The Ultrastructure of Skin in Progressive Systemic Sclerosis (Scleroderma)

I. Dermal Collagen Fibers

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PREVIOUS ULTRASTRUCTURAL STUDIES on the skin of patients with progressive systemic sclerosis (scleroderma) have failed to reveal abnormalities other than an increased number of collagen fibrils with diameters smaller than those found in normal adult skin.[1-6] Bahr [1] described a bimodal distribution of collagen fibril diameters in scleroderma with maxima at 500 and 935 Å. These results were confirmed by Rupec and Braun-Falco,[4] who found maxima at 500 and 800 Å. Fibers outside of this range were relatively infrequent. These distributions are to be compared with collagen fibril diameters of normal adults, which range from 700 to 1400 Å, and exhibit a maximum at 1000 Å.[4,7,8] In none of these studies of scleroderma were alterations observed in the periodicity, cross-banding pattern or internal structure of these fibrils. Sensitivity to collagenase digestion was considered normal.[2]

In an attempt to characterize further the fibril population in scleroderma, we have undertaken an ultrastructural study using negatively stained samples of dermal collagen fibrils. Our findings confirm previous reports concerning the presence of thin fibrils and reveal that these fibrils possess morphologic characteristics typical of embryonic collagen. These include the presence of an especially thin fibril known as the "beaded filamant." [9] The alterations in the fibril population are accompanied by ultrastructural features in the fibrocytes suggestive of active protein synthesis.

Materials and Methods

Skin biopsies were obtained by means of a Keyes punch from the dorsum of the forearms of 7 patients with well-defined systemic sclerosis of 1–7 years duration. All of these patients had diffuse scleroderma, all exhibited Raynaud's phenomenon and

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all had visceral disturbances (predominantly gastrointestinal) characteristic of the disorder.[10] Specimens were also taken from 5 unaffected close relatives of these patients. It can be seen that the weights of the 7-mm skin plug biopsies correlated well with the clinical estimates of the severity of cutaneous involvement (Table 1).

We also examined skin taken from the dorsolateral body wall of fetal pigs (100–150 mm crown-rump length) and from the limbs of therapeutically aborted 8–12 week old human fetuses. All tissues were processed for ultrastructural examination in the same fashion. Fresh dermis was dissected from adjacent subcutaneous as well as epidermal tissue, rinsed in cold isotonic Hank's balanced saline solution and homogenized by a motor-driven ground-glass homogenizer. These preparative procedures were conducted at 4 C to prevent proteolysis. The homogenate was allowed to settle and the residue was deposited upon a freshly carboned, formvar-coated copper grid. The preparation was negatively-stained with 2% aqueous uranyl acetate and examined in a Philips EM-200 or EM-300 electron microscope. Data concerning fibril diameters were obtained by direct measurement from electron photomicrographs of negatively stained collagen fibrils.

