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Adamantinoma of Bone

An Electron Microscopic and Immunohistochemical Study

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Summary. Adamantinoma of bone is a rare tumor, and fine structural analysis has been done in only a few cases. We report four cases studied by electron microscopy and immunohistochemical methods. Ultrastructural evaluation revealed a characteristic constellation of features, including intracellular bundles of type I microfilaments, moderate numbers of evenly dispersed mitochondria, scattered profiles of rough endoplasmic reticulum, occasional Golgi bodies and lysosomes, and scattered glycogen particles. Microvillous processes and desmosomes were identified in all tumors. Well-formed basement membranes enveloped cell clusters but did not surround individual cells. Intercellular basement membrane-like material also was found focally in pools. Ultrastructural features of endothelial differentiation, including Weibel-Palade bodies, micropinocytotic vesicles, and tight junctions, were not identified. Immunoperoxidase stains for coagulation factor VIII (von Willebrand factor) and blood group antigens were negative, whereas similar stains for keratin were positive. Our findings strongly suggest that adamantinoma is a neoplasm expressing definite epithelial, rather than endothelial, characteristics.

Key words: Bone neoplasms – Adamantinoma – Ultrastructure – Immunoenzyme – Tibia/fibula – Keratin – Blood group antigens – Factor VIII-related antigen

Adamantinoma of bone is an uncommon neoplasm. Previous ultrastructural studies of this tumor have purported to show its histogenesis from either angioblastic (Llombart-Bosch and Ortuño-Pacheco 1978) or epithelial tissues (Albores Saavedra et al. 1968; Rosai 1969; Köhler et al. 1974; Unni

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et al. 1974; van Haelst and de Haas van Dorsser 1975; Yoneyama et al. 1977; Ectors et al. 1979). Because of this dichotomy, and the overall paucity of electron microscopic analyses of adamantinoma, we studied four examples of this lesion ultrastructurally and immunohistochemically. We discuss our results in relation to the speculated cellular origins for such tumors.

Methods

Four cases of osseous adamantinoma were identified by light microscopy. In each instance, diced tumor tissue was primarily fixed in 3% phosphate-buffered glutaraldehyde or Trump's solution and was processed routinely for electron microscopy. Multiple, thin sections of each neoplasm were examined with an electron microscope (Philips 201).

Immunoperoxidase studies were performed on undecalcified tumor tissue with rabbit antisera (Immulok) directed against coagulation factor VIII-related antigen (von Willebrand factor). In addition, rabbit antisera directed against blood group antigens A, B, and H and against keratin (kindly provided by Dr. Richard Schlegel, Peter Bent Brigham Hospital, Boston, MA) (Schlegel et al. 1980) were similarly applied to immunohistochemical analysis of the tumors. Positive controls were represented by sections of unrelated vascular neoplasms, erythrocytes contained in sections of tumor tissue from the 4 cases, and normal keratin-containing tissues. Sections of each of the four adamantinomas, stained without inclusion of the various directed antisera, served as negative controls.

Results

Clinical Features. The ages of the patients at diagnosis ranged from 13 to 48 years, and the sex distribution was equal (Table 1). Three tumors arose in the tibia and one in the fibula. In 3 patients, tumor-related symptoms, represented by a slowly growing mass associated with aching pain, had been present from 4 to 15 years before diagnosis. In 1 patient (case

Table 1. Clinical features of 4 patients with adamantinoma of bone

Case	Sex and age, y	Symptoms and duration (y)	Site of tumor growth	Radiologic appearance	Metastases	Treatment	Outcome
1	F, 19	Mass for 4 y; recent aching pain	Distal lt tibia	Cystic, lytic	Pulmonary and inguinal lymph nodes	Above-knee amputation	Dead of tumor 16 y after diagnosis
2	F, 48	Dull aching pain for 15 y	Distal lt tibia	Lytic	None	Below-knee amputation	No evidence of tumor growth 14 mo after surgery
3	M, 26	Mass for 10 y; aching pain for 4 y	Distal lt tibia	Lytic	Inguinal lymph nodes	Local resection	No evidence of tumor growth 6 y after surgery
4	M, 13	None	Distal lt fibula	Lytic	None	Local resection	No evidence of tumor growth 6 y after surgery

