Virchows Arch. A Path. Anat. and Histol. 385, 283–291 (1980)

Virchows Archiv A

On the Pathogenesis of Sclerosis and Nodularity in Nodular Sclerosing Hodgkin's Disease

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Summary. Ten cases of nodular sclerosing Hodgkin's disease involving lymph nodes were studied by electron microscopy to determine the ultrastructural composition of the nodule-stromal interphase and the collagenized regions. In addition to a few lymphocytes, rare eosinophils and neutrophils, abundant filamentous and granular electron dense material, collagen fibers and myofibroblasts were observed in all instances. Since myofibroblasts possess contractile and synthetic properties, it is likely they contribute to the retraction and sclerosis which together represent one of the morphologic hallmarks of the disease. The dense fibrosis and contractile state of such tissue may constitute a beneficial host response to contain and limit local and vascular invasion by the neoplastic cellular population, thus contributing to the relative benignity of this form of Hodgkin's disease.

Key words: Hodgkin's disease – Myofibroblasts – Ultrastructure – Nodular sclerosis – Host response.

Introduction

Over the past several years a considerable body of new information has contributed to a greater understanding of the pathobiology of lymphoreticular malignancies. The rapid evolution of this complex subject has followed the major advances in basic immunology, genetics, immunocytochemistry and cellular biology. A multidisciplinary approach to these conditions has served to better elucidate the morphological, immunological and functional parameters of the neoplastic cellular population.

A potent stimulus for this investigative ferment was provided in 1966 when a new classification for Hodgkin's disease was proposed (Lukes et al., 1966). The ease of applicability, reproducibility, prognostic predictability and universal

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0340-1227/80/0385/0283/$01.80

acceptance of this classification have contributed to major therapeutic advances reflected by increased survival rates. The most common form of Hodgkin's disease was sharply delineated at that time and has been demonstrated to be clinically, pathologically and biologically different from other expressions of the disease. This nodular sclerosing (NS) Hodgkin's is characterized by a propensity for thymic involvement, macroscopic nodularity, firmness and retraction, a distinctive population of marker cells, the lacunar cell, and broad bands of dense birefringent collagen which dissect the involved tissue to impart the nodular and sclerotic pattern. The pathogenetic mechanism(s) responsible for the latter process has not been delineated.

Previous fine-structural studies of Hodgkin's disease, including NS Hodgkin's, were focused on the neoplastic and lymphoreticular cellular components (Dorfman et al., 1973; Glick et al., 1976; Anagnostou et al., 1977). To investigate possible factors which contribute to the desmoplasia and retraction an ultrastructural examination of the nodule-stromal interphase and sclerotic areas from a series of NS Hodgkin's disease was undertaken. The purpose of this paper is to describe the findings of this study which elucidate the cellular mechanism responsible for the macroscopic retraction and sclerosis and to propose that the contractile nature and desmoplastic character of the stroma constitute a host response to the neoplastic process.

Material and Method

Ten surgical biopsies from lymph nodes were available for study. Fresh tissue was examined and immediately sectioned, fixed in 10% buffered formalin and processed in conventional manner for paraffin embedding. Sections were stained with hematoxylin-eosin, methylgreen pyronine and Masson trichrome.

For electron microscopy tissue from each specimen was minced in chilled 3% glutaraldehyde in cacodylate buffer and fixed for four hours. The fragments were then rinsed in buffer, postfixed in 2% osmium tetroxide, dehydrated in graded alcohols and embedded in Epon. Semithin sections (1 μm) from multiple blocks were stained with toluidine blue and examined. From each case several areas containing cellular nodules with adjacent collagenized stroma were selected for study. After appropriate block trimming, semithin sections were recut and stained with toluidine blue to assure that the collagen-nodule interphase had been selected. Ultrathin sections were then cut, stained with uranyl acetate and lead citrate and examined with a transmission electron microscope.

