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Ultrastructure of Ewing's Tumour

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Summary. Tumour tissue surgically excised from 10 patients bearing Ewing's tumour of bones was examined electron microscopically and histoenzymologically. In all cases the tumour was composed of polygonal cells with cytoplasm poor in organelles but with conspicuous aggregates of glycogen particles. There were numerous intercellular connections of desmosomal type and a distinct cell membrane bound positivity for alkaline phosphatase activity. In two cases in which there was a negative reaction for alkaline phosphatase, the lack of enzyme activity might have been related to cytotoxic treatment carried out for several months immediately before excision of the tissue used for histoenzymological studies. The problem of histogenesis of Ewing's tumour remains unresolved although some of the present findings support a haemangiogenic origin of the tumour.

Key words: Ewing's tumour – Ultrastructure – Histochemistry – Histogenesis.

Introduction

The tumour generally recognized as Ewing's sarcoma has been a matter of great interest to clinicians as well as to pathologists, for many years. In the WHO Histological Typing of Bone Tumours Ewing's tumour has been delineated as a separate entity which must be differentiated from retothelosarcoma and metastatic neuroblastoma. In differentiating between these tumours electron microscopy is expected to play a significant role. The first detailed description of the ultrastructure of Ewing's tumour was published by Hou-Jensen et al. (1973). Previous reports (Friedman et al., 1968; Friedman et al., 1971; Kadin et al., 1971; Llombart et al., 1970; Sirsat et al., 1971) did not contain data

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on the presence of desmosomes. Thus despite the fact that electron microscopy is valuable in differentiating between Ewing's tumour and other neoplasms, reports of ultrastructure are few and, to a certain degree, discordant. For this reason we decided to report our experience of the ultrastructure and enzyme histochemistry of Ewing's tumour. The results of electron microscopical examination of an extraskeletal metastasis of the tumour will also be presented.

Material and Methods

The material available for study was obtained from ten patients suffering from Ewing's tumour. The excised tumour tissue was divided in four pieces which were processed as follows:

Tissue blocks for *routine histological processing* were fixed either in 10% neutral formalin or in Gendre's fluid. After embedding into paraffin, histological sections were prepared as usual and stained with haematoxylin and eosin, Masson's blue trichrome stain and impregnated for the demonstration of reticulin fibres according to Gomori. Glycogen was demonstrated with Best's carmine and the periodic-acid Schiff method (P.A.S.) with and without amylase digestion.

For the purpose of *electron microscopy* the material was fixed by immersion in 3% glutaraldehyde with a phosphate buffer (pH 7.2) for 4 h. After rinsing in a pH 7.2 phosphate buffer the blocks were postfixed with 1% osmium tetroxide with phosphate buffer for one hour at 4°C. The tissue blocks were then dehydrated in alcohol and embedded in Epon as usual. Ultrathin sections were produced on a Reichert OmU2 ultramicrotome and after contrasting with lead citrate (according to Reynolds) they were examined with a Tesla BS 500 electron microscope. Semi-thin sections stained with Azur C were used for orientational examination of the sections.

Cryostat sections were used for the following *histoenzymologic examinations*: acid phosphatase, alkaline phosphatase, Naphthol-ASD chloroacetate esterase, DPN and DPNH tetrazolium reductase, lactate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and phosphorylase. All the histoenzymologic examinations were carried out as described by Motlík and Stárka (1972).

Clinical Data

Our collection comprises 10 cases, 7 males and 3 females (Table 1), aged 13–33 years, who had been examined and treated at our Institutes during 1975–1976. In 7 cases the tumour was situated in the bones of the lower extremities, in 2 it occurred in the pelvic bones and in 1 case the upper extremity was affected. The period from the first manifestation of symptoms to the moment when a histologically verified diagnosis was established varied between 2–8 months. In one case (No. 6) there was a history of tarsal pain of six years duration, but roentgenological signs indicating tumour growth were discernible as little as 7 months before the diagnosis was made. All the patients had had an X-ray film and an arteriographic examination of the respective part of the skeleton in two projections. The roentgenological alterations in the bone structure were always in agreement with the diagnosis of Ewing's sarcoma. In 9 cases, arteriography showed a progression of the tumour into the soft parts. In all these 9 cases, malignant-type vascularisation was recorded. In patient No. 9 the tumour was limited to the medullary space. On admission, none of the patients showed obvious pulmonary metastases on X-ray film. Laboratory examinations, namely the urine-analysis, blood cell counts and alkaline phosphatase levels were within normal limits, but the blood sedimentation rate was increased in all cases. Catecholamines and their derivatives were absent from the urine in all cases.

The patients received combined surgical, radiation and cytotoxic treatment. Exceptionally, case No. 6 was given chemotherapy only following biopsy, because of the advanced stage and rapid course of the disease. In the majority of patients the sequence was as follows: diagnostic excision, partial or radical resection, irradiation with ^{60}Co, and chemotherapy with a combination of Vincristine, Cyclophosphamide, Actinomycin-D, Adriamycin and Cytembena. In patient No. 1

Table 1. Clinical data of 10 patients with Ewing's sarcoma and results of histochemical alkaline phosphatase

Case	Name	Sex	Age	Localisation	Anamnesis in months	Therapy	Surviving (in months)	Died (in months)	Alkaline phosphatase
1	R.P.	F	13	Distal tibia dx	4	PE, P-RES CH, RA, M-RES		11	negative
2	V.H.	M	20	Proximal tibia dx	3	PE, P-RES CH, RA		10	
3	J.R.	M	28	Pelvis dx	6	PE, CH, P-RES CH, RA		2.5	positive
4	M.R.	M	15	Proximal femur sin	3	PE, CH, RA	32		positive
5	L.R.	M	29	Pelvis sin	8	PE, CH		2.5	positive
6	E.A.	F	33	I. metatarsus dx	7	R-RES CH, RA	4		
7	J.A.	M	27	Distal femur sin	4	PE, CH, AMP CH, RA	3		negative
8	P.P.	M	15	Fibula dx	2	R-RES CH, RA	4		positive
9	J.D.	F	13	Proximal tibia dx	3	PE, CH, RA	3		
10	V.P.	M	16	Proximal radius dx	3	R-RES CH, RA	2		positive

