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The 'spindle-shaped' body in fibroblasts: intracellular collagen fibrils

F. FERNANDEZ-MADRID, SHARON NOONAN AND JEANNE RIDDLE*

Wayne State University, School of Medicine, Departments of Medicine, Biochemistry and Pathology, Detroit, Michigan

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INTRODUCTION

In a fine structural study of fibroblasts from various tissues, Movat & Fernando (1962) described a spindle-shaped organelle occurring within the cytoplasm, mainly in the Golgi region and in the peripheral portions of the cell. The inner structure of this organelle was apparently formed by bundles of fibrils embedded in an electron-dense material and enveloped by a membrane. Staining with phosphotungstic acid showed that the intracellular fibrils had high electron opacity, though no cross banding could be observed. The nature of these intracellular inclusions remains unknown. During the course of an ultrastructural study on procollagen secretion, using inhibitors of the intracellular transport of this macromolecule, we have repeatedly observed typical 'spindle-shaped' bodies similar to those previously reported. In this communication we have described the collagenous nature of the fibrillar contents of these cellular organelles, recognized by their periodicity and the frequent finding of other fibrillar products of tropocollagen interconversions, such as SLS (segment long spacing) and FLS (fibrous long spacing) in cytoplasmic vacuoles. A preliminary report of this work has been presented in abstract form (Fernadez-Madrid, Noonan & Riddle, 1979).

MATERIALS AND METHODS

Eggs from White Leghorn chickens obtained from Holtzapple's Hatcheries (Romeo, Michigan) were incubated in a moist atmosphere at 37 °C until the chick embryos were 11 days old. Corium fragments were prepared and collected as previously described (Fernandez-Madrid, 1967) at room temperature in Earle's minimal essential medium (MEM). After collecting two groups separately, the corium fragments were washed once with fresh MEM. Since in a previous study (Fernandez-Madrid *et al.* 1980) we found that 2·5 μM/ml colchicine consistently inhibited the secretion of collagen *in vitro*, we used this concentration in our experiments. Alternatively, corium fragments were incubated under identical conditions in medium containing 10 μM/ml vinblastine. For the *in vivo* experiments, viable 11 day old chick embryos were intravenously injected in the yolk sac with 2·5 μM of colchicine or 10 μM vinblastine in 0·2 ml 0·9 % NaCl. Injections were performed with glass microneedles through a window in the shell which was closed and sealed with paraffin, and *in vivo* incubation was continued at 37 °C. The embryos were killed after three hours and the corium fragments were immediately fixed for ultrastructural studies.

* Present address: Henry Ford Hospital, Division of Rheumatology, Detroit, Michigan 48202.

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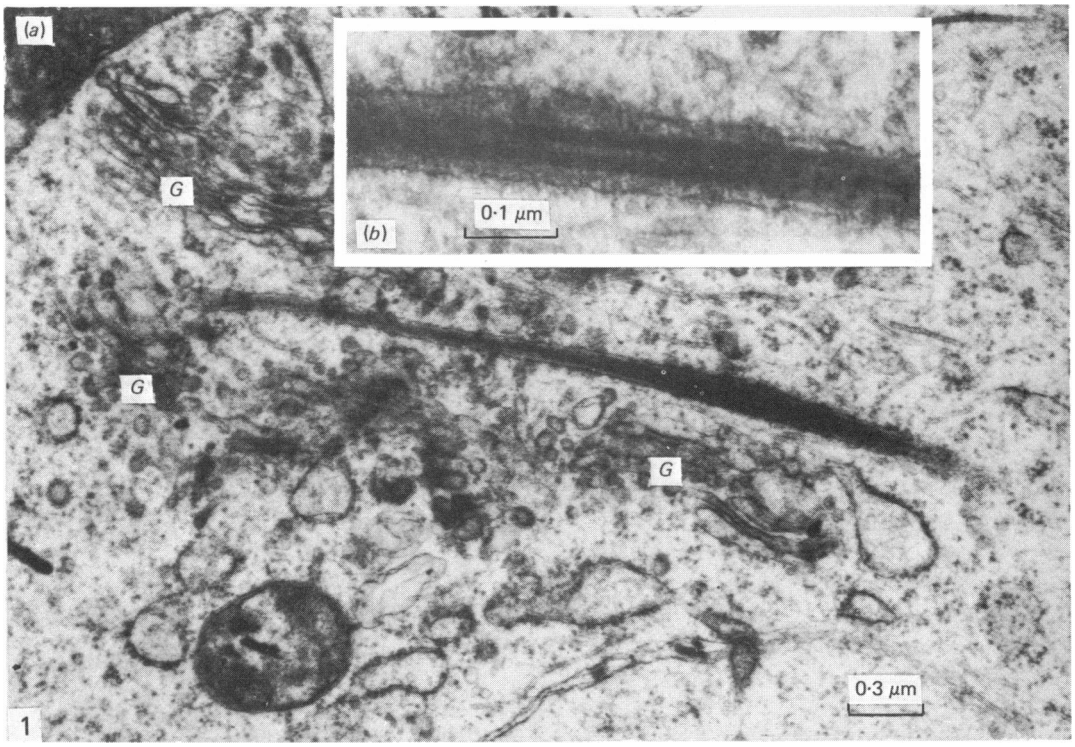

Fig. 1. Fibroblast from chick embryo corium treated with 2·5 μM/ml colchicine, *in vivo*. (*a*) Typical 'fusiform' body in the midst of two active Golgi (*G*) complexes. (*b*) The electron-dense material and the fibrillar nature of the contents are apparent in the unit-membrane enclosed body.

