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Ultrastructure of Adamantinoma of Long Bones

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Summary. Electron microscopic examination of three adamantinomas of long bones revealed structures usually observed in both mesenchymal and epithelial tumours. Tumour cells showing high alkaline phosphatase activity frequently tended to line clefts in connective tissue, resembling endothelial cells. The long processes of such cells showed fenestration. In areas showing fibre production the tumour cells were in close relationship to collagen fibrils. The latter were found, together with microfibrils, between the processes and above the basement membranes. The tumour cells were interconnected by desmosomes with tonofilaments and contained numerous bundles of microfilaments. All three cases revealed tiny intracytoplasmic inclusions resembling Weibel-Palade endothelial bodies. In addition, some of the structures in the lumena contained definite acid mucosubstances. A squamous cell pattern was present in only one of the three specimens.

The coincidence of divergent structures in a single specimen has led us to the conclusion that the so-called adamantinoma of long bone might be possibly related to tumours of mesodermal or mesectodermal origin.

Key words: Adamantinoma of long bone – Ultrastructure – Histogenesis – Mesodermal or mesectodermal origin

Introduction

Adamantinoma of long bones is a rare tumour, typically occurring in the tibia. It has been generally acknowledged to be a slowly growing malignant neoplasm with a marked tendency to recur locally after surgery and showing, in addition, a definite potency for distatnt metastasis. The microscopic appearances of the tumour differ markedly in various specimens (Weiss et al. 1977), the histogenesis of this type of adamantinoma remains a matter of controversy. The biphasic character of the tumour has made several authorities classify adamantinoma with synovial

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sarcomas (Hicks 1954; Lederer et al. 1954; Uehlinger 1957). Others have speculated on its possible angiogenic origin (Changus et al. 1957; Elliot 1963; Gikas et al. 1963; Huvos et al. 1975). Weiss et al. (1977) in their study arrived at the conclusion that the tumour represents a peculiar entity showing a capability to differentiate along epithelial as well as mesenchymal structural lines.

Recent electron microscopic studies have accumulated a considerable amount of information in support of the epithelial origin of adamantinoma (Köhler et al. 1974; Rosai 1969; Saavedra et al. 1968; Unni et al. 1974; Yoneyama et al. 1977). Such conclusions supported the hypothesis proposed by Ryrie (1939) suggesting the development of adamantinoma from epithelial inclusions of traumatic origin within the tibia. Llombart-Bosch et al. (1978), however, suggested that adamantinomas of long bones should be classified with angiosarcomas, on the basis of ultrastructural findings obtained in one case observed personally.

The data on the ultrastructure of adamantinoma have formerly been based on case reports only, with the tumour tissue inadequately fixed in formalin in the majority. No ultrastructural study comparing several cases of adamantinoma by one author has been available till now. We therefore decided to present the results of our ultrastructural study of three adamantinomas, differing structurally from one another in several respects. However, all of them showed structures typical of mesenchymal tumours together with appearances allegedly characteristic for epithelial ones. From this we regard adamantinoma of long bones to be a tumour of mesodermal origin.

Material and Methods

In all three cases the tumour tissue removed was fixed in 10 % neutral formalin for the purpose of routine histological examination. Sections obtained from paraffin blocks were stained with haematoxylin and eosin, Masson's trichrome, the P.A.S. method without and after amylase digestion, alcian blue at pH 2.5 and impregnated for reticulin fibres according to Gomori.

Tissue samples for electron microscopy were obtained at operation, fixed in glutaraldehyde and processed as reported in our previous paper (Povýšil et al. 1977). Semithin as well as ultrathin sections were obtained in a similar way. The sections were examined under a Tesla BS 500 electron microscope.

Cryostat sections of unfixed tissue were used for histoenzymologic examinations. Activities of the following enzymes were studied: Acid phosphatase, alkaline phosphatase, naphtol-ASD chloracetate esterase, DPN- and DPNH-tetrazolium reductase, lactate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase, 6-phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase, phosphorylase. All the histoenzymological examinations were carried out as described earlier (Povýšil et al. 1977).

