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The Fibroblastic Nature of Dermatofibrosarcoma Protuberans

A Tissue Culture and Ultrastructural Study

Julio Escalona-Zapata, Emilio Alvarez Fernandez, and Francisco Llorca Escuin

Ciudad Sanitaria Provincial, Servicio de Anatomia Patologica, c/ Doctor Esquerdo 46, Madrid 30, Spain

Summary. Dermatofibrosarcoma protuberans has been considered to be of fibrohistiocytic or fibroblastic origin. The purpose of this paper is to identify the original cell strain from which this neoplasm derives, using tissue culture and electron microscopic methods. Thirteen cases of DFSP characterised by clinical, topographical, histological and behavioral criteria were explanted. The emigrating cells were bipolar with two opposed processed and showed a radial arrangement in respect to the explants. After the second week the distal processes tended to curve back towards the cell body forming flame-like structures. This cell morphology and cellular orientation persisted during the whole life of the culture. Electron microscopy was performed in three cases; the newly grown cells maintained an electron microscopic picture similar to that found in the original tumors.

This pattern of behaviour is characteristic of fibroblastic tumors and has been found in explants of normal fibroblasts, of fibromatosis and of fibrosarcomas used as controls. On this basis, we believe that DFSP is a fibrosarcoma of the skin of low grade malignancy.

Key words: Dermatofibrosarcoma protuberans – Tissue culture – Electron microscopy – Fibroblasts –Fibrosarcoma

Introduction

The nature of the dermatofibrosarcoma protuberans of Darier-Ferrand has been interpreted in several ways with four proposed origins: a) vascular, b) neural, c) histiocytic and d) fibroblastic. Only two of these theories – histiocytic and fibroblastic – are discussed. The Stout school defends a histiocytic origin based on conventional morphological studies (Kaufmann and Stout 1961; O'Brien and Stout 1964) the specific nature of the storiform pattern (Bednar

Offprint requests to: Prof. Dr. J. Escalona-Zapata Servicio de Anatomia Patologica, Ciudad Sanitaria Provincial, c/ Doctor Esquerdo 46, Madrid 30, Spain

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1957), tissue culture studies (Ozzello et al. 1963; Ozzello and Hamels 1976) and designation of so-called facultative fibroblasts (Stout 1948; Stout and Lattes 1967; Hajdu 1980). In contrast, Lund (1957), Taylor and Helwig (1962), Lever (1964) and Montgomery (1968), believe in the fibroblastic origin of DFSP, an opinion based on their monophasic cell population and on the fibrosarcomatous features of their recurrences and metastases. A similar opinion has been supported by Hashimoto et al. (1974) and Alguacil-Garcia et al. (1978) who have considered this tumor to be a derived from a modified fibroblast of nerve endings of the skin.

Tissue culture has rarely been employed in the study of DFSP and only the paper of Ozzello and Hamels (1976) could be found in the literature. These authors found an initial emigration of histiocytic-like cells followed by fibroblast-like cells after the 2nd week of cultivation and defend the fibrohistiocytic origin of DFSP. Our results are based on a tissue culture study of these tumors, especially devoted to the definition of their original cell strain.

Material and Methods

This work is based on the study of 13 cases of DFSP whose clinical and historical characteristics are summarised in Table 1. They were studied with routine methods: H-E., Van Gieson's trichrome, P.A.S., Scarlet Red for fat.

Fresh material of all these tumors was obtained under sterile conditions. It was minced in 1 mm^3. fragments, which were explanted on coverslides with heparinized chicken plasma and chick embryo extract in equal parts and incubated at 36.5–37° C, with 199 medium supplemented with fetal calf serum and antibiotics. Roller tubes were used applaying the Gey's technic modified by Kersting (1961), using 10 tubes per case with an average of 4 to 6 explants in each one, in continuous rotation at about 4 to 8 turns per hour. Every third day, one tube was removed and observed by phase-contrast microscopy, photographed and then stained with H-E, after being fixed in alcohol-formalin. The results were evaluated according to Willmer's criteria (Willmer 1965).

