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A Morphologic Study of a Mild Form of Ovine Dermatosparaxis

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A mild form of dermatosparaxis has recently been identified in sheep from several properties in central Victoria. Examination of the skin of affected animals by both transmission and scanning electron microscopy has shown that the structure of the majority of the collagen fibrils is irregular and the distorted fibrils do not pack into tight and well-ordered fiber bundles. Examination of the skin by light microscopy has shown that the fiber bundles are not compact and interwoven, and that there is a tendency for the collagen to form layered sheets in the reticular dermis. These studies also show that there is no buildup in the elastic tissue component, but that there is an increased population of fibroblasts in the affected animals.

Dermatosparaxis is caused by a deficiency in the conversion of pN-collagen to collagen and produces skin fragility in affected animals. The disease has been reported to occur in sheep [1–4], cattle [5], a dog [6], and a Himalayan cat [7,8]. It has been proposed [9] that the disease is caused by a deficiency in the procollagen aminopropeptidase, and the recessive inheritance of the condition is consistent with this proposal [1,10]. A biochemically analogous recessive disease which occurs in humans is Ehlers-Danlos syndrome type VII (ED-VII) [11] but this disease differs phenotypically from dermatosparaxis, being characterized by skin elasticity, joint hypermobility, and dislocations [6,11,12]. A dominant form of ED-VII has also been reported in which a structural mutation affecting the α_2[I] collagen chain has been proposed as the biochemical defect [13]. A variety of similar fragile skin conditions have been reported in other animals [14–20] but they have not been characterized fully at a biochemical level. In many of these cases the skin also exhibits hyperelasticity, but this condition is not always observed in dermatosparaxis [14–17,20].

Initial reports of the occurrence of dermatosparaxis in sheep describe a severe condition affecting newborn lambs [1,2] but a mild form of the disease has recently been reported [3,4] and found to occur on several properties in central Victoria. This mild form of the disease has not been observed in lambs, but is usually first noticed when adult sheep (1–4 years old) are shorn. It was shown that this mild form of dermatosparaxis was due to a defect in the processing of procollagen which leads to an accumulation of pNα_1[I] and pNα_2[I] chains in the skin. The identity of these pNα chains was demonstrated by polyacrylamide gel electrophoresis, amino acid analyses, segment-long-spacing aggregates, and susceptibility to pepsin and collagenase digestions [4]. In the previous severe form of ovine dermatosparaxis, all the type I collagen was present as pNα chains. In the present mild form, however, a reasonable proportion (>60%) of normally processed collagen is present in the skin of affected animals [4]. With respect to the collagen composition of its skin, this case is therefore more closely related to the mild form of dermatosparaxis found in the Himalayan cat [7,8] than to the previous severe case of ovine dermatosparaxis [1,2]. However, in contrast to all previous cases of dermatosparaxis, some procollagen aminopeptidase activity has been observed in the present mild ovine case. Extracts from the skin of affected animals were shown to contain an aminopropeptidase activity which had about 25% of the activity found in similar extracts from normal animals [4].

Lapière and coworkers have recently shown that fibroblasts from dermatosparactic calves have lost their ability to retract a collagen gel [21]. They have proposed that the absence of procollagen aminopropeptidase activity in previous cases of dermatosparaxis was due to a defective metabolic pathway for intracellular processing of mannose-containing oligosaccharide side chains which indirectly eliminates the peptidase activity [21]. Since in the present mild form of ovine dermatosparaxis, aminopropeptidase activity is present [4], this new case may represent a biochemically distinct form of the disease. This present report describes the skin morphology for the mild form of ovine dermatosparaxis so that comparisons may be made with other forms of dermatosparaxis and similar heritable skin disorders.

MATERIALS AND METHODS

Biological Samples

Skin samples were taken from mature (1- to 4-year-old) live animals from 3 different properties in central Victoria by means of a 9-mm biopsy gun [22]. Samples were fixed in half-strength phosphate-buffered Karnovsky fixative [8,23].