Control preparations consisted of 11 days old chick embryos which had been injected *in vivo* with 0·2 ml saline, killed at identical intervals and handled in the same way as those treated with the alkaloids. For the *in vitro* experiments, chick embryo corium fragments were incubated under identical conditions except that the alkaloids were omitted. The corium fragments from both the *in vivo* and *in vitro* experiments were processed identically for electron microscopy. They were immediately placed in 0·6 M cacodylate-buffered (1 % w/v) glutaraldehyde (pH 7·2–7·4) and allowed to fix for 24 hours at 4 °C (Sabatini, Miller & Barnett, 1964). Following fixation, the samples were washed overnight with 0·2 M cacodylate-buffered sucrose (pH 7·2–7·4), post-fixed and stained for 2 hours in 1 % veronal acetate-buffered osmium tetroxide (pH 7·2–7·4), progressively dehydrated with ethanol and embedded in Maraglas (Freeman & Spurlock, 1962). Ultrathin sections, 20 to 40 nm thick, were obtained using a Porter-Blum MT-1 ultramicrotome equipped with a diamond knife. The sections were mounted on unsupported 300-mesh copper grids and doubly stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963). Examinations and photography were performed using an RCA EMU-4 transmission electron microscope operated at 50 kV.

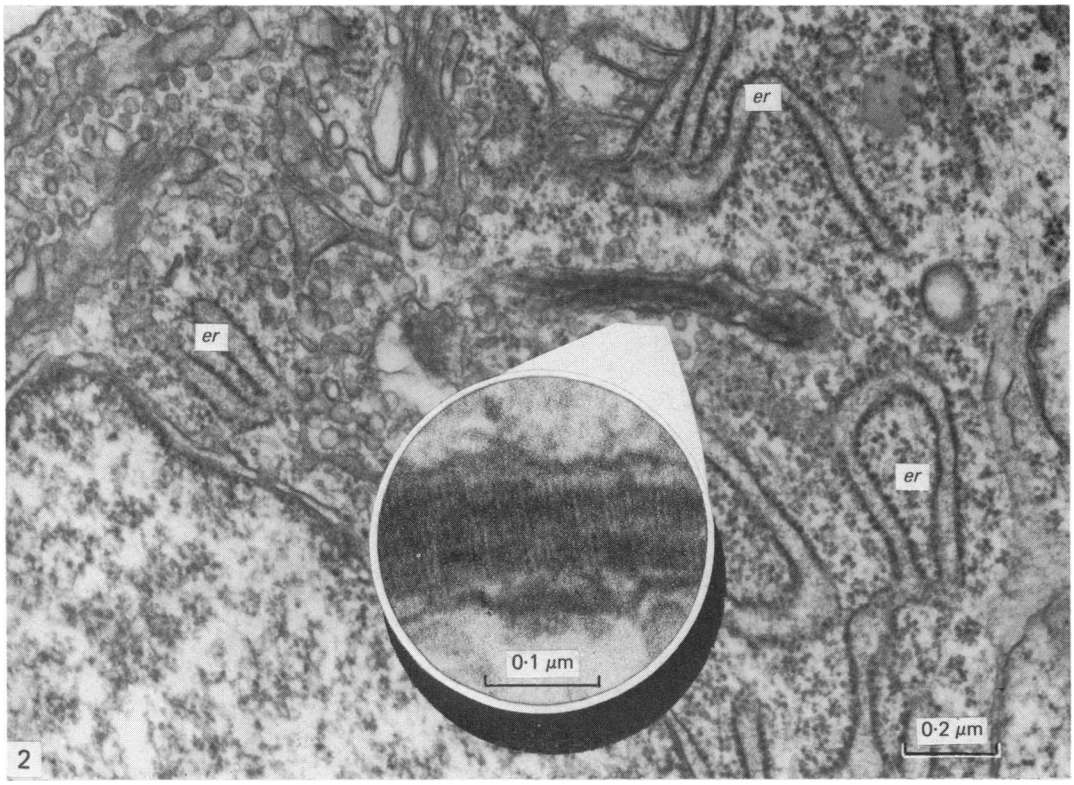

Fig. 2. Chick embryo corium fibroblasts from a preparation treated with 2·5 μM/ml colchicine *in vivo*. 'Fusiform' body in the midst of a very active endoplasmic reticulum (*er*), showing fibrils with 55 nm periodicity.