Table 1. Dermatofibrosarcoma protuberans

Case	Age	Sex	Location	Preoperative course	Evolution
1.	56	M	back	18 years (1)	2 recurrences
2.	54	M	back	16 years	–
3.	46	M	back	3 years (2)	3 recurrences in 9 years
4.	60	M	back	10 years	–
5.	57	F	back	22 years	–
6.	36	F	abdominal wall	4 years	–
7.	39	F	abdominal wall	8 years (3)	–
8.	24	M	thigh	10 years	2 recurrences
9.	62	M	thigh	8 years	1 recurrence
10.	48	M	knee	5 years	–
11.	65	M	shoulder	20 years (4)	4 recurrences
12.	39	M	supraclavicular	2 years	–
13.	56	M	interscapular	19 years (4)	4 recurrences

(1) Cultured from the 3rd recurrence
(2) Cultured from the 1st recurrence
(3) Cultured from the 2nd recurrence
(4) Cultured from the 4th recurrence

Cultures of two fibromatoses, one cellular fibroma and six fibrosarcomas and some explants of normal fibroblasts procured from different organs were utilized as controls for the evaluation of the growth pattern of these 13 tumors.

For electron microscopy, fragments of tumor tissue from cases 4, 5 and 13 and some explants from case 4 were cut immediately after surgical excision, fixed in cold 2.5% glutaraldehyde in Milloning's phosphate buffer, postfixed in osmium tetroxide, dehydrated in graded alcohols and embedded in Epon. Thick sections were stained with alkaline toluidine blue. Thin sections were mounted on copper grids, stained with lead citrate and uranyl acetate and examined with a Siemens 102 electron microscope.

Results

The clinical, topographical and follow-up data are summarised in Table 1. The topographical location, the development of several local recurrences in some of the cases and the histological picture conformed to the commonly described features of these tumors and allowed us to distinguish them from the common fibrosarcoma (Fig. 1).

The cultures were prepared from both original tumors (7 cases) and from recurrences (6 cases). The 13th case is especially important because it was an example of a recurrence with "myxoid change".

Tissue Culture Findings. All tumors showed the same behaviour in vitro. From the first days of explantation spindle-shaped bipolar cells emigrated from the explant, so that by the sixth day of cultivation a growth halo of 1 to 2 milimeters in width developed arround the explants (Fig. 2). The cells had narrow flat and eosinophilic cytoplasm, which constituted two opposite processes on either

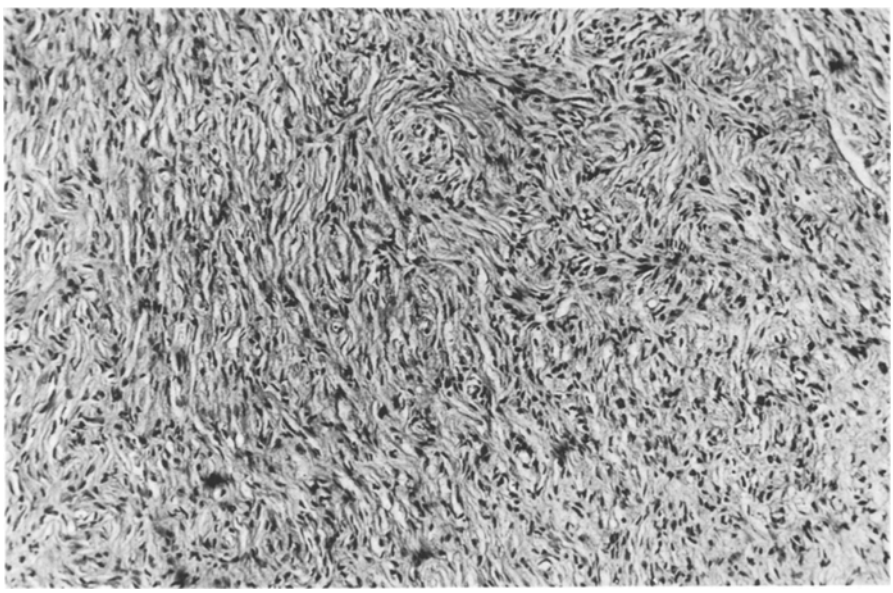

Fig. 1. Case 5. H-E. 100×. Classic features of dermatofibrosarcoma showing long, isomorphic, spindle-shaped cells with a prominent storiform pattern