Electron Microscopy

For transmission electron microscopy samples were prepared as described previously [4] and examined on a Hitachi HS8 microscope at 50 kV. For scanning electron microscopy, the fixed samples were taken through a graded series of alcohols to absolute alcohol, transferred through a series to amyl acetate, and critical point dried using liquid CO_2. The samples were then sputter-coated with gold and examined on a JEOL JSM T20 microscope at 19 kV in the secondary electron mode.

Light Microscopy

Fixed samples for light microscopy were either sectioned directly or after embedding in 20–25% w/v gum arabic solution (R.I. 1.380) using a cryotome at −20°C. Thin (10 μm) sections were stained with Weigert's elastin stain, Harris' hematoxylin and eosin, Masson's trichrome [24], or with picrosirius red [25]. Samples were examined using an Olympus BHS microscope with either normal or differential interference contrast optical systems. Samples stained with picrosirius red were examined under polarized light.

RESULTS

The clinical condition of dermatosparaxis is characterized by fragile skin and is caused by a processing disorder in the conversion of procollagen to collagen. Previous cases of dermatosparaxis have shown that when the deficiency in this conversion is greatest, the clinical condition is the most severe [2,7]. Biochemical studies on this mild case of dermatosparaxis have confirmed that this processing deficiency is present [4]. The deficiency, however, is only partial and fully processed collagen is present in the skin of affected animals [4], which is consistent with the mild form of the disease.

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Abbreviations:
ED-VII: Ehlers-Danlos syndrome type VII

The present mild form of ovine dermatosparaxis has been observed in several fine wool merino flocks in central Victoria. The genetic origins of each of these flocks suggest that all instances of the disease are related by a single common bloodline, although this has not been confirmed. In the previous severe form of ovine dermatosparaxis there was extensive skin tearing at birth [1,2]. In this mild case, tearing has been observed only in older animals, and in all cases the tearing was induced by the handling when attempts were made to shear the animals. The youngest animal in which the condition has been observed was about 10 months, and affected animals up to 6–7 years old have been observed.

The skin of affected sheep tears with mild application of force, such as may occur while the animal is restrained, and a finger can easily be pushed through the skin by accident. Animals have been seen where the skin has torn through its full thickness, and in such cases a flap of skin up to 150 × 150 mm has been observed to be displaced. In other animals, larger areas of skin have separated below the level of the wool follicles and in these cases the papillary dermis and the fleece were lost from the animal, leaving the reticular dermis as the new surface. In both types of tearing there was only a very slight amount of bleeding.

When affected sheep are kept in pens, the tearing heals and new skin and fleece form if infection is eliminated. In the field, however, infections and fly-strike cannot be as readily controlled and the healing is poor and areas devoid of fleece remain.

Apart from the fragile skin, the affected sheep had no other readily observable abnormalities. Thus there was no evidence for either skin hyperelasticity or for joint hypermobility. However autopsies of 2 affected animals indicated that the trachea collapsed more readily and some muscle tissues showed tearing damage (Dr. R. T. Badman, personal communication). The skin of affected animals was thicker (1.5–2.5 times) than that of normal sheep of the same breed, this being particularly evident in the whole skins derived from autopsy.

At the optical microscopy level, neither differential interference contrast nor stains for elastin showed any difference between normal and dermatosparactic skin, either in the amount or the organization of the elastin. This contrasts with the report of excessive elastin fibrils in dermatosparactic tissue [8], although the method used to see the elastic component may have been other than light microscopy [8].