RESULTS

'Spindle-shaped' bodies were frequently observed within fibroblasts from preparations treated *in vivo* or *in vitro* with colchicine or vinblastine. Some were typically 'spindle-shaped' or fusiform (Fig. 1), but many were rod-like in shape as described by Voelz (1964). Many of the 'spindle-shaped' bodies had a width of 50–60 nm, consistent with that previously reported (Movat & Fernando, 1962; Voelz, 1964), but some of these organelles, containing more than one fibril, were considerably wider (Figs. 1, 2). The length of the 'spindle-shaped' bodies was undetermined, since it was not possible to visualize the entire organelle in one plane. Often they were seen in the midst of the rough endoplasmic reticulum (Fig. 2) or very close to the Golgi complex (Fig. 1). The intravacuolar fibrils were surrounded by electron-dense amorphous material (Fig. 1*b*) and some of them showed clear cross banding (50–55 nm), thus establishing their collagenous nature (Fig. 2). Similar organelles were seen very frequently also in the cytoplasm of chick embryo corium fibroblasts treated with vinblastine, and occasionally in untreated fibroblasts. The finding of 'spindle-shaped' bodies was often accompanied by the presence of membrane-bound segment long-spacing (Fig. 3) and fibrous long-spacing crystallites (Figs. 3–5) in the treated preparations. Usually the vacuoles contained one type of crystallite or the other, but an occasional vacuole contained SLS as well as FLS aggregates (Fig. 3). The FLS aggregates were all located intracellularly in the membrane-bound vacuoles containing an electron-dense material (Figs. 3–5) and showed symmetrical banding with

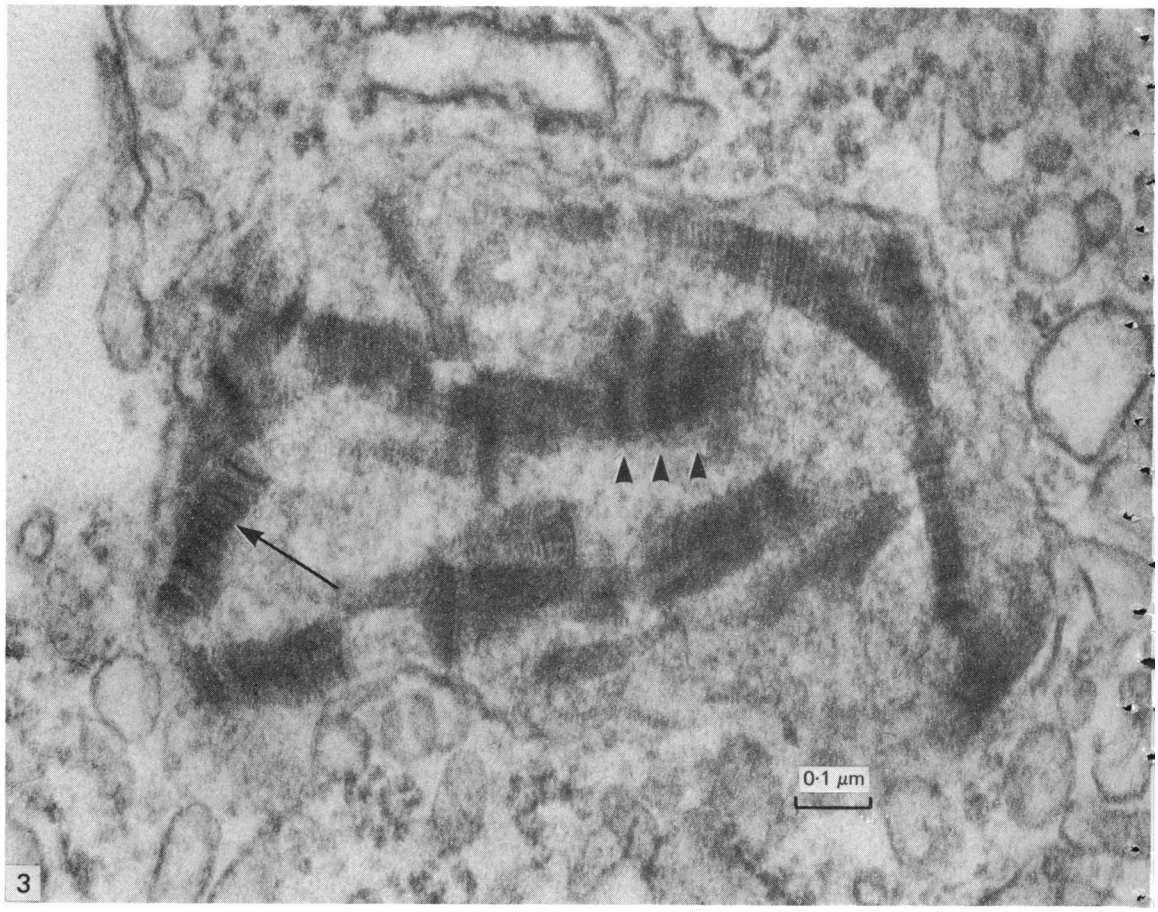

Fig. 3. Multiple fibrillar aggregates in a large cytoplasmic vacuole of a fibroblast from a vinblastine-treated preparation (10 μM/ml *in vitro*). Asymmetric banding can be observed in SLS crystallites (arrow). Some aggregates show alternation of dark and clear bands with symmetrical banding (arrowheads).

alternation of dark and clear bands. The uncorrected periodicity observed was variable, oscillating from 60 to 225 nm in agreement with values reported in the literature (Highberger, Gross & Schmitt, 1951). In many of these structures, longitudinally arranged filaments could be observed. Neither SLS nor FLS aggregates were observed outside the cell membrane. By far the most frequent collagenous fibrillar aggregates found in cytoplasmic vacuoles in the cells treated with the alkaloids were collagen fibrils with native or near native periodicity. SLS aggregates were nearly ten times more frequent than FLS aggregates in our preparations induced by either colchicine or vinblastine.

