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Collagen secretion granules in reactive stromal myofibroblasts, with preliminary observations on their occurrence in spindle cell tumours

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Summary. Collagen secretion granules, representing stages in the intracellular packaging, transport and secretion of collagen-fibril precursor, have been studied by transmission electron microscopy in non-neoplastic human myofibroblasts and in neoplastic cells from a preliminary study of tumours exclusively or partly of spindle cell type. Vesicles, newly separated from Golgi saccules and containing finely fibrillar material, were identified as early presecretory granules, the most immature type of granule. Later stages exhibited longitudinally arranged, densely fibrillar bundles. Subsequently, secretory granules developed more homogeneously dense content. Fibril-containing cisternae near the plasma membrane were interpreted as either endocytotic or lysosomal structures, or as participants in the final stages of secretion. The features by which collagen secretion granules can be distinguished from other Golgi products, in particular melanosomes, Weibel-Palade bodies and lysosomes, are pointed out. The significance of these organelles for cell identification and tumour diagnosis is discussed.

Key words: Collagen secretion granule – Myofibroblast – Spindle cell tumour – Electron microscopy – Diagnosis

Introduction

The ultrastructural basis for the packaging and transport of collagen-fibril precursor before its appearance in the extracellular space as discrete fibrils has been documented in experimental animal tissues and cells by many authors. Organelles believed to be involved in this process have been described in tissue odontoblasts, osteoblasts and fibroblasts in the rat (Weinstock 1972; Weinstock and Leblond 1974; Marchi and Leblond 1983); fibroblasts in the periodontal ligament in the mouse (Cho and Garant 1981); cultured mouse osteoblasts (Scherft and Heersche 1975) and fibroblasts (Goldberg and Green 1964; Scherft and Heersche 1975); chondrocytes from rabbit semilunar and ear cartilage (Sheldon and Kimball 1962; Ghadially et al. 1978); and, in the chick embryo, corneal epithelium (Trelstad 1971; Hay and Dodson 1973; Trelstad et al. 1974) as well as corium, tendon and corneal fibroblasts (Nist et al. 1975; Fernandez-Madrid et al. 1981). Few descriptions exist, however, of such granules in human cells, and it is the object of this paper to document their ultrastructural appearances in the normal hu-

Table 1. Lesions containing non-neoplastic myofibroblasts in which collagen secretion granules and related structures have been identified

Case	1	metastatic adenocarcinoma – cervical node
	2	infiltrating duct carcinoma – breast
	3	keratinising squamous cell carcinoma – neck
	4	metastatic fibrolamellar hepatocellular carcinoma – coeliac node
	5	mesothelioma – omentum
	6	multiple myeloma – subcutis, loin
	7	pleomorphic non-Hodgkin's lymphoma – pelvic region
	8	granulocytic sarcoma – cervix
	9	epithelioid sarcoma – scalp
	10	pleomorphic sarcoma – knee
	11	granulation tissue following excision of malignant fibrous histiocytoma – knee
	12	granulation/fibrous tissue at site of periosteal sarcoma – leg
	13	foreign body (suture) granuloma – groin
	14	metastatic malignant melanoma – lymph node
	15	nodular sclerosing Hodgkin's disease – cervical node

