MORPHOLOGICAL AND CHEMICAL STUDIES OF COLLAGEN FORMATION

I. The Fine Structure of Guinea Pig Granulomata

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ABSTRACT

This paper describes electron microscopic studies of developing connective tissue in granulomata induced by the subcutaneous injection of carrageenin into guinea pigs. Seven days after injection the granulomata contained many fibroblasts and exhibited rapid production of collagen. The fibroblasts were characterised by an extensively developed endoplasmic reticulum and showed numbers of fine, unstriated filaments in the outer regions of the cytoplasm. The filaments, about 50 A in diameter, tended to lie parallel to and closely adjacent to the cell boundary. The cytoplasmic membrane was frequently ill defined or disrupted, particularly bordering regions in which filaments occurred. In longitudinal sections of extended cell processes, filaments were abundant and, in some instances, the cytoplasmic membrane was barely detectable. In the extracellular space striated collagen fibrils were usually accompanied by filaments, 50 to 100 A in diameter, and these often exhibited the characteristic periodicity of collagen, particularly after intense electron bombardment. Much cellular debris was present in the extracellular space. These observations have led to the suggestion that connective tissue precursors are released from fibroblasts by the disintegration or dissolution of the cytoplasmic membrane and the shedding of cytoplasmic material, as in the apocrine gland cells. In some instances this release may take the form of the elongation from the cell of extended processes; disintegration of the cytoplasmic membrane surrounding these processes then leaves the contents in the extracellular phase.

INTRODUCTION

It is generally recognised that the fibroblast plays an essential role in the production of collagen fibrils and of other extracellular components of connective tissues. Despite a considerable volume of work on the subject the precise nature of the mechanisms involved has yet to be satisfactorily established. Present studies are directed primarily at defining the role of the fibroblast at the biochemical and ultrastructural levels, and the work described in this paper is the morphological part of such a combined study.

The biochemical evidence presented in Paper II (Lowther, Green, and Chapman, 12) confirms that the synthesis of collagen protein follows a similar pattern to that of other proteins. The high radioactivity of neutral salt-soluble collagen from the microsomal fraction (from tissue slices incubated with labeled amino acids) indicates that the newly synthesised collagen protein is found intracellularly and is closely associated with the endoplasmic reticulum and its attached particles.

In vitro studies on the precipitation of fibrils from solutions of collagen (Nageotte, 15) have focused attention on the later stages of develop-

ment and have led to the suggestion that the cell secretes monomeric collagen which aggregates into fibrils in the medium of the extracellular space (Gross *et al.*, 7; Fitton Jackson, 3). The mechanism in the living tissue is unlikely to be as simple as this. The observations of Porter and Pappas (19) on collagen formation in tissue-cultured fibroblasts indicate that the cell not only is involved in producing the fibril, but is to some extent responsible for its initial orientation. These authors suggest that the unit fibrils of collagen form in close association with the cell surface and, from here, are shed by the cells into the extracellular phase.

This paper is concerned with the intermediate stages in the formative process and, in particular, with the manner in which the collagen protein is released from the cell. The evidence presented here indicates that the cell loses cytoplasmic material during fibrogenesis and that the secretory mechanism is similar to that occurring in apocrine gland cells.

As a source of rapidly developing connective tissue suitable for electron microscopic study, the carrageenin-induced granuloma in the guinea pig was used (Robertson and Schwartz, 20). The choice of this material was partly dictated by the concurrent biochemical studies, where a relatively large amount of rapidly developing tissue was required. Other *in vivo* sources of developing connective tissue are being studied, and accounts of this work will shortly be published.

MATERIALS AND METHODS

Granulomata were induced in the lower abdominal region of guinea pigs by the subcutaneous injection of carrageenin, a sulfated polygalactose, using the techniques described by Jackson (10). The animals were sacrificed 7 days after the injection of the carrageenin. This interval of time was chosen because the biochemical studies of Jackson (10) showed that most of the insoluble collagen formed between the 5th and 9th days. Preliminary electron microscopic investigations of granulomata of varying ages, using shadow-casting techniques on macerated tissue, also demonstrated the rapid production of striated collagen fibrils during the same period.

The animals were killed by a blow on the head and tissue was selected from several different regions of each granuloma. Fixation was commenced within 90 seconds of death, and the tissue was prepared for electron microscopic examination by standard methods. The fixative was 1 per cent ice-cold osmium tetroxide solution, veronal acetate–buffered at pH 7.4, the osmolar concentration being adjusted to isotonicity by the addition of NaCl. A small concentration of calcium ions was present in the fixing solution (Palade, 16; Porter and Pappas, 19); the calcium, in the form of chloride, was added to the fixative to give a final concentration of 0.02 per cent. Fixation was carried out for 1 hour. After dehydration in alcohol most of the tissue blocks were allowed to stand for 2 to 4 hours in a 1 per cent solution of phosphotungstic acid (PTA) in absolute alcohol (Huxley, 9). This was found to result in marked enhancement of contrast, particularly of the collagen fibrils and other fibrillar components. Following a further brief washing in absolute alcohol the tissue blocks were embedded in araldite, using the techniques employed by Glauert and Glauert (6) but impregnating for considerably longer times (up to 2 or 3 days). Sections 300 to 500 A in thickness were cut on a Huxley ultramicrotome, mounted on carbon-filmed grids, and examined in a Siemens & Halske Elmiskop I electron microscope operating at 80 kv., using double condenser illumination and at instrumental magnifications ranging from 2,000 to 80,000.