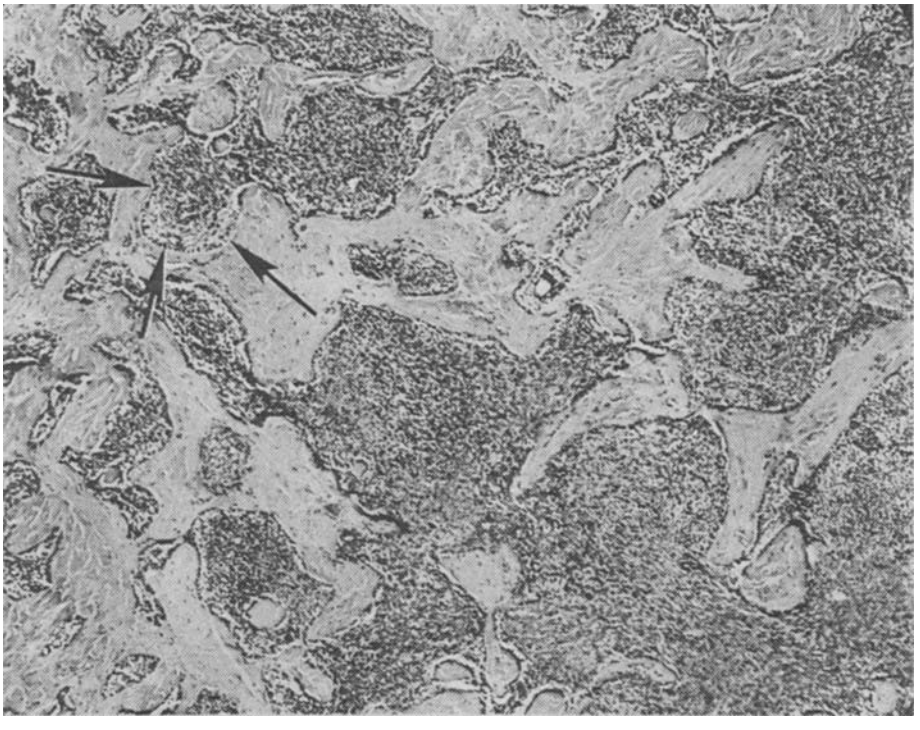

Fig. 1. Adamantinoma of long bone showing islands and sheets of small basaloid cells connected by anastomosing cords of cells. Note pallisading of tumor cells at periphery of tumor islands (*arrows*). (Hematoxylin and eosin; ×160.)

4), the tumor was discovered incidentally on a radiograph taken for unrelated reasons. Two patients (cases 1 and 2) underwent amputation as the primary mode of therapy; 1 of these patients died with pulmonary metastasis 16 years later, and the other has remained well 14 months after surgery. Two patients (cases 3 and 4) had local resection; both patients are disease-free 6 years after operation.

Light Microscopic Findings. All 4 tumors consisted of small basaloid cells disposed in nests, islands, and sheets separated by a fibrous stroma (Fig. 1). Peripheral nuclear palisading was visible within the cell nests (Fig. 2). Individual cells had scant amphophilic cytoplasm and contained little glycogen, as indicated by the periodic acid-Schiff stain. The nuclei were round to oval and occasionally spindled (Fig. 3). Nuclear membranes were delicate, and the chromatin was finely dispersed. Small, round, centrally situated nucleoli were present but inconspicuous. Mitotic figures were rare. The reticulin content of the cellular islands was variable; islands composed of epithelial-like cells were nearly totally devoid of reticulin, whereas areas of spindle cells showed a dense intercellular reticulin pattern (Fig. 4). Numerous cell nests contained strands and pools of basement membrane mate-

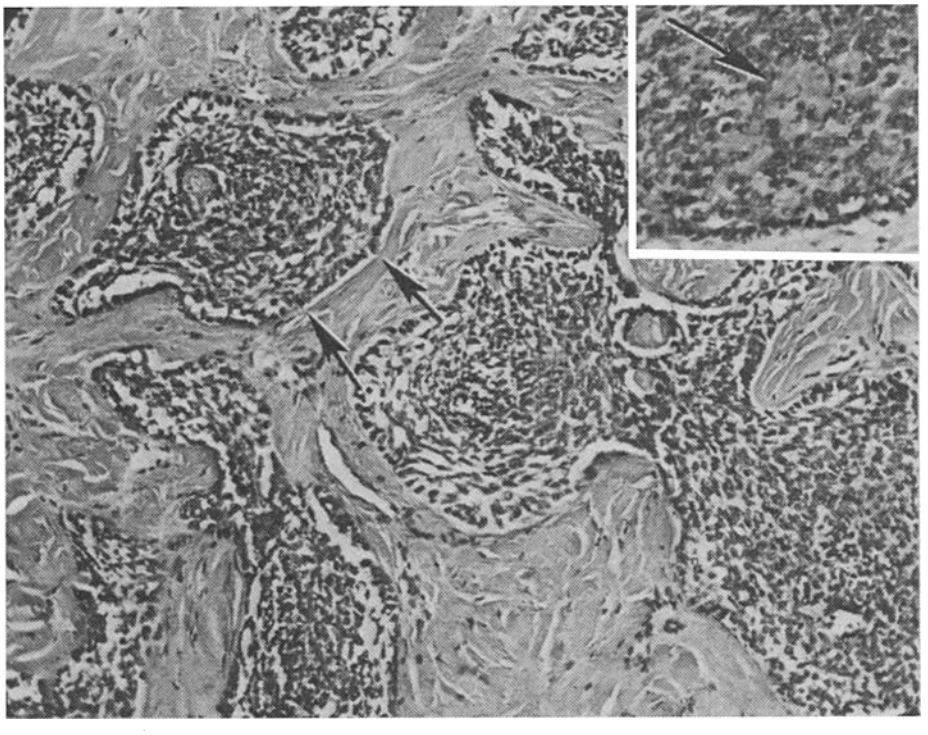

Fig. 2. Adamantinoma showing tumor cell islands and fibrous stroma. Pallisading of peripheral cells is clearly demonstrated (*arrows*). (Hematoxylin and eoxin; × 160.) *Inset:* Clusters of tumor cells with pools of basement membrane (*arrow*). (Hematoxylin and eosin; × 200.)

