicate that ascorbic acid can modify the architectural tissue pattern of the tumor. The roles of ascorbic acid in several important physiological functions have been known for a number of years. Relevant to the work presented here is the fact that ascorbic acid is essential for the formation and maintenance of collagen and the stability of connective tissue in general. It is also important in the formation of ground substance and inter-cellular cement [13, 14]. Vitamin C can improve tissue efficiency and integrity when it is available in sufficient quantity [15]. Ultrastructurally, ascorbic acid plays an important role in the maintenance of desmosomes and tonofilaments in buccal epithelial cells [16].

It has been proposed that proliferating cells 'escape' from their immediate environment via release of hyaluronidase which hydrolyzes the surrounding intercellular ground substance [17]. In health serum concentrations of physiological hyaluronidase inhibitor (PHI), a protein glycosaminoglycan complex, keep hyaluronidase levels in check. There is evidence that ascorbic acid may be needed for PHI synthesis [18]. Under conditions of ascorbic acid deficiency or stress, serum PHI falls with the result that enzymatic depolymerization of intercellular material continues. Thus, one hypothesis suggests that neoplastic cells produce, or can produce, hyaluronidase, and that ascorbic acid may have a potential role in anti-cancer therapy.

Several investigators have documented altered collagen metabolism and increased collagen degradation accompanying neoplasia. The solid form of the Ehrlich ascites tumor exhibits a ten-fold increase in collagen peptidase activity over muscle cells and ascites cells grown in the peritoneum [19]. Ascorbic acid addition reduces the collagen peptidase activity of cultured primary mouse fibroblasts [19].

Undoubtedly, the fibroblasts–collagen response was a major factor in the altered tumor tissue pattern observed in the present study, although the tumor cells responded as reflected in closer cell contact, decreased migration of daughter cells, deposition of material between the cells, and basement membrane formation.

Whether greater cell contact and deposition of material between cells affected cellular adhesion is uncertain. Basement membrane formation and a greater connective tissue stroma evident in some tumors may explain the decreased migration of tumor daughter cells into the surrounding tissue.