In the case of tumours, myofibroblasts were found in the supporting stroma

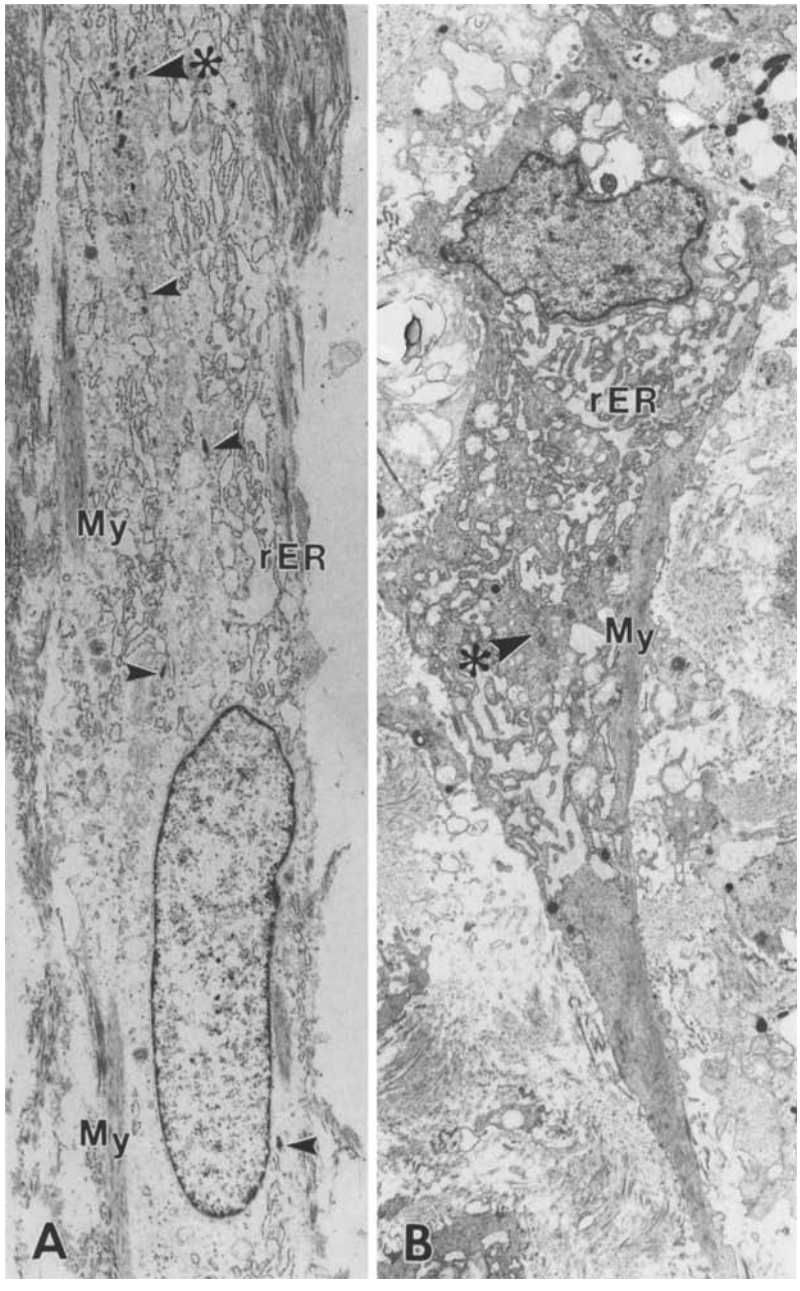

Fig. 1 A, B. (Non-neoplastic myofibroblasts). Abundant rER and myofilament bundles (My) in cells from cases 11 (**A**) and 13 (**B**) (Table 1). Collagen secretion granules are indicated by *arrowheads*; those with an adjacent asterisk are detailed in Fig. 2. Both × 5100

man myofibroblast – a cell with a demonstrated role in fibril secretion (Gabbiani et al. 1976) – as well as in neoplastic cells from a selected number of tumours. This communication also draws attention to the value of these organelles in cell identification and their possible significance in tumour diagnosis.

Material and methods

Tables 1 and 2 list human specimens which provided non-neoplastic myofibroblasts and neoplastic cells respectively for the study of collagen secretion granules by transmission electron microscopy. Fixation in 10% buffered histological formalin or cacodylate-buffered 2.5% glutaraldehyde was followed by conventional processing into Agar 100 (Epon-type) epoxy resin. Ultrathin sections were stained in 5% aqueous uranyl acetate and Reynolds' lead citrate, and photographed in an AEI 801 electron microscope.

Results

With respect to nomenclature, the term *collagen secretion granule* (Cho and Garant 1981) is used as a general term to encompass *presecretory* and

Table 2. Collagen secretion granules in selected problematical spindle cell tumours

		Light microscopy	Electron microscopy
Cellular, myofibroblastic			
Case 1	thyroid	spindle/giant cell carcinoma	rER, CSG[a], FMFD[b]
2	soft tissue, scapula	undifferentiated	rER, CSG, FMFD
3	skin, parietal area	? leiomyoma	rER, CSG, FMFD
4	skin, parietal area	? atypical fibroxanthoma ? leiomyosarcoma	rER, CSG, FMFD, lysosomes[c]
5	skin, pinna	? atypical fibroxanthoma ? malignant melanoma	rER, CSG, FMFD; no melanosomes
Cellular, non-myofibroblastic			
6	mesentery	resembling fibromatosis but with foamy histiocytes	rER, CSG
7	soft tissue, thigh	? malignant fibrous histiocytoma ? malignant schwannoma	rER, CSG, lysosomes

[a] collagen secretion granule; [b] fine myofilaments with focal densities; [c] see Fig. 4A and B

secretory granules as well as fibril-containing cisternae. The precedent term *presecretory* (Cho and Garant 1981) is preferred to *prosecretory* granule (Marchi and Leblond 1983). Presecretory and secretory granules represent a convenient designation for immature and mature forms of collagen secretion granule, between which, however, there is believed to be a continuous transition.