rial that was positive for periodic acid-Schiff stain; some of this material surrounded groups of cells.

Electron Microscopic Findings. Ultrastructurally, the tumor cells had irregularly folded nuclear membranes, with unevenly dispersed chromatin. Some of the cells had prominent nucleoli, with occasionally well-developed nucleolonemata (Fig. 5). The cytoplasm was modest in amount and contained moderate numbers of evenly distributed mitochondria. Profiles of rough endoplasmic reticulum were scattered throughout and were focally dilated; some were rarely arranged in concentric whorls (Fig. 6). Golgi bodies, lysosomes, lipid droplets, and glycogen particles were sparse. A few type I microfilaments (tonofilaments), 15–20 nm in width, were evident in broad bundles with frayed ends (Fig. 7 upper). The microfilaments were moderately prominent in two lesions. Cell membranes were generally smooth, but in each instance, variably developed microvillous processes could be discerned, which projected into small extracellular spaces not bound by junctional complexes. Desmosomes were found in all 4 tumors, though in one, they were rare; tonofilaments did not insert into the desmosomes with any frequency (Fig. 7 lower). Weibel-Palade bodies, micropinocytotic vesicles,

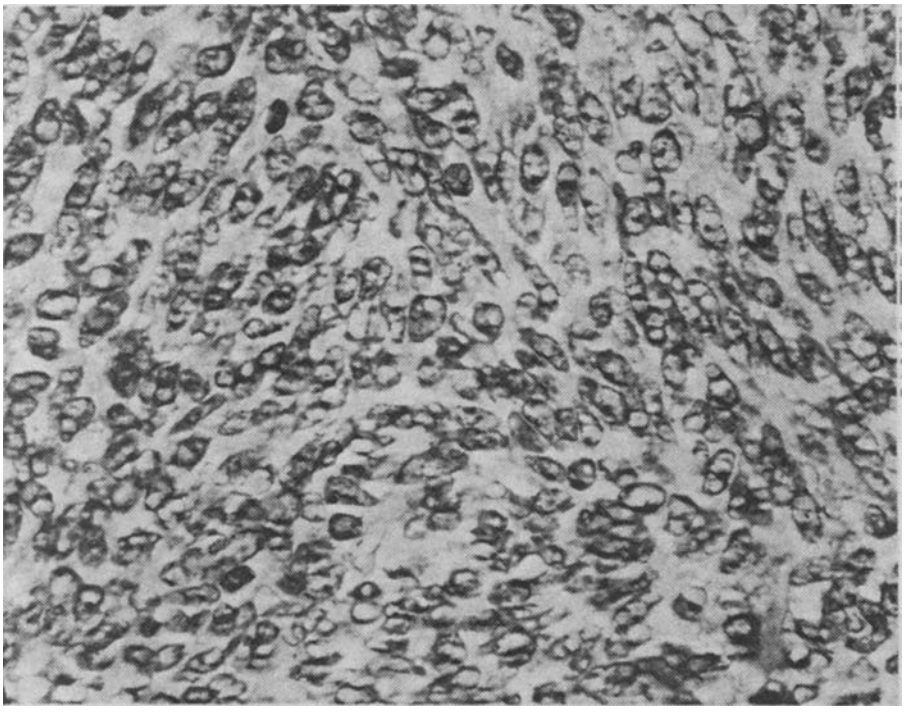

Fig. 3. Nuclei are round to oval and occasionally spindled in this adamantinoma. Their membranes are delicate and nucleoli are inconspicuous. Cytoplasm is scant. (Hematoxylin and eosin; ×640.)

and multivesicular bodies were not observed. Intercellular areas contained flocculent, granular basement membrane material, which occasionally pooled and did not uniformly or completely surround the tumor cells. Well-formed basement membranes encircled cell clusters and separated them from the collagenous stroma (Fig. 8).

Immunohistochemical Findings. Immunoperoxidase strains for coagulation factor VIII-related antigen were negative in all four tumors, except in endothelial cells of the tumor stroma. Tumor cells in the four cases were negative for the expression of appropriate blood group antigens, and three of three tumors tested were positive for keratin content (Fig. 9). Positive and negative controls for these substances stained appropriately.