Duration of anamnesis is given in months from onset of symptoms. Survival and death are given in months from histological confirmation of diagnosis. Therapy: PE-probatory excision, P-RES-partial resection, R-RES-radical resection, AMP-amputation, CH-chemotherapy, RA-radiation

a metastasis was excised from the left breast in the stage of tumour generalization. The tissue obtained was subjected to ultrastructural and histoenzymatic studies. Patient No. 4 had been subjected to a diagnostic excision and had received chemotherapy in another institution. One year later the tumor recurred and was removed by a radical resection of the upper half of the femur with a subsequent application of a special total hip endoprosthesis. Tissue for ultrastructural and histoenzymatic studies was obtained from the resected recurrence of the tumour. It should be stressed that this patient did not receive any chemotherapy three months prior to operation. Patients No. 6, 8 and 10 had had immediate radical resection of the tumour affected bone. Four patiens died of tumour generalization, 6 patients were living at the end of 1976.

Results

Histological Examination. In all the ten cases the pattern characteristic of Ewing's tumour was revealed. Medium sized cells with ovoid or spherical nuclei of uneven size formed solid areas (Fig. 1) lined with reticulin fibres. Sections from tissue blocks which had been fixed in Gendre's fluid contained a considerable amount of glycogen histochemically demonstrable in all the cases. In contrast, formalin-

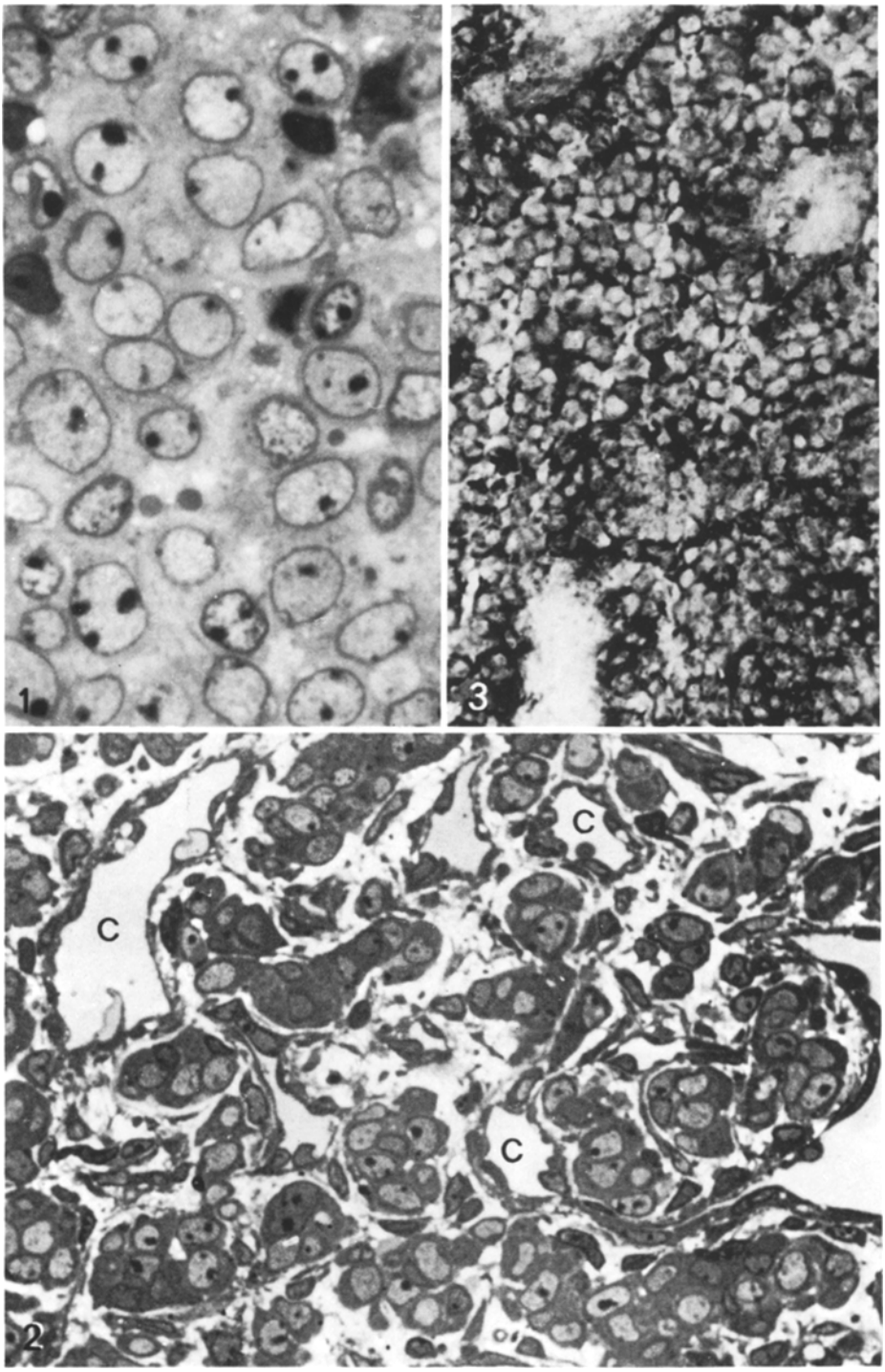

Fig. 1. Solid area of Ewing's tumour as shown in a semithin section. Azur C, ×1100

Fig. 2. A semi-thin section from the tumour of Patient No. 4 showing haemangiopericytoma-like structures. Larger groups of tumour cells were seen between numerous capillaries (*C*). Azur C, ×620

Fig. 3. Distinct cell membrane bound activity for alkaline phosphatase in cells of Ewing's tumour. ×150

fixed tissue blocks were positive for glycogen in some cases only and the results in one section were rather variable. In Case 4 the tumour showed occasional patterns reminiscent of haemangiopericytoma (Fig. 2). In such areas the tumour cells were found between numerous capillaries which seemed to be a part of the tumour.