In dermatosparactic sheep, in contrast to normal animals, two types of architecture of the collagen fiber bundles are observed in different positions on the same animal by light microscopy. Although it has not been established conclusively whether these 2 structural types occur at specific sites on the body, they are sufficiently distinct to be described separately. The first structural type (Fig 1*B*) is similar to that observed in normal skin (Fig 1*A*) although most fiber bundles are smaller than those of normal skin and appear to be more contorted (Fig 1*B*). In addition, the entire structure appears to be significantly more open than normal skin with more space between the fiber bundles. The second structural type (Fig 1*C*) comprises sheets of fiber bundles which generally lie parallel to the skin surface and to each other. In this structure also, the sheets are well separated from each other and the overall appearance is of a very open structure (Fig 1*C*). To demonstrate that the sheet structure was not caused by fiber bundles lying parallel to the sectioning plane, sections were cut at right angles and these also showed the sheet structure. The layered structure of the collagen may explain the observed splitting of the skin at the middermis level. The layered structure is particularly in evidence in sections where the skin is thickest, such as the flanks.

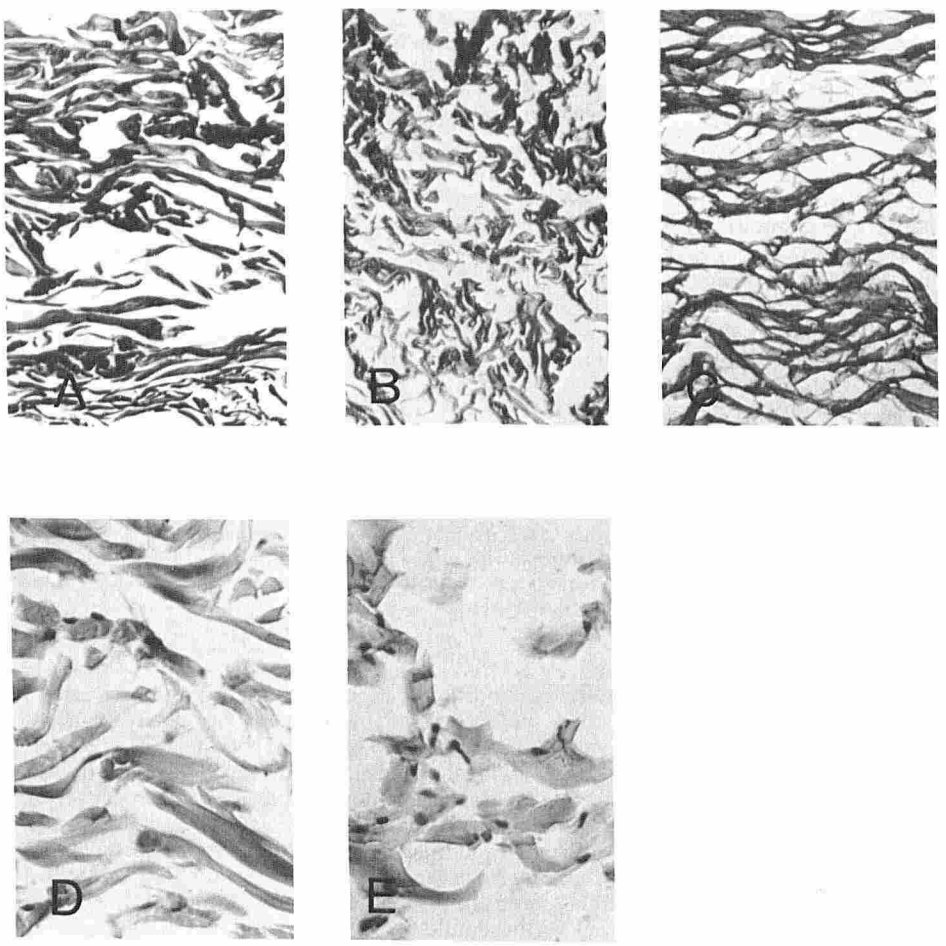

FIG 1. Light microscopy of skin from normal (*A*,*D*) and affected sheep (*B*,*C*, *E*). The two structural types in affected sheep (*B*,*C*) contrast with the normal skin structure (*A*) (picrosirius red, ×100). The differences in fibroblast population and density of collagen fiber bundles between normal (*D*) and affected skin (*E*) are evident at higher magnification (hematoxylin and eosin, ×250).