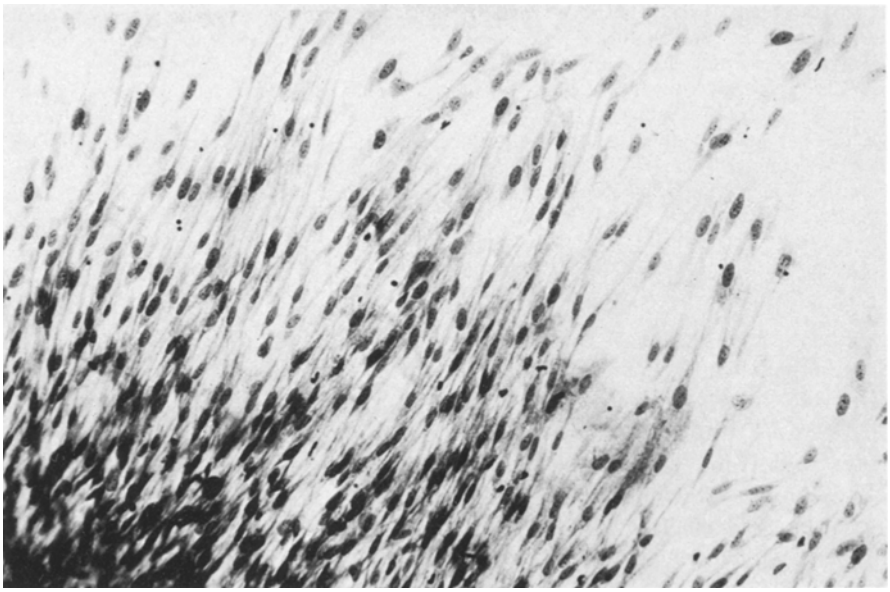

Fig. 2. Case 11. H-E. 100×. Six day old culture. The cells emigrate independently from the explant, showing a radial arrangement and a spindle-shaped morphology

pole of the nucleus (Fig. 3). Sometimes, the distal expansion divided, resulting in cells with a typical "arrow" shape. The nuclei were oval and pale, with a diffuse chromatin network and 1 to 2 nucleoli, surrounded by a clear zone (Fig. 4). A high number of mitotic figures was generally present. The cells remained isolated from each other and emigrated from the explant in a radial pattern, showing a slight tendency to digest the plasma clot.

During following weeks this growth pattern persisted with only some minimal variations, but at the outer margin of the explants the peripheral cell processes tended to curve back, forming flame-like structures (Fig. 5). Macrophages, intermingled with a small number of lymphocytes were found superimposed on the main spindle cell proliferation only during the initial days of explantation.

According to Willmer's criteria, the growth pattern of the tumors was considered to be of fibroblastic type, one of the variants of the mecanocitic group.

Electron Microscopical Findings. From the ultrastructural point of view the neoplastic cells of case 5 appeared as elongated spindle-shaped elements with very thin undulating cytoplasmic expansions which extended for a very long distance from the cell body. The nucleous was large, deeply idented and with a moderate amount of elongated mitochondria and a well developed Golgi apparatus, and abundant rough endoplasmic reticulum and some residual dense bodies. In some places there were some filaments running parallel to the long axis of the cell. Occasional cilia were also found emerging to the cellular surface. There were also some small areas of basal-membrane-like material surrounding

