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Clear-cell sarcoma of tendons and aponeuroses

An immunohistochemical and electron microscopic analysis indicating neural crest origin

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Summary. A clinico-pathological, light microscopic and immunohistochemical study of 15 clear-cell sarcomas, with an ultrastructural analysis of 6 of the tumors, is presented. The tumors showed a strong predilection for tendons and aponeuroses of the extremities in predominantly young and middle-aged people. The clinical setting, course and light microscopic appearance agree well with the original description by Enzinger (1965). Nine of the 15 patients developed metastases, most of them including lymph nodes, and 8 of the patients had died at the time of follow-up (median follow-up time 4.8 years). Reducing pigment was demonstrated within the cells of 2 tumors. Ultrastructurally the 6 tumors studied had a uniform appearance with characteristically rounded or oval tumor cells with a single nucleus containing one or two very prominent nucleoli, a light-staining cytoplasm with a moderate amount of organelles and a variable content of glycogen. Polymorphic melanosomes were seen in the cells of one of the tumors. External laminas enclosed groups of tumor cells and invested parts of individual tumor cells. With immunoperoxidase analysis for S-100 protein positive staining was observed in the vast majority of the tumor cells of all 15 clear-cell sarcomas. Metastases appearing in 9 of the 15 cases showed positive staining for S-100 protein. There was a strong staining of the cytoplasm and generally a weak and varying staining of nuclei. The immunohistochemical and electron microscopic findings indicate that clear-cell sarcoma is a homogenous entity among soft tissue sarcomas, of probable neural crest derivation.

Key words: Clear-cell sarcoma – Electron microscopy – Immunohistochemistry – Neural crest – S-100 protein

Since Enzinger in 1965 described clear-cell sarcoma as a distinct entity, several reports on small series and occasional cases of this tumor have

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appeared in the literature (Rousselot et al. 1968; Angervall and Stener 1969; Dutra 1970; Mackenzie 1974; Alitalo et al. 1977; Chung and Enzinger 1978; Mukherjee and Gupta 1978; Gupta et al. 1978; Johnson and Uhlman 1980). Most of Enzinger's 21 cases were originally diagnosed as synovial sarcoma or fibrosarcoma. At the 67th Annual Meeting of IAP in Atlanta (1978), Chung and Enzinger presented the experience of 112 cases diagnosed as clear-cell sarcoma at the AFIP over a period of 35 years. Despite the fact that the light microscopic appearance and the clinical presentation and course have been well documented, there is a divergence of opinion about the histogenesis, and whether the clear cell sarcoma represents a homogeneous entity or is merely a clinico-pathologic presentation common to a number of sarcomas when they grow in tendons and aponeuroses (Bearman et al. 1975). In many instances, reducing pigment has been observed. In 42% of the cases presented by Chung and Enzinger (1978) there were neoplastic cells bearing a melanin pigment. Melanosomes have been demonstrated ultrastructurally in most of the few clear-cell sarcomas studied hitherto (Hoffman and Carter 1973; Bearman et al. 1975; Boudreaux and Waisman 1978; Tsuneyoshi et al. 1978; Bednar 1979; Clark Raynor et al. 1979; Ekfors and Rantakokko 1979; Choux et al. 1981). This has led some authors to suggest that the tumor may represent a variant of malignant melanoma (Bearman et al. 1975; Clark Raynor et al. 1979; Ekfors and Rantakokko 1969). The lack of melanin in many tumors, however, and the ultrastructural appearance of occasional cases has resulted in a suggestion of synovial origin (Kubo 1979). Based on a comparative light microscopic study of 13 clear-cell sarcomas, and an electron microscopic study of two of these, Tsuneyoshi et al. (1978) proposed a subgrouping into melanotic and synovial types.

S-100 protein is a specific protein which was isolated from the central nervous system (CNS) more than 15 years ago (Moore 1965). Within the CNS, S-100 has been demonstrated in astrocytes and oligodendrocytes by immunohistochemical techniques (Hydén and McEwen 1966). S-100 has also been found in human gliomas (Haglid et al. 1973). Recently S-100 has also been found outside the CNS in Schwann cells (Stefansson et al. 1982b and melanocytes (Gaynor et al. 1980), two closely related subclasses of cells derived from the neural crest (Weston 1970). From the studies hitherto performed, S-100 seems to be a highly selective marker of Schwann cells and neoplastic melanocytes. Utilizing immunohistochemical techniques it has also been possible to localize S-100 in benign tumors of Schwann cell origin and in melanocytic tumors including malignant melanoma (Stefansson et al. 1982a; Nakajima et al. 1981; Nakajima et al. 1982). The demonstration of this protein has helped to establish a Schwann cell origin for granular cell tumor (myoblastoma) (Nakazato et al. 1982; Stefansson and Wollmann 1982) and plexiform nerve sheath myxoma (Angervall et al. 1983).

The aim of the present investigation is to contribute to the question of the histogenesis of clear-cell sarcoma of tendons and aponeuroses by means of an immunoperoxidase study of the presence of S-100 protein. Ultrastructural studies were also performed.

Material

The series consisted of 15 cases of clear-cell sarcoma of tendons and aponeuroses. Clinical records and follow-up data were available in all cases. All cases were studied light microscopically and immunohistochemically and 6 were studied ultrastructurally. Three of the cases have been previously published (Angervall and Stener 1969). For comparison, 10 cases of classical synovial sarcoma were also analysed immunohistochemically.

Methods

Light microscopy

From paraffin blocks, 4–5 μ thick sections were stained according to van Gieson and with haematoxylin-eosin. For the demonstration of reducing pigment, the Masson-Fontana method was used, and Prussian blue was used for the demonstration of haemosiderin. The PAS-staining, with and without prior diastase (Merck, Darmstadt, FRG) digestion, was performed to visualize glycogen. Silver impregnation according to Gordon and Sweet was used to demonstrate reticulin.

Electron microscopy

Small pieces from 4 of the tumors (cases 6, 13, 14 and 15) were immediately put into 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2 for 2–4 h, at 4° C, washed in cold buffer, postfixed with 1% OsO_4 for 1 h, and thereafter dehydrated in ethanol, embedded in Epon 812, and cut in an LKB Ultratome III. They were then examined under a Philips 400 electron microscope.

Some pieces of tumor were embedded without postfixation with OsO_4, and thin sections of these blocks were stained using the PATCH-SP-method (Thiery 1967).

From 2 cases (cases 4 and 7), small selected pieces from the paraffin blocks were cut out, carefully deparaffinized in xylene, rehydrated in decreasing concentrations of ethanol, and finally washed in cacodylate buffer and fixed in 1% OsO_4. They were thereafter dehydrated in ethanol, embedded in Epon and processed and examined as described above.