Clinical Data. The present series comprises 2 males and 1 female aged 52 to 61 years (Table 1) in whom the disease presented with local pain and a slight swelling of the tibia. In none of the cases was there a history of trauma. On X-ray films there were rather typical cyst-like osteolytic foci partially destroying the cortical bone. In all the three patients the tumour was removed by enucleation and the defect was filled with grafts. The two male patients have remained well for 2 and 3 years, respectively. In the female, the tumour recurred 6 years after the operation, requiring a block resection and substitution of the tibia by an autograft from the fibula. For more than two years the patient has remained symptom free.

Results

Histological Examination

In all the tumours studied there were tubular, basaloid and spindle-cell structures contained in a cellular connective tissue resembling that found in fibrous dysplasia.

Table 1. Summary of clinical and morphological data

Case	Age	Sex	Location	TP	AP	BP	SP	SqP	Des	Mf	Fen	WPb	Mv	Muc
1	55	m	Tibia	+	+	+	+	−	+	+	+	+	±	+
2	52	m	Tibia	+	±	±	+	−	+	+	−	+	+	+
3	61	f	Tibia	+	±	±	+	+	+	+	−	+	±	+

TP – Tubular pattern; AP – Angiomatoid pattern; BP – Basaloid pattern; SP – Spindled pattern; SqP – Squamoid pattern; Des – Desmosomes; Mf – Bundles of intracytoplasmic microfibrils; Fen – Fenestrated cell processes; WPb – Inclusions resembling Weibel-Palade bodies; Mv – Microvillous processes; Muc – Mucosubstances in the lumina

The so-called tubular structures frequently contained red blood cells (Fig. 1). Their lining varied in height throughout the tumour. In areas with flat cells the pattern not infrequently resembled that of mature angiosarcoma (Fig. 1) notably in case 1. In such areas intraluminal papillary proliferation was a frequent finding. There were small amounts of basophilic material, stained by alcian blue at pH 2.5 in the lumina or on the surface of the cells lining the tubular formations.

Tubular structures showed frequent transitions into solid strands or larger solid foci (Fig. 2). In some of these the peripheral cell layer assumed a palisade-like arrangement. Such foci resembled the structures seen in basalioma to some extent (Fig. 3) but, in our opinion, such a similarity was not close. We were frequently able to demonstrate bundles of collagen fibres in the intercellular spaces of such seemingly solid areas. The greatest amount of collagen was found in areas with prevailing fusiform cells (Fig. 4). In some instances such areas were related to areas of hyalinized connective tissue with scattered tumour cells. In other cases, such as our case No. 2, nodular formations developed in a rather loose connective tissue. In such nodes the difference between the tumour cells proper and the stromal cells virtually disappeared. Few of them had hyperchromatic nuclei.

In case 3 there occurred the three basic patterns and areas showing signs of differentiation towards squamous-cell epithelium (Fig. 5). In the central areas of many neoplastic foci the majority of tumour cells assumed the appearence of rather large and light cells, polygonal or fusiform in shape. In places these cells, showing a well developed cell membrane, produced spherical parakeratotic pearls.

Rarely, cylindromatous formations were found in the tumour.

Histoenzymatic Examination

This has been carried out in tumours Nos. 1 and 2 only. In all the cells there was considerable activity of the two tetrazolium reductases, Krebs cycle dehydrogenases, pentose cycle dehydrogenases and a marked membrane-bound activity of alkaline phosphatase (Fig. 6). Activities of acid phosphatase, beta-glucuronidase, and Naphtol-ASD chloracetate esterase was present in single tumour cells only. No activity of phosphorylase was demonstrated.

Electron Microscopic Examination

The spindle-shaped cells of the connective tissue stroma resembled fibroblasts (Fig. 12). In some cells, their elongated nuclei contained a thickened lamina fibrosa.