Histoenzymatic Examinations were carried out in the mammary metastatic tumour of Case 1, and in the tumour tissue of Cases 3–5, 7, 8 and 10 (Table 1). The tumour cells were always positive for the two diaphorases, the pentose and Krebs cycle dehydrogenases and the phosphorylase. Acid phosphatase and Naphthol-ASD chloroacetate esterase, which appeared to be lysosome-bound, was present in a few tumour cells only. In the tumours obtained from patients 3–5, 8 and 10 there was a marked cell membrane bound activity of alkaline phosphatase (Fig. 3). The same finding was made in the tumour tissue excised during the reoperation of Case 3, carried out after a course of chemotherapy of one month's duration. In the metastatic tumour tissue obtained from the breast of Case 1 and in the tumour of Case 7 the results of the alkaline phosphatase reaction were completely negative. Those two cases received a course of chemotherapy of several months' duration prior to biopsy and histoenzymologic studies.

Electron Microscopic Examinations. All the tumours examined showed an identical structure. They were composed of light ovoid or polygonal tumour cells of variable size with centrally placed spherical or ovoid nuclei (Fig. 4). In the nuclei, the chromatin was usually at the nuclear membrane and conspicuous nucleoli were always present.

Individual tumour cells were situated close to one another and were separated by narrow intercellular spaces (Fig. 5). Intercellular connections resembling primitive desmosomes were frequent (Figs. 5 and 6). At the site of these connections a thin layer of electron dense substance was found on the inner side of the parallel cell membranes. The intercellular space between the desmosomes either remained empty or contained an electron dense substance (Fig. 6). The cell membrane of the tumour cells produced pseudopodia-like projections which were cut longitudinally in some sections. The projections of neighbouring cells were interwoven so that they resembled interdigitations. Some of the spaces between the cellular projections contained amorphous masses of variable density which could have been interpreted either as fibrin remnants or basement membrane-like material.

The cytoplasm of the tumour cells was rather scanty and contained numerous free ribosomes and polyribosomes, a few ovoid mitochondria, rough endoplasmic reticulum (Figs. 5 and 7), centrioles, occasional intracytoplasmic microfilaments and a Golgi apparatus consisting of fine cisternae and vesicles. Rather large aggregates of glycogen particles were a regular finding and were conspicuous (Figs. 4 and 8). At times the cytoplasm contained membrane-bound dense bodies of variable size. Such bodies, most probably lysosomes, were occasionally placed in the vicinity of phagosomes containing rests of nucleus containing cells (Fig. 7) or glycogen particles.