Results

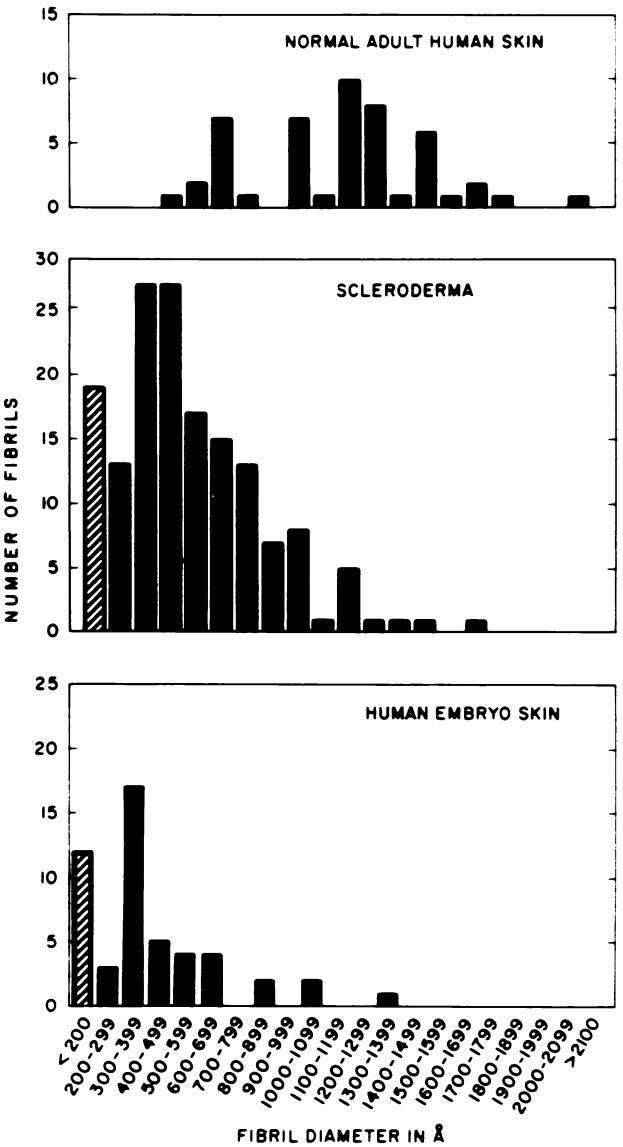
Fibril Diameters

In Text-fig 1, the diameters of collagen fibrils are recorded as a function of their numerical representation in homogenates of skin from the patients with scleroderma, unaffected relatives and from porcine embryos and human fetuses. The fibril population from the normal adults ranged from 400 to 2100 Å in diameter, with a mean value of 1200 Å. Human fetal fibrils ranged from 100 to 1400 Å in diameter, with a mean 400–500 Å. Although not shown in this figure, fibrils from the

Table 1—Clinical Histories of Patients and Normal Adults from Whom Skin Biopsies Were Taken.

Patients and relatives*	Age, sex	Duration of scleroderma (years)	Severity of scleroderma	Weight of 7-mm skin plug (mg)
1. EB	55, F	4	++	49
[HB (s)]	52, F	—	—	35
2. NP	20, F	2	+++	49
[RK (m)]	58, F	—	—	24
3. BC	29, F	5	++	52
[HC (m)]	49, F	—	—	41
4. RV	50, F	1½	++++	108
[BM (s)]	40, F	—	—	36
5. DB	36, M	1	++	48
[WB (b)]	38, M	—	—	39
6. RP	52, F	4	+	36
7. AE	71, M	7	+	39

* Normal adult relatives of patients are indicated in brackets (s, sister; m, mother; b, brother).



TEXT-FIG 1—Profiles of populations of pooled collagen fibrils from the skins of (1) normal adults, (2) patients from Table 1, and (3) the pooled population from 3 therapeutically aborted human fetuses. Measurements were taken from electron micrographs of negatively stained unfixed dermal homogenates.

pig embryo were distributed similarly around a mean of 300–400 Å, with a narrower range of 100–500 Å. In comparison to these values, the fibrils from the patients with scleroderma, which were distributed over a wide range of 100–1700 Å, all contained a significant number of fibrils under 900 Å in diameter, comprising up to 80% of the total population. The mean diameter for the apparently unimodal fibril population in these individuals was 400–500 Å. Thirty-eight percent of the fibrils measured were 400 Å or less in diameter, a size notably ab-

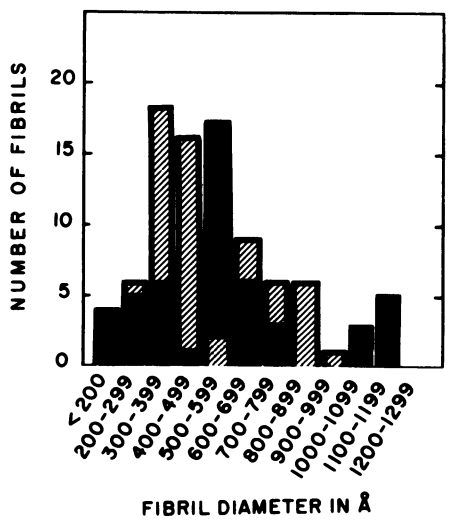
sent from the normal adult fibril population, but comprising the significant component of the embryonic populations.

Comparison of the profiles of collagen fibrils from 2 patients with scleroderma (Text-fig 2) indicates that the characteristics of the fibril population may vary in different stages of the disease. Thus, the percentage of smaller fibrils (200–400 Å) was considerably greater (63% smaller fibrils) in patient RP, who exhibited mild scleroderma of long duration, than in patient RV (24% smaller fibrils), who had very active disease of short duration.

Cross-Banding Patterns of Native Collagen Fibrils

The cross striations observed on negatively stained collagen fibrils of 500 Å or greater diameter in specimens from the normal adults (Fig 1A) were indistinguishable from those in patients with scleroderma (Fig 1B and 1C). Both sets of fibrils revealed a major repeat interval of 640 Å in which at least eight cross bands were clearly visible.