In several cases thick (about 1 μ) sections of large areas of some of the blocks were cut in the Huxley ultramicrotome and were mounted for examination by phase-contrast microscopy. Thin sections were then cut from selected regions of these blocks.

OBSERVATIONS

Phase-contrast observations and low-magnification electron micrographs of sections of 7-day granuloma showed the presence of several cell types, differing in over-all shape, cytoplasmic features, and shape of the nucleus. Some of the cells could be identified as leucocytes, erythrocytes, and muscle cells. The remainder, forming the bulk of the cellular part of the tissue, appeared to be fibroblasts, intimately mixed with numerous histiocytes. Fibroblasts and histiocytes are not easily differentiated, although certain staining properties can serve to identify them in the light microscope (Williams, 26). At the electron microscopic level, in the absence of cell-by-cell correlation with histological observations on adjacent thick sections, it was not always possible to distinguish between the two cell types. In the regions chosen for electron microscopic study a high proportion of the cells (not less than 60 to 70 per cent) showed common features and were taken to be fibroblasts. In 7-day tissue these cells invariably occurred in close proximity to bundles of collagen fibrils; they were frequently

elongated and possessed moderate-sized round or oval nuclei with one or more nucleoli. The cells in the 7-day carrageenin granuloma tended to be less closely packed than the cells in other rapidly developing connective tissues (for example, regenerating tendon) and it was usually possible to trace the outlines of individual cells. In this respect the tissue proved to be a very satisfactory one for electron microscopic investigation.

Intracellular Features: The cytoplasm of the fibroblasts was characterised by an extensive development of the endoplasmic reticulum (Figs. 1*a*, 1*b*). The profiles showed some tendency to be arranged in ordered parallel arrays but were mostly irregular and distended, occasionally to such an extent that the vesicles occupied a considerable part of the cytoplasmic volume. Similar distended vesicles of the endoplasmic reticulum in actively synthesising fibroblasts have been reported by other workers (18, 21, 24, 25). The vesicles were observed to be filled with a fine filamentous material, consisting of a mass of short, randomly orientated filaments less than 50 A in diameter. The characteristic association of the outer surface of the limiting membrane of the system with small osmiophilic particles, presumably ribonucleoprotein (17), was noted. Isolated particles of similar appearance occurred in the cytoplasm. In some instances continuity between the limiting membrane of the endoplasmic reticulum and the outer nuclear membrane was seen. In no case was continuity observed between the limiting membrane of the endoplasmic reticulum and the cytoplasmic membrane.

Mitochondria of typical appearance were abundant. Most were small and round, or oval in section although some elongated forms were present. Internal cristae and limiting double membranes were clearly resolved and in some cases it was possible to distinguish the layered structure of a single 70 A thick membrane (4).

A prominent feature of many of these cells was the presence in the cytoplasm of numerous fine filaments, about 50 A in diameter (Figs. 1*b*, 1*c*). There was no clear indication of striations or periodic structure in these filaments although irregularities in outline and electron density sometimes gave rise to a nodular appearance. They rarely exhibited pronounced curvature, and appeared as semi-rigid, rather than flexible, structures. In most cells in which they occurred the filaments were concentrated in the outer regions of the cytoplasm with a marked tendency to orientate parallel to the cell boundary and closely adjacent to it. Intracellular filaments similar to those described in the present study have been observed by Wassermann (23) in regenerating rat tendon and by Wessel (25) in decidual cells of the human endometrium. Fitton Jackson (2) describes parallel filaments within the cytoplasm of fowl osteoblasts and suggests that these may be concerned with fibrogenesis.

The cytoplasmic membrane of the fibroblasts showed considerable variation in appearance, even in a single cell. It was frequently ill defined or disrupted and sometimes barely discernible for appreciable lengths of the cell boundary. In such regions where the limiting membrane between the cytoplasm and the extracellular space was poorly defined, intracellular filaments were frequently observed (as in Fig. 1*b*). The possibility that the cytoplasmic membrane may have been damaged during the preparative procedures cannot be ignored, and it is therefore important to consider the appearance of the outer limiting membranes of other cell types present in the same preparations. Muscle cells showed a prominent complex membrane; no damage to this structure was observed, even when it was adjacent to the process of a fibroblast with a poorly defined membrane (Fig. 2). In thicker sections muscle cell membranes were frequently broad and blurred, but this appearance could always be attributed to oblique sectioning. Endothelial cells of capillaries possessed well marked continuous membranes. The thin limiting membrane of leucocytes was normally distinct and continuous although most of these cells showed some ruptures in the membrane (Fig. 3); such discontinuities were not accompanied by the release of cell contents and it seems reasonable to suppose that they were artefactual in origin. Similar damage, in the form of breaks in an otherwise distinct cytoplasmic membrane, was encountered in fibroblasts (see, for example, Fig. 4*b*), and it is probable that this appearance is also attributable to artifacts of specimen preparation. The ill defined and sometimes barely discernible limiting membrane, commonly encountered in the vicinity of accumulations of intracellular filaments, was peculiar to fibroblasts. It seems improbable that this appearance could result merely from damage during specimen preparation.