Discussion

Adamantinomas are slow-growing tumors, as evidenced by symptoms of long duration and prolonged postoperative disease-free intervals in most patients. The lesion is most common in young adulthood and occurs most frequently in the tibia. Metastatic spread can occur. Despite the characteristic clinical and pathologic features of these tumors, their origin is in question.

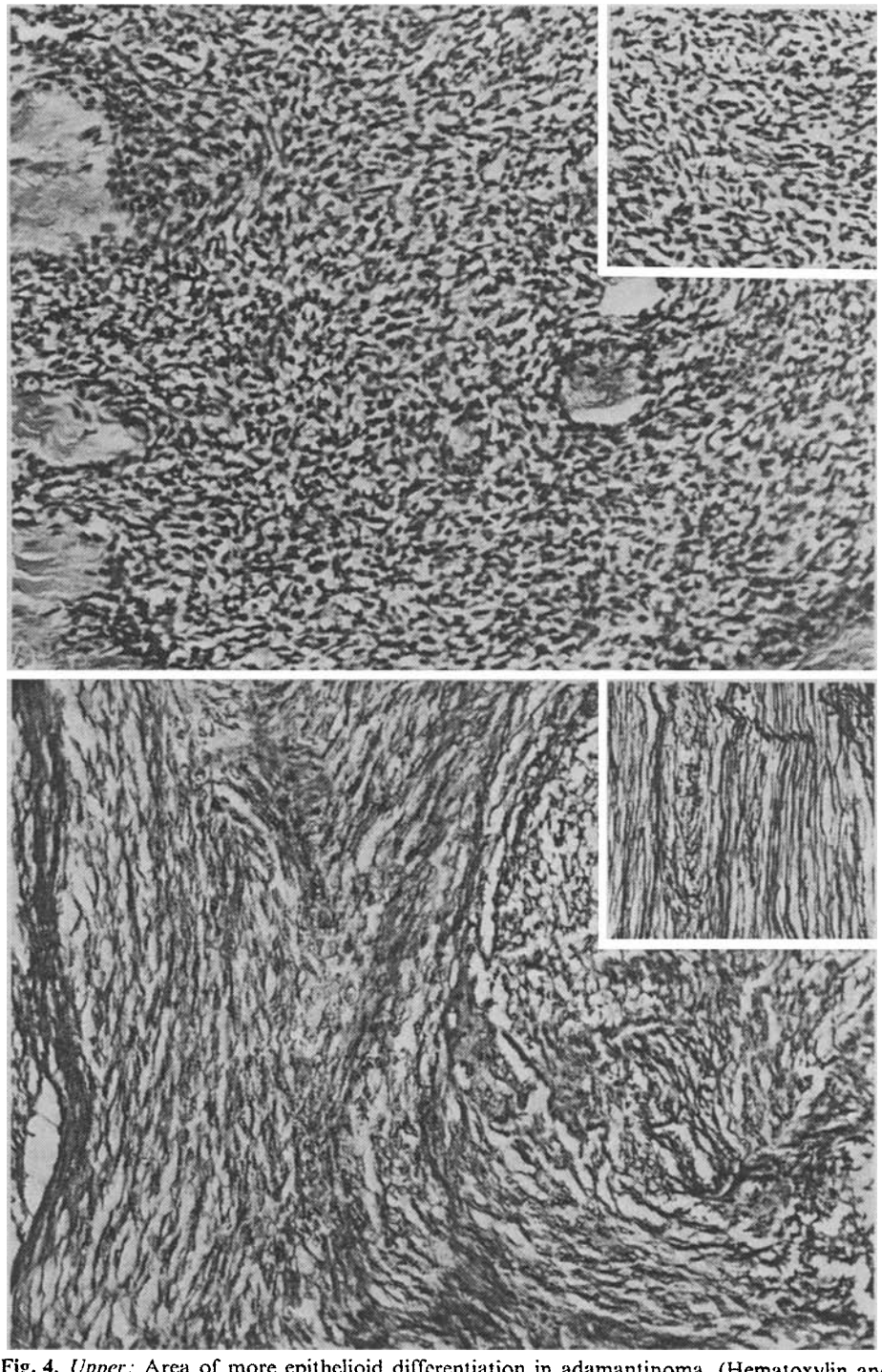

Fig. 4. *Upper:* Area of more epithelioid differentiation in adamantinoma. (Hematoxylin and eosin; ×250.) *Upper inset:* Reticulin stain showing epithelioid areas devoid of reticulin. (Reticulin stain; ×250.) *Lower:* Area of spindling cells in adamantinoma. (Hematoxylin and eosin; ×250.) *Lower inset:* Reticulin stain of spindling area showing reticulin between groups of cells but not surrounding individual cells. (Reticulin stain; ×250.)