DISCUSSION

As part of the morphological spectrum of vacuoles induced massively in fibroblasts by the action of colchicine or vinblastine, we have found organelles identical to those described by Movat & Fernando (Figs. 1, 2). These authors suggested a possible relation of the organelles with cellular secretory processes because of their association with the Golgi region. Although cross banding was not detected, it was suggested

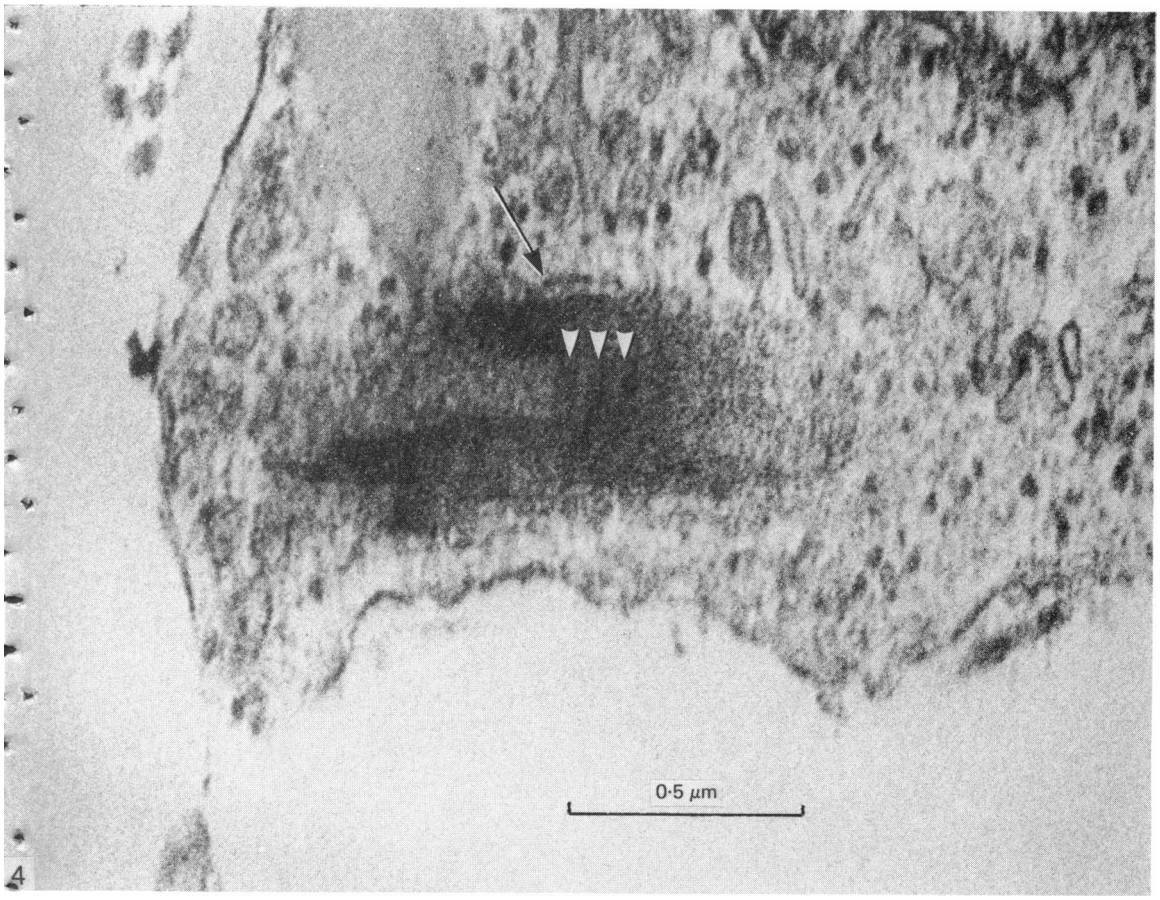

Fig. 4. Cortex of a fibroblast from a colchicine-treated preparation (2·5 μM/ml *in vitro*) showing a fibrillar aggregate with symmetric banding embedded in electron-dense material (arrowheads). A segment of limiting membrane (arrow) is visualized.

that the organelles might represent phagocytosed collagen. In a study on chicken fibroblasts, Voelz (1964) suggested that these structures were formed by the endoplasmic reticulum. Other workers have also reported intracellular 'rod-like' or cigar-shaped organelles which we believe to be similar in nature (Price & Silk, 1967; Trelstad, 1971). In our work the membrane-bound fibrils can be recognized as collagenous because of their periodicity (Fig. 2). As opposed to the exceptionally infrequent presence of these 'spindle-shaped' bodies in previous reports, in our study they were extremely frequent in all the preparations treated with colchicine or vinblastine which we examined. Several studies have reported that drugs which interfere with the action of microtubules are able to block the secretion of procollagen (Diegelmann & Peterkofsky, 1972; Dehm & Prockop, 1972; Ehrlich, Ross & Bornstein, 1974; Ehrlich & Bornstein, 1972). Recently we have reported that when colchicine blocks the secretion of procollagen, a variety of vacuolar formations appears in the cytoplasm of the fibroblasts, which contains non-striated fibrils, collagen fibrils with native periodicity and segment long-spacing crystallites (Fernandez-Madrid *et al.* 1980). The central aspect of that work was the report of massive formation of intracellular SLS, as a part of the spectrum of changes induced