Immunohistochemistry

The procedure for purifying S-100 protein, and the production of antiserum, has previously been described in detail (Haglid et al. 1973; Angervall et al. 1983).[1]

Immunoperoxidase staining

The peroxidase-antiperoxidase methods of Mason and co-workers (Mason et al. 1969) and Sternberger and co-workers (Sternberger et al. 1970; Sternberger 1979) were employed with slight modifications. The sections were deparaffinized in xylene, hydrated through a decreasing concentration of ethanol down to 70% ethanol, from which the slides were put into 3% H_2O_2 in 0.15 M NaCl – 0.05 M Tris HCl buffer at pH 7.6 for 30 min. All sections were examined with and without prior treatment with trypsin 0.1% in 0.2 M Tris saline buffer, pH 7.8, containing 0.1% $CaCl_2$, at 37° C for 15–30 minutes. The slides were then washed in 0.15 M NaCl – 0.05 M Tris-HCl at pH 7.6 which was the buffer used in all the subsequent stages of the procedure. This wash and all subsequent washes were done with four changes of buffer for 7 min each. The sections were next overlayed for 15 min with normal swine serum (NSS), diluted 1:10 in the buffer and then blotted but not washed. This was followed by a 30 min overlay with rabbit antiserum to bovine S-100 at 1:80 dilution in 1:10 NSS or an overlay with one of the control rabbit sera also in 1:10 NSS and at 1:80 dilution; this was followed by a wash. Next came a 20 min overlay with a 1:20 dilution (in 1:10 NSS) of swine antiserum against rabbit immunoglobulins (DAKO, Copenhagen, Denmark), subsequently the slides were

[1] The antiserum was made available to us through a gift from Associate Professor Kenneth Haglid, Department of Histology, University of Göteborg.

Table 1. Summary of clinical data and follow-up in 15 patients with clear-cell sarcoma

Case no.	Sex	Age at diagnosis	Duration of symptoms (years)	Anatomic location	Tumor size (cm)
1	F	22	2	Dorsal aspect of right lower arm	1–2 ∅
2	M	29	1	Distal medial aspect of right thigh	7 × 5 × 5
3	M	44	14	Plantar aspect of left hallux	$1 \times 3 \times {}^{1}/_{2}$
4	M	39	2	Medial aspect of left knee	not given
5	F	13	1	Dorsal aspect of right thumb	1 ∅
6	M	25	0.5	Anterior aspect of right thigh	3–4 ∅
7	M	79	3	Right elbow	10
8	F	37	not given	Right thigh	not given
9	F	14	0.1	Left ankle	11 × 7 × 4
10	M	64	10	Volar aspect of left hand	4 ∅
11	M	33	0.5	Medial aspect of right foot	not given
12	F	33	1	Plantar aspect of right hallux	3 ∅
13	M	18	2–3	Behind right ear	1 ∅
14	M	35	1.5	Dorsal aspect of right foot	3 × 3 × 2
15	M	60	0.2	Anterior muscle group of the left lower leg	6 × 4 × 2

washed. The last overlay was for 30 min with a solution of horseradish peroxidase rabbit anti-peroxidase immunocomplexes (DAKO) diluted 1:20 in 1:10 NSS; this was followed by a wash. Finally the slides were put into a solution containing 0.50 mg/ml of 3,3′-diaminobenzidine ·4 HCl (Sigma Chemicals, St. Louis, MA, USA) and 0.01% H_2O_2 v/v in saline Tris-HCl buffer pH 7.6 for 4 min and then washed. Some sections were counterstained with haematoxylin; others were not counterstained. For control staining the specific antiserum was replaced by the immunoglobulin fraction of serum from the non-immunized rabbit as well as Tris buffer. Furthermore, the specific antiserum was replaced by an antiserum absorbed with S-100 protein.

Type of treatment	Recurrence	Follow up	
		Duration (years)	Course
Local excision	–	13	Alive
Local excision	–	2	Dead with lymph node, bone, pericardium, myocardium, and adrenal gland metastases
Local excision, followed by amputation	–	2.5	Dead with lymph node, heart, bladder, spleen, and diaphragm metastases
Radiotherapy, followed by local excision	–	0.5	Dead with lymph node, bone and lung metastases
Local excision, followed by amputation	×5	26	Alive
Local excision, followed by radio- and chemotherapy	–	4	Dead with lymph node and pulmonary metastases
Local excision, followed by chemotherapy	–	1	Alive, with bilateral pulmonary metastases
Local excision	×1	20	Pulmonary metastasis surgically removed after 14 ys. Currently alive and well
Local excision	–	6.5	Alive, well
Local excision	–	5.5	Dead, with femur, diaphragm and left upper arm metastases
Amputation	–	7	Dead, with bone and liver metastases
Amputation, followed by chemo- and radiotherapy	–	2	Dead, with lymph node, pancreas, pulmonary and skin metastases
Local excision	–	6	Alive, well
Local excision	–	1	Alive, well
Local excision	–	8.5	Dead, with lymph node and pulmonary metastases

Results

Clinical data

The pertinent clinical data is summarized in the table. Of the 15 patients, 10 (67%) were males, and 5 (33%) were females. In 9 cases (60%) the diagnosis of clear-cell sarcoma was made during the third or fourth decade of life. The age range was 13–79, and the median age at the time of diagnosis was 33. Only 4 patients reported a duration of symptoms of less than 1 year,

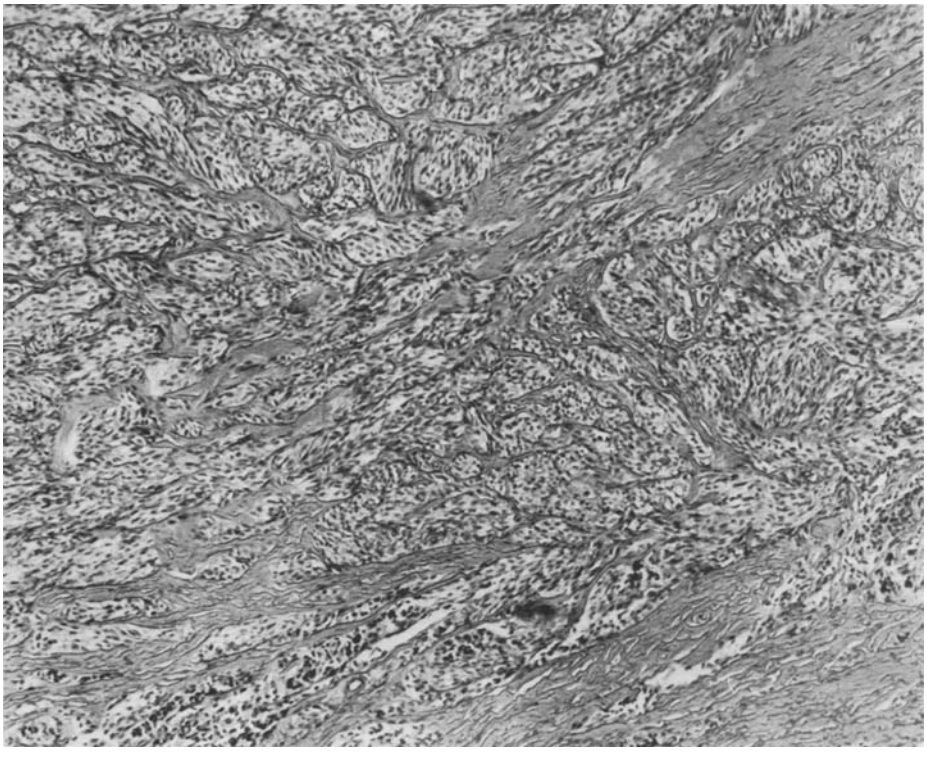


Fig. 1. Characteristic pattern of clear-cell sarcoma splitting up the collagen of a tendon. The tumor cells are arranged in small fascicles or nests. H & E, ×90

while two patients had observed a tumor mass for 10 years or more. Mostly, the tumors were fairly small and well delimited at the gross examination. In 8 cases, the tumors were smaller than 5 cm at the largest diameter and only two of the tumors were 10 cm or larger; these were located in the elbow and the thigh. The tumors were located in the extremities in all cases except one, which occurred behind the right ear. Nine occurred in the lower extremity (60%) and 5 in the upper extremity (33%). All patients were treated surgically. Radiotherapy and/or chemotherapy was attempted only when the tumor had metastasized. Follow-up data was obtained for periods ranging from 0.5 to 26 years, with a median of 4.8 years. Of the 15 patients, 8 had died with distant metastasis at the time of follow-up. One patient was alive but had multiple pulmonary metastases. Six were alive and apparently free from tumor disease; one of them had had a solitary pulmonary metastasis removed surgically 6 years earlier.