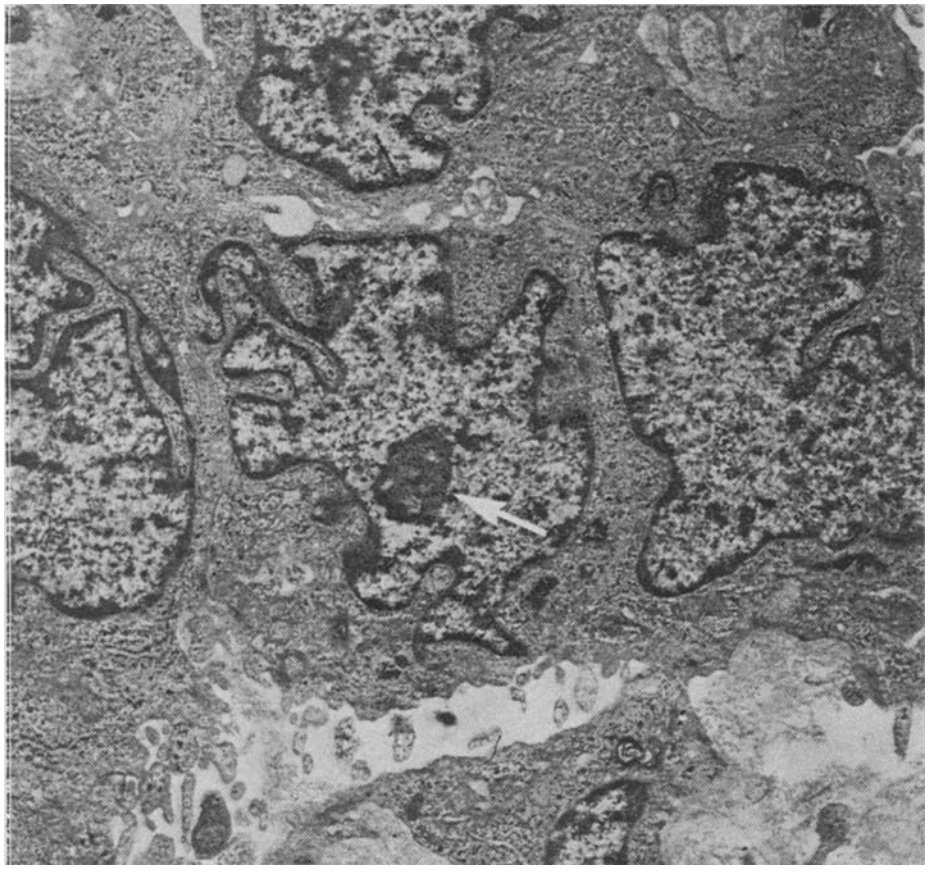

Fig. 5. Nuclei have irregular folded nuclear membranes in adamantinoma. Some cells have prominent nucleoli and occasional well-developed nucleolonemata (*arrow*). (Uranyl acetate-lead citrate; ×4,500.)

Theories suggesting either an angioblastic or epithelial histogenesis have been proposed, based on ultrastructural studies done in a total of nine cases (Albores Saavedra et al. 1968; Rosai 1969; Köhler et al. 1974; Unni et al. 1974; van Haelst and de Haas van Dorsser 1975; Yoneyama et al. 1977; Llombart-Bosch and Ortuño-Pacheco 1978; Ectors et al. 1979; Mori et al. 1981). All have been individual case reports, and only a few utilized tissue optimally fixed for electron microscopy. None have included immunohistochemical studies for the expression of blood group antigens in tumor tissue or for keratin content in the neoplastic cells.

Only one of these studies supports an angioblastic theory of histogenesis (Llombart-Bosch and Ortuño-Pacheco 1978). In that report, interdigitating microvillous processes were seen as modifications of tumor cell membranes, along with prominent pinocytosis and cytoplasmic rod-shaped structures interpreted as Weibel-Palade bodies. These features have been characteristically seen in several tumors of endothelial origin, such as hemangioma,