At the light microscope level fibroblasts are

characterised as spindle-shaped or stellate cells with the cytoplasm elongated into a number of cell processes. This shape is less readily observed in the electron microscope owing to the thinness of the sections employed, and relatively few micrographs can be expected to show an appreciable length of a process in continuity with its parent cell. Nevertheless careful observation of a number of sections revealed several cells with elongated cytoplasmic processes. Such a cell is shown in Figs. 4*a*, 4*b*, 4*c*. Many 50 A filaments occur in the cytoplasm of the process. These are orientated in the direction of the process and, as before, tend to occur in regions where the cytoplasmic membrane is least distinct. Cross-sections through processes of other cells appear in the same micrograph.

Figs. 5*a*, 5*b*, and 5*c* show an elongated structure, presumed to be a cell process, consisting largely of filaments together with a few mitochondria and vesicles of the endoplasmic reticulum. There is little indication of a cytoplasmic membrane surrounding the process, apart from an ill defined and discontinuous outline in a few places. As before, the filaments have an over-all orientation in the direction of the process. The relative distribution of the cytoplasmic organelles and the filaments suggests an intracellular origin of the filaments despite the absence of a limiting membrane and even though, in this case, the parent cell was out of the field of view. Suggestions of a periodic structure (periodicity about 100 A) are evident in a few of the intracellular filaments (Fig. 5*c*).

Extracellular Features: Typical striated collagen fibrils occurring singly and in randomly orientated bundles were widely dispersed in the extracellular space. The fibrils showed a marked degree of uniformity in diameter and, in the 7-day granuloma, most of the clearly striated fibrils occurred in the range 300 to 500 A. These fibrils will be referred to as "mature" collagen fibrils. Their periodicity had a mean value close to 500 A, as compared with the expected 640 A, probably owing to shrinkage during preparation and to compression of the sections on cutting. Some preparations contained a small number of isolated bundles of collagen fibrils of larger diameter

Explanation of Figures

All figures are electron micrographs of preparations fixed with 1 per cent osmium tetroxide and stained before embedding in a 1 per cent solution of phosphotungstic acid in absolute alcohol.

KEY TO SYMBOLS

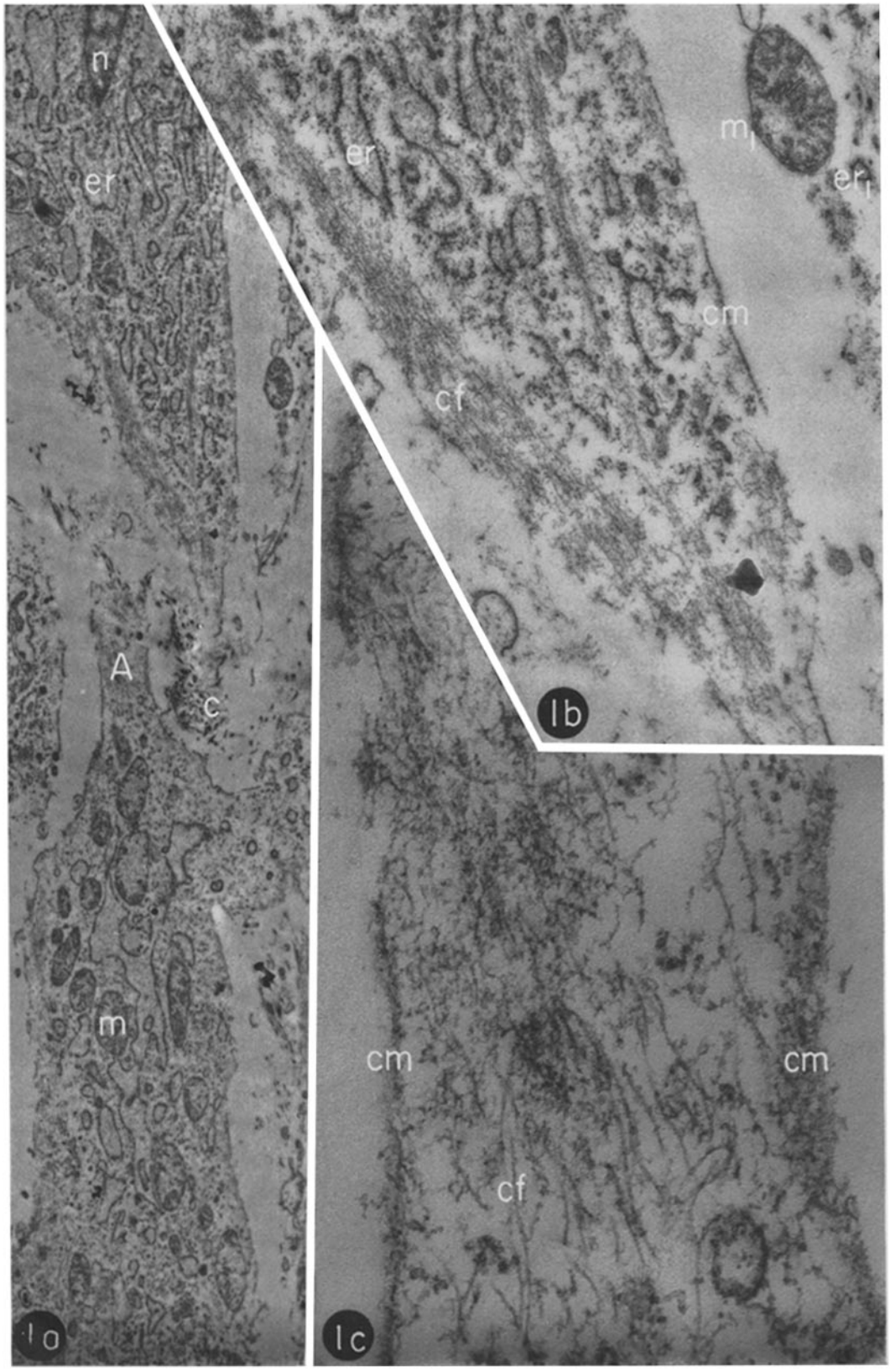
c, collagen fibrils
cf, cytoplasmic filaments
cm, cytoplasmic membrane
ef, extracellular filaments
er, particle-studded vesicles of the endoplasmic reticulum
m, mitochondrion
mc, muscle cell
n, cell nucleus
p, elongated cytoplasmic process
s, densely stained clumps of extracellular material