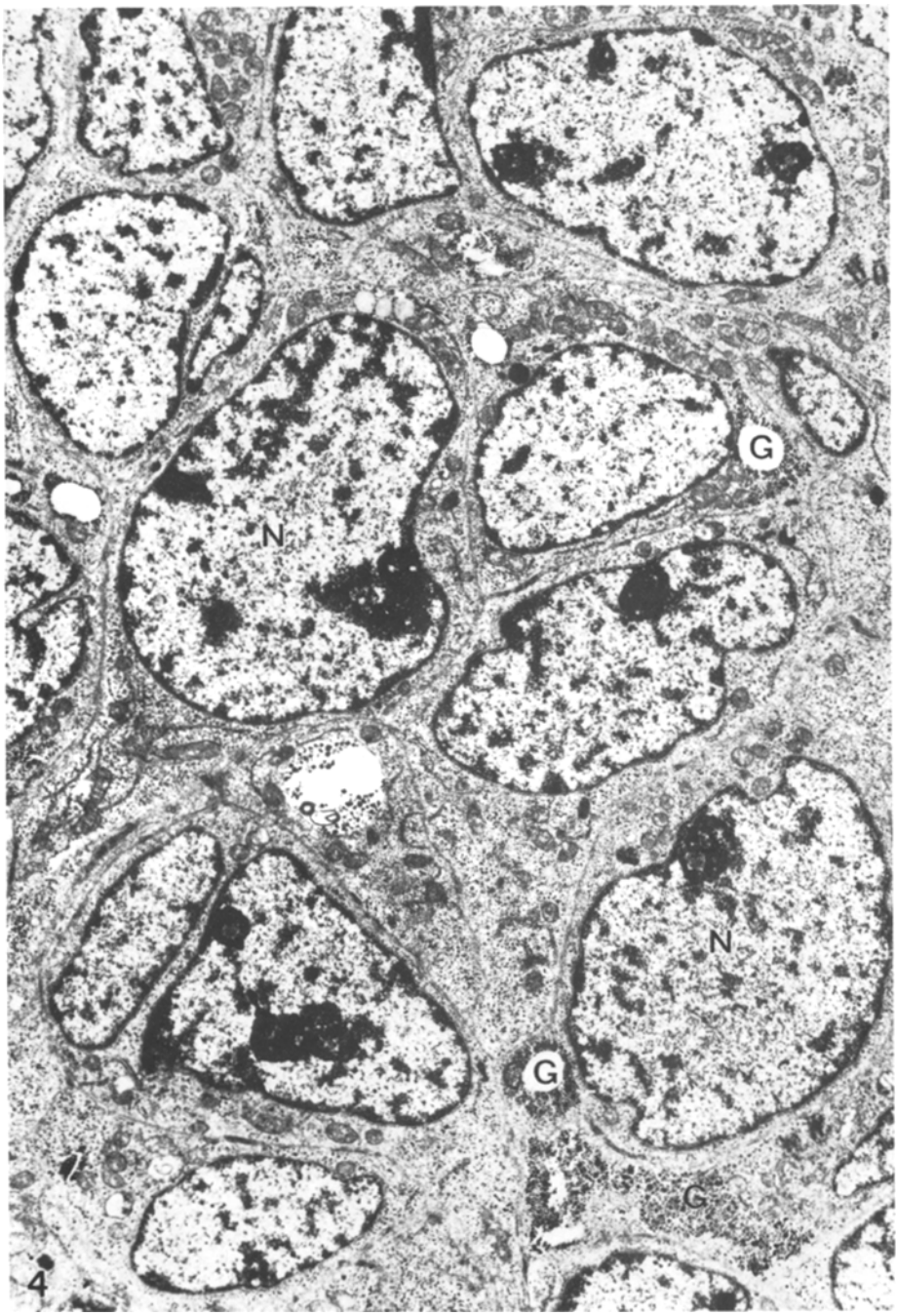

Fig. 4. Survey electron micrograph of Ewing's tumour composed of polygonal cells with nuclei (*N*) of variable size containing nucleoli. Individual cells are closely related to one another. Their cytoplasm contains aggregates of glycogen particles (*G*) in addition to several organelles. ×7500

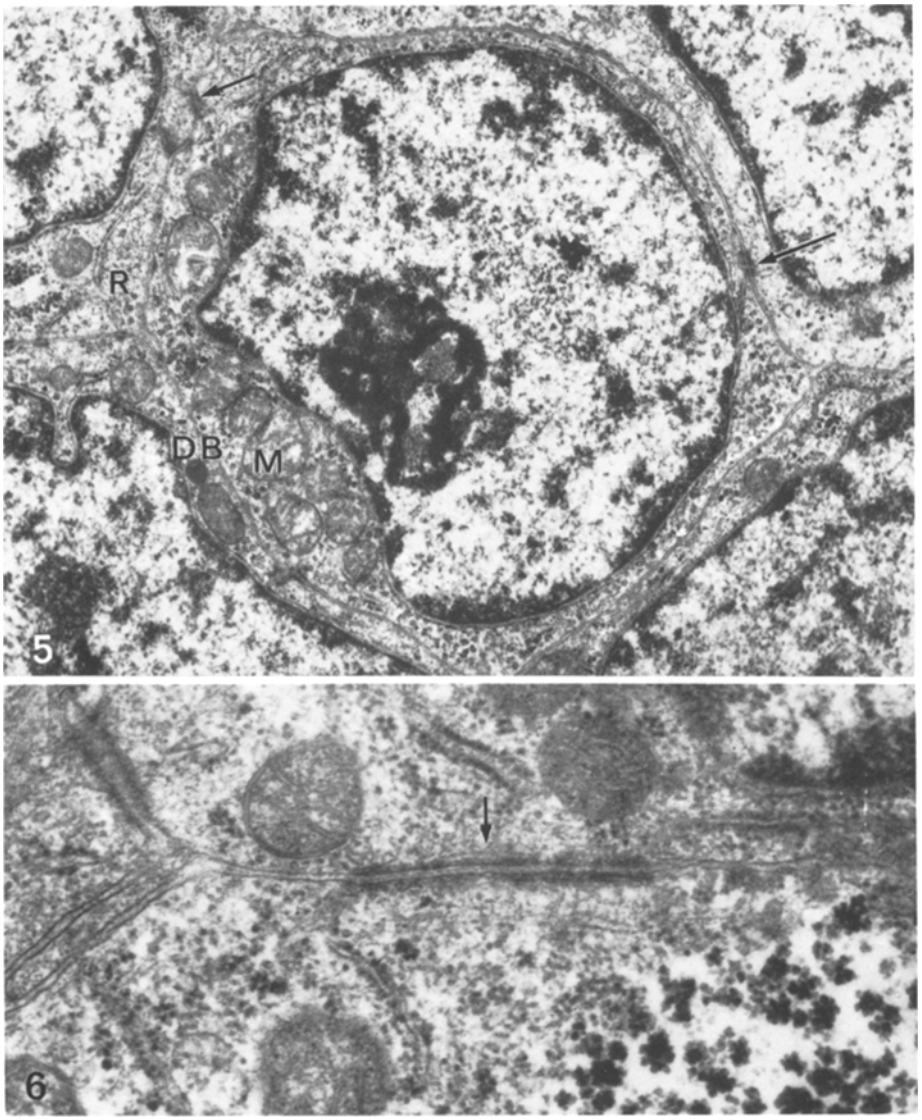

Fig. 5. One of the tumour cells with a distinct cell membrane and several intercellular connections of the desmosome type (↗). The cell organelles visible in the field include mitochondria (*M*), rough endoplasmic reticulum (*R*), ribosomes and dense body (*DB*). ×13,600

Fig. 6. Detail of a desmosomal connection (↗) with a slightly electron dense material contained in the intercellular space. ×42,000