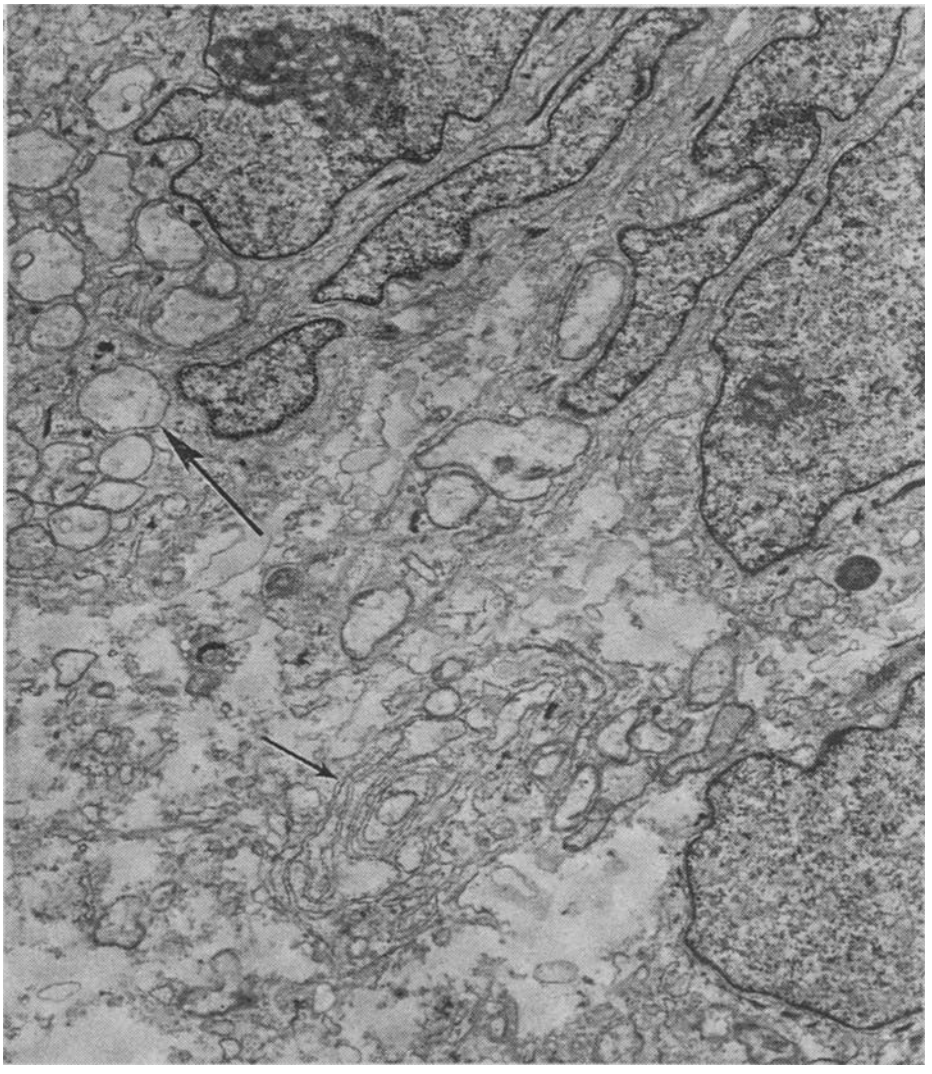

Fig. 6. Dilated rough endoplasmic reticulin (*large arrow*) is apparent in cells of adamantinoma. Rough endoplasmic reticulin is occasionally arranged in concentric whorls (*small arrow*). (Uranyl acetate-lead citrate; ×2,000.)

hemangioendothelioma, and angiosarcoma (Steiner and Dorfman 1972; Rosai et al. 1976); however, aside from Weibel-Palade bodies, such markers also may be found in various other neoplasms, most notably epithelioid leiomyosarcomas (Wick et al. 1981).

The remaining eight studies demonstrated desmosomes and type I microfilaments in adamantinomas (Albores Saavedra et al. 1978; Rosai 1969; Köhler et al. 1974; Unni et al. 1974; van Haelst and de Haas van Dorsser 1975; Yoneyama et al. 1977; Ectors et al. 1979; Mori et al. 1981). These are accepted as features of epithelial neoplasms; nevertheless they have not