FIGURE 1

1*a*. Low-magnification micrograph showing typical elongated fibroblasts in a 7-day carrageenin-induced guinea pig granuloma. The profiles of the endoplasmic reticulum are irregular and distended. Numerous mitochondria are present. × 11,000.

1*b*. Enlargement of part of the upper fibroblast of Fig. 1*a*. Many fine filaments occur in the cytoplasm, particularly in the outer regions of the cell. The cytoplasmic membrane adjacent to these filaments is ill defined and disrupted. Isolated mitochondria and particle-studded vesicles of the endoplasmic reticulum (such as those marked m_1 and er_1) occur freely in the extracellular phase. × 35,000.

1*c*. An enlargement of the region marked *A* in the lower fibroblast of Fig. 1*a*, showing the appearance of the cytoplasmic filaments and the ill defined nature of the cytoplasmic membrane. × 100,000.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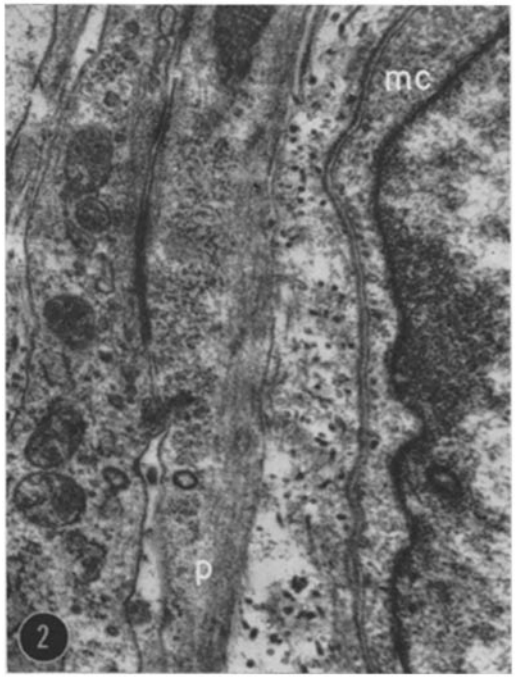


FIGURE 2

Part of a muscle cell (*mc*) showing a typical well defined limiting membrane system. Near by is an elongated cytoplasmic process (*p*) of a fibroblast with intracellular filaments and a poorly defined cytoplasmic membrane. × 25,000.

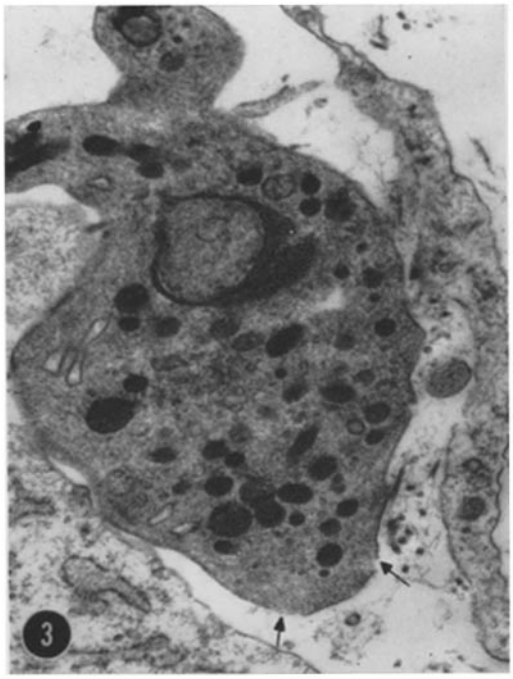


FIGURE 3

A leucocyte and parts of several fibroblasts. Apart from isolated breaks (arrows), the limiting membrane of the leucocyte is continuous and clearly defined. × 20,000.

(1,000 A); these were thought to be derived from pre-existing subcutaneous tissue. In view of the biochemical (Jackson, 10) and histological (Williams, 26) evidence and the almost complete absence of striated collagen fibrils in macerated specimens of 3-day granulomata, it is reasonable to suppose that most, if not all, of the fibrils 300 to 500 A in diameter were formed during the development of the granulomata.