Results

The relevant clinical data are summarized in Table 1. In most instances the lymph nodes were moderately firm and displayed macroscopic nodularity with alternating irregular retracted areas on cut section. Histologically most nodes were surrounded by a thick fibrous capsule and replaced by cellular nodules enveloped by concentrically disposed collagen. The latter was birefringent with polarization microscopy. The extent of collagen formation varied, yet, each biopsy denoted nodal collagenization sufficient to justify the diagnosis. The nodules were composed of lymphocytes, immunoblasts, mononuclear "Hodgkin's" cells, occasional Reed-Sternberg cells, plasma cells and eosinophils, and

Table 1

Case number	Sex	Age	Biopsy site	Clinical stage at time of diagnosis	Treatment	Follow-up
1	F	31	(L) Supraclavicular	IA	Radiation	Alive 8 months
2	F	40	(L) Axilla	IIB	MOPP Radiation	Expired $2\,^1/_2$ years
3	F	27	(L) Cervical	IA	–	Lost to follow-up
4	M	21	Mediastinum	IA	–	Lost to follow-up
5	F	27	(L) Scalene	IIIB	MOPP Radiation	Alive $4\,^1/_2$ years
6	F	27	(R) Cervical	IIA	MOPP Radiation	Alive 3 years
7	F	18	(R) Cervical	IIA	Radiation MOPP	Alive 5 years
8	M	35	(R) Subclavian	IIA	Radiation	Alive 3 years
9	F	22	(R) Axilla	IIB	MOPP Radiation	Alive 2 years
10	F	13	(L) Cervical	IIIA	MOPP Radiation	Alive 1 year

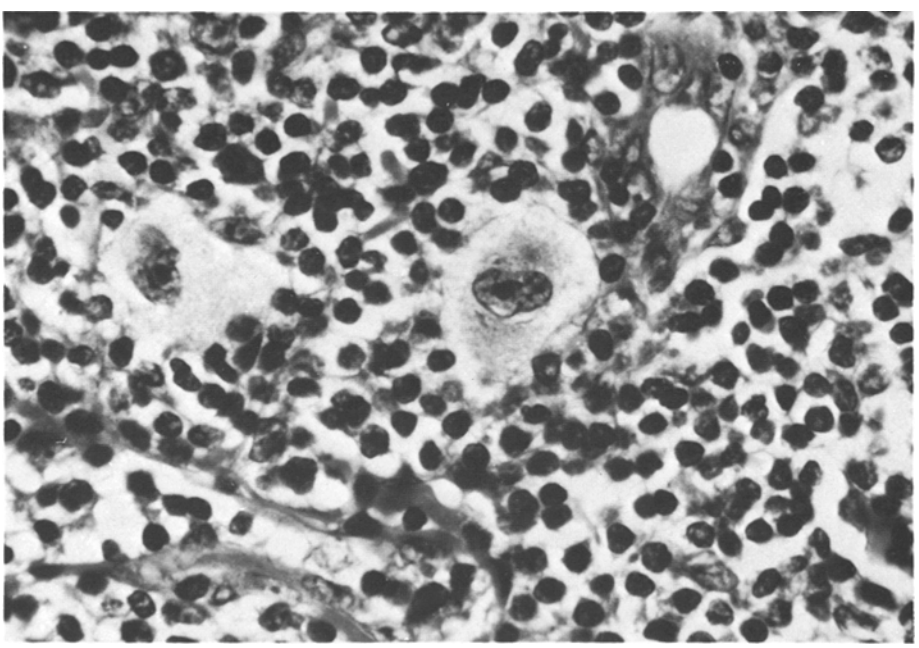

Fig. 1. Photomicrograph of nodule in NS Hodgkin's illustrating the cellular composition and two lacunar cells. (Hematoxylin-Eosin ×400)