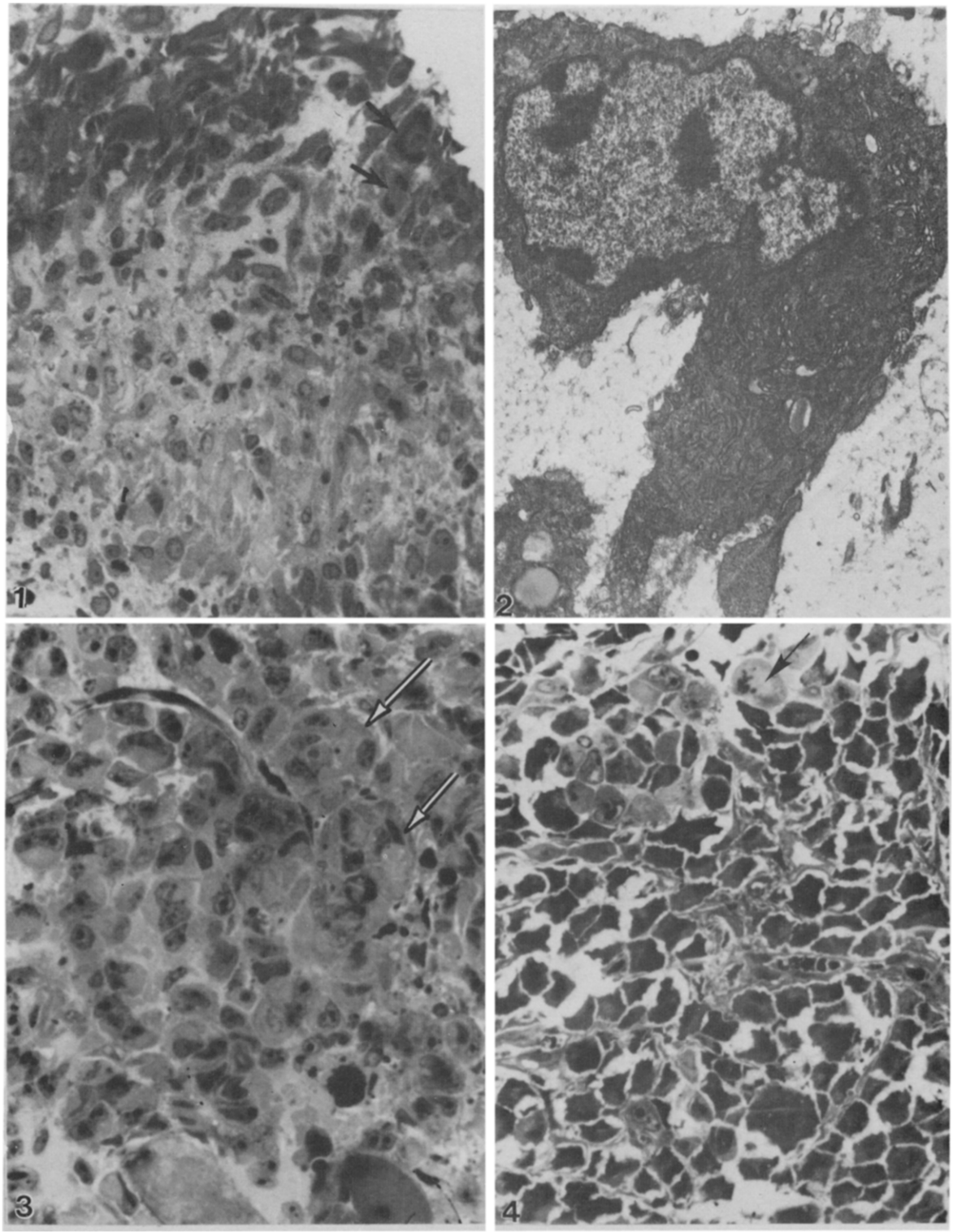
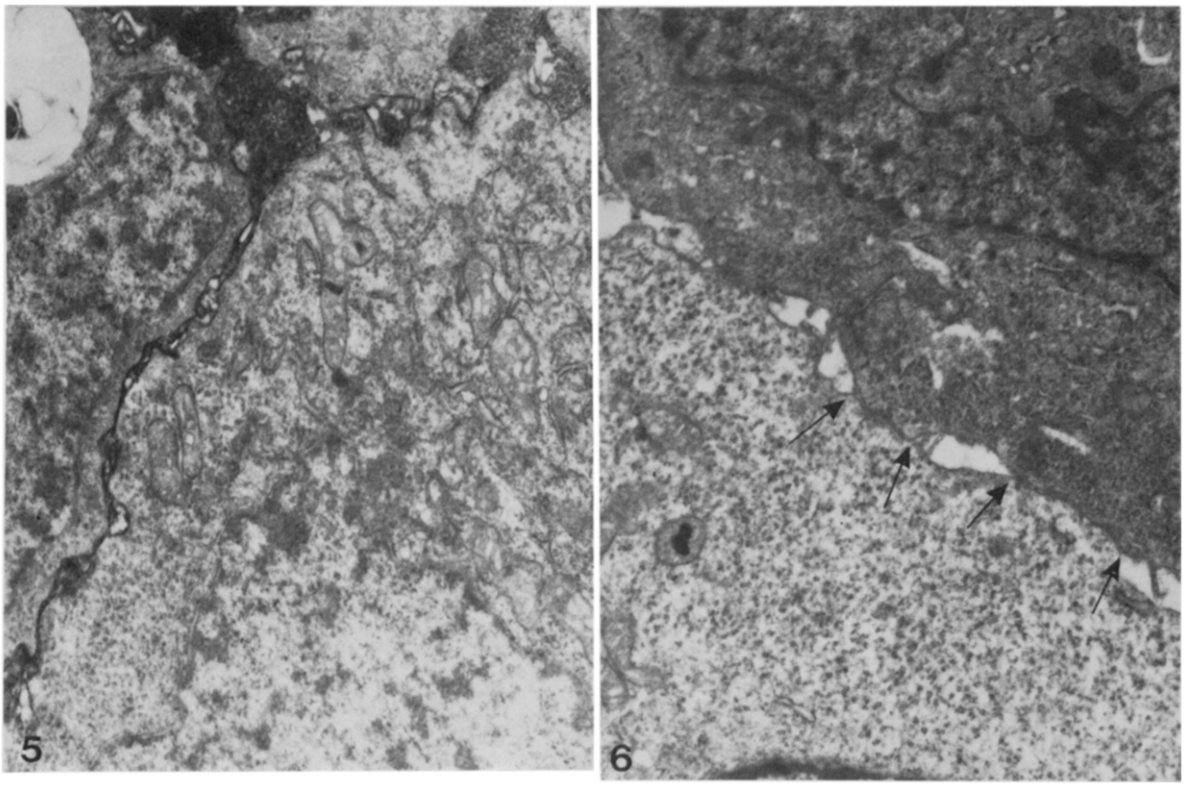