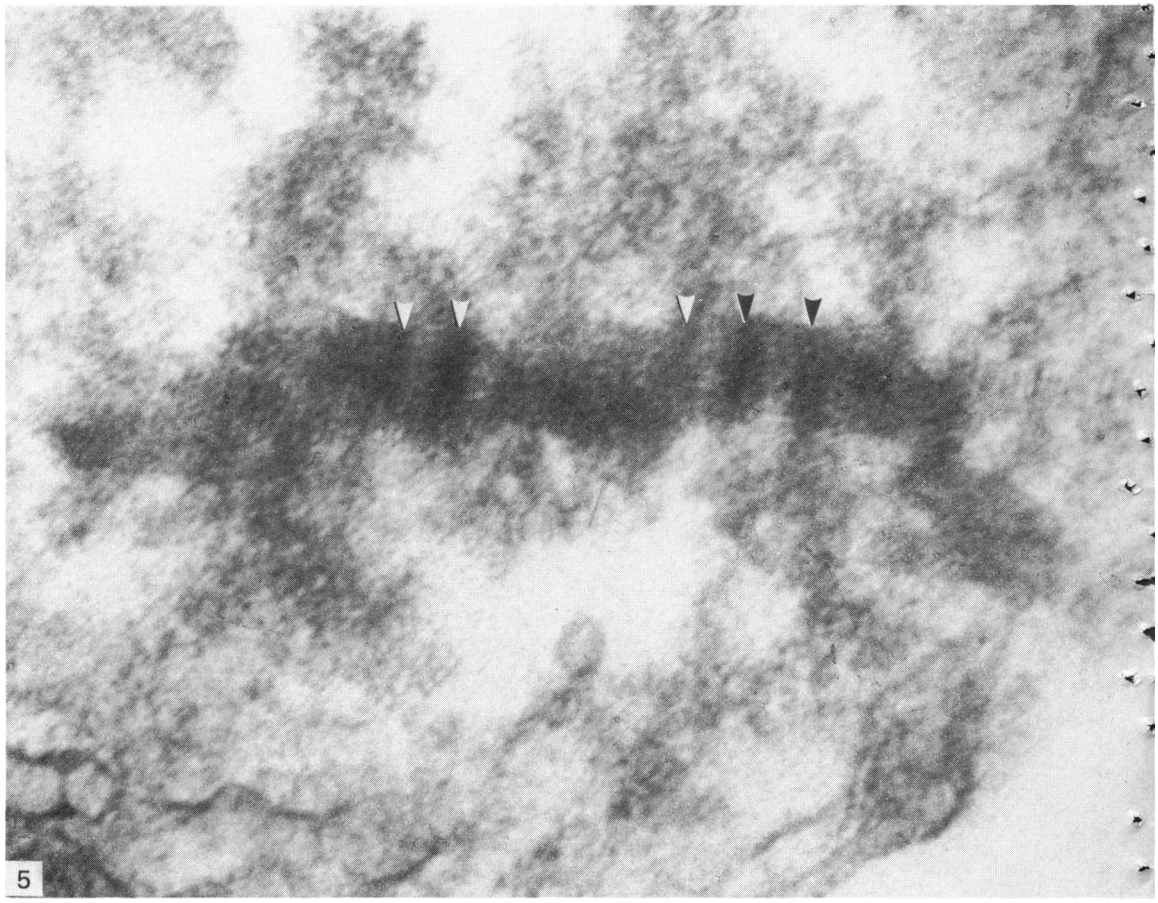

Fig. 5. Colchicine-treated (2·5 μM/ml *in vivo*) fibrillar structure with symmetric banding embedded in electron-dense material (arrowheads).

by colchicine. In this work, we have reported similar results by inhibition of transport with vinblastine and, in addition to the findings of intravacuolar SLS, we have reported the presence of FLS crystallites in cytoplasmic vacuoles from colchicine- or vinblastine-treated cells (Figs. 3–5). Though FLS aggregates have been reported many times in the extracellular space (Jakus, 1956), intracellular FLS aggregates have occasionally been described in cytoplasmic vacuoles (Gokel & Hubner, 1977; Navas-Palacio, 1978; Sheldon & Kimball, 1962). The presence of tropocollagen in the vacuoles induced by these alkaloids is suggested by the presence of SLS as well as FLS (Figs. 3–5) and collagen fibrils with native periodicity (Fig. 2). Schmitt, Gross & Highberger (1953) postulated a common macromolecular precursor, tropocollagen, based on the reversible interconversions of SLS, FLS, non-striated fibrils and fibrils with native periodicity from solutions of collagen, adjusting pH and the ionic strength of the medium. We propose that a similar phenomenon occurs within the large vacuoles formed in the fibroblasts treated with these alkaloids. The presence of processed procollagen in cytoplasmic vacuoles may also explain the intravacuolar appearance of other fibrillary collagenous aggregates, such as non-striated fibrils and collagen fibrils with native periodicity.