Myofibroblasts were identified in granulation and granulomatous tissue, and the supporting stroma of a number of tumours (Table 1) as spindle cells containing numerous cisternae of rough endoplasmic reticulum (rER) and conspicuous bundles of fine myofilaments with focal densities (Fig. 1A, B). They contained several ultrastructurally distinct forms of collagen secretion granule, examples of which were also seen in neoplastic cells from a selection of tumours, exclusively or in part of spindle cell type (Table 2).

The earliest sign of secretory product elaboration was a finely granular or filamentous material in the peripheral dilatations of Golgi saccules (Fig. 2A). A similar meshwork or fibrillar bundle was found in rounded, ovoid, angular or somewhat elongate vesicles[1] in the Golgi zone (Fig. 2A, D, E). These structures were 300–400 nm across and were interpreted as early presecretory granules. The fibrillar bundle was usually solitary, longitudinally orientated, surrounded by an essentially electron-lucent matrix and lacked periodicity (Fig. 2D). On a rare occasion, several bundles were found in an abnormally rounded presecretory granule (Fig. 2E). Also in the Golgi zone, as well as in more peripheral regions of cytoplasm, presecretory granules of more mature type were identified. In these, a broader mass of filamentous material occupied most of the granule interior (Fig. 2A, B), leaving a narrow but irregular electron-lucent space beneath the limiting membrane. In some of these late presecretory granules the fibrillar bundle exhibited coarse longitudinal densities, 15–20 nm in diameter, corresponding to the *aggregates* of Marchi and Leblond (1983). Areas of the limiting membrane of both early and more mature presecretory granules were often focally coated and occasionally possessed almost fully formed coated vesicles (Fig. 2A, B, D).

Secretory granules were identified by an absence of coated areas from the limiting membrane and by a finely textured and homogeneous interior with a minimal submembrane space (Fig. 2F). They were found as commonly in the Golgi zone as in more peripheral cytoplasmic regions and most measured 300–500 nm long, with an occasional example reaching 800 nm. Some organelles, containing polar densities with fine interconnecting strands (Fig. 2C), were difficult to classify. Such an appearance resembles the presecretory granules of Cho and Garant (1981) as well as both the presecretory and secretory granules described by Marchi and Leblond (1983). The absence of focally coated regions on the limiting membrane, however, suggests an early type of secretory granule.

Structures considered to be functionally related to presecretory and secretory granules were ob-

[1] It is recognised that the structures being described here as "vesicles" may be in continuity with Golgi saccules in other planes of section; in this context, however, organelles are being defined in terms of their contour or profile within a single section.