Light microscopic appearance

The tumors consisted of characteristically compact, solid nests and fascicles of rounded or spindle-shaped tumor cells, with a pale and often vacuolated

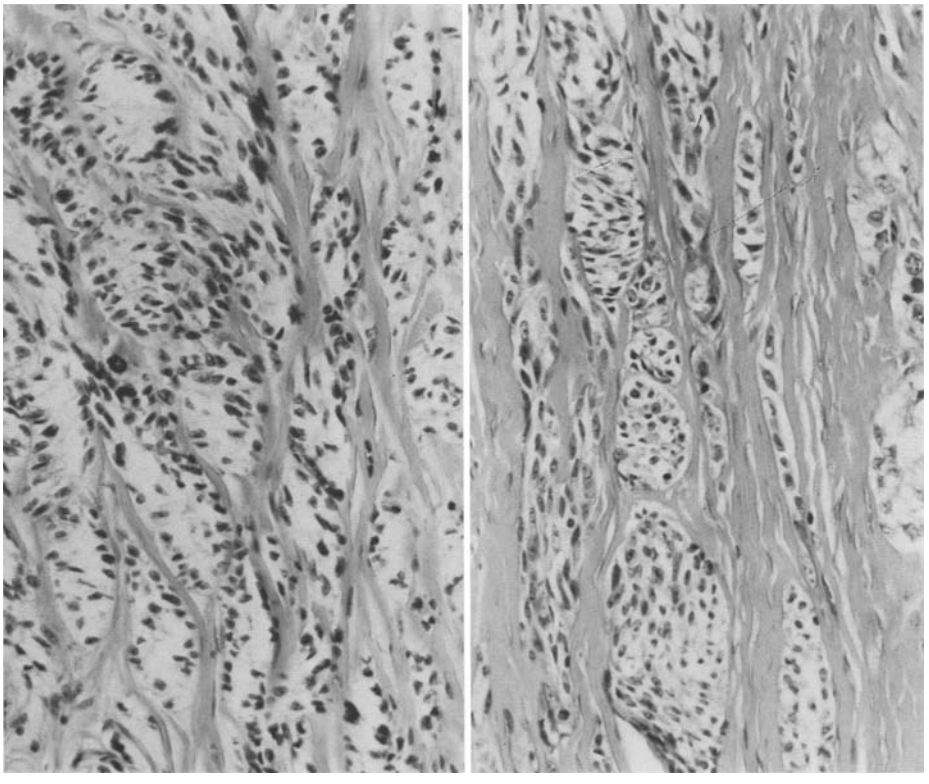


Fig. 2A, B. Nests of tumor cells with a poorly defined clear cytoplasm, growing infiltratively within fascia **A** and tendon **B**. H & E, ×180

or water-clear cytoplasm (Figs. 1 and 2). The cytoplasmic borders were indistinct. The tumors infiltrated tendons or aponeuroses, splitting up the collagen bundles (Figs. 1 and 2). In 3 tumors, all located in the hand or foot, the skin was also involved, but no invasion of the covering epidermis was observed.

The tumor cells contained a single, round or slightly ovoid, light-staining nucleus, with a distinct nuclear membrane. Generally one or rarely two prominent nucleoli were observed (Fig. 3). Usually, the nuclear polymorphism was slight, and the mitotic activity low. Small areas of some tumors, however, revealed tumor cells with larger, darker, more chromatindense nuclei, and a more pronounced polymorphism. In 7 cases, a few multinucleated giant cells were observed, mostly appearing singly. The nuclei of these giant cells were peripherally arranged, and the individual nuclei were usually smaller than those of the mononucleated cells.

Some tumor cell nests showed central empty spaces, surrounded by an irregular rim of tumor cells, giving them a pseudo-alveolar appearance. Silver impregnation technique gave prominence to the finely nodular architecture by clearly visualizing the reticulin septa which separated the well defined nests of varying size (Fig. 4). Reducing pigment was found in only

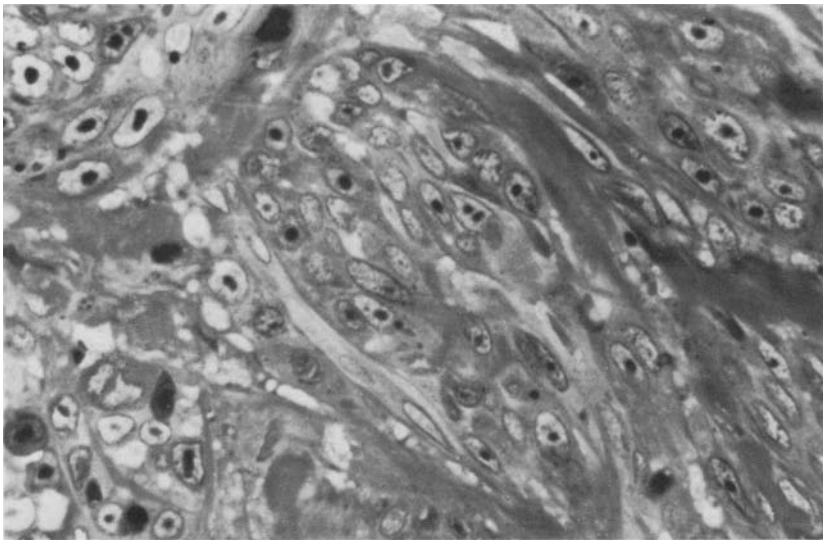
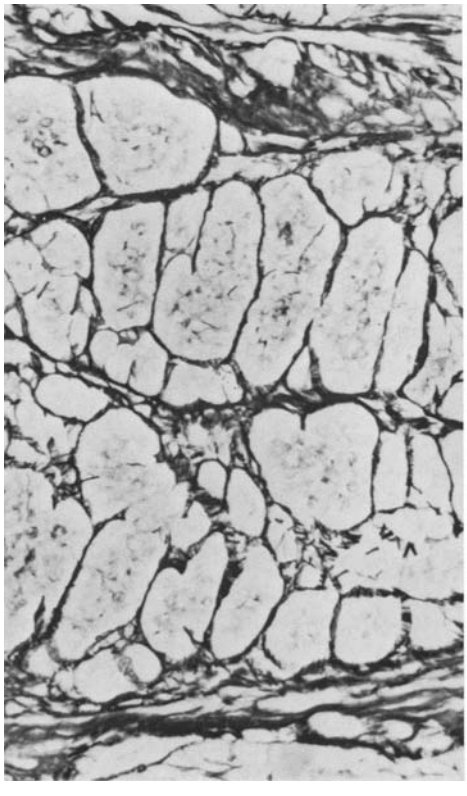