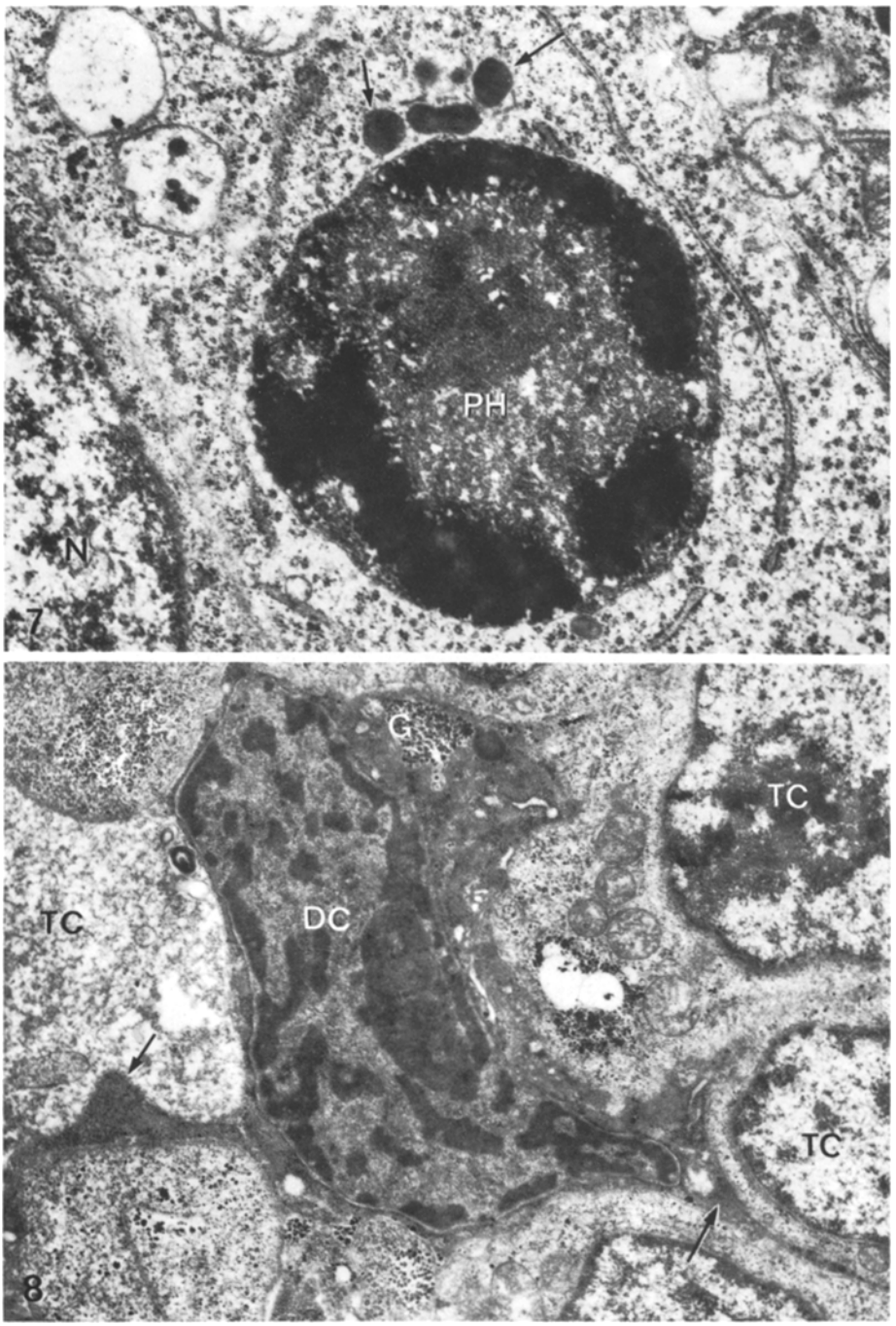

Fig. 7. A phagosome (*PH*) with cell remnants contained in the cytoplasm of a tumour cell. Several tiny dense bodies (↗) can be seen in its vicinity. *N* nucleus. ×22,000

Fig. 8. A dark cell (*DC*) closely related to the light tumour cells (*TC*). Its dense cytoplasm produces narrow projections (↗) and contains glycogen (*G*). Nuclear chromatin shows some condensation. ×12,000