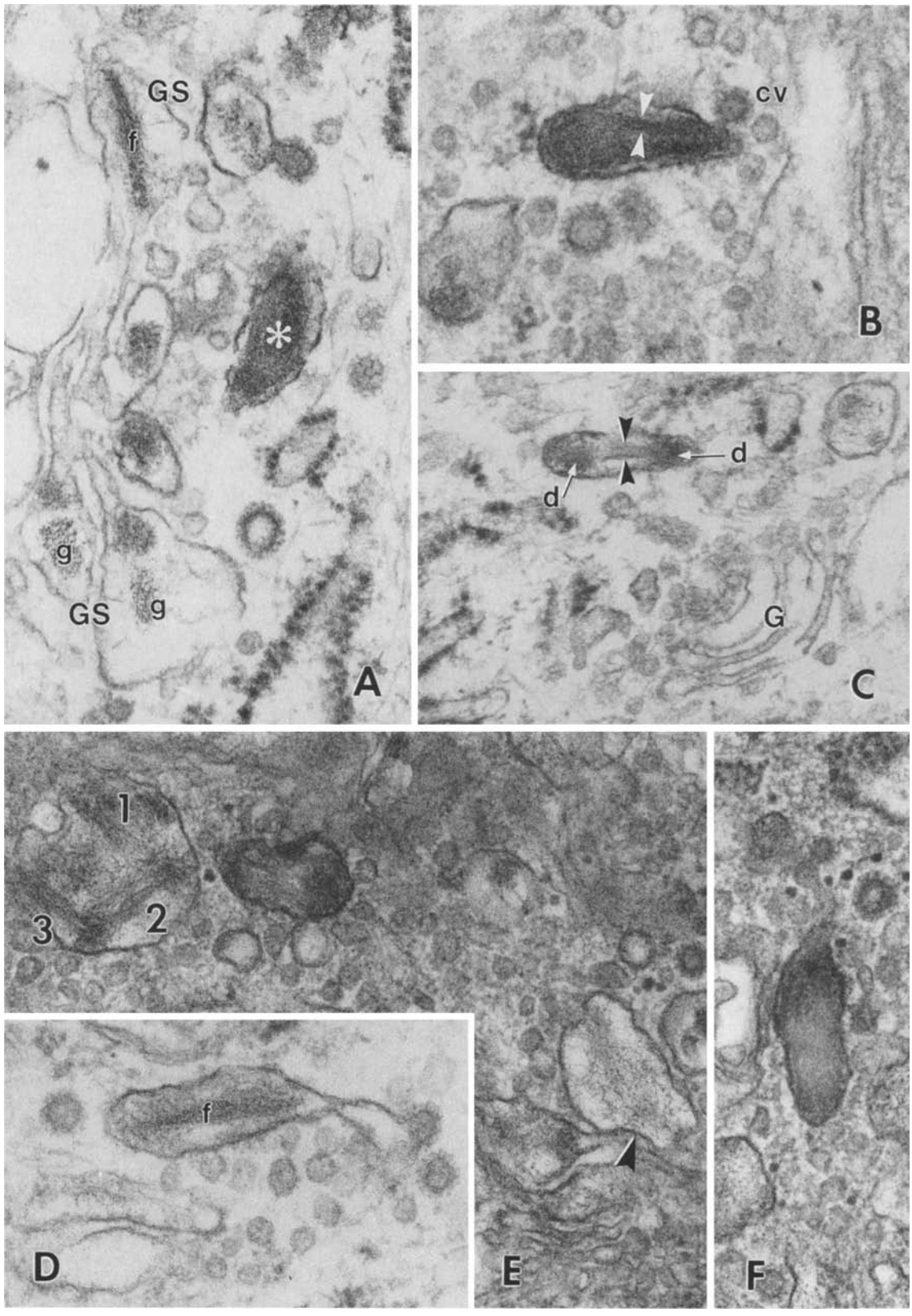

Fig. 2 A–F. (Non-neoplastic myofibroblasts). Collagen secretion granules from cases 11 (**A, B, D**), 10 (**C**), and 13 (**E, F**) (Table 1). **A** Detail from Fig. 1 A showing Golgi saccules (GS) with granular (g) and fibrillar (f) content, and an adjacent late presecretory granule (*). × 76000. **B** Late presecretory granule with internal aggregates (*arrowheads*) and a coated vesicle (cv) attached to the limiting membrane. × 98000. **C** Granule of uncertain status (? secretory granule) with central filamentous material (*arrowheads*) connecting two polar densities (d). G, Golgi apparatus. × 76000. **D** Early presecretory granule with a single fibrillar bundle (f). × 98000. **E** Abnormally rounded presecretory granule with three fibrillar bundles; at right, an early presecretory granule (*arrowhead*). × 76000. **F** Secretory granule with finely textured homogeneous content. × 76000