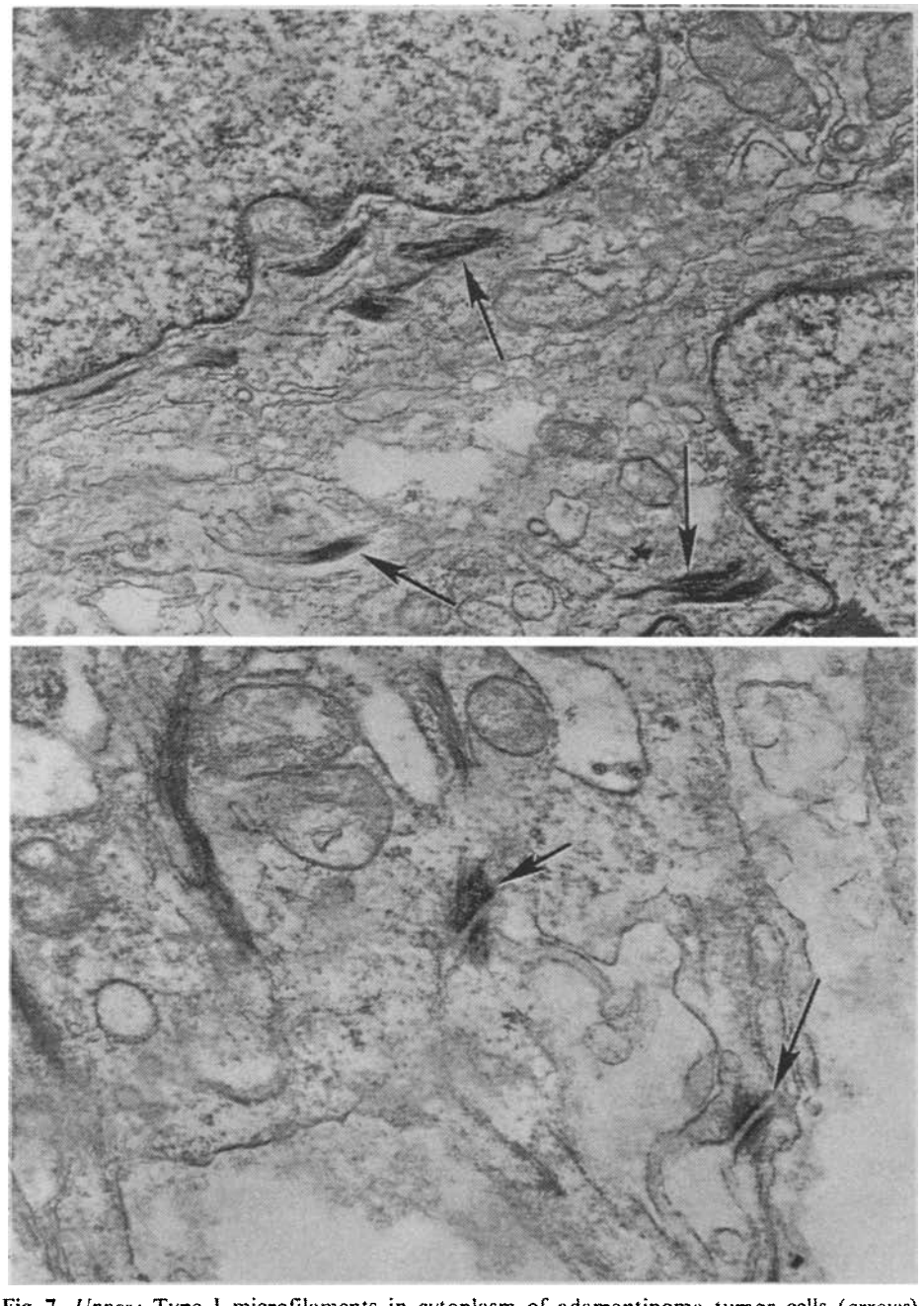

Fig. 7. *Upper:* Type I microfilaments in cytoplasm of adamantinoma tumor cells (*arrows*). (Uranyl acetate-lead citrate; ×10,000.) *Lower:* Type I microfilaments and desmosomes are evident in this view of osseous adamantinoma (*arrows*). (Uranyl acetate-lead citrate: ×15,000.)

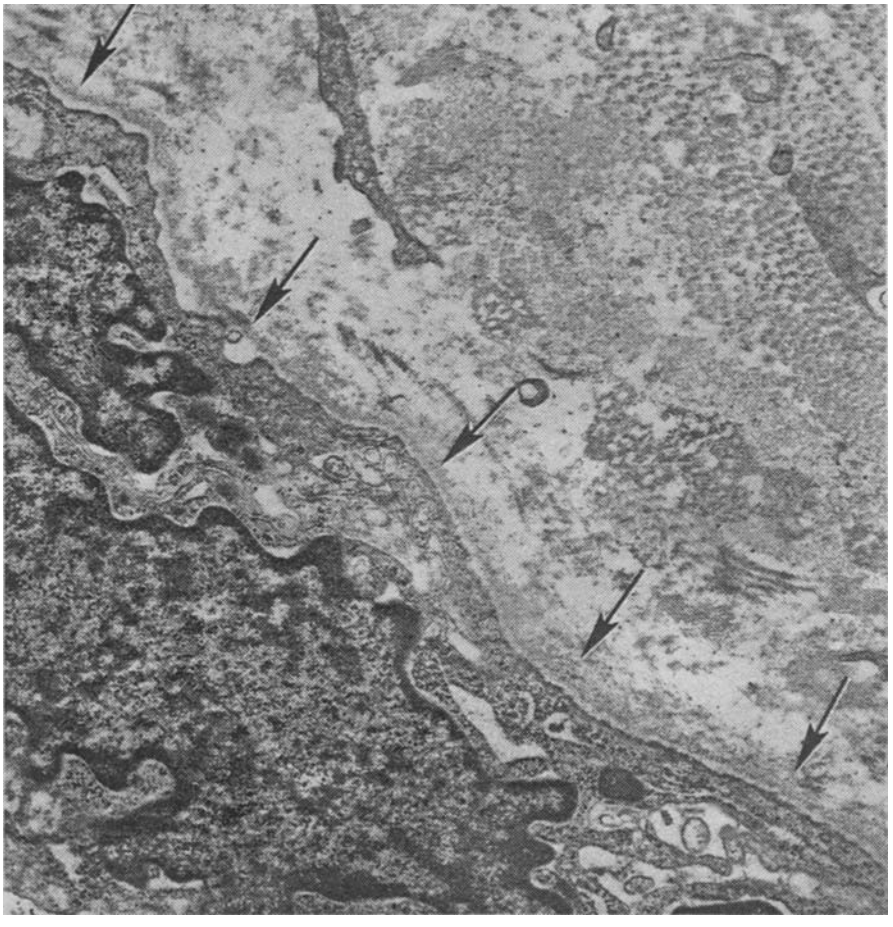

Fig. 8. Well-formed basement membrane encircles cell clusters and separates them from collagenous stroma (*arrows*). (Uranyl acetate-lead citrate; × 7,000.)

been regarded as definitive proof for an epithelial derivation of adamantinomas.