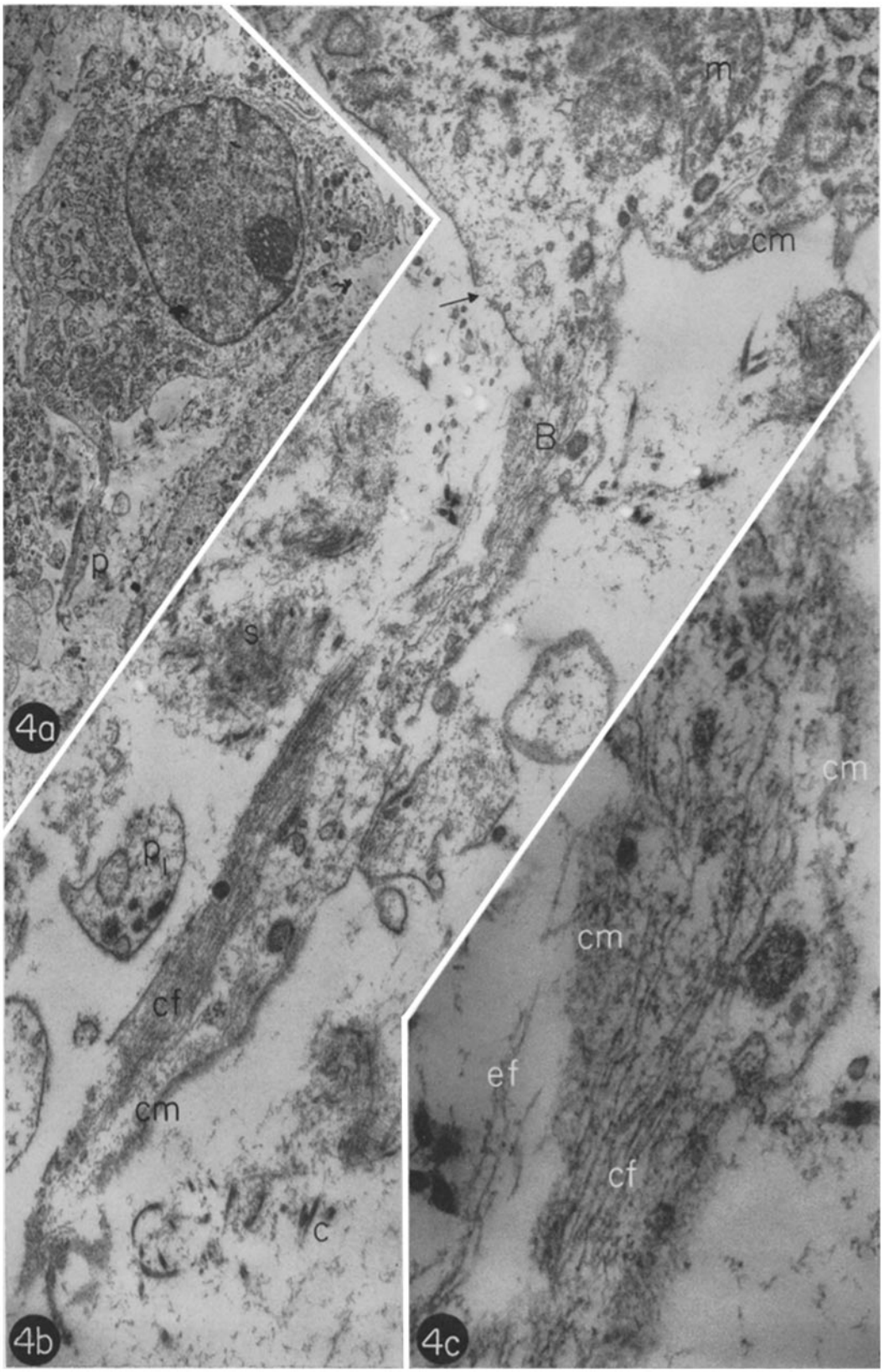
Numerous fine filaments, 50 to 100 A in diameter, were frequently observed in close proximity to mature collagen fibrils (Fig. 6). Most of these filaments showed some irregularities in structure,

FIGURE 4

4*a*. Low-magnification micrograph in which the plane of the section passes through an elongated cytoplasmic process (*p*) extending from a fibroblast. × 6,000.

4*b*. An enlargement of the cytoplasmic process shown in Fig. 4*a*. Fine filaments are abundant, and these show an over-all orientation in the direction of the process. Much of the surrounding cytoplasmic membrane is indistinct, particularly bordering regions in which the filaments occur. The occasional break in the cytoplasmic membrane (as at arrow) is probably an artifact of specimen preparation. Elements such as p_1 probably represent cross-sections through other cytoplasmic processes. Densely stained clumps of partly filamentous, partly amorphous material (*s*) occur in the extracellular phase. × 40,000.

4*c*. A further enlargement of the region marked *B* in Fig. 4*b*, showing the ill defined cytoplasmic membrane and intracellular filaments. Some filaments of similar appearance are apparently located in the extracellular phase. × 100,000.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and in those with larger diameter there was evidence of an indistinct 500 A beading as observed by Karrer (11). Confirmation that the filaments were collagenous came from an accidental observation. It was noticed that a momentary exposure to a relatively high-intensity electron beam—for example, by removing the condenser aperture for a second or less—produced marked enhancement of contrast in the specimen, apparently due to aggregation of electron-dense components. Care was necessary to avoid a too intense beam, as this merely gave rise to gross changes in all parts of the specimen; the effect was less easily observed after the specimen had been examined at a normal beam intensity. Under favourable conditions the effect was most noticeable in collagen fibrils and in the associated filaments, probably as a result of the aggregation of tungsten in these structures. The periodic banding and more prominent interbands of collagen were rendered more conspicuous; Hofmann *et al.* (8) have described similar changes in the appearance of PTA-stained collagen fibrils after intense electron bombardment. The most marked change occurred in the thin filaments, many of which developed pronounced beading with a periodicity of about 500 A (Figs. 7, 8). In some filaments fainter "beads" corresponding roughly in position to interbands could be distinguished and occasional coalescence between beaded filaments and mature collagen fibrils was observed. These observations indicate that the extracellular filaments, often found in close proximity to mature collagen fibrils, are themselves collagenous in nature and probably represent an intermediate stage in the formation of collagen.