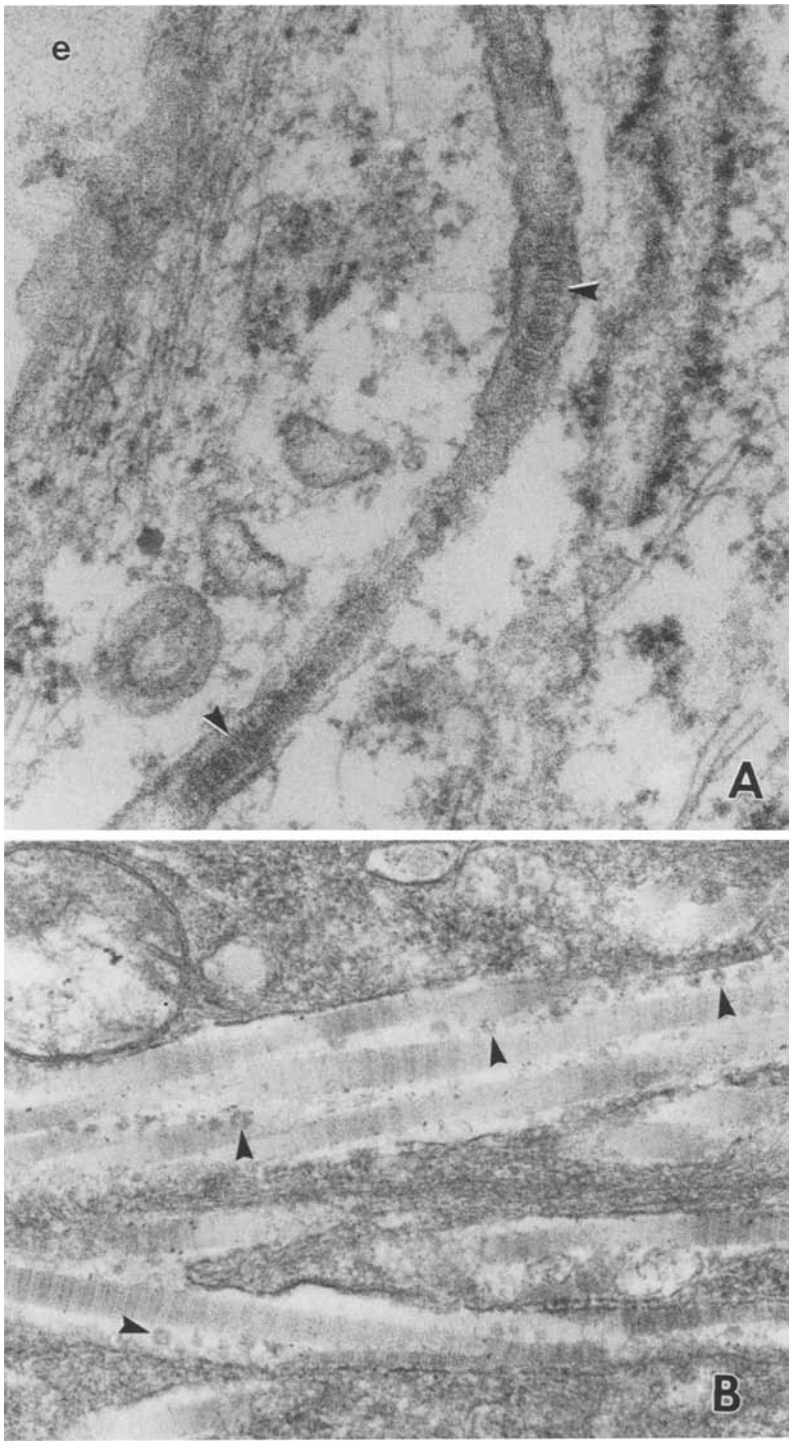

Fig. 3A, B. (Non-neoplastic myofibroblasts). Collagen fibrils within membranous cisternae near the plasma membrane. **A** Case 5. Note laterally striated areas of fibril (*arrowheads*). e, extracellular space. ×72000. **B** Case 9. *Arrowheads*, microvesicles. ×76000

served in the vicinity of the plasma membrane. They consisted of straight or curving smooth-membraned cisternae, 60–80 nm in diameter, and often exceeding 1 μm in length. Each contained a 20–40 nm diameter fibril (Fig. 3A) lying in an electron-lucent background and sometimes exhibiting the same kind of fine lateral periodicity seen in collagen fibrils in the extracellular space. On occasion, several fibrils were found within a single cisterna which also contained small membranous microvesicles (Fig. 3B). Collagen secretion granules, mainly of presecretory type, were seen in small numbers in diagnostically secure tumours of spindle cell type (fibromatoses) or where there was