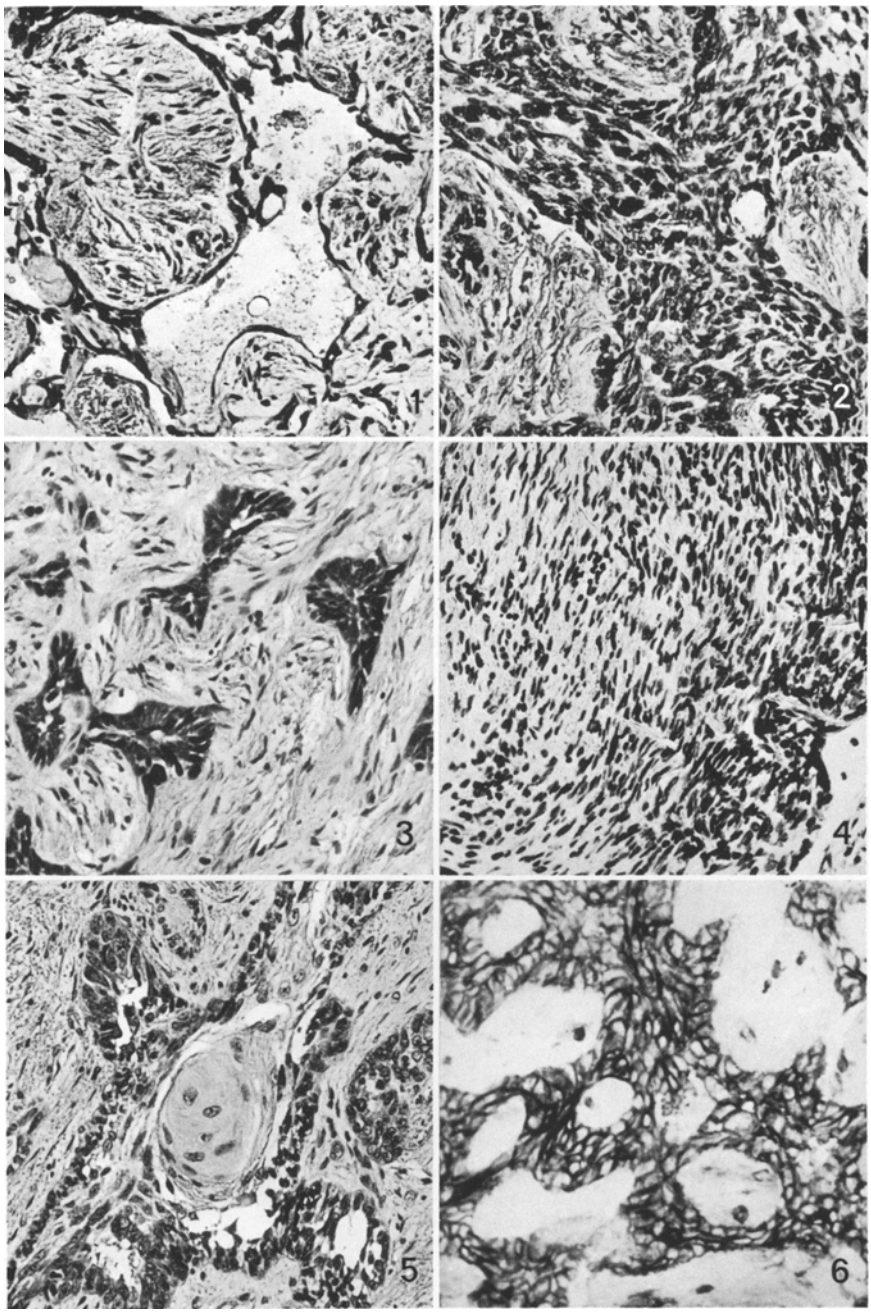

Fig. 1. Wide erythrocyte-containing spaces lined with flat tumour cells. Such a pattern resembles that encountered in vascular tumours (Case 1). Haematoxylin and eosin. ×230

Fig. 2. Solid areas in adamantinoma of tibia (Case 1). Haematoxylin and eosin, ×230

Fig. 3. Basaloid pattern in tumour tissue (Case 1). Haematoxylin and eosin, ×230

Fig. 4. An area of adamantinoma, case 3, composed of spindle-shaped cells. Haematoxylin and eosin, ×230

Fig. 5. A tumour area with squamous epithelial structures (Case 3). Haematoxylin and eosin, ×230

Fig. 6. High alkaline phosphatase activity in the tumour cells of solid areas. ×230

In their cytoplasm were conspicuous profiles of rough endoplasmic reticulum. The processes of these cells were never found to contact the tumour cells proper.