Fig. 1. *Histological appearance of a* 13-day-*old Ehrlich ascites tumor implanted in the thigh of a* CF_1 *mouse drinking distilled water only. No cellular clusters are present; two mitotic figures are present in the upper right (arrows). Epoxy section, methylene blue–azure* II. ×500.

Fig. 2. *Electron micrograph of a representative tumor cell from a* 13-day *implant in a* CF_1 *mouse drinking distilled water. Note the lobulated nucleus, and extensive endoplasmic reticulum in the lower portion of the cell.* ×6320.

Figs. 3 *and* 4. *Sections from tumors implanted in ascorbic acid-supplemented Swiss mice. Fig.* 3. *Two smaller cell clusters are marked by the arrows; a larger grouping lies immediately to the left of these two clusters. Fig.* 4. *Regions of more pronounced connective tissue were occasionally observed. A mitotic figure is marked by the arrow; a cell cluster is present to the left of this mitosis. Epoxy sections, methylene blue–azure* II. ×525.



Figs. 5 *and* 6. *Ultrastructural features of cells in clusters in ascorbic acid-supplemented Swiss mice. Fig.* 5. *A dense deposit fills the intercellular spaces between three adjacent tumor cells.* ×15,600. *Fig.* 6. *Cell membranes were often closely apposed, as in these two regions (marked by arrows) involving light and dark staining tumor cells.* ×15,600.