The finding of intracellular tropocollagen rather than procollagen in cytoplasmic vacuoles is surprising, since present views on the conversion of procollagen to collagen support an extracellular process (Fessler & Fessler, 1978). This finding apparently also conflicts with the suggestion of the presence of procollagen in Golgi-derived vacuoles obtained by subcellular fractionation or by using ferritin antibodies to procollagen (Olsen & Prockop, 1974; Nist *et al.* 1975; Harwood, Grant & Jackson, 1976). It also conflicts with the EM description of small vesicles containing parallel arrays of filaments by Weinstock (1972), and by Weinstock & Leblond (1974) in odontoblasts and by Ehrlich *et al.* (1974) and Olsen & Prockop (1974) in colchicine-treated cells, and more recently by Bruns *et al* (1979) in fibroblasts in tissue culture. The reported electron microscopic studies assumed that the fibrillar structures in cytoplasmic vacuoles were procollagen, but identification based on the analysis of the band pattern was lacking. Our data are not incompatible with intracellular accumulation of procollagen in colchicine-treated cells (Ehrlich & Bornstein, 1972) since they do not indicate that all procollagen is converted to collagen intracellularly. It is interesting that Ehrlich & Bornstein, in their study on microtubules and transcellular movements of procollagen, found that cytochalasin B did not affect the conversion of procollagen to collagen, although it did inhibit collagen synthesis. They suggested that their results were compatible with some intracellular processing of procollagen.

The previously reported findings (Fernandez-Madrid *et al.* 1980) which we now extend in this paper can be interpreted as an example of autophagocytosis with intracellular processing of procollagen, occurring in chick embryo fibroblasts when the secretion of procollagen is blocked by colchicine or vinblastine. This process may be related to the observation of cellular autophagocytosis reported in secretory cells (Ericcson, 1969; De Duve & Wattiaux, 1966). Our observations suggest that a portion of the procollagen synthesized under the influence of colchicine or vinblastine is processed intracellularly and may be rapidly degraded. Earlier studies on collagen metabolism suggested that the turnover of this macromolecule is considerably slower than that of other proteins (Neuberger, Perrone & Slack, 1951) but subsequent studies have shown that some fractions of collagen do have significant rates of metabolic turnover (Harkness, Marko, Muir & Neuberger, 1954; Jackson, 1957) and that part of the hydroxyproline excreted in the urine must originate from the degradation of newly synthesized collagen (Lindstedt & Prockop, 1961; Prockop & Kivirikko, 1967). Lapière, Onkelinx & Richelle (1966) reported that a large fraction of the newly synthesized hydroxyproline does not appear in mature calcified matrix of rat bones. Other biochemical studies have suggested that a portion of the collagen synthesized may be immediately destroyed by the cell (Krane, Muñoz & Harris, 1967, 1970). It has also been shown that colchicine treatment significantly reduces the bursting strength of scar tissue in rats (Morton *et al.* 1974), an observation which has been explained as being due to rapid turnover of collagen without a purposeful incorporation of new molecules into scar tissue (Blau, Peacock, Carlson & Chvapil, 1975). More recently, evidence was provided that 30–40% of collagen from rabbit lung explants is rapidly degraded after synthesis (Bienkowski, Cowan, McDonald & Crystal, 1978*a*) and that lung fibroblasts degrade newly synthesized collagen within the cell before secretion (Bienkowski, Baum & Crystal 1978*b*). Our observations may represent the morphological counterpart of those findings (Lapière, Onkelinx & Richelle, 1966; Krane *et al.* 1967, 1970; Morton *et al.* 1974; Blau *et al.* 1975; Bienkowski *et al.* 1978*a*, *b*). When the secretory process is inhibited by the action of

colchicine or vinblastine, an autophagocytic mechanism would contribute to degrade the excess procollagen retained within the cell. One may speculate that such a process constitutes a control mechanism, operative under normal conditions to remove the product which cannot be secreted, perhaps when it is produced in excess of physiological needs.

The possible meaning of our findings in terms of packaging of monomers into fibrillar forms under normal conditions is intriguing. Many fixatives, including glutaraldehyde and osmium tetroxide, penetrate tissues relatively slowly. During fixation, changes are inevitably introduced; material is extracted, dimensions are altered and molecular rearrangements occur (Glauert, 1975). We interpret the formation of SLS as well as of FLS as artefacts of preparation resulting from the changes of micro-environment in the vacuoles, including the presence of other macromolecules, or changes of the pH; and that the cellular changes which occur during fixation merely reflect the presence of free, intact tropocollagen molecules within the vacuoles. Since secretion is not completely blocked by these agents (Diegelmann & Peterkofsky, 1972; Dehm & Prockop, 1972; Ehrlich & Bornstein, 1972) we have to consider the possibility that some of the fibrillar structures described in this work may find their way out and be extruded, thus contributing to the process of fibrillogenesis. Some reports in the literature are compatible with this interpretation (Price & Silk, 1967; Trelstad, 1971).