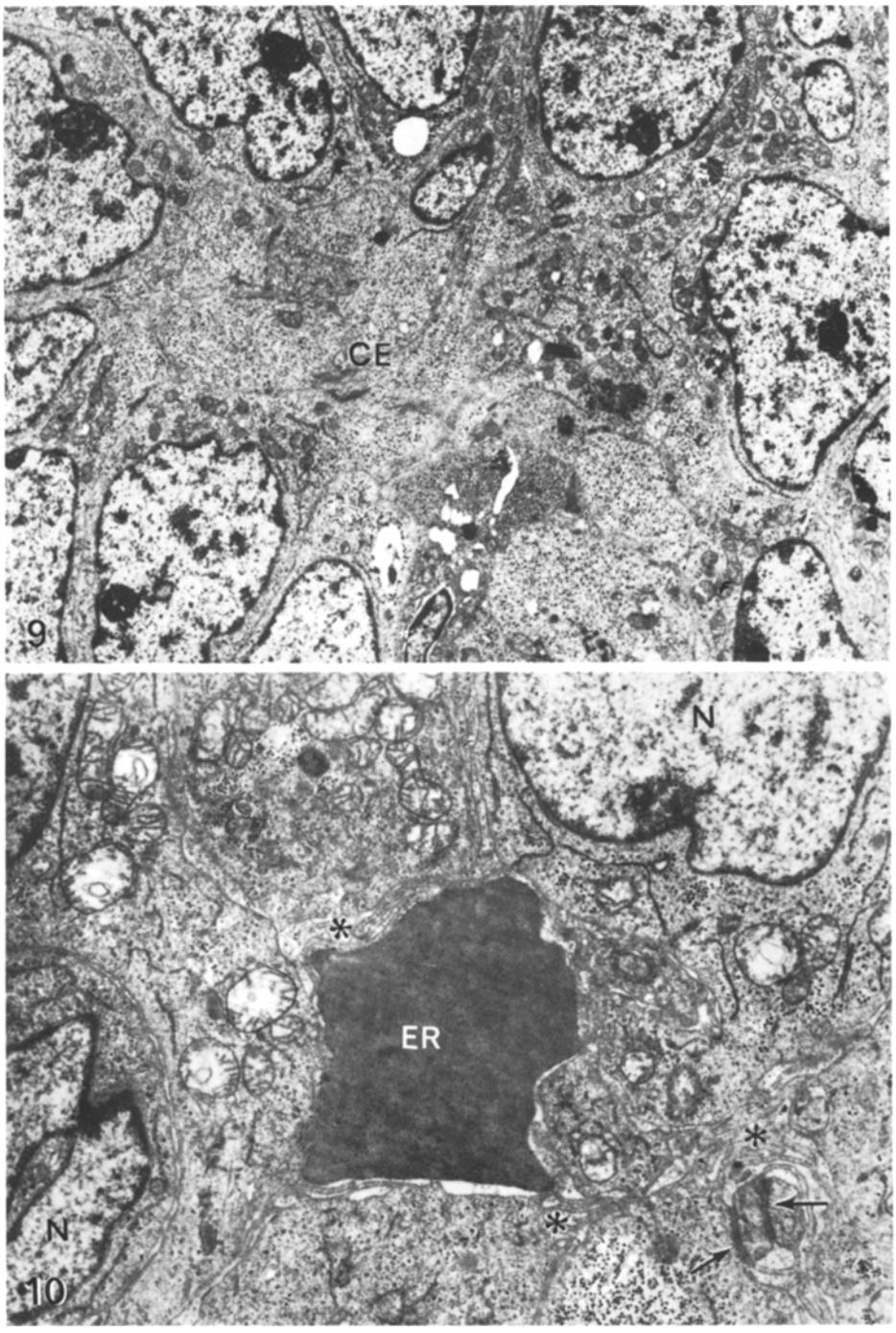

Fig. 9. A rosette-like formation composed of several tumour cells assuming a pallisade-like arrangement. Parts of cell bodies can be seen in centre (*CE*) of the formation. × 5800

Fig. 10. An "angiomatous formation" permitting poor topographic orientation. Tumour cells (*TC*) with cytoplasmic projections (*) embrace a narrow space containing erythrocyte (*ER*). *N* nucleus, ↗ desmosome. × 6500

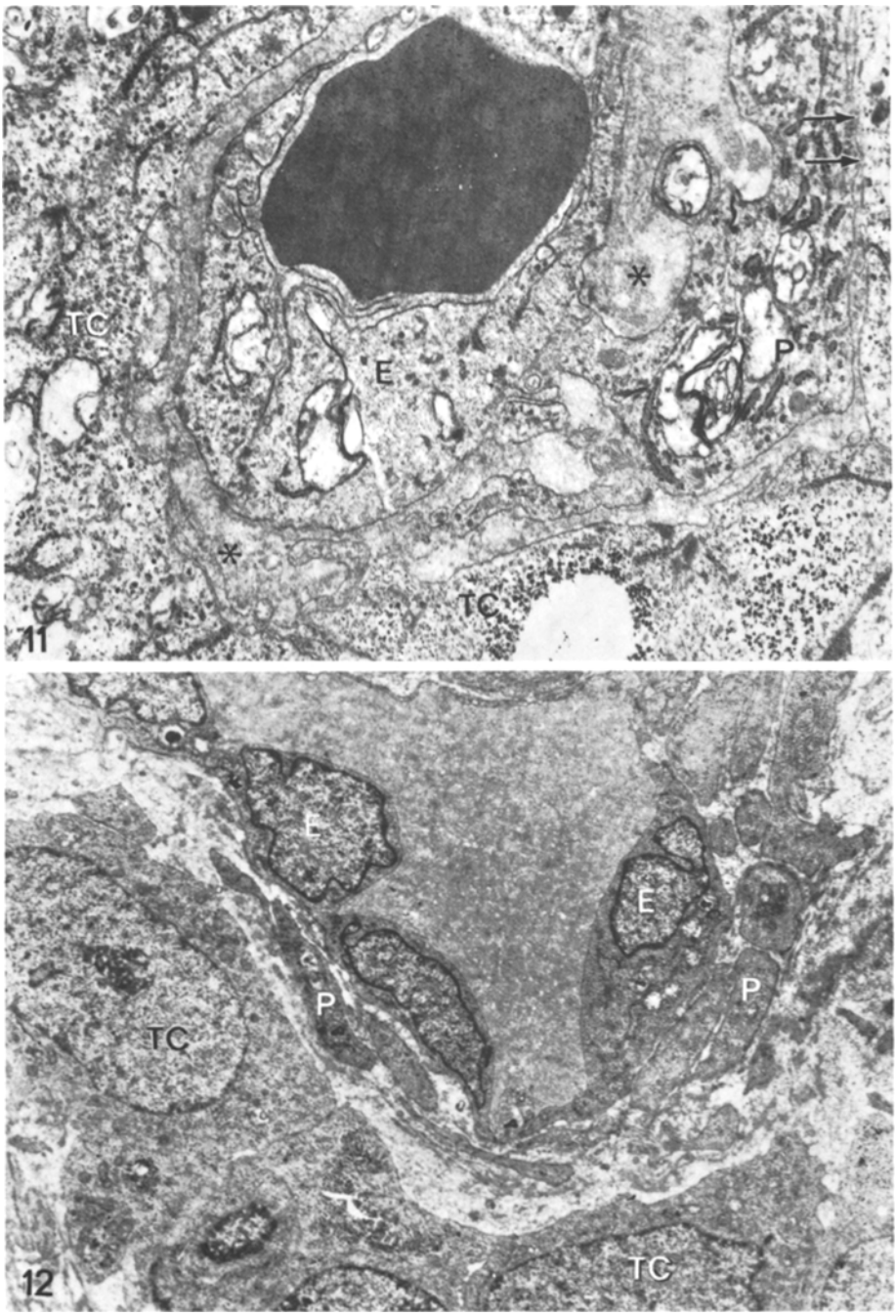

Fig. 11. A small capillary, the endothelia (*E*) of which are separated from the pericyte (*P*) as well as from the immediately neighbouring tumour cells (*TC*) by an amorphous material (*) of the basement membrane. At two points the pericyte is in a close contact with the tumour cell (↗). × 19,000

Fig. 12. Detail from Figure 2. Neither endothelia (*E*) nor pericytes (*P*) of the vessel (*C*) are joined with the tumour cells (*TC*). × 5100

In addition to the above light cells the clusters of tumour cells contained a few dark cells with narrow long processes (Fig. 8). Their conspicuously electron dense cytoplasm contained almost identical organelles as the first type of tumour cells except for the finding of more numerous lysosomes.