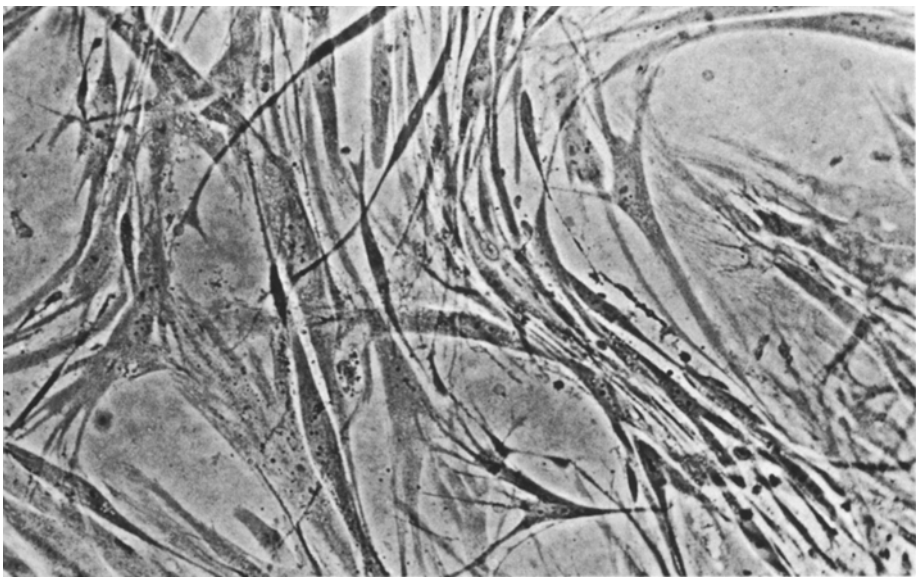

Fig. 3. Case 4. Phase contrast microscopy. 200×. Nine day old culture. The cells are fusiform and bipolar. Only in some ones one of the processes bifurcate into two or three branches

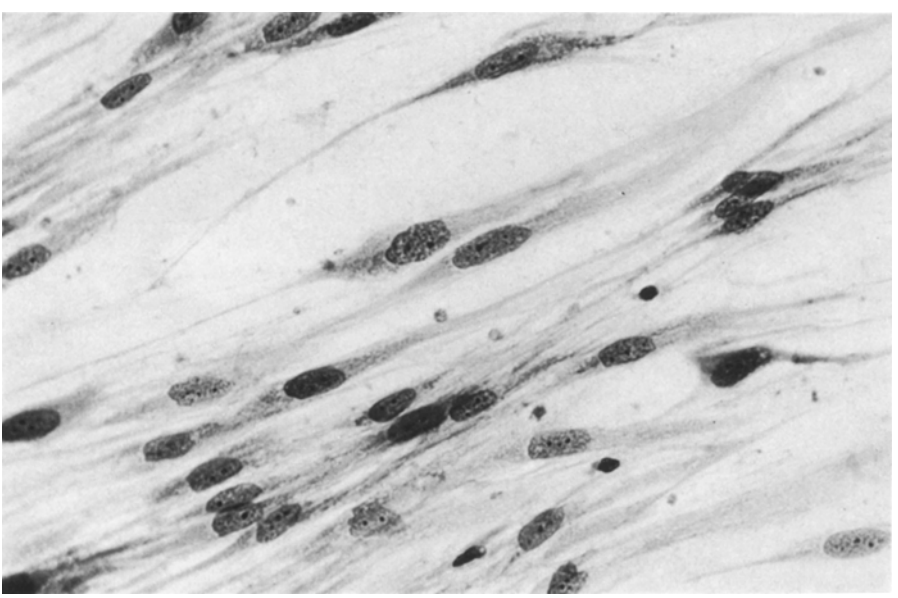

Fig. 4. Case 4. H-E. 400×. Twelwe day old culture. The nuclei are oval with two or three prominent nucleoli. The cytoplasm is filamentous, bipolar and flat