SUMMARY

The 'spindle-shaped' or 'fusiform' bodies of fibroblasts are membrane-bound collagen fibrils. These cellular organelles, also described in the literature as rods or cigars, can be induced massively in chick embryo fibroblasts by treatment with colchicine or vinblastine. These alkaloids also induce massively the presence of non-striated fibrils, segment long-spacing (SLS) and fibrous long-spacing (FLS) crystallites in cytoplasmic vacuoles.

The intracellular fibrillar structures described within the cytoplasmic vacuoles suggest the occurrence of tropocollagen interconversions, indicating that under the experimental conditions used in this work some procollagen is processed intracellularly. Our findings may represent the first step of autophagocytosis of collagenous products when the secretory process is blocked. It is possible that these agents, by blocking secretion, magnify a normal process, and that under normal conditions a population of procollagen molecules may be processed intracellularly, thus contributing to the process of fibrillogenesis.

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REFERENCES

BLAU, S. A., PEACOCK, E. E. Jr, CARLSON, E. C. & CHVAPIL, M. (1975). Effect of colchicine on cross-linking of collagen in rats. *Surgical Forum* **26**, 65–67.

BIENKOWSKI, R. S., COWAN, M. J., MCDONALD, J. A. & CRYSTAL, R. G. (1978*a*). Degradation of newly synthesized collagen. *Journal of Biological Chemistry* **253**, 4356–4363.

BIENKOWSKI, R. S., BAUM, B. J. & CRYSTAL, R. G. (1978*b*). Fibroblasts degrade newly synthesized collagen within the cell before secretion. *Nature* **276**, 413–416.

BRUNS, R. R., HULMES, D. J., THERRIEN, S. F. & GROSS, J. (1979). Procollagen segment-long-spacing crystallites: their role in collagen fibrillogenesis. *Proceedings of the National Academy of Sciences of the U.S.A.* **76**, 313-317.

DE DUVE, C. & WATTIAUX, R. (1966). Function of lysosomes. *Annual Review of Physiology* **28**, 435–492.

DEHM, P. & PROCKOP, D. J. (1972). Time lag in the secretion of collagen by matrix-free tendon cells and inhibition of the secretory process by colchicine and vinblastine. *Biochemical and Biophysical Acta* **264**, 375–382.

DEHM, P., JIMENEZ, S. A., OLSEN, B. R. & PROCKOP, D. J. (1972). A transport form of collagen from embryonic tendon. Electron microscopic demonstration of an NH_2-terminal extension and evidence suggesting the presence of cystine in the molecule. *Proceedings of the National Academy of Sciences of the U.S.A.* **69**, 60–64.

DIEGELMANN, R. F. & PETERKOFSKY, B. (1972). Inhibition of collagen secretion from bone and cultured fibroblasts by microtubular disruptive drugs. *Proceedings of the National Academy of Sciences of the U.S.A.* **69**, 892–896.

EHRLICH, H. P. & BORNSTEIN, P. (1972). Microtubules in transcellular movements of procollagen. *Nature, New Biology* **238**, 257–260.

EHRLICH, H. P., ROSS, R. & BORNSTEIN, P. (1974). Effects of antimicrotubular agents on the secretion of collagen. A biochemical and morphological study. *Journal of Cell Biology* **62**, 390–405.

ERICCSON, J. L. E. (1969). Mechanism of Cellular Autophagy. In *Lysosomes in Biology and Pathology* (ed. J. T. Dingle & H. B. Fell), vol. 2. Amsterdam: North-Holland Publishing Co.

FERNANDEZ-MADRID, F. (1967). Biosynthesis of collagen. *Journal of Cell Biology* **33**, 27.

FERNANDEZ-MADRID, F., NOONAN, S., RIDDLE, J., KARVONEN, R. & SASAKI, D. (1980). Intracellular processing of procollagen induced by the action of colchicine. *Journal of Anatomy* **130**, 229–242.

FERNANDEZ-MADRID, F., NOONAN, S. & RIDDLE, J. (1979). Intracellular tropocollagen interconversions induced by colchicine. *Federation Proceedings* **38**, 413.

FESSLER, J. H. & FESSLER, L. I. (1978). Biosynthesis of procollagen. *Annual Review of Biochemistry* **47**, 129–162.

FREEMAN, J. A. & SPURLOCK, B. O. (1962). A new epoxy embedment for electron microscopy. *Journal of Cell Biology* **13**, 437–443.

GLAUERT, A. M. (1975). Fixation, dehydration and embedding of biological specimens. In *Practical Methods in Electron Microscopy* (ed. A. M. Glauert), pp. 1–4. Amsterdam: North-Holland Publishing Co.

GOKEL, J. M, & HUBNER, G. (1977). Intracellular 'fibrous long spacing' collagen in morbus Dupuytren (Dupuytren's contracture). *Beiträge zur Pathologie* **161**, 176.

HARKNESS, R. D., MARKO, A. M., MUIR, H. M. & NEUBERGER, A. (1954). The metabolism of collagen and other proteins of the skin of rabbits. *Biochemical Journal* **56**, 558–569.