Fig. 3. One μ thick Epon section showing tumor cells with oval or rounded vesicular nuclei, distinct nuclear membranes and 1 or 2 prominent nucleoli. Toluidine blue. $\times 450$



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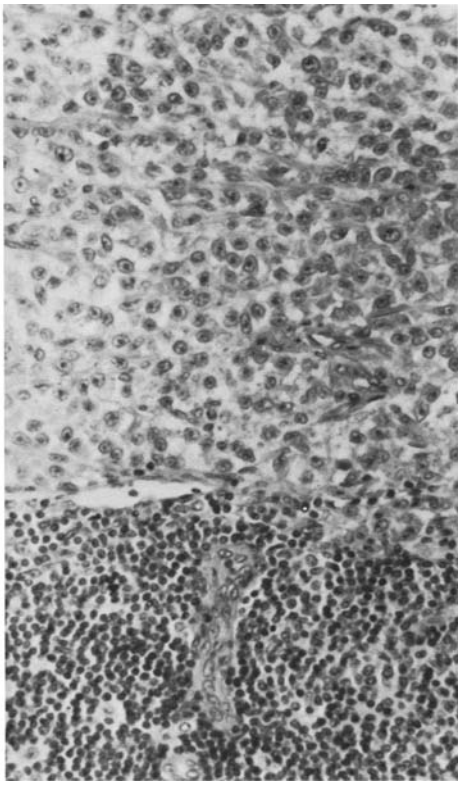

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Fig. 4. Well defined nests of tumor cells separated by silver-impregnated septa. Gordon's and Sweet's reticulin stain. $\times 180$

Fig. 5. Lymph node showing tumor tissue above, and lymphoid tissue below. The cellular appearance is similar to that of the primary tumor. H & E, $\times 225$

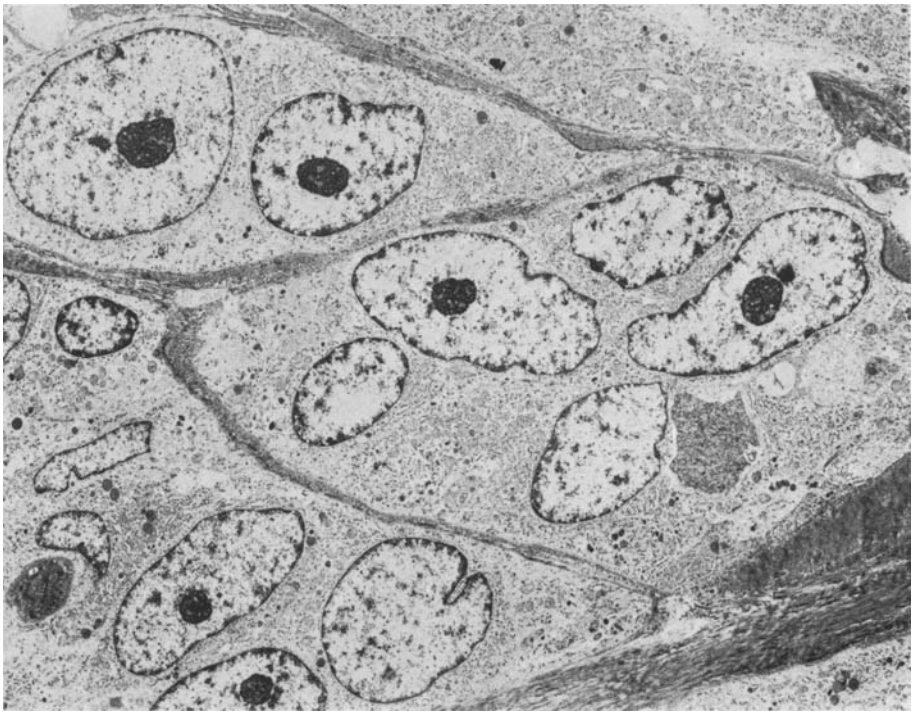

Fig. 6. Groups of closely associated tumor cells enclosed by collagen bundles of varying size. ×2,900

two tumors (cases 4 and 7), and was in these cases seen as scattered deposits. Usually slight amounts of intracytoplasmic PAS-positive diastase digestible material, indicating the presence of glycogen, could be demonstrated.

Necrosis or haemorrhage were never prominent features in the primary tumors. The lymph node and pulmonary metastases had a similar cellular appearance. However, the general architecture of the metastases differed from that of the primary tumors in its frequent lack of distinct fibrous dividing septa (Fig. 5). This deprived the metastases of the characteristic finely nodular and nest-like appearance. Especially in the lymph node metastases, the tumor cells were more dissociated, and sometimes lacked the clear-cell appearance. In one of the tumors (case 6) the pulmonary and lymph node metastases revealed a much more prominent polymorphism, including some multinucleated bizarre giant cells.

Electron microscopic appearance

The 6 tumors studied were characteristically composed of groups of very closely associated tumor cells, with no significant intercellular substance within the tumor cell groups (Fig. 6).

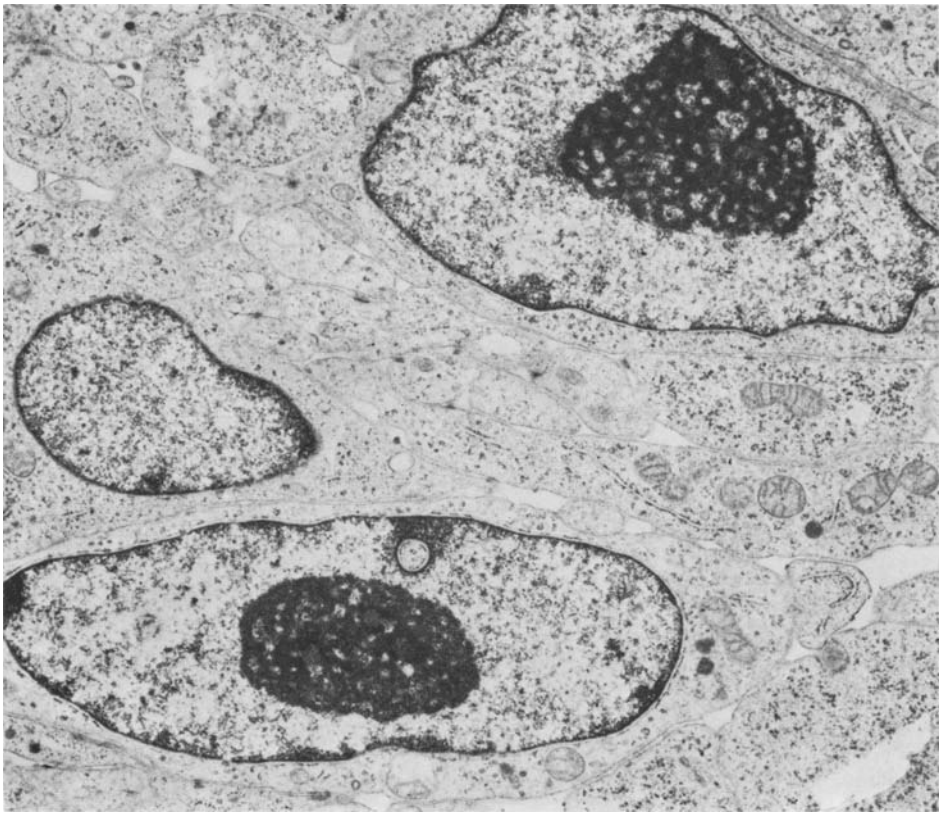

Fig. 7. Tumor cells showing oval nuclei with fairly evenly dispersed chromatin, a zone of condensed heterochromatin at the periphery and one large, reticular nucleolus. Sparse amounts of organelles are seen within the cytoplasm. The tumor cells are intimately associated and no significant intercellular substance is observed. × 10,500