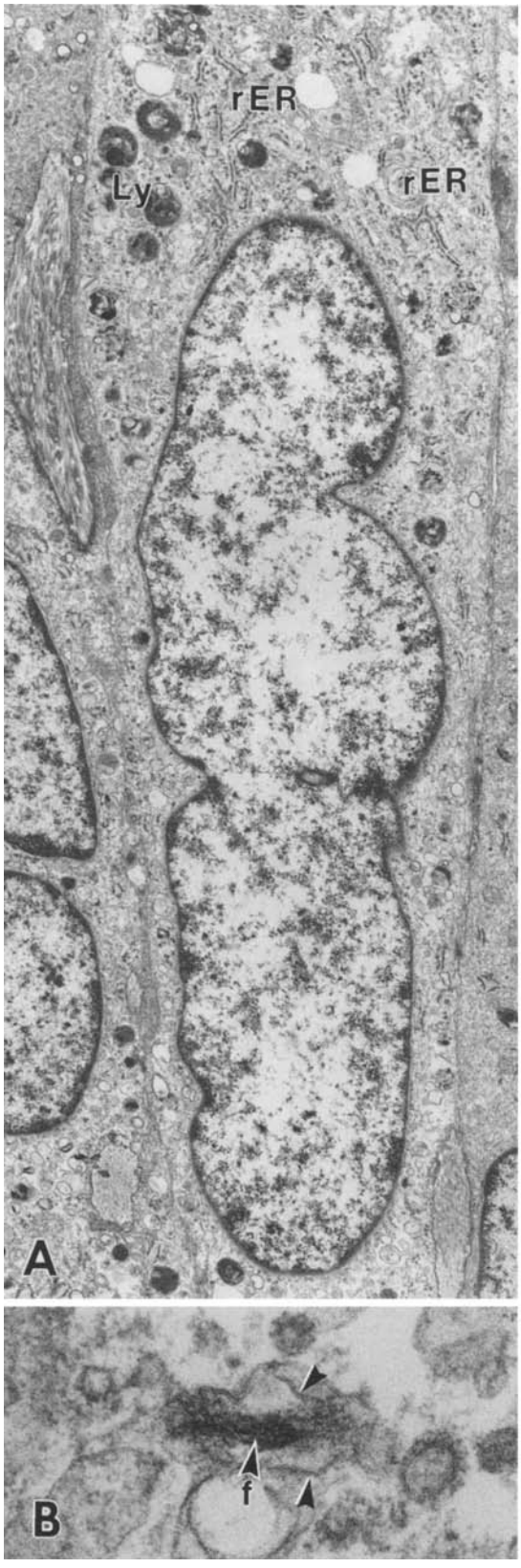

Fig. 4A, B. Problematical case 4 (Table 2). **A** Fibrohistiocyte showing abundant lysosomes and conspicuous rER. ×8600. **B** Presecretory granule exhibiting internal fibrillar bundle (f) and limiting membrane (*arrowheads*). ×71000