The solid and the luminized tumour foci were mostly bounded by a basement membrane at their periphery (Figs. 7, 11 and 12). In close proximity to the basement membrane collagen fibres, microfibrils and cell processes of the fibroblasts frequently occured (Fig. 11). The tumour cells contiguous with the basement membrane showed numerous intercellular connections of the desmosome type (Figs. 7 and 9). In such areas, a few dense microfibrils were occasionally seen to join at the site of the dense plate of the cell membrane. The cells were often columnar or cuboid in shape, sometimes, however, they were markedly flattened with long processes covering the inner side of the basement membrane (Fig. 12). They rarely developed scanty digit-like processes of variable length on their surface. Their cytoplasm occasionally contained vacuole most probably corresponding to the intracytoplasmic lumen. The space above the basement membrane contained cell processes with collagen fiber bundles intermingled with microfibrils (Fig. 11). The lumina of tubular formations contained a thin amorphous material of medium electron density (Figs. 11, 12) and occasional erythrocytes.

In some areas the tumour cells tended to line cleft-like spaces in the connective tissue resembling endothelial cells or small vessels (Fig. 12). Quite exceptionally structures typical of fenestrated capillaries were also found (Fig. 13). In such cases the basement membrane was covered by a thin layer of cytoplasm containing irregularly distributed pores of variable size.

In areas where the non-luminized tumour strands lined with the basement membrane merged with the fibrous areas, the tumour cells came into close contact with collagen (Fig. 14). In such areas the bundles of collagen fibres were intercalated between cell processes, mutually interconnected with desmosomes and occasionally lined with an incomplete basement membrane from the periphery (Fig. 14). In the intercellular substance of such areas there was sometimes a prevalence of fine microfibrils with an amorphous material of medium electron density. A few microfibrils and pro-collagen fibres were also seen in the intercellular spaces of solid areas bounded with a basement membrane at the periphery. The presence of intercellular substance was also noted in a similar localisation in case 3 showing areas of squamous cell epithelium.

The tumour cells obtained from various areas of the tumours resembled one another cytologically in all three cases. Their nuclei (Figs. 7, 12, 14) with chromatin clumped at the nuclear membrane, were variable in shape, but most were elongated or markedly irregular owing to the presence of deep indentations. In the cytoplasm (Figs. 7, 11) we found the ordinary organelles, mitochondria, Golgi apparatus, occasional centrioles, membrane-bound dense bodies, ribosomes, glycogen particles, and rough endoplasmatic reticulum, usually well developed and with dilated cisternae. Microfibrils were regularly found within the cytoplasm (Fig. 8). In the cells of the tubular structures, microfibrils mostly followed a course parallel with long axis of the respective cell. More often they were placed in that part of the cytoplasm furthest from the lumen and only rarely became crowded to form dense bundles. In the epithelial-looking areas, in contrast to this, they formed bundles with frayed ends of various thickness. Bundles of microfilaments were most numerous within the cells of tumour No. 2. Several variously oriented bundles