The intracellular cytoplasmic filaments showed no clear evidence of periodic beading after exposure to an electron beam sufficiently intense to produce beading in extracellular filaments. A further increase in beam intensity merely gave rise to random aggregation effects also produced in other structures.

A noticeable feature of many extracellular regions was the presence of densely stained clumps of partly amorphous, partly filamentous material (Figs. 4*b*, 7). Short lengths of striated collagen fibrils could be recognised in some of these clumps.

A considerable amount of cytoplasmic debris was observed in the extracellular space. Isolated fragments and particle-studded vesicles of the endoplasmic reticulum were of frequent occurrence together with free mitochondria (Figs. 1*b*, 5*a*). Isolated nuclei or disintegrated nuclear material were, however, never observed. The normal appearance of other tissue components suggests that these phenomena are unlikely to be explicable in terms of postmortem, fixation, or other procedural changes. Preliminary studies using permanganate as a fixative (Luft, 13) indicate a similar distribution of cell debris in extracellular locations and appreciable disruption of the cytoplasmic membrane.

DISCUSSION

These investigations have been carried out with the object of defining the relationship between cell and fibril during the early stages of collagen morphogenesis. Several new features have emerged from these studies. Thus, fine cytoplasmic filaments have been shown to occur in relative abundance in the outer regions of cells and in elongated cell processes. Moreover, fibrogenesis appears to be accompanied by appreciable cellular disintegration.

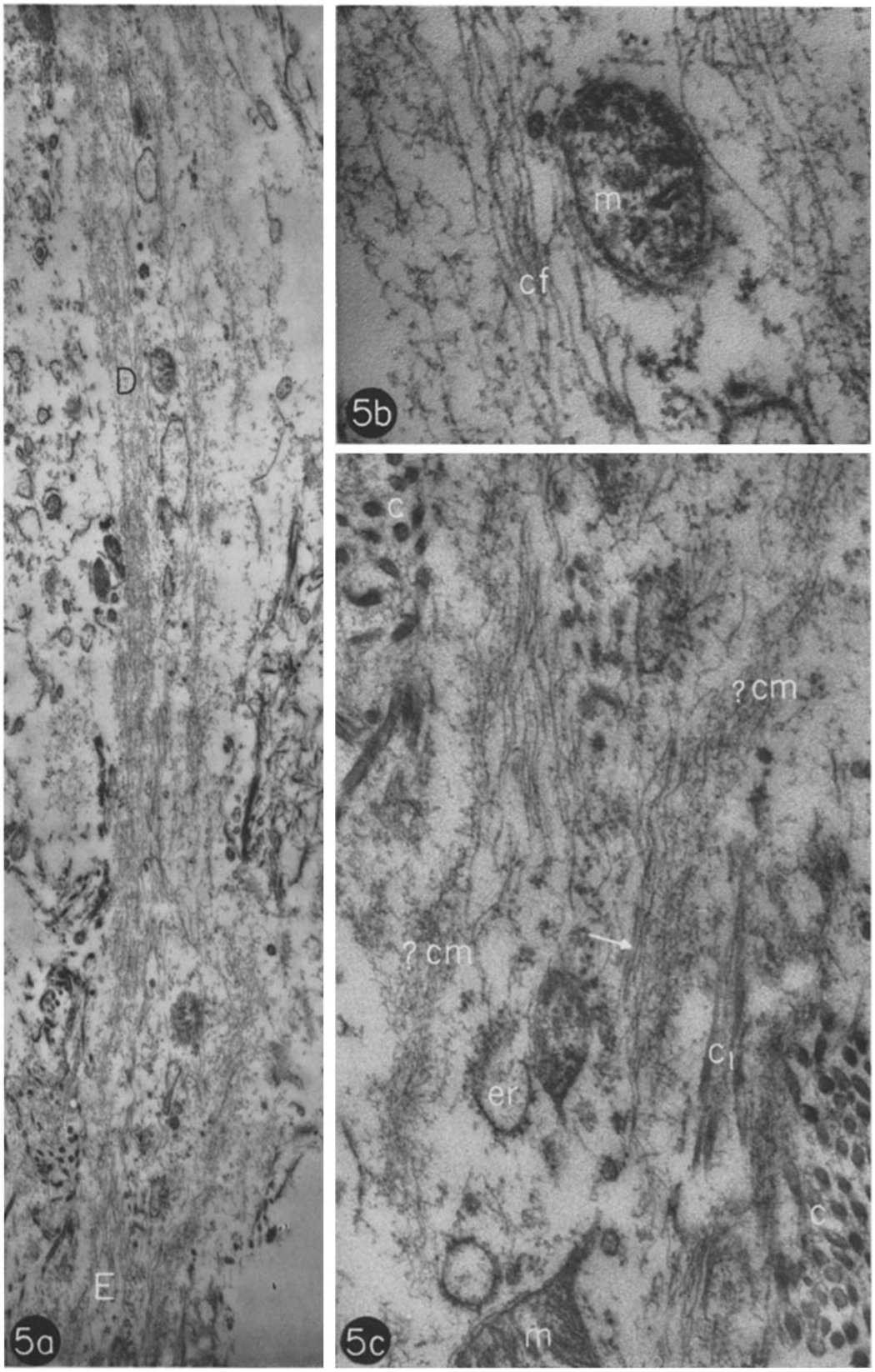
The 50 A cytoplasmic filaments may represent an early stage in the aggregation of collagen

FIGURE 5

5*a*. A cell process consisting largely of cytoplasmic filaments together with a few mitochondria and vesicles of the endoplasmic reticulum. There is little indication of any definite cytoplasmic membrane surrounding the process. The extracellular space outside the process contains collagen fibrils and cytoplasmic debris. × 20,000.

