NORMAL CHARACTERISTICS OF DERMATOSPARACTIC CALF SKIN COLLAGEN FIBERS FOLLOWING THEIR SUBCUTANEOUS IMPLANTATION WITHIN A DIFFUSION CHAMBER INTO A NORMAL CALF

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1. Introduction

The heritable connective tissue disorder in cattle, dermatosparaxis, is characterized by a conspicuous fragility of the skin due to the formation of disorganized collagen bundles in the dermis and impaired alignment of the individual polymers [1, 2]. It has been shown that the dermatosparactic skin contains increased amounts of precursor molecules, procollagen, which have a characteristic non-helical cysteine-containing polypeptide extension at the N-terminus of each of the alpha-chains [3–5]. It has also been shown that this alteration results from the lack of an enzyme activity that normally catalyzes the excision of the additional peptides. The absence or reduction in conversion of procollagen to collagen leads to the accumulation of the former under its polymeric form in the extracellular space [3, 6]. Beside two possible roles of the extension peptides of procollagen in a biological system, namely coordination of the assembly of the three alpha-chains into a triple helical conformation [7], and transport of the molecule out of the cell into the extracellular space where the collagen fibers are ultimately formed [8], a third function, outside the cell, has been suggested, namely control of fibril formation [9–11].

In this article we present experimental results supporting the involvement of the extension peptides of procollagen in the physiological process of collagen fibers formation. Obvious modifications have been observed by electrophoretic, microscopic, and X-ray diffraction studies when comparing fibers derived from the dermis of dermatosparactic calves, before and after being implanted under the skin of a normal calf using the diffusion chamber technique [12].

2. Methods

Samples of dermatosparactic and normal calf skin were cut into squares of about 1.5 × 1.5 cm, sectioned on a cryostat and both the epidermis and subcutis removed under observation in the microscope. The remaining thin corium was placed in a diffusion chamber made of millipore filters, pore size 0.45 μ, glued on plexiglass rings (I.D. 2.5 cm) as described earlier [12]. The sealed chambers were implanted under the back skin of a normal calf and removed after 4–8 days. All procedures were carried out under strictly aseptic conditions. The tissue slices retrieved from the chambers were analyzed as follows. A fragment of each tissue slice was processed for histological examination; another fragment was cut into a thin strip, stretched and air-dried for X-ray diffraction studies; the remaining parts of the slices were pooled and ground for extraction of collagen with 0.5 M acetic acid and further purification for polyacrylamide gel electrophoresis as previously described [3]. Quantitative studies