In the case of both sets of prenatal fibrils, however, the cross-striation pattern was incomplete, revealing only two of the intraperiod cross-bands (Fig 2A and 2B). A similar incomplete pattern was found in the thin fibril population (200–400 Å) from patients with scleroderma, as indicated in Fig 2C. This pattern, which was not encountered in larger fibrils, is not in itself abnormal since it is regularly encountered



TEXT-FIG 2—Profiles of collagen fibrils from the skins of 2 patients with scleroderma. Solid bars represent the fibril counts from a patient (RV) with rapidly progressing disease (n=64). Hatched bars represent profile of a patient with an arrested case of long duration (n=51).

in normal embryonic tissue. Reference to Text-fig 1 indicates that this pattern rarely is represented in the normal adult because of the infrequency of small-diameter fibrils.

Beaded Embryonic Filaments

In Text-fig 1 and 2, the hatched bars represent an especially thin fibril found in tissue from 5 of the 7 patients with scleroderma and shared by embryonic tissues. This fibril measures less than 200 Å. It is not found in normal adult skin. In negatively stained specimens, this structure appears as a beaded filament consisting of two parallel strands, each 30–40 Å in diameter, linked by roughly spherical beads, 120–140 Å in diameter, at intervals of 640 Å. As can be seen in Fig 3, the fine structure of the filament found in embryonic tissues (Fig 3A and 3B) is identical to that noted in patients with scleroderma (Fig 2C and 2D). These filaments comprised 5–15% of the total fibril population in the skins of the 5 patients with scleroderma in which they were identified. All of these 5 exhibited evidence of active and severe cutaneous disease. The 2 patients in whom beaded filaments were not found had had scleroderma for 4 and 7 years duration and the degree of skin involvement at the time of biopsy was considered relatively mild.

Discussion

The majority of collagen fibrils in normal adult human skin have been found to range from 700 to 1400 Å in diameter, with a maximum of from 900 to 1000 Å.[4,7,11] In close agreement with these estimates, we have noted an average diameter of 1000–1100 Å, using unfixed material viewed by negative-staining technic.

In the patients with progressive systemic sclerosis, we and others have found that there is a decided increase in the proportion of thinner fibrils, with a corresponding shift in maxima. In the present study, 38% of a total of 217 fibrils measured were less than 500 Å in diameter, and the peak value lay in the range of 300–500 Å. We did not observe the bimodal distribution in fibril diameter reported by Bahr[1] and by Rupec and Braun-Falco.[4] There are several possible reasons for this discrepancy, over and beyond differences in technical approach. In out study, we pooled data from a group of 7 patients, the majority of whom had severe and rapidly progressive disease, while the other studies mentioned above involved a total of only 3 patients with disease of unstated severity.

This preponderance of thin fibrils (less than 400 Å) is characteristic

of recently deposited collagen populations, as seen in embryonic and infant (human) skin,[7,8] and establishes that the thickening of the skin in systemic sclerosis is attributable to an abundance of newly formed collagen fibrils. This conclusion is further supported by our identification of the beaded filament, which comprises up to 15% of the fibril population in rapidly progressing cases of scleroderma. This extremely fine fibril, which has been observed in embryonic dermis from the chick, rat and pig,[10] and which we have found in embryonic human skin, is notably absent from normal human adult skin. This element is believed to be a protofibril of embryonic collagen. On addition, the cross-banding pattern of the (200–400 Å) fibrils present in scleroderma is incomplete. Such thin fibrils are abundant in embryonic material.[10,12,13]

Our preliminary investigation of the fibrocyte in sclerodermatous skin has revealed that these cells display a morphologic appearance indicative of active protein synthesis.[14] These fibrocytes exhibit a well-developed endoplasmic reticulum with dilated cisternae filled with an electron-dense material. Although we have no direct evidence that these cells are specifically engaged in the synthesis of collagen, they are undoubtedly active in the synthesis of some proteinaceous material, which may well be collagen.

Recent studies of the metabolic capacity of skin from patients with scleroderma lend further support to our morphologic evidence of deposition of new collagen fibers Uitto and co-workers have described enhanced activity of protocollagen proline hydroxylase, the enzyme responsible for the hydroxylation of peptide-bound proline.[15]Keiser and Sjoerdsma [16] concluded that the incorporation of labeled proline into collagen in the dermis from patients with scleroderma was increased above normal values. The elevation of hydroxyproline-containing plasma proteins and the increased excretion of hydroxyproline-rich urinary peptides in patients with rapidly progressing disease [17,18] also are in keeping with the conclusion that there is increased collagen synthesis in this disease.

Summary

In a study of the ultrastructure of negatively stained collagen fibrils from the thickened skin (forearm) of 7 patients with progressive systemic sclerosis (scleroderma), we have noted an abnormally great number of thin fibrils (200–400 Å in diameter) with incomplete cross-banding pattern and the appearance of embryonic "beaded filaments." These features are shared by the collagen fibril population of fetal human and pig skin, but not by normal adult human dermis.