The tumor cells appeared rounded when cut transverselly, and oval or short and spindle-shaped in longitudinal sections. The majority of the cells contained one large nucleus, mostly rounded or oval in shape, with even contours (Fig. 7). A few nuclei were deeply cleft and revealed a very irregular shape; some of the invaginations appeared as pseudo-inclusions in the sections. The chromatin was generally finely dispersed, with a thin zone of heterochromatin condensed at the inner surface of the nuclear membrane. In all the tumor cells, the nuclei contained one, or rarely two very prominent nucleoli. The nucleoli were most often centrally or excentrally located, had a rounded shape and a trabecular structure. A few marginally located nucleoli were observed. The nucleoli were often very large, some-times occupying more than half of the nucleus. In one tumor (case 15), many nuclei contained numerous perichromatin granules, 30–35 nm in diameter, and surrounded by an electron lucent halo; most of these granules were closely associated with the peripheral heterochromatin zone. In this case, there were also many

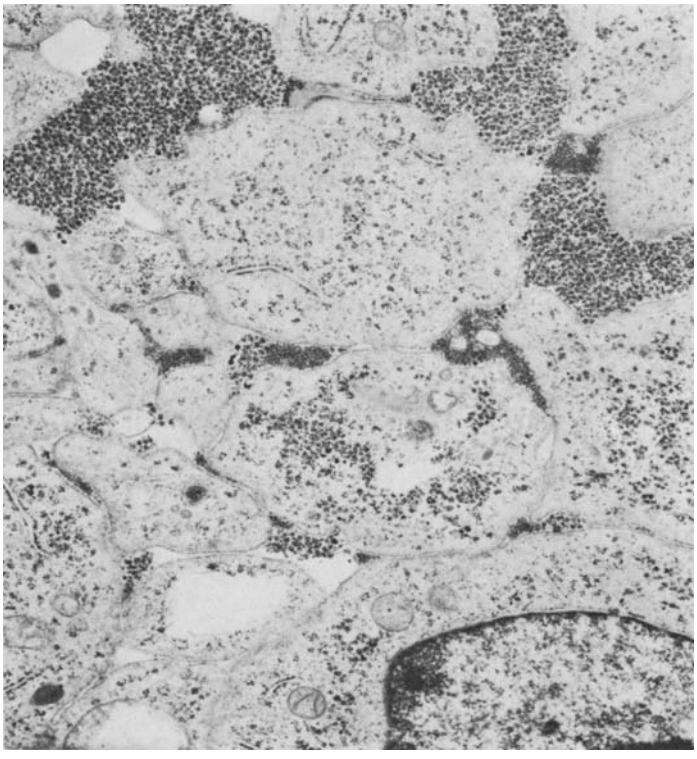

Fig. 8. Detail of tumor cells containing abundant cytoplasmic glycogen. Glycogen is present also in intercellular spaces. × 14,000

nuclei which contained large inclusions, which were only partly surrounded or devoid of a nuclear membrane, thus suggesting that they represented true inclusions. These inclusions were predominantly composed of multilayered membrane structures, partly forming myelin-like figures.

The cytoplasmic matrix was generally light-staining and contained a moderate amount of organelles. Of the rather sparse endoplasmic reticulum, a considerable part appeared smooth, and only fairly small segments were clearly granular. Numerous free ribosomes and polysomes were observed in most cells. The Golgi zones were rarely clearly distinguishable. Most mitochondria were small and had a round shape. Many cells contained small amounts of filament, mostly loosely arranged and of intermediate size (10 nm diameter). A few dark-staining, rounded bodies of lysosome type were observed. Occasionally, tumor cells contained small lipid droplets. There were tumor cells with delicate villus-like cytoplasmic projections, containing tightly packed intermediate filaments.

In one tumor (case 6), the tumor cells revealed abundant accumulations of glycogen. This glycogen appeared mostly in rosette-formation (alpha-type), but also in monoparticulate form (beta-type). Some of the glycogen was associated with cytoplasmic vacuoles, which were probably caused by

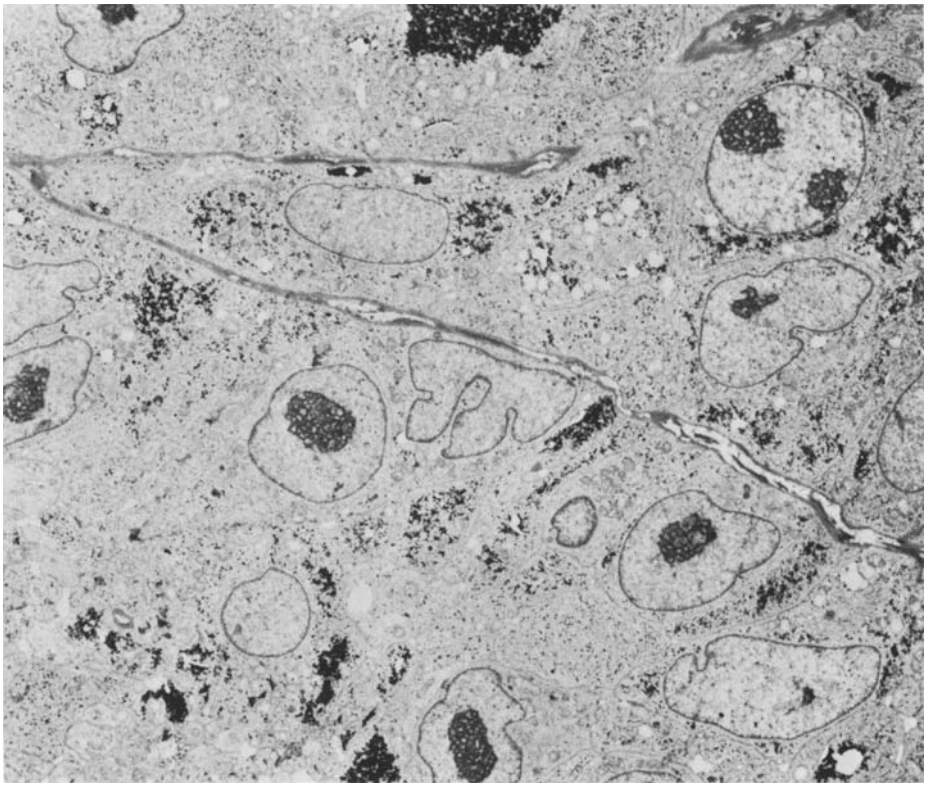

Fig. 9. Varying amounts of glycogen visualized as black deposits in most tumor cells by the PATCH-SP staining technique. × 2,900

the dissolution of some of the glycogen. Glycogen was also observed extracellularly (Fig. 8). The cytoplasmic membranes in the surroundings of these extracellular glycogen deposits appeared to be intact. In the remaining 4 tumors, glycogen was less prominent, or could not be clearly distinguished at all in the routinely stained EM sections. However, after the PATCH-SP-staining, it could be demonstrated that these tumors contained glycogen, mostly of the monoparticular type (Fig. 9). In these tumors, the glycogen material was partly located adjacent to vacuolized areas of the cytoplasm.