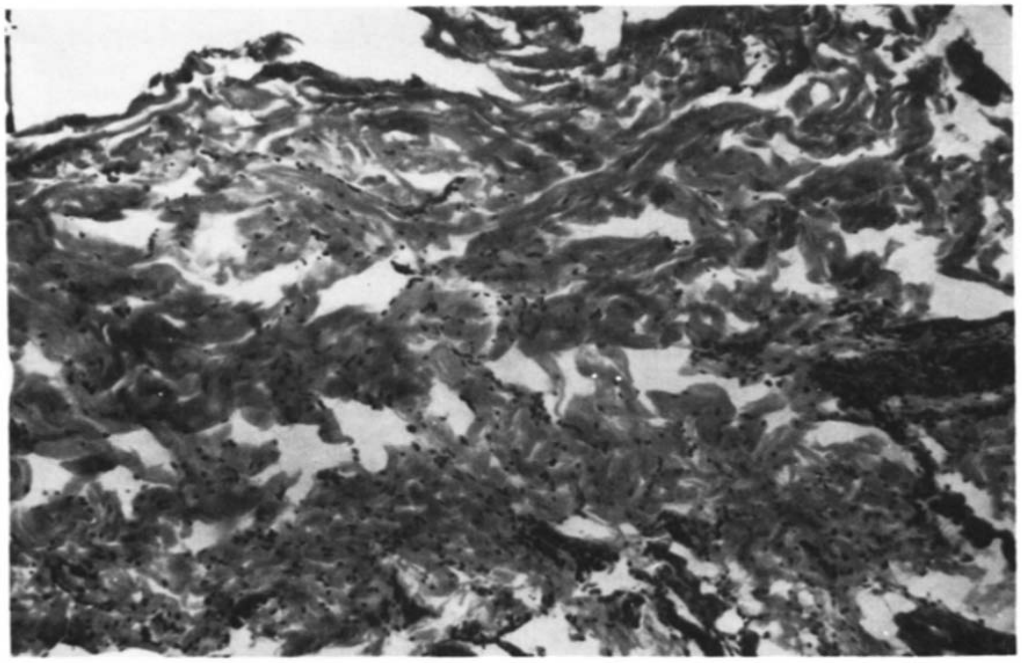

Fig. 1. Photomicrograph of a normal skin preparation before implantation inside the diffusion chamber. Note the density of the collagen fibers and the absence of epithelium and subcutaneous tissue components. Hematoxylin-Eosin × 105.

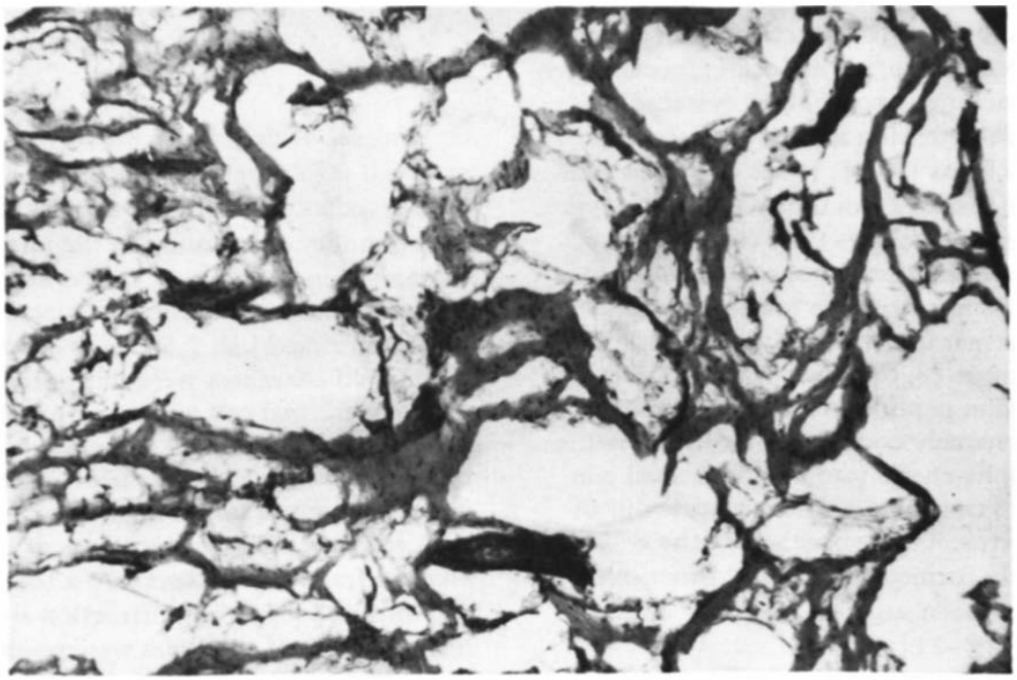

Fig. 2. Photomicrograph of dermatosparactic dermis before implantation into the subcutis of a normal calf. Note the spongy appearance of the dermal collagen framework and the abundance of thin collagen fibers. H.-E. × 105.

to determine the degree of solubility of dermatosparactic skin collagen before and after implantation were not carried out in this series of experiments.

X-ray diffraction photographs were taken with a Chesley fiber microcamera adapted for specimen-to-film distance of 20 mm and 50 mm for high and low-angle patterns respectively. Additional low-angle photographs, not shown in this paper, were taken on a Rigaku-Denki camera at a specimen-to-film distance of 270 mm. The specimen was kept stretched and in a helium atmosphere at room humidity.

3. Results and discussion

The photomicrographs of the dermatosparactic dermis before implantation clearly demonstrate the distorted organization and the conspicuously reduced thickness of the collagen bundles, as compared to sections prepared from the skin of normal calves. They also showed that clean corium preparations were obtained by the cryostat technique (figs. 1, 2).