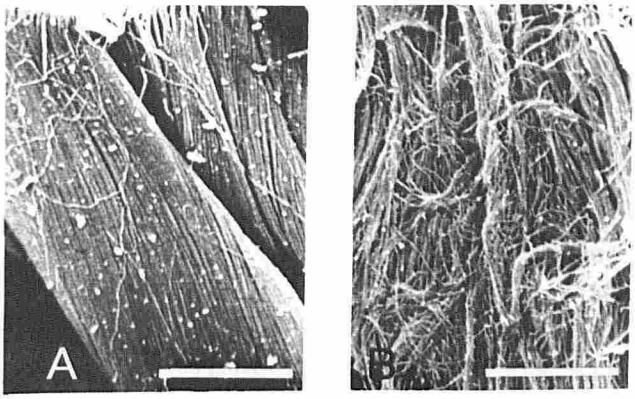

FIG 2. Scanning electron microscopy of normal (*A*) and affected (*B*) skin showing the loose, convoluted nature of the fibrils. *Bars* = 0.01 mm.

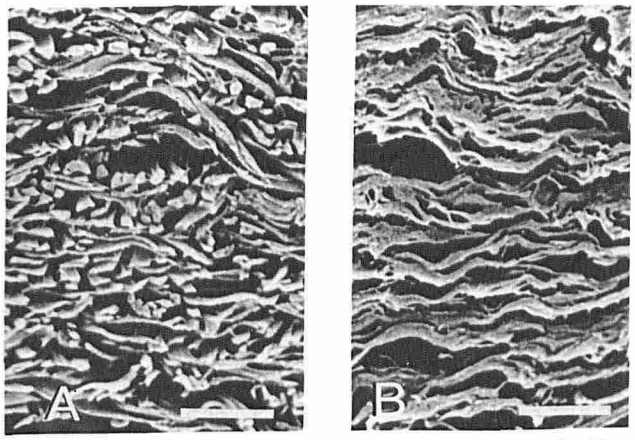

FIG 3. Scanning electron microscopy of normal (*A*) and affected (*B*) skin showing the sheet structure in dermatosparactic skin. *Bars* = 0.1 mm.

In both structural types, dermatosparactic skin has about 3–4 times as many fibroblasts per unit cross section as does normal skin. This was observed by counting fibroblasts, stained with hematoxylin and eosin, in fields under the microscope or in photographs of such fields (Fig 1*D*,*E*).

The intensity of staining of the collagen of skin from affected animals with hematoxylin and eosin is noticeably decreased; normal skin collagen is stained a strong pink but dermatosparactic skin collagen is stained only faintly. Others have observed a similar faint staining of the collagen from dermatosparactic skin using van Geison's picrofuchsin or hematoxylin and eosin [2]. In the present study, sections of the same thickness (10 μm) of both normal and dermatosparactic skin were stained for the same time with eosin (2 min). Extension of the staining time to 12–17 min did not result in any greater uptake of eosin by the affected skin. This effect was present in both the papillary and the reticular dermis.

The different skin types were also compared under polarization microscopy after staining the collagen with picrosirius red. No significant differences were noted between the various samples of dermatosparactic and normal skin when stained with picrosirius red or with Masson's trichrome apart from the very obvious layered structure in the skin of affected animals.

Scanning electron microscopy (Fig 2) shows that the fibrils that make up the fiber bundles in dermatosparactic skin are loosely packed (Fig 2*B*) when compared with the tight packing observed in normal skin (Fig 2*A*). Also, as part of this more open structure, the fibrils follow convoluted rather than straight paths through the fiber bundles. At lower magnification levels, scanning electron microscopy also shows (Fig 3) that fiber bundles in the dermatosparactic skin are less distinctly organized and smaller (Fig 3*B*) than the fiber bundles present in normal skin (Fig 3*A*). In the dermatosparactic skin the sheet structure is seen clearly with few fiber bundles extending vertically through the skin.