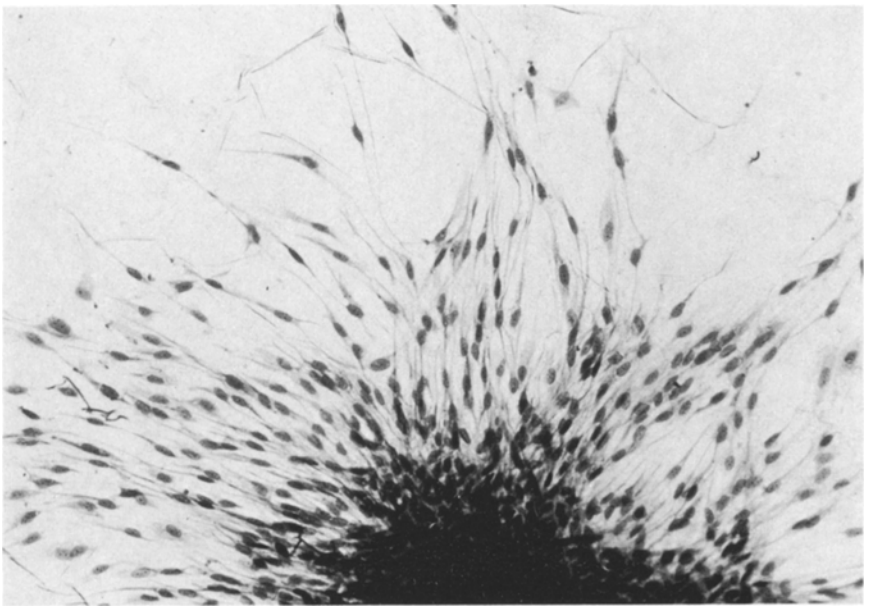

Fig. 5. Case 9. H-E. 40×. Twenty-four day old culture. At the end of the cultivation, the cells remain with the same growth pattern: spindle-shaped, independent and radially. In the periphery they tend to incurvate forming pinsel- or flame-like structures

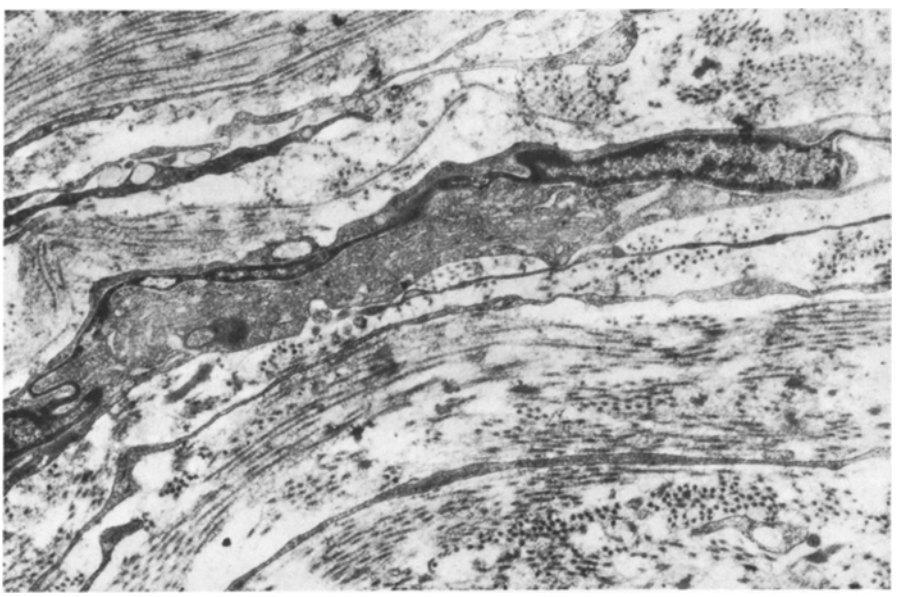

Fig. 6. Case 4. Fusiform neoplastic cell showing a deeply lobulated nucleus. In the background, abundant collagen fibers and slender cellular expansions can be seen. Uranyl nitrate and lead citrate. 3,000×