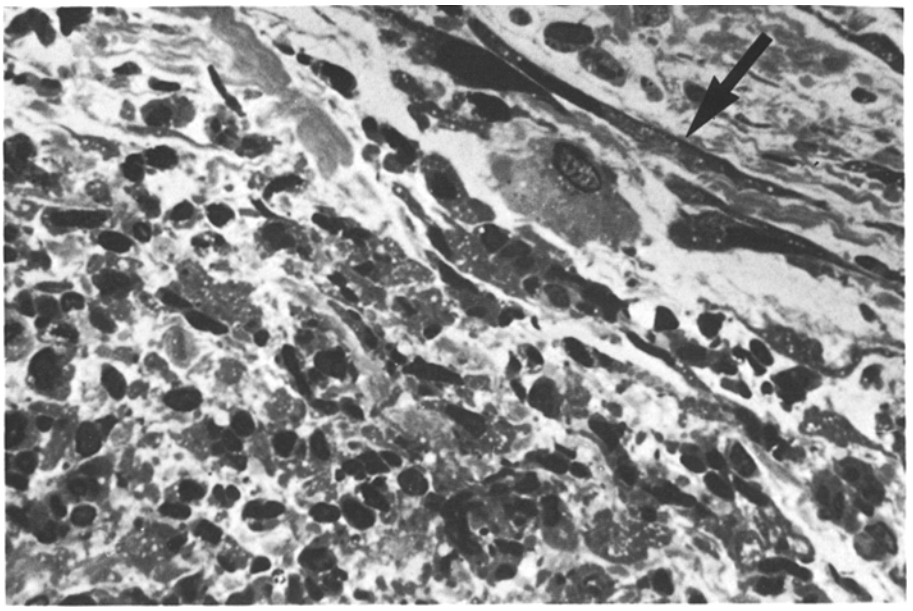

Fig. 2. Photomicrograph of stromal-nodular interphase. Note elongated cells (*arrow*) at periphery of nodule. (Toluidine-Blue stain, Epon-embedded ×400)

above all, numerous lacunar cells (Fig. 1). In several cases areas of irregular hyalinization were noted within the cellular nodules.

Toluidine blue stained Epon sections disclosed a distinct population of elongated fusiform cells in the collagenous stroma, often adjacent to the cellular nodules (Fig. 2). Review of corresponding paraffin sections confirmed this observation although these cells were better appreciated in the Epon-embedded material.

Ultrastructural examination of the stroma and nodule-stroma interphase revealed finely granular and filamentous electron dense material, collagen fibers with 640 Å periodicity, moderate numbers of small and transformed lymphocytes and occasional eosinophils and neutrophils (Fig. 3). The spindle-shaped cells observed in toluidine blue-stained sections were characterized by fusiform, often notched nuclei containing a prominent nucleolus and nuclear bodies, well-developed Golgi zones and rough endoplasmic reticulum and bundles of cytofilaments dispersed within elongated cytoplasmic extensions (Fig. 4). The filaments generally measured 40–60 Å in diameter, were often grouped to form dense bodies, were localized in greatest concentration beneath the plasma membrane and occasionally extended into the contiguous stroma to form "microtendons" (Fig. 5). An irregular focal deposition of basement membrane-like material, occasional cellular junctions of the macular adherentes type and rare pinocytotic activity were observed. In all ten cases examined the nodule-stromal interphase and stroma contained this unique cell although their numbers varied. The fine structural features were deemed sufficient to categorize these cells as myofibroblasts.