5*b*. An enlargement of the region marked *D* in Fig. 5*a*, showing filaments and other cytoplasmic components. × 100,000.

5*c*. An enlargement of the region marked *E* in Fig. 5*a* (part of Fig. 5*c* is beyond the field of view of Fig. 5*a*). Various cytoplasmic components are present. Suggestions of a periodic structure are visible in the cytoplasmic filaments marked with the arrow. Extracellular collagen fibrils (c_1) occur near by. The remnants of the cytoplasmic membrane probably occur between these collagen fibrils and the filaments. × 60,000.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macromolecules, but the evidence on this point is inconclusive. Some similarities exist between these filaments and the extracellular beaded filaments, but periodic structure was not normally detectable in the intracellular filaments, apart from one instance in which suggestions of such a structure were found (Fig. 5*c*). Filaments could not be identified in significant numbers in any of the centrifuged fractions of homogenised tissue, even after density-gradient centrifugation (Lowther, Green, and Chapman, 12), so that no biochemical evidence was available. The filaments may be an "artifact of fixation" in the sense that fixation may itself bring about some aggregation of fibrogenic materials in the cytoplasm. However, cells other than fibroblasts do not exhibit structures of similar appearance and location when fixed and processed by the same methods. Thus the presence of the filaments in fibroblasts, whether resulting from fixation or not, can certainly be regarded as significant.

The ill defined nature of the cytoplasmic membrane bordering those regions of the cell in which cytoplasmic filaments were abundant, particularly around elongated cell processes, suggests that these phenomena are related and that they represent essential steps in the process of fibrogenesis. Some modification of the cytoplasmic membrane to allow the passage of collagen macromolecules or aggregates thereof seems inevitable, and the present results indicate that both dissolution of the membrane and release of cell contents take place. This release appears, in some instances at any rate, to take the form of the elongation and perhaps even separation from the cell of masses of cytoplasmic material containing thin filaments probably collagenous in nature. Disintegration or dissolution of the cytoplasmic membrane leaves the filaments, together with other cytoplasmic components, in the extracellular phase. Other connective tissue precursors, including polysaccharides, may be released at the same time.

The presence in the extracellular phase of many isolated fragments of the endoplasmic reticulum and occasional mitochondria might be taken as further evidence in support of this mechanism. Such evidence must be regarded with caution, however, as Jackson (10) found that the wet weight of carrageenin-induced granulation tissue decreased after the 7th to 9th day and deduced that synthesis and breakdown must take place simultaneously at least from this time. Thus it is conceivable that some, or all, of the cellular disintegration may be peculiar to carrageenin granulomata and may not be typical of developing connective tissue in other situations. On the other hand, it is significant that the disintegration was apparently limited to fibroblasts and to the loss of cytoplasmic material and that isolated or disintegrating nuclei were never observed. Nevertheless it could be argued that fibroblasts may be more susceptible than other cells to disruption, either by inflammatory products present in the granuloma or by the fixation and dehydration techniques used; this susceptibility to breakdown may even be greater in regions where filamentous elements are present within the cell.

The strongest evidence in support of the hypothesis that extrusion of cytoplasm is an essential step in fibrogenesis comes from the optical studies of Stearns (22) on living fibroblasts in transparent chambers in the rabbit's ear. She noted constant modifications in the form of the cells with conspicuous activity of the cytoplasm immediately