Coupled with other morphologic and biochemical evidence of protein synthesis by fibroblasts in the dermis of patients with scleroderma, these characteristics of the collagen indicate that these fibrils are newly deposited as a consequence of increased fibrillogenesis in this disease.

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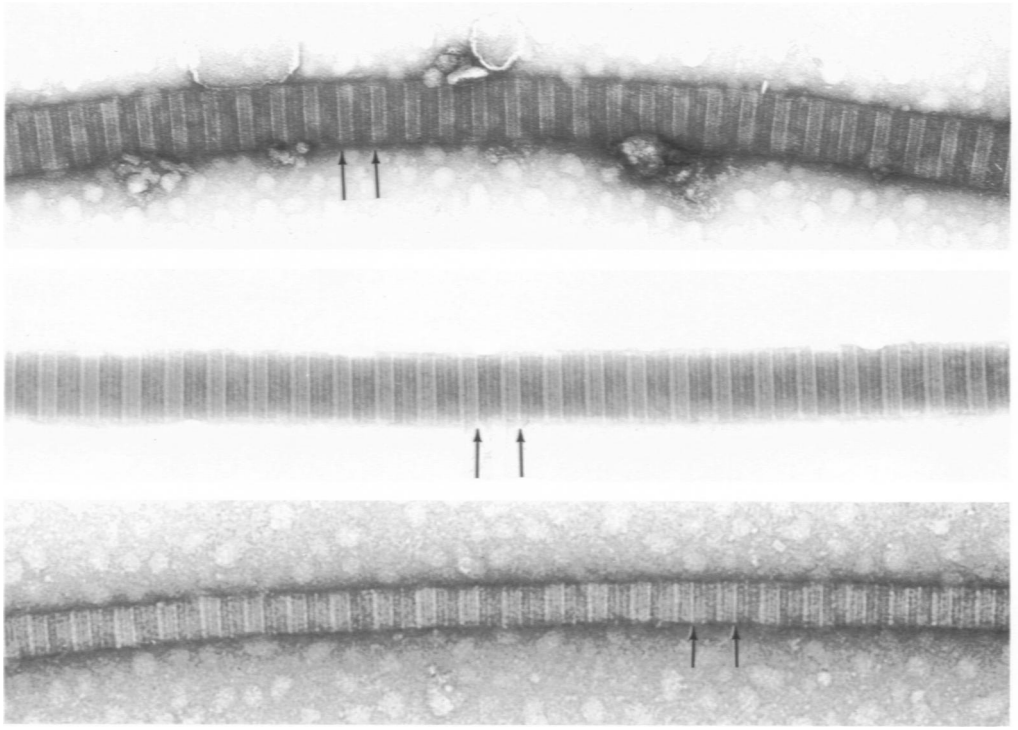

Fig 1—Mature collagen fibrils from skin biopsies revealed by negative staining of unfixed dermal homogenates. **A**—Control, 38-year-old normal male. **B**—20-year-old female with scleroderma. **C**—55-year-old female with scleroderma. All fibrils reveal normal 640-Å periodic cross-banding pattern (*arrows*) with normal intraperiod cross striations (× 76,250).

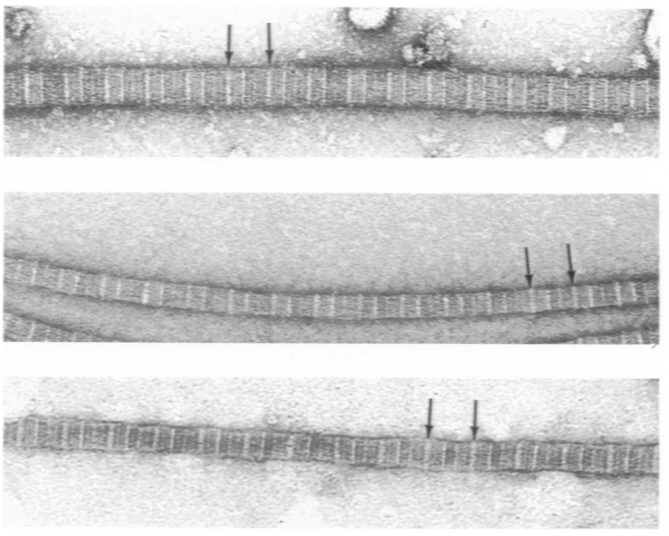

Fig 2—Thin collagen fibrils (200–500 Å diameter) from skin biopsies revealed by negative staining of unfixed dermal homogenates. **A** — 10-week human fetus. **B** — 150-mm fetal pig. **C** — 29-year-old female with scleroderma. These were not observed in adult control tissues. Although 640-Å periodic striation pattern is established (*arrows*), intraperiod cross banding is incomplete in these small fibrils (× 76,250).