The morphology of the dermatosparactic corium was entirely changed after implantation under the skin of a normal calf (fig. 3). The thin spongy structures of the affected corium are replaced by denser and thicker collagen bundles differing only slightly from those of the normal dermis. It is evident that the affected corium inside the diffusion chamber underwent organizational changes due to some diffusible active factor, since no cells penetrated the diffusion chambers. The disc electrophoretic pattern of the denatured dermatosparactic skin collagen after implantation showed a disappearance of the two additional bands characteristic of pro-alpha$_1$ and pro-alpha$_2$ present before implantation (fig. 4). The proportion of precursor polypeptides is similar to that observed earlier in an acetic acid extract [3]. Several dimers (beta's) are also present. After implantation, both precursors of alpha-chains are no longer present and the pattern of the dimers (beta's) has become that observed in normal skin. The relative proportion of dimers is slightly increased. The disappearance of pro-alpha chains after implantation and the increased proportion of dimers are consistent with the conversion of procollagen to collagen by procollagen pepti-

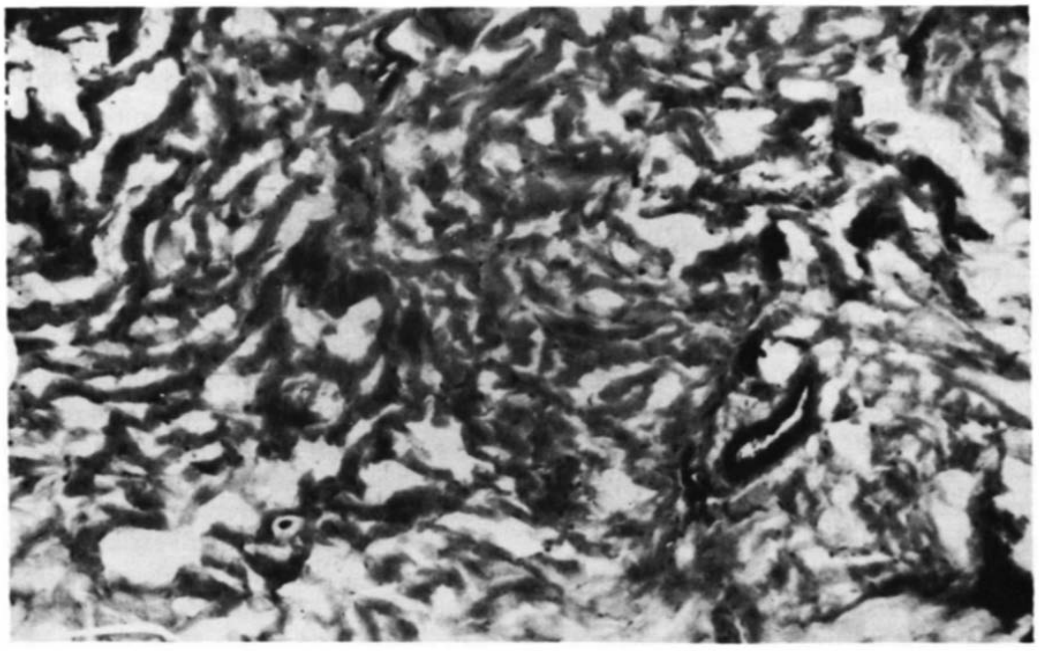

Fig. 3. Photomicrograph of dermatosparactic skin following implantation with a diffusion chamber into the subcutis of a normal calf. Note the increased density of the tissue and the thickness of the collagen bundles. H.-E. × 105.