Transmission electron micrographs of the reticular dermis of the affected animals (Fig 4) show the distorted collagen fibrils that are characteristic of dermatosparaxis [2,6,8,26]. A proportion of the fibrils visible in cross section (Fig 4*A*) are almost circular, like the fibrils from normal animals. This combination of distorted and apparently normal fibrils is similar to the appearance of the fibrils in a dermatosparactic cat [8] which also had a mild form of the disease, but contrasts greatly with the fibrils from the previous severe case of ovine dermatosparaxis in which all of the fibrils were highly distorted [2]. Longitudinal views of the collagen fibrils (Fig 4*B*) show that the fibrils are formed of twisted ribbons, and that these ribbons twist in both right- and left-handed directions.

DISCUSSION

The present case of ovine dermatosparaxis is mild and has not been seen in newborn or young animals [3,4]. Previous studies have shown that it is caused by a deficiency in the processing of procollagen which leads to an accumulation of some pNα_1[I] and pNα_2[I] collagen chains in the skin [4], and that this processing deficiency is associated with a reduced level of aminopropeptidase activity [4]. Although the clinical condition is mild compared to other cases of dermatosparaxis, the observed changes to the skin show that a small change in the collagen biosynthesis can have quite distinct effects on the structure and function of the skin.

In animals affected with this mild form of ovine dermatosparaxis, there is a tendency for the collagen to form layered sheets in the reticular dermis and to be loosely packed; this gives a much more open appearance than that observed for normal skin. A similar sheet structure has been previously observed in a case of bovine dermatosparaxis [27] and in EDS-VII [28]. Light microscopy demonstrates an increased population of fibroblasts in the skin of diseased animals, but it has not been determined whether this leads to an increased level of synthesis of collagen, proteoglycan, or other tissue components. Previous reports [6,29] have indicated that there is an increase in the amount of mucopolysaccharide, particularly hyaluronic acid [29], present in the skin of dermatosparactic animals but no compositional data are available for the animals in the present study.

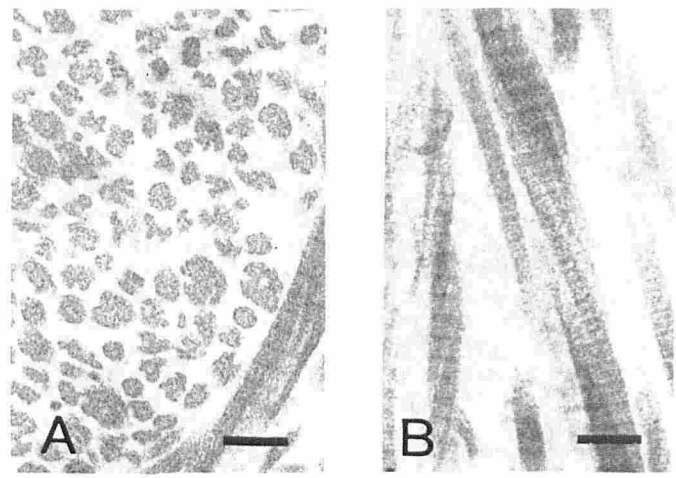

FIG 4. Transmission electron microscopy of skin from affected animals showing a small proportion of apparently normal circular fibrils (*A*) and ribbons (*B*) twisting in both directions. *Bars* = 200 nm.

Dermatosparaxis is caused by a deficiency in the conversion of pN-collagen to collagen and results in an accumulation of pN-collagen in the tissues [9,30]. In all cases of dermatosparaxis reported previously, the accumulation of pN-collagen has caused the development of irregular hieroglyph-like collagen fibrils [2–6,8]. In addition to being irregular in form, the fibrils and the resulting fibril bundles are loosely packed in the tissue and this can be observed in both the transmission and scanning electron microscopes [3,8,27]. In ED-VII, ultrastructural examination shows a high proportion of fibrils which have an apparently normal circular cross section together with some collagen "flowers" [6]. Between the various cases of dermatosparaxis, the proportion and extent to which fibrils show these irregularities of structure increase with both the biochemical and clinical severity of the condition [2–4,7,8,26].