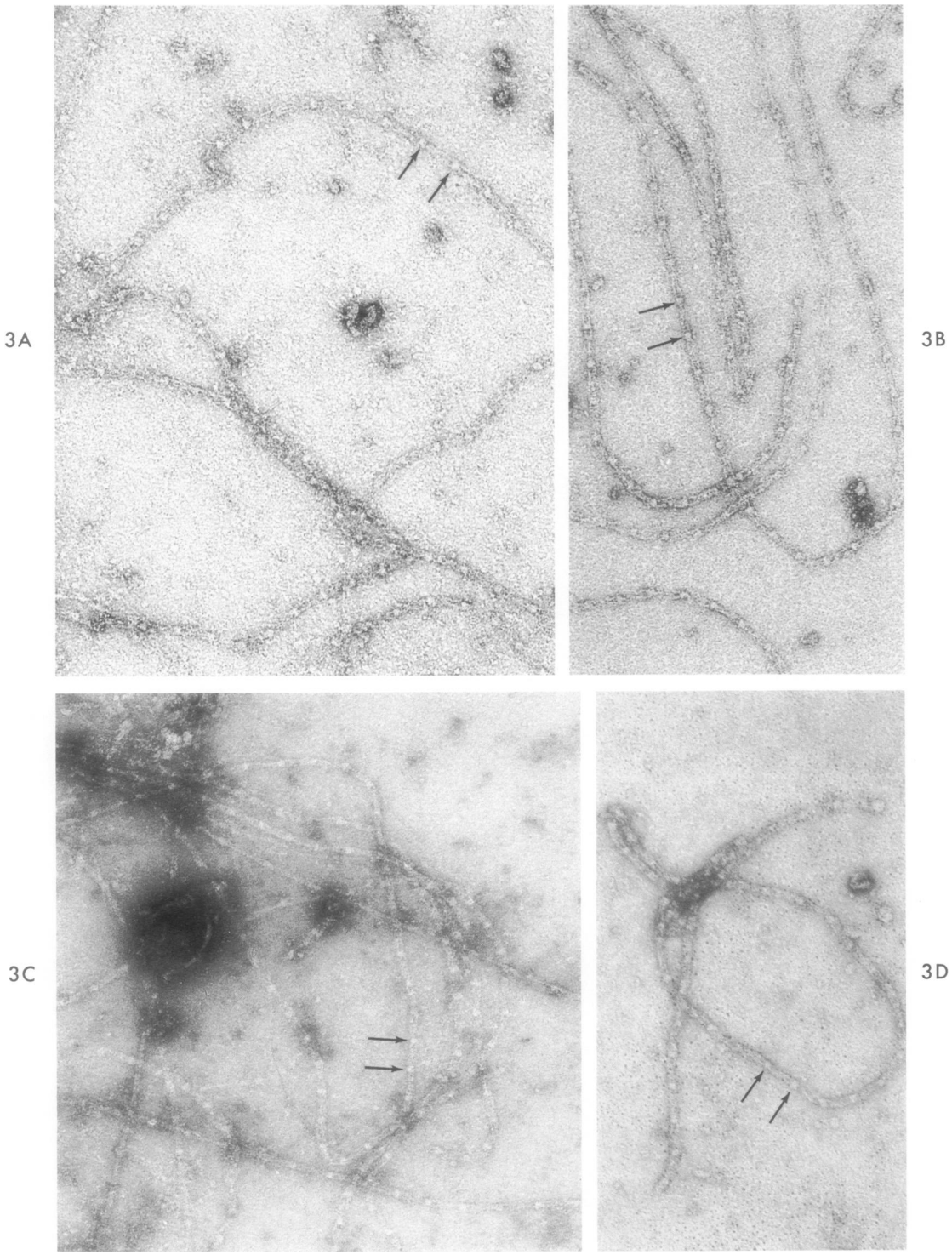

Fig 3—Beaded filaments, revealed by negative staining of unfixed dermal homogenates. **A**—10-week human fetus. **B**—115-mm fetal pig. **C**—50-year-old female with scleroderma. **D**—29-year-old female with scleroderma. Note doublet strands, 30–40 Å in diameter, joined at 640-Å intervals (*arrows*) by single spherical beads 120–140 Å in diameter. These structures are not seen in homogenates of adult control tissues (× 76,250).