In one tumor (case 7), groups of tumor cells contained abundant melanosomes. Otherwise these cells had general features similar to those of the majority of the tumor cells. The melanosomes were highly pleomorphic, with very variable sizes, shapes, patterns of internal structure and degree of melanization. They filled most of the cytoplasm of the cells (Fig. 10).

A few desmosome-like and hemidesmosome-like junctions were found, although no well-developed desmosomes were observed. Bands of collagen fibers, of mature, ordinary cross-banded appearance, were often seen surrounding the tumor cell groups. In 3 tumors (cases 7, 14 and 15), the collagen material partly consisted of broader fibers with a periodicity of about

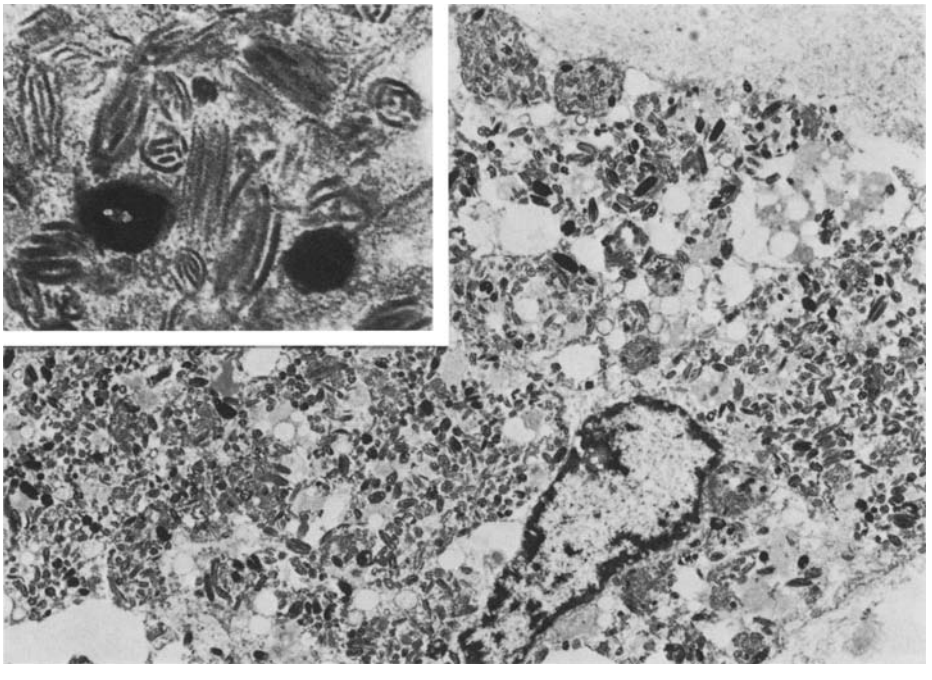

Fig. 10. Tumor cell filled with melanosomes of varying degree of melanization. × 8,500. Inserted is a high magnification of the melanosomes which appear pleomorphic with varying internal structure. × 37,000

130 nm, i.e. collagen of the so-called long-spacing type (Fig. 11). Generally, a distinct external lamina surrounded the outer surface of the cell groups (Fig. 11). Within the cell nests, segments of tumor cells were sometimes invested by the same kind of external lamina. In some areas of the tumors, slit-like spaces had formed between the tumor cell groups. These spaces contained some bundles of collagen at the centre, and were lined at the periphery by thick, dark-staining, external lamina-like material (Fig. 12).

Immunohistochemical analysis

All 15 clear-cell sarcomas contained tumor cells positively stained for S-100 protein (Figs. 13, 14A and B). In 13 of them, all or the vast majority of the cells were strongly positive. In one of them, the staining prior to trypsin treatment was weak. In the two remaining cases, some areas of the tumors were negative, while others were positive, often with mingling positive and negative tumor cells. In most tumor cells, the granular dark brown reaction product was distributed throughout the cytoplasm (Fig. 15). Usually, some degree of positive staining was also noted in the nuclei. The stain for S-100 protein gave prominence to peripherally located, infiltrating tumor cells, singly or in minute groups, enclosed within the tendons or aponeuroses, which could otherwise be difficult to identify as tumor cells. In all the

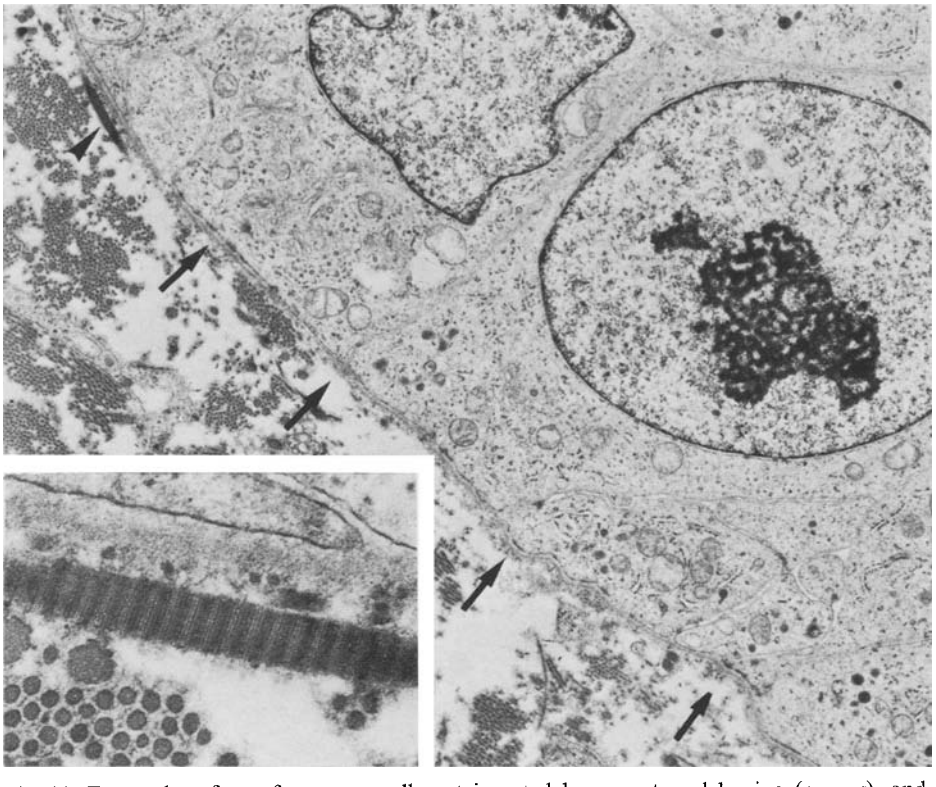

Fig. 11. External surface of a tumor cell nest invested by an external lamina (*arrows*), and surrounded by collagen. A broad collagen fiber (*arrowhead*) is seen. × 8,000. At high magnification (*inserted*) the fiber is found to be of the so-called long-spacing type. × 48,000