HARWOOD, R., GRANT, N. E. & JACKSON, D. S. (1976). The route of secretion of procollagen. The influence of α,α'-bipyridyl, colchicine and antimycin A on the secretory process in embryonic tendon and cartilage cells. *Biochemical Journal* **156**, 81–90.

HIGHBERGER, J. H., GROSS, J. & SCHMITT, F. O. (1951). The interaction of mucoprotein with soluble collagen: an electron microscope study. *Proceedings of the National Academy of Sciences of the U.S.A.* **37**, 286-291.

JACKSON, D. S. (1957). Connective tissue growth stimulated by carrageenin. I. The formation and removal of collagen. *Biochemical Journal* **65**, 277-284.

JAKUS, M. A. (1956). The fine structure of Descemet's membrane. *Journal of Biophysical and Biochemical Cytology*, Suppl. **2**, 243-252.

KRANE, S. M., MUÑOZ, A. J. & HARRIS, E. D. Jr. (1967). Collagen-like fragments: excretion in urine of patients with Paget's disease of bone. *Science* **157**, 713–716.

KRANE, S. M., MUÑOZ, A. J. & HARRIS, E. D. Jr. (1970). Urinary polypeptides related to collagen synthesis. *Journal of Clinical Investigation* **49**, 716–729.

LAPIÈRE, C. M., ONKELINX, C. & RICHELLE, L. J. (1966). Collagen metabolism in skin and bone. In *Biochemie et Physiologie du Tissue Conjonctif* (ed. P. Comte), p. 505. Lyon: Société Ormecc et Imprimerie du Sud-Est.

LINDSTEDT, S. & PROCKOP, D. J. (1961). Isotopic studies on urinary hydroxyproline as evidence of rapidly catabolized forms of collagen in the young rat. *Journal of Biological Chemistry* **236**, 1399–1403.

MORTON, D., STEINBRONN, K., LATO, M., CHVAPIL, M. & PEACOCK, E. E. Jr. (1974). Effect of colchicine on wound healing in rats. *Surgical Forum* **25**, 47–48.

MOVAT, H. Z. & FERNANDO, N. V. P. (1962). The fine structure of connective tissue. I. The fibroblasts. *Experimental and Molecular Pathology* **1**, 509–534.

NAVAS-PALACIO, J. J. (1978). Characteristics, incidence and significance of fibrous long spacing collagen (FLSC). *Morfologia normal y patologica, Sec. B.* **2**, 123–133.

NEUBERGER, A., PERRONE, J. C. & SLACK, H. G. B. (1951). The relative metabolic inertia of tendon collagen in the rat. *Biochemical Journal* **49**, 199–204.

NIST, C., VON DER MARK, K., HAY, E. D., OLSEN, B. R., BORNSTEIN, P., ROSS, R. & DEHM, P. (1975). Location of procollagen in chick corneal and tendon fibroblasts by use of ferritin-conjugated antibodies. *Journal of Cell Biology* **65**, 75–87.

OLSEN, B. R. & PROCKOP, D. J. (1974). Ferritin-conjugated antibodies used for labeling of organelles involved in the cellular synthesis and transport of procollagen. *Proceedings of the National Academy of Sciences of the U.S.A.* **71**, 2033–2037.

PRICE, Z. H. & SILK, M. H. (1967). Observations of filamentous material in cultures of human skin fibroblasts. *Journal of the Royal Microscopical Society* **86**, 349–357.

PROCKOP, D. J. & KIVIRIKKO, K. I. (1967). Relationship of hydroxyproline excretion in urine to collagen metabolism. Biochemistry and clinical applications. *Annals of Internal Medicine* **66**, 1243–1266.

REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology* **17**, 208–212.

SABATINI, D. D., MILLER, F. & BARNETT, R. J. (1964). Aldehyde fixation for morphological and enzyme histochemical studies with the electron microscope. *Journal of Histochemistry and Cytochemistry* **12**, 57–71.

SCHMITT, F. O., GROSS, J. & HIGHBERGER, J. H. (1953). A new particle type in certain connective tissue extracts. *Proceedings of the National Academy of Sciences of the U.S.A.* **39**, 459–470.

SHELDON, H. & KIMBALL, F. B. (1962). Studies on cartilage. III. The occurrence of collagen within the vacuoles of the Golgi apparatus. *Journal of Cell Biology* **12**, 599–613.

TRELSTAD, R. I. (1971). Vacuoles in the embryonic chick corneal epithelium. An epithelium which produces collagen. *Journal of Cell Biology* **48**, 689–694.

VOELZ, H. (1964). The 'spindle-shaped body' in fibroblasts. *Journal of Cell Biology* **20**, 33–37.

WATSON, M. L. (1958). Staining of tissue sections for electron microscopy with heavy metals. *Journal of Biophysical and Biochemical Cytology* **4**, 465–468.

WEINSTOCK, M. (1972). Collagen formation observations on its intracellular packaging and transport. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **129**, 455–470.

WEINSTOCK, M. & LEBLOND, L. P. (1974). Synthesis, migration and release of precursor collagen by odontoblast as visualized by radioautography after [^{3}H] proline administration. *Journal of Cell Biology* **60**, 92–127.