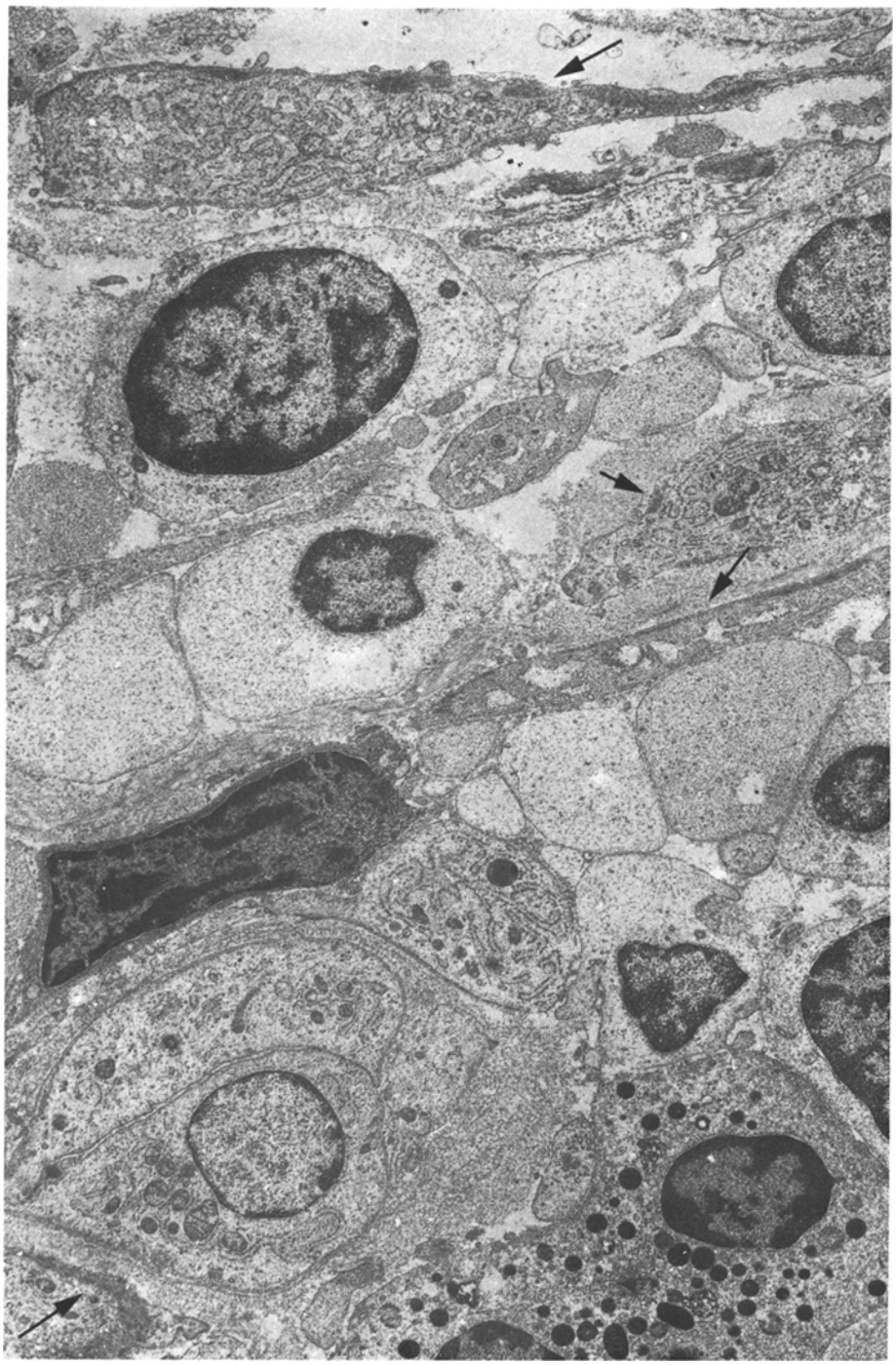

Fig. 3. Electronmicrograph of stromal-nodular interphase illustrating myofibroblasts *(arrows)* admixed with lymphocytes and neutrophil. ($\times$ 1,400)