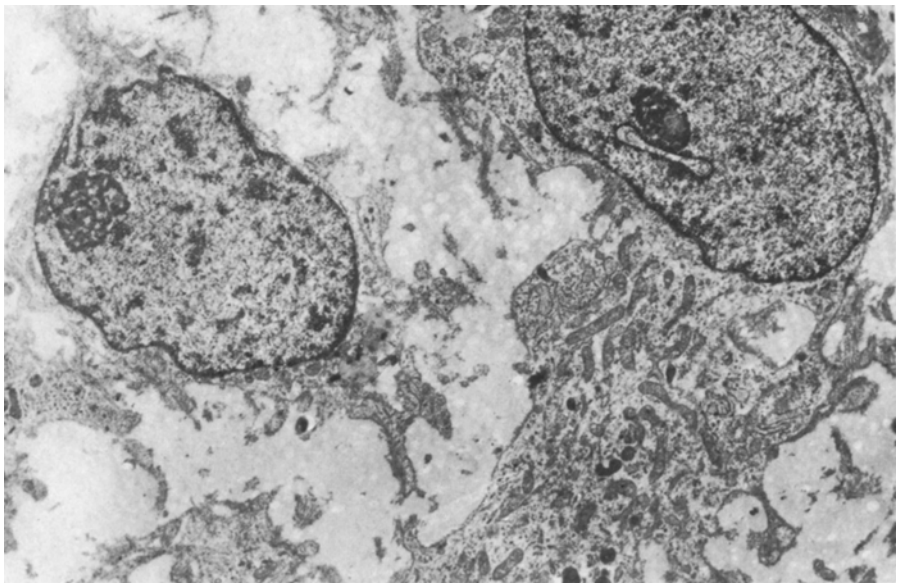

Fig. 7. Case 13. Myxoid recurrence of DFS. The neoplastic cells show a more abundant cytoplasm with numerous organellae, specially mitochondriae. The nuclei are roundish with few indentations. Uranyl nitrate and lead citrate. 5,000 ×

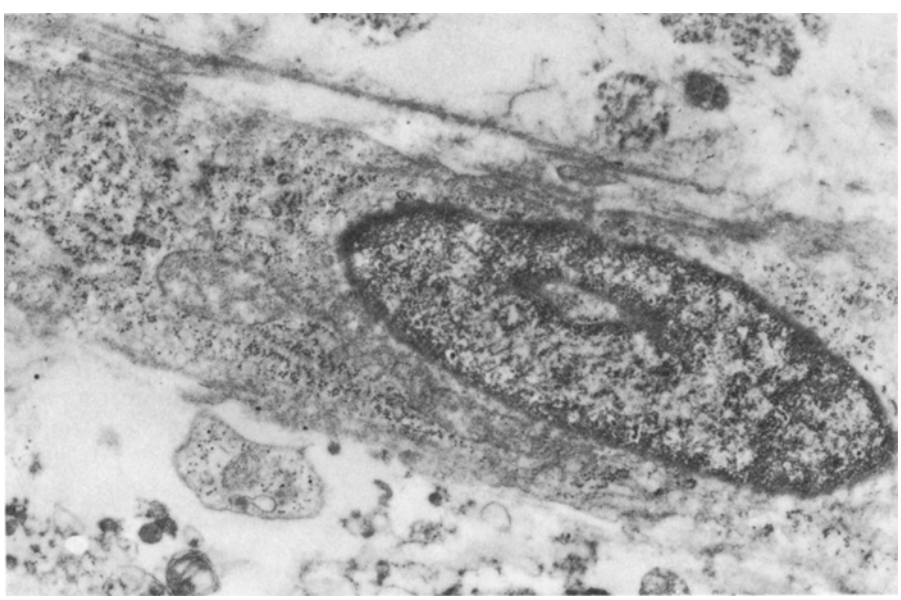

Fig. 8. Case 4. Cultured cell from case 4. The nucleus shows some small lobulations. The cytoplasm of the cell shows abundant profiles of rough endoplasmic reticulum, mitochondriae and microfilaments. Uranyl nitrate and lead citrate. 4,000 ×