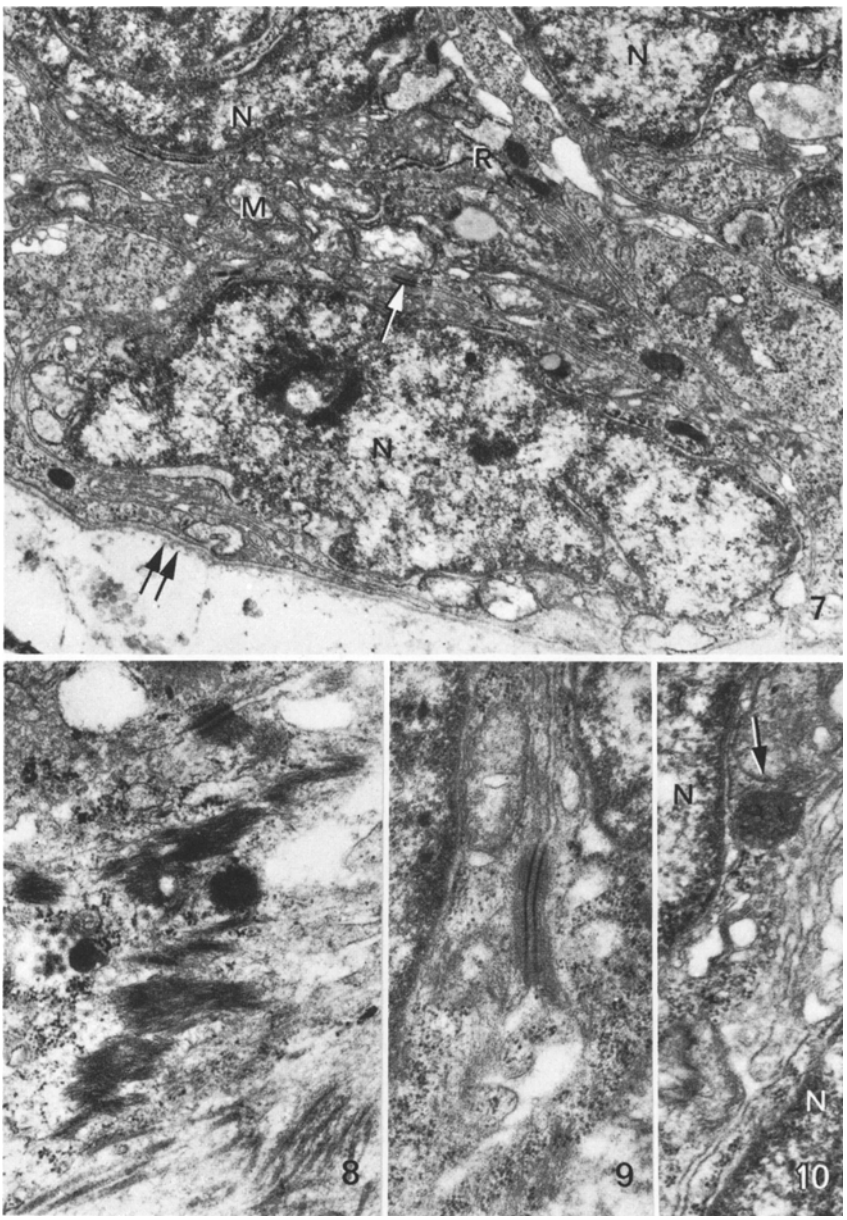

Fig. 7. A solid tumour focus bounded with a basement membrane ( ). The tumour cells are interconnected with desmosomes ( ). In their cytoplasm, they contain mitochondria (*M*), rough endoplasmatic reticulum (*R*), and dense bodies. *N* nucleus. ×14,000

Fig. 8. Bundles of dense microfilaments as viewed in detail. ×22,000

Fig. 9. Desmosomes as viewed in detail. ×30,000

Fig. 10. A dense inclusion with tiny microtubules resembling a Weibel-Palade body ( ). *N*, nucleus. ×21,000