The tumour cells were usually arranged in solid clusters in a haphazard manner (Fig. 4). Occasionally, however, they formed rosette-like formations with radially oriented nuclei (Fig. 9). The centres of such formations usually contained the remaining parts of the tumour-cells bodies or the processes of such cells cut transversely. In other cases there were unidentifiable spaces containing occasional red blood cells between the tumour cells (Fig. 10). The origin and character of the structures described cannot be satisfactorily explained at present although they might be regarded as angiogenic formations at first glance (Fig. 10). On several occasions there occurred basement membrane-like structures in the vicinity of those formations. In view of the difficulties in assessing the topography of the respective area, remnants of a vessel invaded by the tumour could not be ruled out. Rarely the solid tumour nests contained capillaries (Fig. 11) with distinct basement membrane and a few pericytes. The tumour cells were frequently situated close to the basement membrane and the pericytes but they were only exceptionally in a close contact with them (Fig. 11).

In case 4 some sections contained structures resembling tumours of the haemangiopericytoma type (Figs. 2 and 12). There were groups of tumour cells between numerous capillaries. They were not however connected with the endothelial cells nor with the occasional pericytes present (Fig. 12), but were always separated from endothelial cells by a distinct basement membrane. No transitional cell forms between the tumour cells and pericyte-type cells were observed.

The findings which obtained in the case of that tumour metastatic to the mammary gland did not show any differences when compared with the first excision. There was only a focal increase in lysosomes and phagosomes in tumour cells. The same was true of the tumour obtained after reoperation on patient No. 3 after a one month's cytotoxic treatment.

Discussion

The findings presented here agree with the previously published data on the problems of the ultrastructure of Ewing's tumour (Friedman et al., 1968; Friedman et al., 1971; Hou-Jensen et al., 1972; Kadin et al., 1971; Llombart et al., 1970; Nakayama et al., 1975; Takahashi et al., 1976). All the tumours previously described in the literature were essentially identical in structure. They were composed of polygonal cells, the cytoplasm of which was rather poor in organelles, but contained conspicuous glycogen aggregates. The picture is completed by the presence of primitive desmosomes or so-called atypical junction complexes and the presence of a "second type" of tumour cell. The tumour retains its ultrastructural features even in extraskeletal metastases as shown by the findings in patient No. 1. This phenomenon, hitherto undescribed in the literature, indicates the possibility of establishing a reliable diagnosis with

the electron microscope in patients in whom the neoplastic disease presents with an extraskeletal metastasis. Cytotoxic treatment of several weeks' duration did not substantially alter the basic tumour pattern, despite an increase in phagosomes and more marked necroses.

Histochemical examinations of Ewing's tumour have not yielded significant findings as yet. However, the presence of cell-membrane bound alkaline phosphatase activity has been demonstrated beyond any doubt. This fact has been mentioned by Kadin and Bensch (1971) and by French authors (Llombart et al., 1970) whose results were not as distinctly positive as ours. They showed alkaline phosphatase positivity only in the vicinity of stromal vessels, and on the basis of such findings, considered the possibility of a haemangiogenic origin of the tumour. The negative results of alkaline phosphatase activity in the mammary metastasis of the tumour from patient No. 1 and in the tumour of Case 7 is interesting but its interpretation remains doubtful. It might have been due to the dedifferentiation of the tumour, but could also have been related to the long-term cytotoxic treatment.

The majority of authors were able to discern two cell types in the tumour tissue. The second-type cells, the so-called dark cells, are usually present in small numbers. Their relationship to the prevailing chief cells is unclear. They were thought to represent stromal cells by some authors (Hou-Jensen et al., 1972), an idea we regard as improbable. Others (Friedman et al., 1968; Nakayama et al., 1975) think that the cells were related to the chief tumour cells but that they differed by their degree of differentiation. In our opinion the dark cells most probably represent regressive tumour cells. Their presence appears to be of importance in view of the possible histogenetic relationship of Ewing's tumour to malignant lymphomas, which are known regularly to contain such cells (Molo et al., 1969).

Differentiating between Ewing's tumour and a metastasis of neuroblastoma or malignant lymphoma may be extremely difficult with conventional methods. Such a differential diagnosis may be significantly easier by electron microscopy (Friedman et al., 1971; Nakayama et al., 1975). Nakayama et al. (1975), comparing Ewing's tumour with neuroblastoma, disclosed in the latter, processes containing microtubules, mitochondria and secretion granules. None of these findings, regarded as characteristic of neuroblastoma, were disclosed in Ewing's tumours as we have shown. In contrast, glycogen accumulation (Schajowitz, 1959) and positivity for alkaline phosphatase is found only in Ewing's tumour. The significance of the finding of glycogen in the diagnosis of Ewing's tumour remains unsolved. Recently, some authors (Macintosh et al., 1975) have suggested the posibility that there occur Ewing's tumours, the cells of which are devoid of glycogen, but such a supposition has not been confirmed electron microscopically. All the cases reported in the literature have been found to contain glycogen (Friedman et al., 1968; Friedman et al., 1971; Hou-Jensen et al., 1972; Kadin et al., 1971; Llombart et al., 1970; Nakayama et al., 1975; Takahashi et al., 1976).

The positive finding of glycogen permits us to differentiate between Ewing's tumour, neuroblastoma as well as malignant lymphomas (Schajowitz, 1959). In malignant lymphomas electron microscopical patterns in bone have not been

studied in detail. In our experience such tumours are devoid of desmosomes (except for germinoblastoma) and glycogen. Moreover, the tumour cells are not so closely apposed and some of them show collagen formation. The results of enzyme reactions might lead to erroneous diagnosis, because some malignant lymphomas are also positive for alkaline phosphatase (Namba et al., 1975).