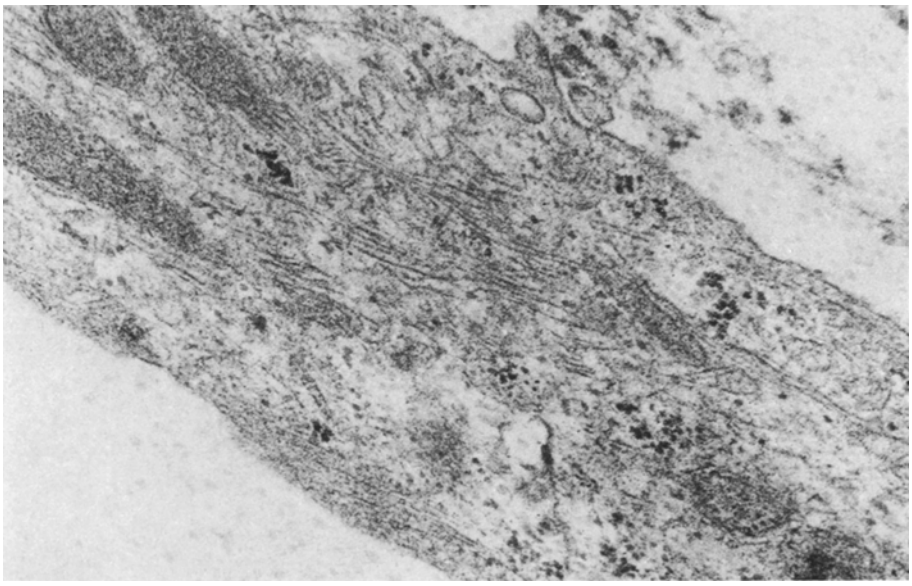

Fig. 9. Cultured cell from case 4. The cytoplasm shows bundles of filaments running parallelly, pinocytotic vesicles and profiles of rough endoplasmic reticulum. Uranyl nitrate and lead citrate. 30,000 ×

the cells. The interstitial tissue was composed of amorphous ground substance and collagen fibers with the usual morphology (Fig. 6).

The cells of the myxoid recurrence of case 13 showed an oval or roundish nucleus with scanty indentations. The chromatin was less dense than in the usual form of this tumor and nucleoli were large. The cells showed a greater amount of cytoplasm, with a larger number of mitochondria, RER cisternae filled occasionally by electron dense material, residual bodies and free ribosomes. Cellular expansions were shorter and blunt (Fig. 7).

The cultivated cells of case 4 reproduced most of the features found in the electron microscopical estudies of conventional tumors. The cells were elongated, the nucleus was less indentated than in the original neoplasms, but showing clear lobulations. There were abundant RER and mitochondria and some residual osmiophilic bodies. There were some filaments which were more abundant near the cytoplasmic membrane. Free ribosomes were very numerous and there were some micropinocytotic vesicles and scanty short cytoplasmic expansions (Figs. 8 and 9).

Discussion

Stout (1948) and Stout and Lattes (1967) suggested that the histiocyte could be transformed under special conditions into a spindle cell similar to the fibroblast. These "facultative fibroblasts" could explain the appearance of a double cell population in fibrous xanthomas and fibrohistiocytomas and the two forms, lipid-laden histiocytes and fibroblasts ussually arranged themselves in a cart-

wheel or a storiform pattern. The inclusion of DFSP in the group of fibrous histiocytomas would be then justified by the presence of the storiform pattern, this tumor being a special type of fibrohistiocytic neoplasm in which only the fibroblastic phase appears.

However, in recent years, some doubts have arisen concerning the validity of Stout's conception as far as DFSP is concerned. The constant spindle shaped cell population without histiocytes and the progressive loss of the storiform pattern with a more "fibromatous" picture in the recurrences (McPeack et al. 1967; Hagedorn et al. 1974 Brenner et al. 1975) and noteably, the appearance of myxoid recurrent tumors are arguments that speak against a histiocytic origin. From the ultrastructural point of view, all the reported cases have shown a similar structure (Fisher and Vuzevski 1968; Hashimoto et al. 1974; Auböck 1975; Ozzello and Hamels 1976; Alguacil-Garcia et al. 1978). The data provided by the electron microscope point to an origin from a special type of fibroblast with "sheath forming" differentiation (Alguacil-Garcia et al. 1978), probably derived from the perineural or endoneural cells of the skin.