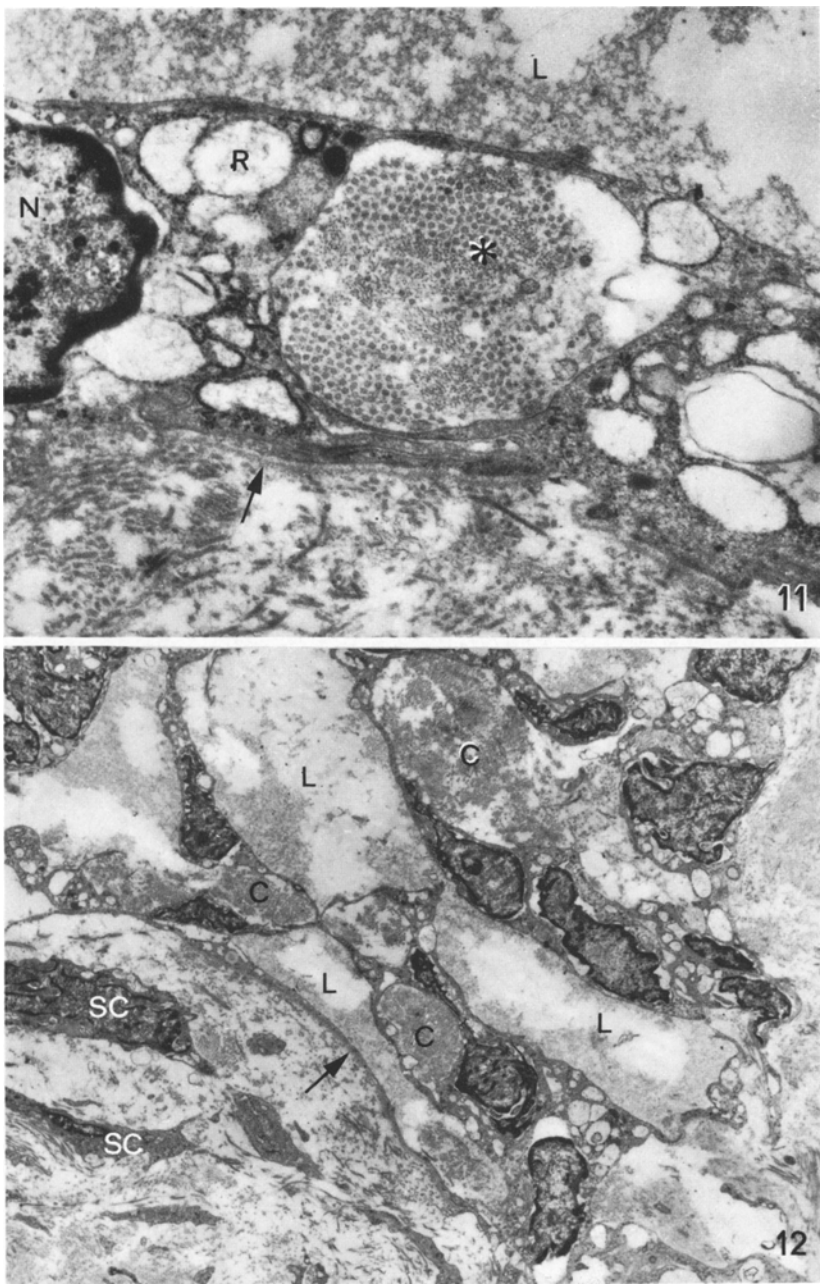

Fig. 11. A high power view of a cell lining a lumen-containing neoplastic structure. Above the basement membrane (↗) there is a bundle of collagen fibrils and fine microfibrils (*) between processes of tumour cells. Dilated profiles of rough endoplasmatic reticulum are seen (*R*). *N*, nucleus; *L*, lumen. ×18,000

Fig. 12. Tumour cells lining clefts in collagen connective tissue in an endothelial-like fashion. The fine processes of these cells rest upon the basement membrane (↗). *L*, lumen; *C*, collagen; *SC*, stromal cells. ×4,800