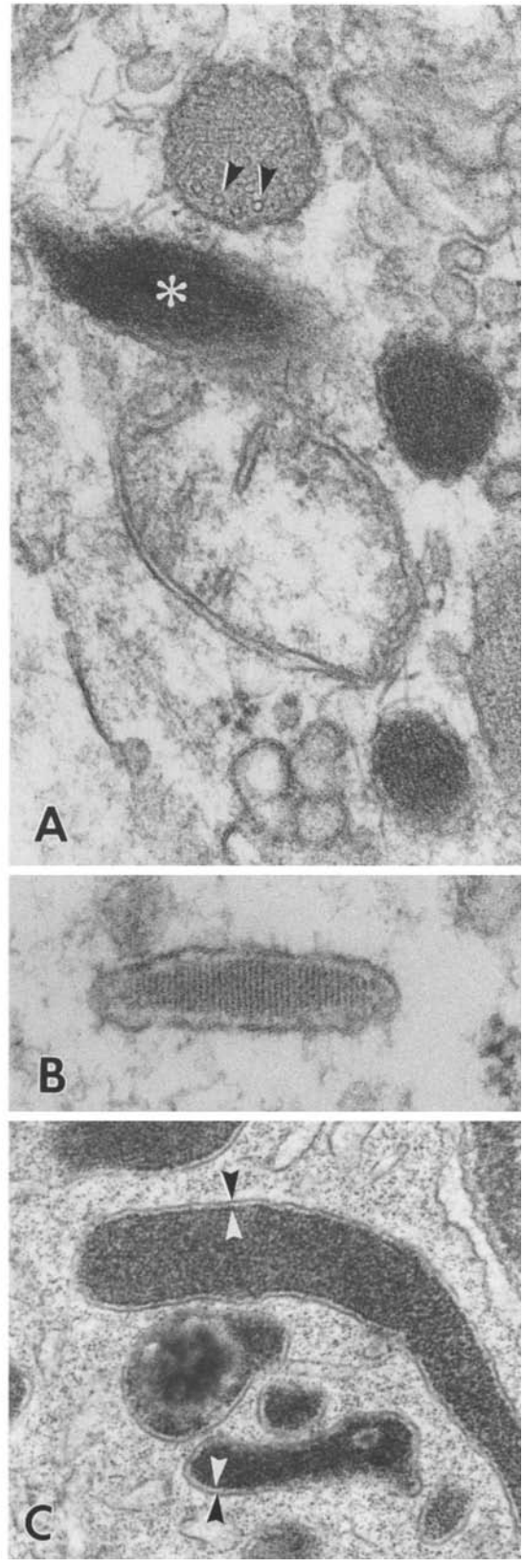

Fig. 5A–C. Organelles with the potential for confusion with collagen secretion granules. **A** Weibel Palade bodies in endothelium from case 5 (Table 1). *Asterisk*, organelle resembling collagen secretion granule in Fig. 2A; *arrowheads* indicate internal microtubular structures. ×98000. **B** Melanosome. ×115000. **C** Lysosomes. Note coarely granular interior and uniform submembrane halo (*arrowheads*). ×63000

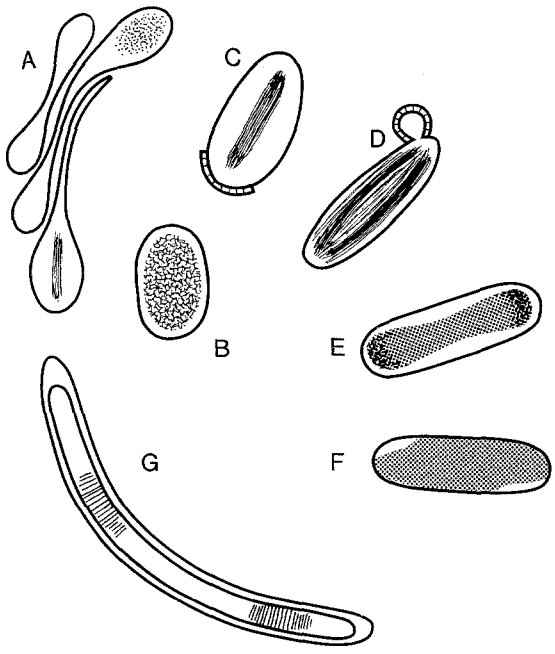

Fig. 6A–G. Diagrammatic reepresentation of collagen secretion granules. **A** Golgi; **B** earliest presecretory granule; **C, D** presecretory granules with fibrillar content and coated areas on limiting membrane; **E** probable early secretory granule; **F** mature secretory granule; **G** fibril-containing cisterna

a prominent spindle cell component (fibroadenoma). They were also detected in a number of problematical tumours of which brief details are given in Table 2. Figure 6 summarises the principal ultrastructural features of collagen secretion granules.

Collagen secretion granules were most numerous in granulation tissue where 15–20 granules could be identified in a single myofibroblastic profile. In the granuloma (case 13 – Table 1) clusters of up to about five organelles were commonly seen. In other myofibroblasts and in the neoplastic cells referred to in Table 2 as few as one or two collagen secretion granules per cell profile were detected.

Comparisons were made with other Golgi-derived organelles which usually or occasionally have an elliptical or somewhat elongate profile: Weibel-Palade bodies (Fig. 5A) in endothelium from case 5 (Table 1); type II melanosomes (Fig. 5B) from an axillary node metastasis of an amelanotic malignant melanoma; and primary lysosomes (Fig. 5C) from a human bone marrow cell.

Discussion

In this paper organelles from human non-neoplastic myofibroblasts and from neoplastic cells from certain spindle cell tumours have been identified which are identical in their electron microscopic appearances to structures documented in certain matrix-forming cells in experimental animals (see introduction). Histochemical reactions with phosphotungstic acid and silver methenamine (Weinstock and Leblond 1974; Cho and Garant 1981) and autoradiographic patterns of labelling with tritiated proline (Marchi and Leblond 1983) have provided good evidence for believing that these organelles participate in the intracellular packaging, transport and secretion of collagen-fibril precursor. It is argued, therefore, that the organelles described here for the first time in human myofibroblasts have the same function, a suggestion in keeping with the known role of this cell in matrix formation (Gabbiani et al. 1976).

In cultured human myofibroblasts vesicles designated as *secretory* (Oda et al. 1988) and *filament-containing* (Reger and Dabbous 1988) have been described: these were rounded structures lacking the internal fibrillar organisation seen in the collagen secretion granules described in the present paper. Myofibroblasts produce a variety of collagens (Oda et al. 1988) some of which have a fine, amorphous appearance in comparison with classical type I fibrils, for example. It is not yet clear whether the collagen secretion granules on the one hand, and structures such as the filament-containing vesicles on the other, represent segregated pathways for these structurally different collagen types.