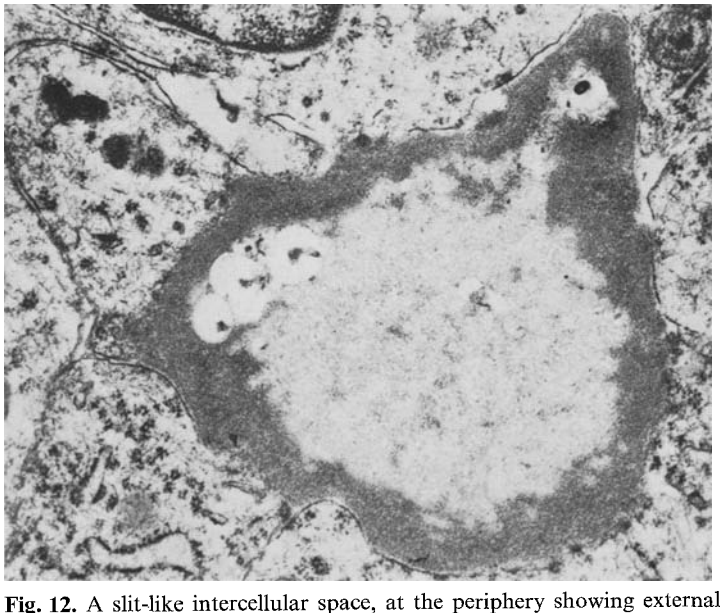

Fig. 12. A slit-like intercellular space, at the periphery showing external lamina-like material and centrally containing pale-staining cross-sectioned collagen. × 23,000

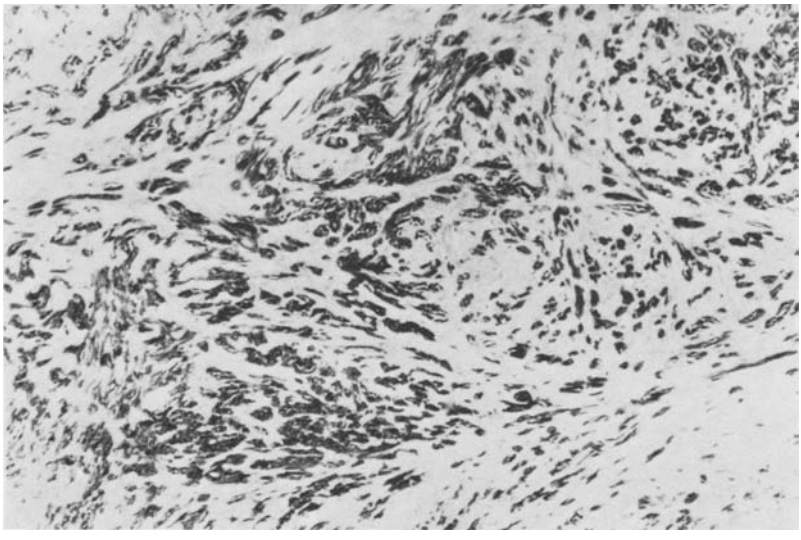

Fig. 13. Low power view of tumor tissue diffusely infiltrating a tendon. The dark stained tumor is sharply distinguished from the unstained tendon tissue. Anti-S-100 protein-immunoperoxidase staining. × 60

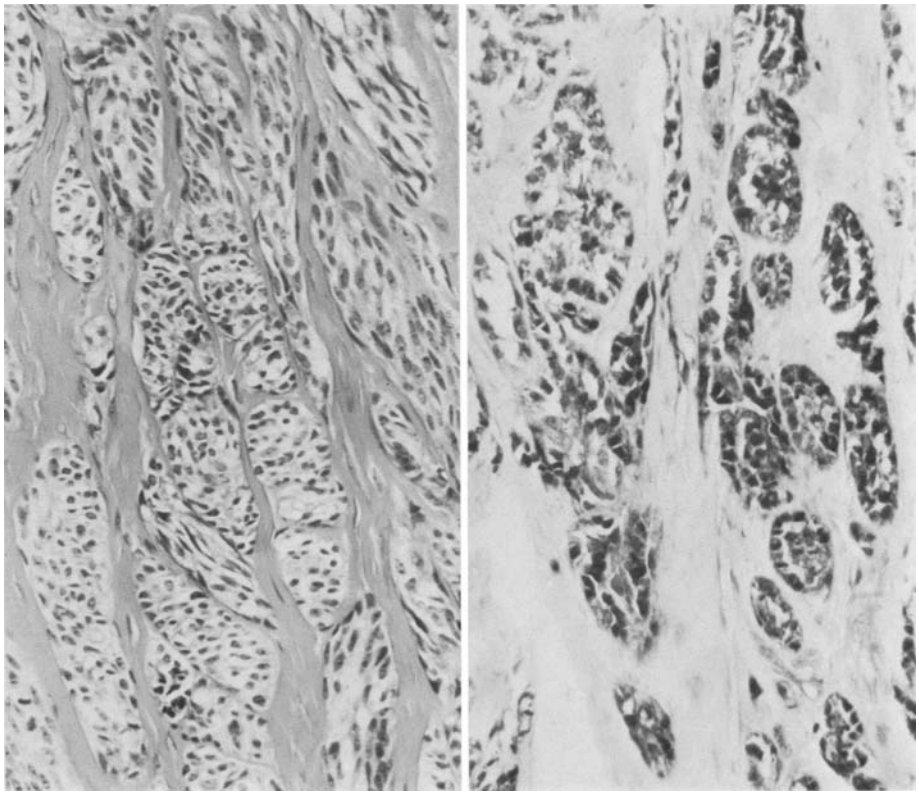

Fig. 14A, B. Detail of tumor infiltrating a tendon **A**. H & E, × 180. A neighboring area with a strong staining for S-100 protein of tumor cells only **B**. × 180