Tissue culture has rarely been employed in this field. The report by Ozzello and Hamels (1976) concerning four cases reported an initial phase of growth of histiocytic-like cell that disappear in the second week and are replaced by fibroblasts. This sequence has been interpreted as an expression of the transformation of the initially histiocytic cell type into fibroblasts.

Our findings differ from those of Ozzello and Hamels (1976) especially in relation to the behaviour of the macrophages. In some of our cases, macrophages have been found during the first few days of cultivation, but in all the tumors spindle cells were the predominant growing element right from the beginning of the culture, and, after the ninth day, they were the only cell type identifiable. These cells showed a striking electron microscopical similarity to the polygonal and spindle cells described by Ozzello and Hamels (1976) and with the neoplastic elements of the original tumors. Electron microscopy showed indented nuclei, abundant RER profiles, filaments and free ribosomes. The spindle-shaped cell morphology, radial arrangement and the cellular independence in our explants of DFSP correspond with the behaviour of neoplastic and non-neoplastic fibroblastic growths (see Willmer 1965). This pattern was present in cases without the common light microscope features. Maintenance of the same appearance in recurrences with loss of storiform pattern (cases 1, 2 and 4) and the myxoid recurrence of case 12 is highly significant. The same growth pattern was also found in our control cases of fibromatosis, cellular fibroma and fibrosarcomas (Escalona 1979). On the other hand, the growth pattern of the fibrohistiocytic tumors described by Ozzello et al. (1963) and found in our laboratory (Escalona-Zapata 1970), differs substantially from that of the fibroblastic one. The histiocytic cells of the cultures of fibrohistiocytoma are star-shaped or polygonal and flat, frequently multinucleated and larger than the macrophages present in the first days of cultivation of DFSP. The later are smaller with a round-shaped dark cytoplasm and lateralized nucleus which are characteristic of the mature, secondary macrophages present in most tumors. As Kersting (1961) demonstrated, all inflammatory cells present in the first week of cultivation must be considered as non-tumoral. Only when

the macrophages persist during several weeks of the development of the culture, as happens in neurinomas (Escalona-Zapata and Diez-Nau 1978), they could be considered as primitive.

The transformation between histiocyte and fibroblast has been accepted as the explanation of the coexistence of both cells in the same tumor. However, Spector and Willoughby (1968) in an experimental study of the inflammatory phenomenon have excluded the transformation of fibroblasts into histiocytes, Ross et al. (1970) denied the possibility of transformation of labelled macrophages into fibroblasts in healing wounds. As far as tissue culture is concerned, the evidence for the transformation between histiocyte and fibroblast found in the literature is derived from three types of experiments, in which fibroblasts are assumed to be derived from histiocytes: a) from different sources (Maximow 1927), b from serous fluids (Moen 1935) and c) from the buffy coat (Fischer 1925; Paul 1958). Jacoby (1965) questioned the validity of these experiments on the basis of the high risk of fibroblastic contamination of the cultures and emphasized that such transformation has never been demonstrated. Fu et al. (1975) have proposed the existence of a primitive stem cell from which the two cell populations – histiocytic and fibroblastic – of fibrohistiocytomas would derive.

It is possible that the macrophages constitute a superimposed cell population which is explanted with the tumoral cells and emigrates during the first days and then dies and dissappears. In our opinion, this is the most reasonable explanation of the presence of macrophages in the cultures of DFSP. It is in accord with two important facts: the scarcity of the macrophages, which have been identified only in some cases and which are present only during the first week, and the abscence of histiocytic cells in the histological pictures of this tumor. On this basis, we believe that DFSP should be considered as a fibrosarcoma of the skin of low grade malignancy.

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