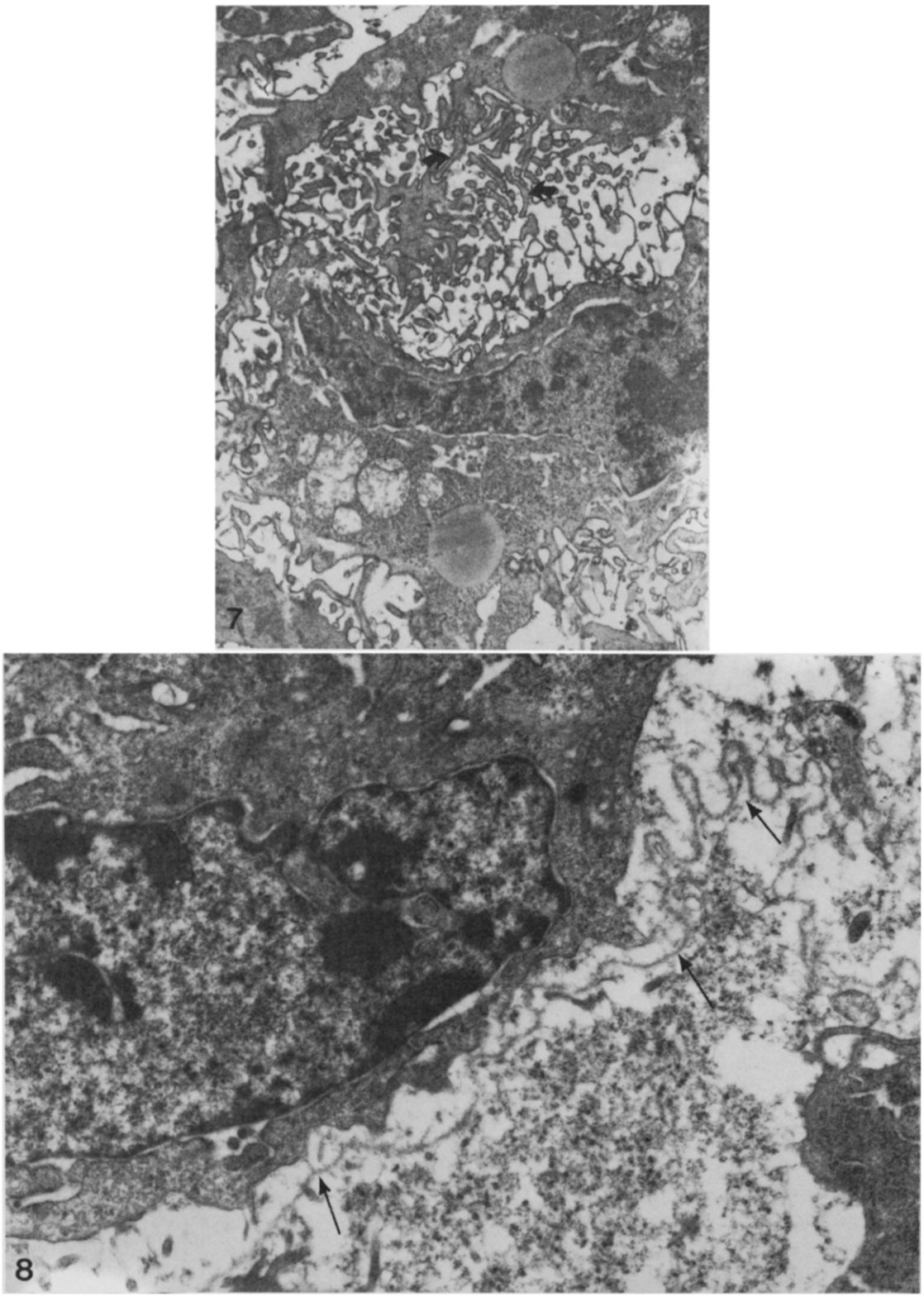


Fig. 7. Tumor cells frequently maintained extensive microvillar projections (arrows); CF_1 mouse supplemented with ascorbic acid. ×8130.

Fig. 8. Basement membrane was present in ascorbic acid-supplemented mice and occasionally formed undulating patterns as illustrated here (arrows, basement membrane). Swiss mouse supplemented with ascorbic acid. ×15,380.

Unfortunately, results of recent studies on the effect of ascorbic acid on neoplastic tissues are often contradictory. Morphologic alterations have been reported in irradiated Chinese hamster ovary cells grown in ascorbic acid-supplemented media [20], and in mesenchymal tumors [21]. Increased tumor growth has been associated with higher amounts of ascorbic acid [21], and tumor tissue appears to take up ascorbic acid to an increased extent [22]; however, in a series of examinations of squamous carcinoma of the skin where ascorbic acid tumor levels of patients were obtained, no alterations in histologic features related to ascorbic acid levels were found [22].

Thus, morphologic changes in tumor architecture may or may not be observed during variation of levels of ascorbic acid in the diet. If tumors do take up ascorbic acid to an increased extent, the amount available to the surrounding normal tissue may be reduced. This, in turn, may affect the efficacy of the host response.

In summary, our study of Ehrlich tumor implanted in ascorbic acid-supplemented mice documented clustering of tumor cells, closer association of tumor cells within these cultures, deposition of cellular product between cells, and the presence of basement membrane. Clusters of tumor cells are significant since they may reflect an improvement in host resistance. Host fibroblasts may be more successful in sequestering tumor cells in mice supplemented with ascorbic acid. This, in turn, may decrease the migration of daughter cells away from the tumor into the surrounding tissue and may help control tumor invasiveness. These changes in histological architecture provide an explanation for the slower growth rate characteristics of tumors in ascorbic acid-supplemented mice.

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