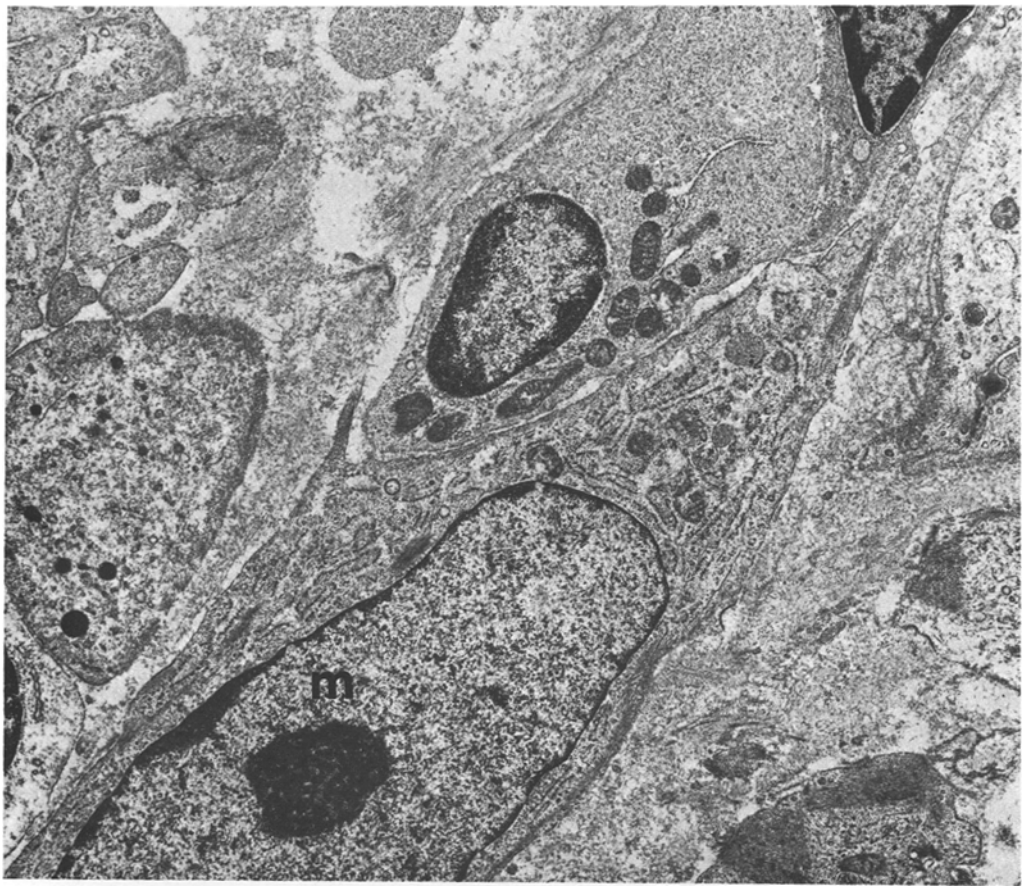

Fig. 4. Electronmicrograph of stromal myofibroblast *(M)* illustrating prominent rough endoplasmic reticulum and bundles of cytofilaments. (×2,600)

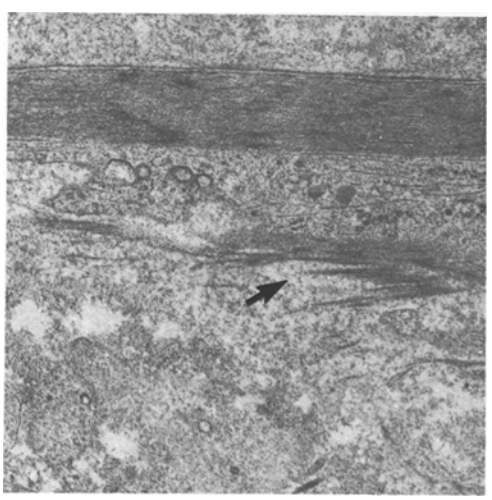

Fig. 5. Electronmicrograph of myofibroblast illustrating abundant grouped cytofilaments some of which appear to extend beyound the plasma membrane into the stroma as "microtendons" *(arrow)*. (×4,700)

Discussion

Fibroblasts with contractile properties were initially described in a series of experiments employing granulation tissue designed to study the cellular mechanism of wound closure (Majno et al., 1971; Gabbiani et al., 1971). In these experiments a unique stromal cell was observed which shared ultrastructural features of fibroblasts and smooth muscle cells and thus contained the morphological requisites to transmit contractile forces. It was later determined that this cell additionally possessed a synthetic capability for type III collagen (Gabbiani et al., 1976). Subsequent immunological, pharmacological and chemical studies have further elucidated the biological nature of this cell, termed a myofibroblast (Gabbiani et al., 1972; Gabbiani et al., 1977). The origin of this "contractile fibroblast" has not been resolved. The existing data suggest that myofibroblasts are derived locally from fibroblasts (modulation) or more primitive mesenchymal tissue cells in response to a variety of stimuli. Their life span appears to be finite except in processes of exuberant scarring in which they may persist indefinitely (Rudolph et al., 1977).

The nodular sclerosing variant of Hodgkin's was initially defined as a specific clinicopathological entity in 1966 (Lukes et al., 1966). The disease has a perplexing topographical distribution with common involvement of lymph nodes of the anterior superior mediastinum, lower cervical, scalene and supraclavicular region as well as thymus. It represents the most common form of Hodgkin's disease both in children and adults and has demonstrated a female predominance in most series (Strum et al., 1970; Dorfman, 1971; Butler, 1971). The involved nodes are firm and often matted together. The cut surface denotes yellowtan soft nodules which alternate with retracted gray-white interconnected bands.