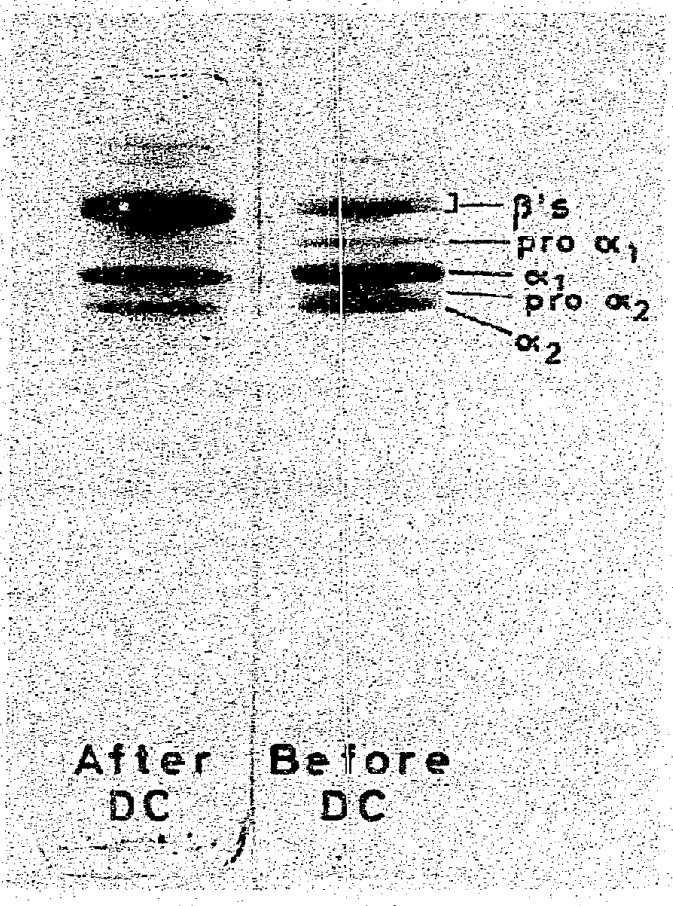

Fig. 4. The band pattern of dermatosparactic collagen polypeptides following disc electrophoretic separation on polyacrylamide gels before and after implantation into the subcutis of a normal calf. Note the disappearance of the bands representing pro-alpha$_1$ and pro-alpha$_2$ following implantation.

dase [6]. Non specific proteolysis would indeed excise the polypeptide extensions but then also the telopeptide regions would be cut off resulting in the conversion of dimers (beta's) into monomers (alpha-chains).

The X-ray diffraction studies showed that collagen from the dermis of dermatosparactic calves exhibited a typical collagen-like high-angle X-ray pattern, including the meridional 2.9 Å reflection (fig. 5). This indicates that the molecules are in the normal triple helix conformation [13]. However, the low-angle meridional X-ray pattern, which comprises the various orders of the 650 Å repeat, was found to be much weaker for dermatosparactic dermis than for a dermis from normal calves. To make this comparison independent of extraneous factors, such as specimen thick-

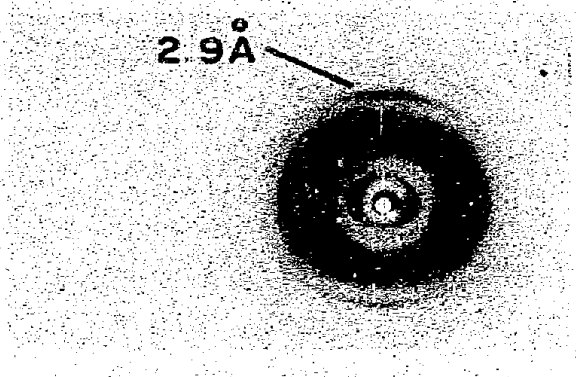

Fig. 5. High-angle X-ray photograph of stretched collagen from dermatosparactic calf skin.

ness or exposure time, X-ray photographs were taken to show features of both the high- and low-angle patterns, so that their relative intensities could be estimated (fig. 6). Comparisons were also facilitated by stretching the specimens in order to orient equally well the normal and the dermatosparactic collagen fibers. The weak low-angle pattern of the latter, therefore, indicates that the 4.4. staggered molecular assembly in fibrils [13] is impaired in the dermatosparactic fibers. However, the specimen of dermatosparactic collagen, photographed after implantation, showed a more normal intensity ratio between the low- and high-angle X-ray patterns (fig. 6c).