The histogenetic classification of Ewing's tumour remains obscure. Potential neurogenic and myelomonocytic origins (Kadin et al., 1971) are improbable in our opinion. Those considered most frequently are the haemangiogenic origin (Ewing, 1921; Llombart et al., 1970; Takahashi et al., 1976) and a possible relationship of the tumour to malignant lymphomas (Oberling, 1928). The first possibility is supported by the presence of numerous desmosomes, alkaline phosphatase positivity and, to a certain degree, angiogenic formations occasionally observed in the tumour. The presence of the basement membrane-like material, might also support the opinion of the Japanese authors (Takahashi et al., 1976). It is our view none of the findings presented to date can be regarded as unequivocal. Firstly, it cannot be entirely ruled out that tumour areas with angiogenic formations, including identifiable basement membranes, represent original vessels infiltrated by tumour cells. Whether the material found in the intercellular spaces is a product of the tumour cells and not a remnant of the original non-neoplastic structures is uncertain. The findings reported here, where areas of a tumour resemble haemangiopericytoma, cannot be regarded as convincing evidence because the vessels demonstrated cannot be definitely regarded as being part of the tumour. Despite this fact this finding deserves further study, because there are other factors, such as the presence of glycogen in the tumour cells of haemangiopericytomas (Battifora et al., 1973), which might indicate a relationship between the two tumours.

A similarity between Ewing's tumour and malignant lymphomas is based on the presence of dark cells, on the positive alkaline phosphatase activity and the findings of desmosomes which have however, been described only in lymphomas originating from the cells of the lymph node germ centers (Lennert et al., 1969). However, all these features are specific neither for the lymphomas nor for the haemangiogenic tumours. In our opinion, for example, the suggestion that Ewing's tumour is composed of immature mesenchymal cells cannot be ruled out (Melnick, 1933). As generally recognized, a number of mesenchymal tumours were shown to contain desmosomes – chondrosarcoma (Erlandson et al., 1974), fibrosarcoma (Hou-Jensen et al., 1972), chondroblastoma (Huvos et al., 1972), osteosarcoma (Paschall et al., 1975), positivity for alkaline phosphatase activity (osteosarcoma) and even glycogen accumulation – chondroblastoma (Huvos et al., 1972). Under such circumstances the divergence of opinions concerning the histogenesis of Ewing's tumour (Nakayama et al., 1975; Takahashi et al., 1976) cannot be regarded as surprising. At present we are unaware of any sufficiently specific feature of Ewing's tumour. We have to content ourselves with the statement that it is a separate oncologic entity which can be unequivocally defined by its morphological features as well as its clinical behaviour. In our opinion the problem of its histogenesis cannot be regarded as solved. However, there seem to be several facts supporting the haemangiogenic origin of the tumour.

References

Battifora, H.: Hemangiopericytoma: Ultrastructural study of five cases. Cancer **31**, 1418–1432 (1973)

Ewing, J.: Diffuse endothelioma of bone. Proc. N.Y. Path. Soc. **21**, 17–24 (1921)

Erlandson, R.A., Huvos, A.G.: Chondrosarcoma: A light and electron microscopic study. Cancer **34**, 1642–1652 (1974)

Friedman, B., Gold, H.: Ultrastructure of Ewing's sarcoma. Cancer **22**, 307–322 (1968)

Friedman, B., Hanaoka, H.: Round cell sarcomas of bone, J. Bone Joint Surg. **53A**, 1118–1135 (1971)

Hou-Jensen, K., Priori, E., Dmochowski, L.: Studies on ultrastructure of Ewing's sarcoma of bone. Cancer **29**, 280–286 (1972)

Huvos, A.G., Marcove, R.C., Erlandson, R.A., Miké, V.: Chondroblastoma of bone. Cancer **29**, 760–771 (1972)

Kadin, M.E., Bensch, K.G.: On the origin of Ewing's tumour. Cancer **27**, 257–273 (1971)

Lennert, K., Niedorf, H.R.: Reticulum cells with desmosomal connections in follicular lymphoma. Virchows Arch. Abt. B Zellpath. **4**, 148–150 (1969)

Llombart, A., Peydro, A., Lopez-Fernandez, A., Zuzuarregui, C.: Sur les sarcomas réticulaires de la moelle osseuse type Ewing. Ann. Anat. Path. **15**, 431–452 (1970)

Macintosh, D.J., Price C.H.G., Jeffree, G.H.: Ewing's tumour. A study of behaviour and treatment in forty seven cases. J. Bone Joint Surg. **57B**, 331–340 (1975)

Melnick, P.J.: Histogenesis of Ewing's sarcoma of bone, with post-mortem report of a case. Amer. J. Cancer **19**, 353–363 (1933)

Molo, F., Monga, A., Stramignoni, A.: Dark reticular cells in human lymphadenitis and lymphomas. Virchows Arch. Abt. B Zellpath. **3**, 117–126 (1969)

Motlík, K., Stárka, L.: Morphological changes in the endocrine glands of female rats injected with aminoglutethimide. Acta Univ. Carolinae Med. **18**, 334–363 (1972)

Namba, K., Itagaki, T., Iijima, S.: Enzyme histochemical investigations of human malignant lymphomas. Beitr. Path. **154**, 233–242 (1975)

Nakayama, I., Tsuda, N., Muta, H., Fujii, H.: Fine structural comparison of Ewing's sarcoma with neuroblastoma. Acta Path. Jap. **25**, 251–268 (1975)

Oberling, C.: Les réticulosarcomes et les réticuloendothéliosarcomes de la moelle osseuse (sarcome d'Ewing). Bull. Ass. Franç. Cancer **17**, 259–296 (1928)

Paschall, H.A., Paschall, M.M.: Electron microscopic observations of 20 human osteosarcomas. Clin. Orthopaedics Rel. Res. **III**, 42–56 (1975)

Schajowitz, F.: Ewing's sarcoma and reticulum cell sarcoma of bone with special reference to the histochemical demonstration of glycogen as an aid to differential diagnosis. J. Bone Joint Surg. **41A**, 349–356 (1959)

Sirsat, S.M., Panicker, K.N.S., Podtar, G.G.: Ultrastructure of Ewing's sarcoma of the bone. Indian J. Cancer **8**, 157–162 (1971)

Takahashi, K., Sato, T., Kojima, M.: Cytological characterization and histogenesis of Ewing's sarcoma. Acta Path. Jap. **26**, 167–190 (1976)

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