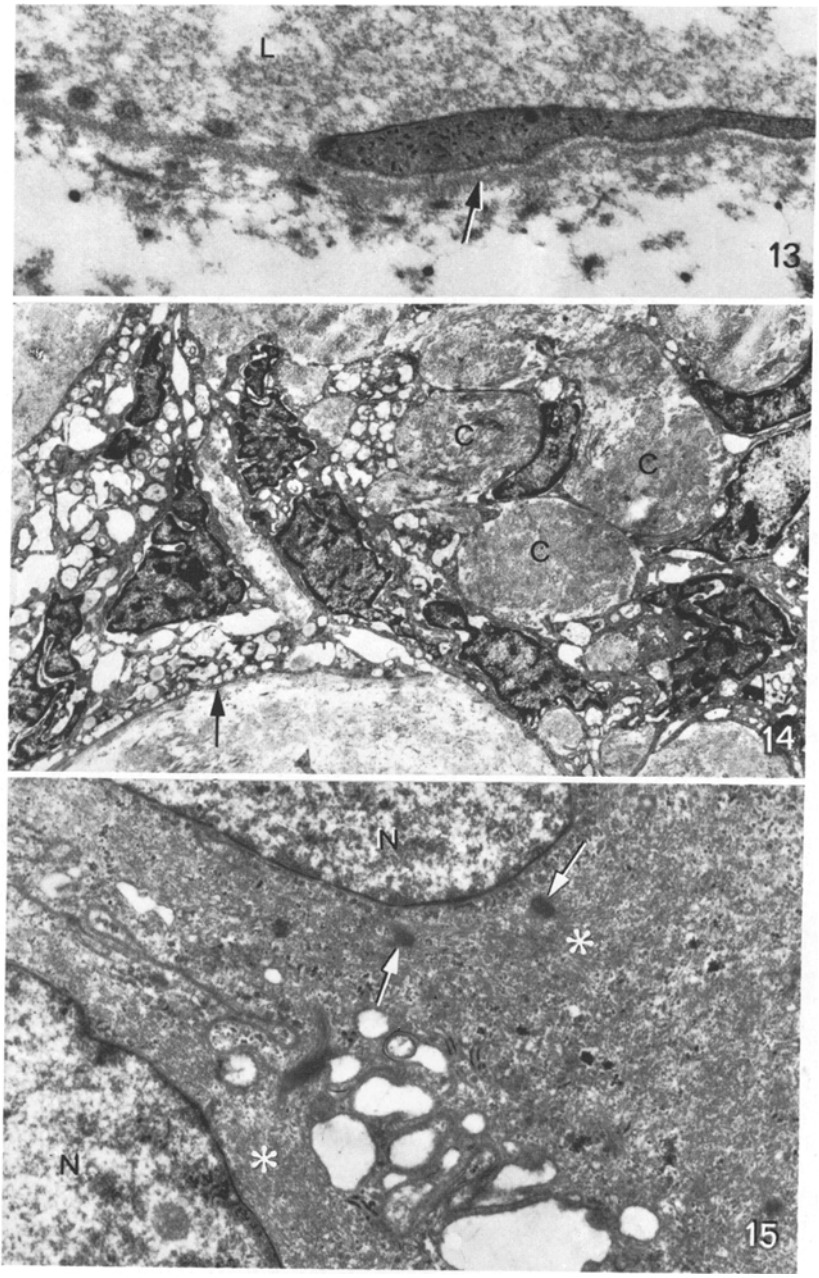

Fig. 13. A fenestrated process of a tumour cell viewed in detail. ↑, basement membrane; *L*, lumen. ×32,000

Fig. 14. A tumour area with exaggerated fibroplasia. The processes of tumour cells embrace bundles of collagen fibrils (*C*). ↑, basement membrane. ×6,000

Fig. 15. A high power view of an area composed of squamous type cells. The cytoplasm of such cells contains numerous fine microfibrils (*) and occasional intracytoplasmic desmosomes (↑). *N*, nucleus. ×17,600

could occasionally be demonstrated in a single cell. The tumour cells of tumour No. 2 also produced numerous microvillous processes on their surfaces.

In tumour No. 3 the central parts of the solid foci tended to differentiate towards squamous epithelium (Fig. 15). Larger cells in these areas were mutually interconnected with desmosomes. Not infrequently, numerous desmosomes were also found within the cytoplasm. Besides numerous organelles, the cytoplasm contained a number of fine microfibrils which, however, did not produce dense bundles (Fig. 15).

Inclusions structurally resembling the endothelial bodies of Weibel-Palade might be regarded as a significant finding with respect to tumour histogenesis and were found in all three specimens (Fig. 10). The inclusions were membrane-bound and contained dense material with several paler tubules embedded within them. Pinocytotic vacuoles were only recorded quite exceptionally in the cells from tubular areas.

Discussion

In our opinion, all the three tumours studied can be safely regarded as typical adamantinomas of long bones. Such a diagnosis receives support by their localisation, X-ray appearances and, above all, the histological findings. Although we do not consider the tumours to be histogenetically different, there were some definite differences between the individual specimens. Cases No. 1 and 2 resembled mesenchymal tumours by their general structure. In contrast, structures of epithelial appearance showing signs of differentiation towards squamous epithelium prevailed in case No. 3. Even here, however, there occured areas composed of fusiform cells and tubular formations containing red blood cells, with a lining of variable height. At the ultrastructural level the tumour cells of the neoplasms showed a number of similar cytological features.

The interpretation of the findings presented here from the histogenetic standpoint appears to be rather uneasy. The structures observed permit us to arrive at various conclusions. No wonder that the authors of other ultrastructural studies have arrived at rather divergent conclusions. Such a situation might suggest that morphologically very similar tumours of different histogenesis have been grouped under one heading of adamantinoma. No valid support has been lended hitherto to such opinion. The comparison of the cases presented here cannot be regarded as supporting a multihistogenetic origin of adamantinoma of long bones, our tumours were virtually identical with regard to their basic cytological features.

Those advocating an epithelial origin for adamantinoma (Köhler et al. 1974; Rosai 1969; Saavedra et al. 1968; Unni et al. 1974; Yoneyama et al. 1977) have supported their opinion by the findings of distinct microfilamentous bundles, epithelial type desmosomes and cells which can be characterized as keratinocytes. In the few available studies on the ultrastructure of angiosarcoma no such structures have been described (Rosai et al. 1974), but despite this fact, we believe that the significance of the "epithelial features" reported has been somewhat overestimated. Squamous cell metaplasia has been exceptionally found in synovial

sarcoma and mesothelioma (Weiss et al. 1977). Desmosomes with attached tonofilaments and intracytoplasmic tonofilaments represent a common feature of pleural mesotheliomas and of adenomatoid tumours in the genital region (Taxy et al. 1974).