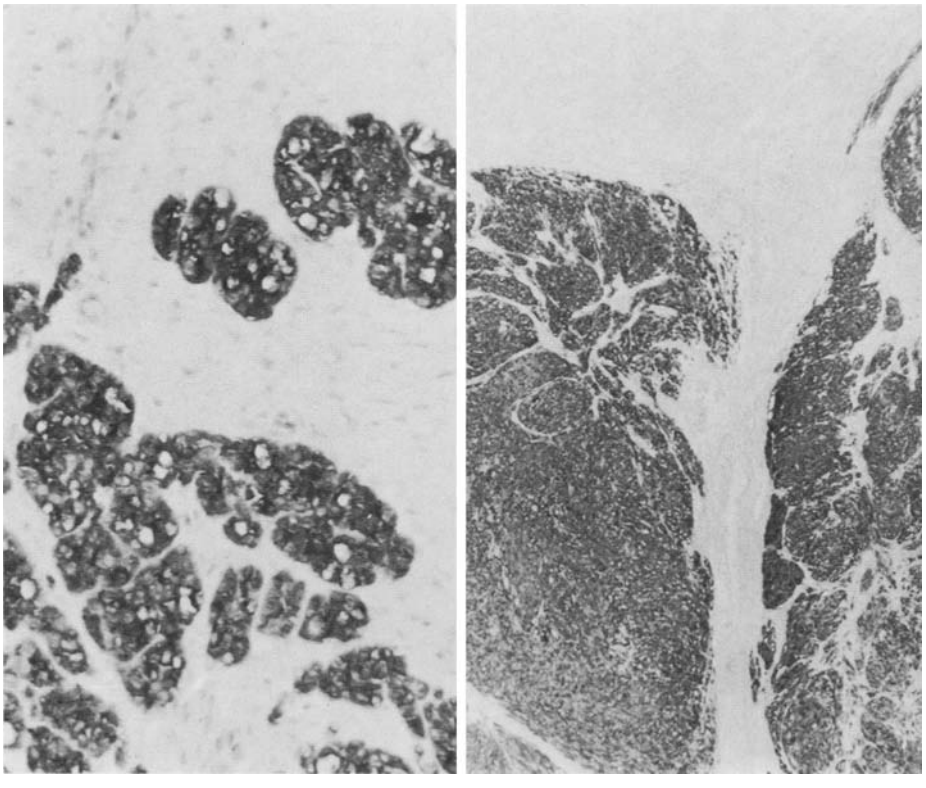

Fig. 15. There is a strong positive staining for S-100 protein in the cytoplasm of all tumor cells, while the nuclei appear unstained or weakly stained. × 250

Fig. 16. Solid metastatic tumor growth within a lymph node. The tumor tissue is strongly stained for S-100 protein as opposed to the unstained surrounding lymphoid tissue. × 60

examined lymph nodes (Fig. 16) and lung metastases, there was a positive staining like that in the primary tumor.

Except for the peripheral nerves, no other structures were positively stained. In the examined synovial sarcomas, there was no positive staining of tumor cells. No control sections revealed positive staining for S-100 protein.

Discussion

The light microscopic appearance of the tumors in the present series corresponds well with the original description by Enzinger (1965) of clear-cell sarcoma as a soft tissue tumor entity. In addition, the clinical setting, with tumors of tendons and aponeuroses which were usually small and appeared in young adults or middle-aged people, a long duration of symptoms prior to diagnosis and a strong predilection for the extremities agree well with previous report of large series (Enzinger 1965; Chung and Enzinger 1978).

A protracted course, with a high frequency of metastasis, especially to the lymph nodes as noted in our series, is characteristic of clear-cell sarcomas (Enzinger 1965; Chung and Enzinger 1978).

Tsuneyoshi and coworkers (1978) proposed a division of clear-cell sarcoma into a melanotic and synovial type, based on an ultrastructural analysis of two cases and a light microscopic review of 11 additional cases. Among others, the light microscopic findings of pseudo-glandular structures and multinucleated tumor giant cells were interpreted as signs of a synovial origin. In the present series there were no ultrastructural differences between tumors which contained pseudo-glandular structures and giant cells light microscopically and those who did not. The two tumors containing melanin both revealed a few multinucleated giant cells and pseudo-glandular structures. Moreover, at the ultrastructural analysis we found slit-like spaces of varying size, lined by external lamina-like material both in tumors with and without pseudo-glandular structures light microscopically. These slit-like spaces seen ultrastructurally may be of the same origin as the larger glandlike structures visible under the light microscope. Our findings and interpretations are thus at variance with those of Tsuneyoshi and coworkers (1978).

The presence of external lamina has previously been considered to advocate a synovial origin (Kubo 1969; Tsuneyoshi et al. 1978). However, our findings of external lamina material partly encircling cell groups or occasional cells and lining slit-like spaces, may also fit well with a neural crest origin. External lamina has been described as typical of peripheral nerve sheath tumors of both Schwann cell and perineurial cell differentiation (Erlandson and Woodruff 1982), and may be seen in malignant melanoma (Curran and McCann 1975). Long-spaced collagen was observed in three cases. This has often been documented in peripheral nerve tumors, although not exclusive for such tumors (Erlandson and Woodruff 1982). The presence of true intranuclear cytoplasmic inclusions, observed in one of the 6 ultrastructurally studied tumors, has been noted in malignant melanoma (Henderson and Papadimitriou 1982).

The reason why it has been suggested that clear-cell sarcoma is related to malignant melanoma, or in fact represents a soft tissue melanoma, is the melanin producing capacity of some of them (Hoffman and Carter 1973; Mackenzie 1974; Bearman et al. 1975; Chung and Enzinger 1978; Toe and Saw 1978; Tsuneyoshi et al. 1978; Bednar 1979; Ekfors and Rantakokko 1979; Choux et al. 1981). Only 2 of the 15 tumors in our series contained reducing pigment in the cells, and only 1 of 6 ultrastructurally analyzed tumors contained melanosomes. This seems to be a lower percentage than that reported by Chung and Enzinger (1978). The fact that most of the single cases previously studied ultrastructurally contained melanin may be due to a selection of such tumors. The electron microscopic analysis of one of our tumors made it evident that the reducing pigment seen light microscopically corresponded to melanin. Apart from the melanosomes, this tumor had a light and electron microscopic appearance similar to the others. The strong polymorphism of the melanosomes and the high degree

of variation of melanization noted in this case has been found to be characteristic of malignant melanoma in contrast to benign pigmented naevi (Drzewiecki 1979; Curran and McCann 1975).

Light microscopically, the vacuolization of the cytoplasm gives the tumor cells a clear-cell appearance. Ultrastructurally, there were no vacuoles corresponding to those seen light microscopically. Possibly the cytoplasmic vacuoles seen light microscopically may represent glycogen (cf. Enzinger 1965), which is dissolved to a large extent by formalin fixation. Also at electron microscopy some of the glycogen appeared to be dissolved. It should be noted that with the PATCH-SP technique glycogen could also be demonstrated ultrastructurally in tumors that lacked glycogen on light microscopic examination.

It is well documented that an intimate relationship exists between tumors of peripheral nerves and tumors of melanocytic origin. Ultrastructural similarities between tumors of these origins have been emphasized, and the ability of some peripheral nerve tumors to produce melanin has been well established (Bednar 1957; Bind and Willis 1969; Abell et al. 1970; Mennemeyer et al. 1979). The resemblance between melanocytic and peripheral nerve tumors is considered to be an expression of their common origin from neural crest derived cells (Mennemeyer et al. 1979; Stefansson et al. 1982). The results of the electron microscopic and immunohistochemical analysis of the 15 clear-cell sarcomas indicate that the tumors are composed of a homogenous cell population of probable neural crest derivation. The finding that synovial sarcomas did not contain S-100 protein suggests a clear distinction between the two entities, contrary to previous suggestions that clear-cell sarcoma represents a form of synovial sarcoma (Kubo 1969), or is a part of a spectrum of tenosynovial sarcomas as presented by Hajdu (1979).

We firmly believe that this type of combined light and electron microscopic and immunohistochemical investigation may provide a valuable contribution to the often intricate problem of the histogenesis of soft tissue tumors.

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