The observed results demonstrate in vivo what has been postulated following earlier studies in vitro [6, 9–11], namely that physiologically functioning collagen fibers with normal mechanical strength are properly organized in the extracellular space after the extension peptides of procollagen have been cleaved off from the precursor molecules. These peptides actually might act as a control in the assembly of the molecules to form fibers. The X-ray diffraction results indicate that the non-helical polypeptide extension at the ends of the molecules in dermatosparactic collagen allows the winding of the crystalline part of the alpha-chain to form the normal collagen triple helix. In the extracellular space, in absence of removal of these bulky globules, the molecules are prevented from approaching each other sufficiently closely (about 10 Å) to form compact fibrils with normal mechanical strength. This ultimately depends upon the formation of covalent crosslinks by reaction of the lysyl aldehydes. It occurs after excision of the peptide extension [14]. The organisation of the procollagen fibers

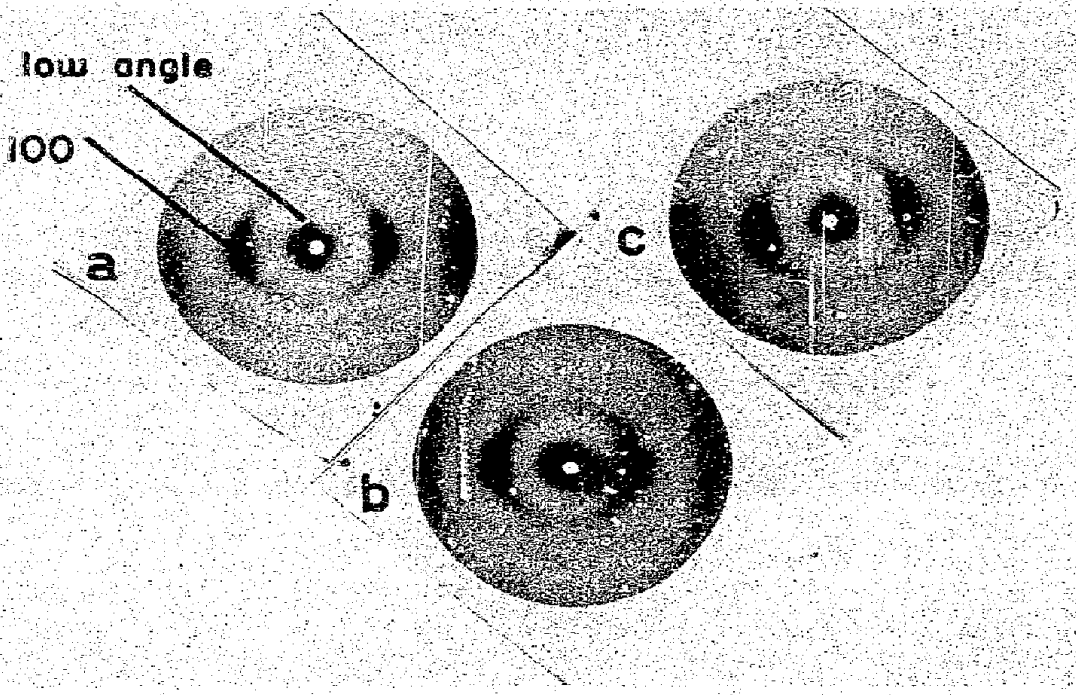

Fig. 6. X-ray photographs of stretched collagen specimens showing the 100 reflection of the high-angle pattern together with several orders of the low-angle pattern. Specimens were (a) normal calf skin, (b) dermatosparatic calf skin and (c) dermatosparatic calf skin after implantation into the subcutis of a normal calf.

has occured in the diffusion chamber most probably after the activity of procollagen peptidase, the specific excising enzyme commonly found in the extracellular space [15] and known to operate upon polymeric procollagen [6].

The most surprising observation is the modification in architecture observed when the procollagen framework is converted to collagen fibers, in absence of living cells. To be explainable one has to accept that the undulating procollagen fibers shrink after removal of the peptide extensions and during the process of completing polymerization. This property would be most interesting to allow the parallel packing of the fibrils to form bundles and to insure a suitable tension in the connective tissue fibers together with their increased resistance to stress.

Initial findings in electronoptical investigations also confirm a higher structure of fibrils after implantation. This will be published elsewhere.

Acknowledgments

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