Although a deficiency in the pN-collagen to collagen conversion is a readily identifiable cause of this disease, it is not certain that a deficiency of the pN-collagen aminopropeptidase itself is the primary cause in all instances. In the case of dermatosparaxis in calves, it has been proposed that the disease may be caused by a defect in the metabolic pathway producing the mannose-containing oligosaccharide side chains, and this then indirectly affects the aminopropeptidase activity [21]. However, in the present mild form of ovine dermatosparaxis it is more likely that the primary defect is in the aminopropeptidase itself since a reduced level of this specific activity is detected in the affected animals [4]. No specific enzyme activity has been detected in any of the previous cases, including the very mild form that was found in the cat [7].

Clearly, the change in collagen biosynthesis, with its effect on the packing of the fibril at a molecular level (Fig 4), is not the only effect, and a variety of concurrent changes occur which affect other skin components. The concurrent changes observed with both dermatosparaxis and ED-VII may reflect, singly or in combination, either the different genetic causes for the disease, the differences in composition of normal skin between species, or differences in the feedback mechanisms that control the biosynthesis of the various tissue components. It is known that the pN-peptide is involved in these feedback mechanisms and affects cellular protein phosphorylation in addition to controlling the overall levels of collagen synthesis [31–33].

While ED-VII is biochemically similar to dermatosparaxis, it is very different phenotypically, exhibiting hyperelasticity of the skin without undue fragility and also showing irregularity in joint function. However, the observed similarity in phenotype between the recessive and dominant forms of ED-VII, which differ in the biochemical mechanism by which the pN-peptide is retained, suggests that the genetic mechanism for the interruption of the feedback mechanisms is of less importance than the interruption itself.

Many of the various concurrent changes that are present are best seen by histology. It is observed that while all the cases of dermatosparaxis and ED-VII show some resemblance to one another, a variety of histologic differences exists between skin samples from these examples.

The differences between the cases of dermatosparaxis found in various species and ED-VII in humans can be illustrated by consideration of certain characteristics of the affected skin when compared to normal skin. Both of the cases with sheep show the development of a thicker skin. The skin may also be thicker in the calves [5,27], but is thinner in the cat [8] and in ED-VII [28]. The sheet structure of the fibril bundles that was observed in the present study is also found in calves [27] and in ED-VII [28] but was not found in the cat [8]. In both cases in sheep, there is no apparent increase or alteration in the elastin component of the skin [2, this work] whereas in both cat [8] and calves [27] there is an increase in the amount of elastin. In ED-VII, there is also a change in elastin morphology [6,28]. Hyperelasticity of the skin was reported with cat and ED-VII (6–8,13,28], but not with either of the cases in sheep or in calves. Both of the sheep examples show a weak staining response with hematoxylin and eosin [2, this work] but for the calves, the staining with hematoxylin and eosin is normal [27]. A significant increase in the number of fibroblasts was noted in the present study with sheep and a similar excess may also occur in calves (see Fig 2 in [27]); a normal level was found in the cat [8]. The cat is apparently unique in that the differences that were observed were confined to the reticular dermis and no changes were observed in the papillary dermis [8].

The present study on a mild form of ovine dermatosparaxis has shown that irregularities in the organization of the collagen occur at a fibrillar level. These are consistent with those observed in other cases of dermatosparaxis [2–6,8], particularly the mild form of the disease found in a cat [8]. A variety of other changes in the skin have also been observed, particularly by the use of light microscopy. These changes were very similar to those observed in the severe form of dermatosparaxis previously reported in sheep [2]. The data from the present study, along with that previously reported for other cases of dermatosparaxis and ED-VII, suggest that two generalizations can be made about the skin morphology in dermatosparaxis and ED-VII. Firstly, comparisons between the species indicate that for a given biochemical defect, the concurrent changes that occur differ between the species. Secondly, comparisons between the two different cases observed in sheep indicate that the concurrent changes are moderately constant within a species and may be independent of the severity of the condition.

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