According to our experience, it is possible to identify in a case of adamantinoma structures typical of epithelial tumours, together with those which can be encountered in mesenchymal tumours, particularly angiosarcomas. In this context, the most conspicuous electron microscopic findings were obtained in the first two observations. In these desmosomes with tonofilaments were found, but simultaneously the tumours contained non epithelial structures. The cells showed a marked tendency to line clefts in the connective tissue, appearing as capillary endothelial cells. The flat processes covering the inner surface of the basement membrane occasionally showed fenestration. Such fenestration, typical of fenestrated capillaries and the cells of angiosarcomas, has not previously been described in an adamantinoma. Findings of collagen bundles between the cells above the basement membrane and the close association between tumour cells and collagen fibres can be regarded as evidence against the epithelial origin of adamantinoma. Scanty amounts of the fibrillary material of the intercellular substance have been observed between the cells of the epithelial-appearing foci in case No. 3. The significance of alkaline phosphatase positivity in adamantinoma cells is difficult to interpret at present. It appears to be of interest, however, that the activity of this enzyme has not been reported in tumours of indisputably epithelial origin, such as ameloblastoma of the jaw (Changus et al. 1957), or adnexal tumours of the skin (Chung-Hong 1978). The finding of inclusions resembling the Weibel-Palade granules, also mentioned by Llombart-Bosch et al. (1978), represents a significant argument in favour of the angiogenic origin of the tumour. In specimens processed in identical ways, no qualitative or quantitative differences were noted between the bodies found in the tumour and those found in the endothelia of vessels in a control case. Therefore we think that these bodies are not identical with the multivesicular bodies occuring in adnexal tumours (Chung-Hong et al. 1978).

All the facts presented here permit a serious argument to be maintained against the hypotheses that adamantinoma of long bones is derived from intraosseal epithelial inclusions. There was no history of injury in any of our three patients. The existence of congenital epithelial inclusions within the tibia cannot be ruled out, but there are not findings supporting their occurrence. But it must also be admitted, that an angiogenic or synovial origin of adamantinoma of long bones has not previously received indisputable support. The extreme variability of tumour structure including a squamous pattern and the presence of intracytoplasmic tonofilaments and a certain amount of intratubular acid mucosubstances does not seem to be in good agreement with the angiogenic concept.

Such seemingly controversial findings made us to look for a concept which might offer an acceptable explanation for the coincidence of the heterogenous structures found in adamantinoma of long bones. In view of the tremendous variability observed it seems reasonable to presume that the tumour might originate from cells capable of differentiating in several directions, thus mimicking structures observed in epithelial as well as mesenchymal tumours. Such properties have been

ascribed to mesotheliomas. It is generally acknowledged, that mesothelium as well as mesenchym is of mesodermal origin. We therefore suggest that adamantinoma of long bones might be a tumour of mesodermal origin.

Recent embryological studies revealed that cells of the end bud (i.e. mesectoderm) and those of the primitive streak (Seichert and Jelínek 1968) participate significantly in the blastema of lower extremities. This pluripotential material gives rise to the caudal parts of the neural tube and at the same time to the axial and paraaxial mesoderm, from which cells migrate into the developing blastema of the limb buds. There they are incorporated, particularly in the proximal segments such as the thigh and crural region (Seichert 1976). In our opinion the areas described above may contain tissues, the stem cells of which might be capable of differentiating into mesodermal and mesenchymal tumours. At the same time, such cells might resemble ectodermal cells in some of their properties. If such a hypothesis were correct, adamantinoma of long bones might originate from cells endowed with the properties of pluripotential mesectoderm. Such a concept would permit us to regard the tubular structures as analogous with the pseudoglandular formations of mesothelioma and the squamous metaplasia as a manifestation of differentiation towards ectodermal structures. Simultaneously, such a concept might well explain a tendency to differentiation along the line of angiosarcoma and the similarity between adamantinoma of long bones and synovial sarcoma.

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