In human cells little attention has been paid to granules of presecretory and secretory type. They seem to have been described only in fibroblasts, from the intralobular stroma of the normal, non-lactating adult breast (Eyden et al. 1986) and cartilage (Ghadially 1988). It might be useful, therefore, to point out features by which collagen secretion granules can be distinguished from other Golgi-derived organelles whose profiles are usually or occasionally elliptical or somewhat elongate – namely, melanosomes, Weibel-Palade bodies and primary lysosomes. Collagen-secretion granules from the present study have never contained either the amorphous and highly electron-dense melanosomal pigment melanin, or the laterally or diagonally periodic inclusion typical of nonpigmented (type II) melanosomes. Conversely, the limiting membrane of melanosomes never seems to possess the coated regions which are common in collagen-secretion granules of presecretory type. While confusion with the melanosome will in most instances be unlikely, comparison of figures 5A and 2A shows that solitary collagen-secretion granules or Weibel-Palade bodies can have an almost indistinguishable appearance. However, the spectrum of

ultrastructure that is seen where numbers of organelles are present will generally reveal distinguishing features. Characteristically, Weibel-Palade bodies contain microtubule-like structures which are observed as circular profiles in cross-section (Fig. 5A) and as fine 4 nm striations in longitudinal section. Circular profiles have never been seen within collagen-secretion granules, and while the latter have dense longitudinal elements (aggregates), these at 15–20 nm thick, are noticeably coarser than the fine striations of the Weibel-Palade body. With respect to lysosomes, these can be pleomorphic and commonly exhibit elliptical or elongate profiles. They generally have a distinct submembrane space of uniform width and a coarsely granular interior which contrasts with the fibrillar content of the presecretory granule and the fine, homogeneous interior of granules of secretory type.

The peripheral fibril-containing cisternae correspond to *intracellular collagen* found widely amongst mesenchymal and non-mesenchymal cells (discussed by Ghadially 1988). Trelstad and Hayashi (1979) interpreted them as deep channels of the extracellular space and sites of exteriorisation of secretory material. Conflicting autoradiographic data, however, have lead some (Marchi and Leblond 1983) to believe that such structures were lysosomal or endocytotic. The fibril-bearing cisternae of figure 3B of this paper might be lysosomal to judge by their content of microvesicles, for the latter are a common intralysosomal component. Where such microvesicles are absent, as in Fig. 3A, uncertainty exists as to whether the cisternae represent a terminal stage of secretion or a nascent endocytotic structure.

The primary significance of collagen secretion granules is that they identify cells actively engaged in the packaging, intracellular transport and secretion of collagen-fibril precursor. While one or other of the collagen proteins is produced by a wide variety of both epithelial and mesenchymal cells, in non-neoplastic cells the elaboration of collagen is of somewhat more restricted occurrence: odontoblasts, osteoblasts, chondroblasts, fibroblasts, myofibroblasts, as well as corneal epithelium. In appropriate settings, therefore, collagen secretion granules can help in cell identification. In this context, it is relevant to point out that none of the many publications on myofibroblast ultrastructure (see reviews by Majno 1979; Lipper et al. 1980; Seemayer et al. 1980; Bhawan 1981; Gabbiani 1981; Ghadially 1985) has referred to these organelles. Hitherto, the ultrastructural definition of the myofibroblast has rested on the finding of abundant rER cisternae and focal densities amongst fine myofilaments. Although not specific, collagen secretion granules provide a further feature by which myofibroblasts can be defined or recognised.

Enhanced cell-type recognition can be expected to be of help in the histopathological diagnosis of tumours, and it has, in fact, been possible to clarify the nature of certain spindle cell tumours by finding collagen secretion granules in them (Table 2). Some of these data will be presented in greater detail elsewhere, but they can be illustrated here by reference to case 4 (Table 2) (Fig. 4A, B). The finding of modest peripheral tracts of fine myofilaments with focal densities (not shown in the cell in Fig. 4A), co-expressed with collagen secretion granules (Fig. 4B) and rER, suggests myofibroblastic phenotype: more abundant myofilaments in the absence of collagen secretion granules would, it is argued, be more consistent with leiomyosarcoma. Furthermore, the presence of conspicuous numbers of lysosomes identifies the cell as a *fibrohistiocyte,* again arguing against leiomyosarcoma and favouring atypical fibroxanthoma. A more complete appreciation, however, of the diagnostic value of collagen secretion granules must await a more comprehensive analysis of their occurrence in both normal and neoplastic cells.

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