Our cases demonstrated certain features in common with all of these previous reports. Cytoplasmic processes and microvillous membrane projections were focally prominent in each tumor. In addition, desmosomes and broad type I microfilaments with frayed ends could be seen in all 4 tumors. Weibel-Palade bodies and micropinocytotic vesicles were not observed, and immunohistochemical stains for coagulation factor VIII-related antigen, a relatively specific cytoplasmic marker for endothelial cells (Mukai and Rosai 1980), were negative. Hence, we are unable to support a vascular origin for adamantinoma. Conversely, the immunohistochemical presence of keratin, a substance unique to epithelial tissues (Battifora et al. 1980; Schlegel et al. 1980; Altmannsberger et al. 1982), in all of the tumors so studied is compelling evidence for an epithelial histogenesis of this neoplasm. The absence of tumor cell positivity for blood group antigenic expression does

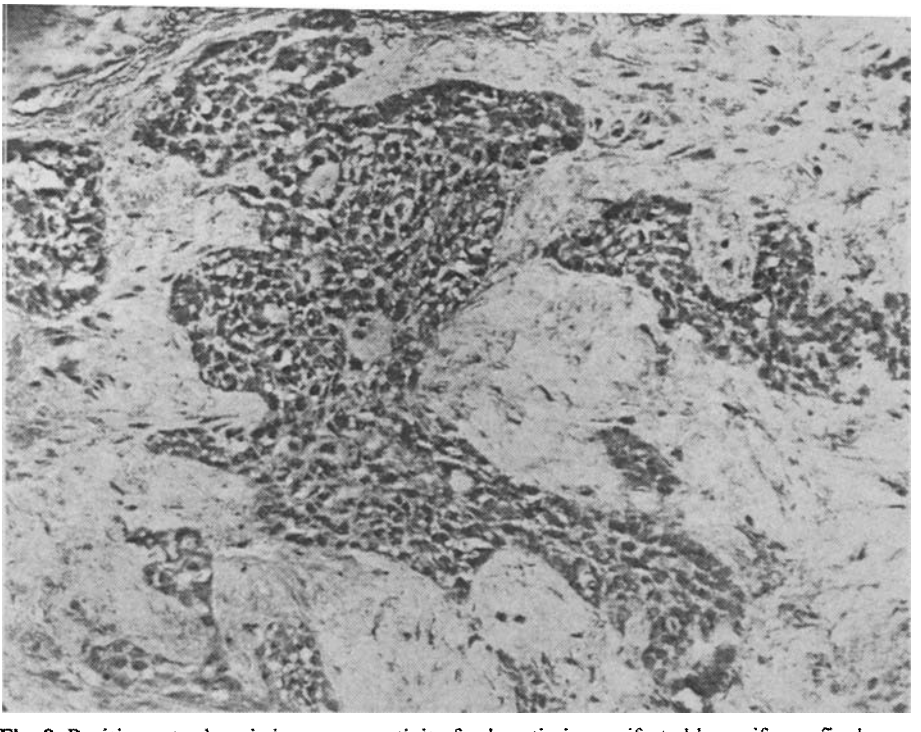

Fig. 9. Positive cytoplasmic immunoreactivity for keratin is manifested by uniform, finely granular staining in tumor cells. (Peroxidase-antiperoxidase; × 250.)

not detract from such a premise; numerous previous reports have documented the loss of these reactants from the cells of clearly epithelial malignancies with decreasinng levels of differentiation (Limas et al. 1979; Newman et al. 1980; Cooper and D'Elia 1982).

In conclusion, the results of our ultrastructural and immunohistochemical analysis of adamantinoma provide strong support for an epithelial derivation of this tumor, albeit with seemingly primitive differentiation. Furthermore, we found no similar substantiation for a previously proposed endothelial histogenesis. However, the mechanisms through which an epithelial neoplasm such as adamantinoma is induced to arise within mesenchymal (osseous) tissue are as yet speculative. Future studies are required in an attempt to dispel this remaining uncertainty in the natural history of a pathogenetically perplexing tumor.

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Note Added in Proof

Since this paper was first submitted for publication, a corroborative study has been published by Rosai J, Pinkus GS (1982) Immunohistochemical demonstration of epithelial differentiation in adamantinomas of the tibia. Am J Surg Pathol 6:427–434