The initial descriptions of NS Hodgkin's delineated a broad spectrum of histologic findings. The early lesion is often focal and cellular characterized by proliferative nodules of lymphoid cells and variants of Reed-Sternberg cells (lacunar) with little or no detectable collagen. Later on capsular fibrosis becomes prominent and the cellular nodules are circumscribed by birefringent collagen. This phase is often associated with the appearance of eosinophils, neutrophils, plasma cells and small blood vessels in the cellular nodules. A more advanced pattern is distinguished by an obliterative sclerosis of the nodules of varying intensity (Lukes et al., 1966; Lukes, 1971). This trend toward progressive sclerosis was confirmed in sequential biopsies from 48 patients with NS Hodgkin's which also demonstrated the persistence of the NS pattern in 91.7% of patients. This relative constancy of pattern contrasts with other variants of Hodgkin's in which change to a more aggressive form is common. In those in whom a cellular variant of NS was initially diagnosed, subsequent biopsies revealed progression to the more classical sclerotic form in 5 of 7 patients (Strum et al., 1971). Thus, in both the typical NS and cellular variant of NS Hodgkin's the involved tissue tends to retain the NS pattern but to become more sclerotic with time. This demosplastic process has been interpreted to constitute a host response to the attempted induction of neoplasia in Hodgkin's disease (Lukes et al., 1966; Lukes, 1971).

The sclerosis in NS Hodgkin's is considerably greater than other forms

of the disease. Possibly this represents a heightened reactivity unique among certain individuals to the basic process. Children possess an accelerated cellular response to injury and infection (Bolande, 1979). Such responsiveness may contribute to the high incidence of NS Hodgkin's in the pediatric population. The loss of this reactivity might also explain the abortive attempt at fibrosis which characterizes lymphocyte-depleted Hodgkin's late in life. In these patients the stroma is amorphous, disorderly, and non-collagenous (Lukes et al., 1966; Neiman et al., 1973). Clearly the intensity of sclerosis, as well as quantity of lymphocytes, relate directly to biological behavior in Hodgkin's. This correlation is not unique to Hodgkin's since two distinct forms of malignant lymphoma (one histiocytic, the other lymphocytic) with a sclerosing stromal response are also associated with a more favorable prognosis than their non-sclerotic counterparts (Rosas-Uribe et al., 1972; Millett et al., 1970).

The process of sclerosis and retraction in neoplasia extends beyond NS Hodgkin's. In a recent ultrastructural study of the stroma of invasive and metastatic carcinomas which displayed gross retraction, firmness and desmoplasia myofibroblasts were consistently observed. In contrast various intraepithelial (non-invasive) carcinomas examined as controls lacked stromal myofibroblasts (Seemayer et al. (in press)). It was suggested that stromal myofibroblasts induction represented a response to invasive and metastatic carcinoma. Moreover, the density of collagen and contractile state of such stroma might serve to retard the local and vascular invasion by neoplastic cells.

This study has demonstrated myofibroblasts in the stroma and stromal-nodular interphase in ten lymph node surgical specimens of NS Hodgkin's disease. The finding is judged to be unique since myofibroblasts are not known to represent constituents of normal lymph nodes or nodular lymphoma (Levine et al., 1975). In eight patients with available follow-up data, seven are alive and well (8 months to $4^1/_2$ years), while one died from disease in two and one-half years. Thus, the biologic nature of the process in these patients conforms to NS Hodgkin's and illustrates its relatively favorable prognosis. The sclerosis which is such an integral feature of this expression of Hodgkin's has been considered to reflect a host response with survival value. The myofibroblasts observed in the stroma of collagenized and retracted regions likely contribute to the sclerosis and retraction and, indirectly, the nodularity of the process. The dense fibrosis and contractile state of such tissue may constitute a physical mechanism, albeit primitive, of containment to limit local and vascular invasion by the neoplastic cellular population. The incidence of vascular invasion in Hodgkin's provides some support for this proposal for a recent study documented invasion in 7.4% of NS Hodgkin's contrasted with 33% in mixed cellularity and 75% in lymphocyte depletion (Naeim et al., 1974).

Factors which induce this myofibroblastic response are unknown but may be related to the age and sex of the individual, the genetic milieu, the biochemical/molecular microenvironment and other factors as yet undefined. It thus appears to be most important that studies be designed to probe the molecular basis of myofibroblast induction. The elucidation of the mechanism(s) responsible for this process is likely to have considerable biological significance.

Acknowledgment. The authors gratefully acknowledge the contribution of cases 1–6 from Dr. Marie L. Brisson de Champlain, Department of Pathology, Jewish General Hospital, Montreal.

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Received July 30, 1979