FIGURE 6

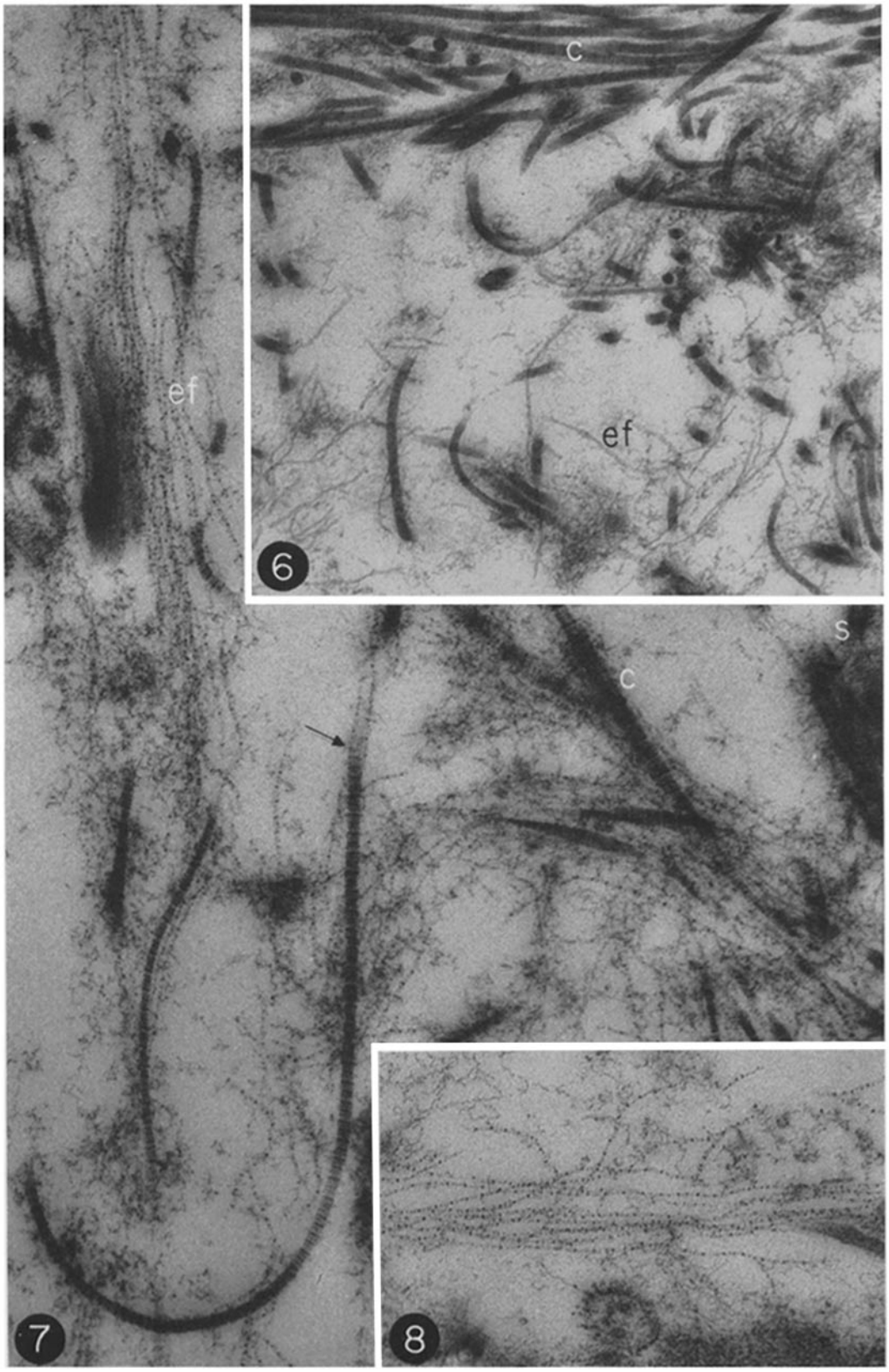
Extracellular filaments and striated collagen fibrils. This specimen was exposed only to a low-intensity electron beam. × 60,000.

FIGURE 7

Extracellular filaments and collagen fibrils after exposure to a moderately intense electron beam. The periodicity of the beading in the filaments is about 500 A, approximately the same as that in the collagen fibrils. In some instances coalescence between beaded filaments and striated collagen fibrils was observed (arrow). × 60,000.

FIGURE 8

Beaded filaments, showing some evidence of interperiod beading. × 60,000.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preceding the development of fibrils. The process of fibril formation was associated with "the projection from the surface of vesicular masses of cytoplasmic material which may become actually separated from the main body of the fibroblasts. This cytoplasmic material is apparently utilised in the production of fibrils, for it diminishes and eventually disappears as the fibrils form."

Electron microscopic evidence presented by several other workers may be cited in support of the hypothesis. The work of Porter and Pappas (19) on tissue-cultured fibroblasts suggests that the fibrogenic material accumulates at the cell surface and is then shed into the extracellular space. The importance of the cell surface has also been stressed by Bradbury and Meek (1), who, in a study of fibrogenesis in the leech, have demonstrated that long processes of the fibrocytes are surrounded by a collagenous cortex comprising many small fibrils in parallel array. They suggest that these fibrils form by "shredding off" from the cell surface. Yardley *et al.* (27), from studies of thin sections of fibroblasts from tissue cultures, conclude that collagen production is related to a "condensation" occurring at the margin of the cells; associated with these marginal condensations is a loss of the usual distinct outer cell membrane. These authors put forward the hypothesis that the material released from the cell may be cytoplasmic and that the process is an apocrine type of secretion in which part of the cell is relinquished to release possible collagen precursors and constituents of the ground substance. The precise relationship between these marginal condensations and the masses of 50 A cytoplasmic filaments observed in the present study near cell boundaries and in extended cell processes is not yet clear. In a recent electron optical study of collagen formation by fibroblasts in chick embryo dermis, Gieseking (5) suggests that the liberation of fibrillar material takes place by the disintegration and dissolution of the peripheral cytoplasmic zone of the fibroblasts. Her micrographs show appreciable cytoplasmic debris in the extracellular spaces of the tissue during fibrogenesis.

The release of connective tissue precursors from fibroblasts by the disruption or dissolution of the cytoplasmic membrane and the shedding of cytoplasmic material is analogous to the secretory mechanism of apocrine gland cells. In glands of this type (*e.g.*, the mammary gland) the secretion accumulates in part of the cytoplasm and this portion is later pinched off; most of the cell is undamaged and after a period of recovery the process is repeated (Maximow and Bloom, 14). The evidence submitted here, together with that described by these other workers, suggests that the apocrine secretory process may be a more common biological phenomenon than has hitherto been supposed.

The extracellular development of the collagen fibrils has not been studied in detail in the present investigation. This development may take place by the aggregation of the smaller filaments or, more probably, by the accretion of material from the surrounding milieu, possibly released from the fibroblasts by the same mechanism. In the latter case the cytoplasmic fibrils shed into the extracellular space may act as nuclei for the formation of fibrils. The cell may thus be capable of exercising some control over the initial orientation of the fibrils.

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