

The Ultrastructure of Collagen in the Dermis of Tight-skin (Tsk) Mutant Mice

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A recently discovered dominant mutation in C57/B10 mice called tight-skin (Tsk) results in hypertrophy of certain collagenous tissues including the dermis and hypodermis. The skin of heterozygotes (Tsk/+) is indurated and substantially stiffer than that of the normal animals (+/+). In this study, an electron microscopical comparison of the skin of these animals revealed that the fibrous architecture of the hypertrophic reticular dermis of Tsk/+ mice is more disorganized than that of the +/+ mice and in many areas, the collagen fibrils are more densely packed. The abundance of fibroblasts with distended endoplasmic reticulum in both the dermis and hypodermis of Tsk/+ mice is consistent with increased collagen synthesis. Several of the changes in the dermis and hypodermis of the Tsk/+ mice are similar to changes reported in scleroderma skin of man. Surprisingly, an apparent abnormality in the morphology of some of collagen fibrils in the skin of Tsk/+ mice was found to be at least as prevalent in the "normal" +/+ mice. The reticular dermis of both animals contain scattered fibrils which are much larger in diameter than normal and often have a twisted appearance resulting from either helical grooves in the surface of the fibril or discrete branches which twist about one another.

A dominant mutation in the mouse called "tight-skin" (Tsk) was discovered in 1967 by Helen Bunker in the B10.D2/Sn strain at the Jackson Laboratory. The integument of animals heterozygous (Tsk/+) for this mutant gene lacks the pliability, resilience and deformability typical of normal skin. Green, Sweet, and Bunker [1] have shown that the Tsk mutation primarily affects the connective tissues and results in hypertrophy of small tendons, tendon sheaths, cartilage, bone and loose connective tissue. The subcutaneous connective tissue in particular was reported to be both thicker and more cellular than normal. Electron microscopical study of the hypodermal areas revealed scattered collagen fibrils in the midst of masses of fine "microfibrils" measuring about 10 nm in diameter and lacking a banded periodicity [1]. No abnormalities were reported in the dermis itself.

In a recent light microscopical and rheological study of the skin of Tsk/+ mice, the dermis was found to be abnormal in both structural and tensile properties compared to that of normal siblings (+/+) [2]. The dermis from the ear, back, and abdomen, for example, proved to be nearly twice as thick as normal and appeared to lack the regular weave pattern typical of normal dermal collagen. Instead the fibrous architecture of the Tsk/+ dermis is generally disorganized and in some areas appears amorphous or hyaline in the superficial half of the

dermis. In addition, the Tsk dermis is usually more cellular than normal.

Rheological studies have shown that the Tsk/+ dermis is indurated and substantially stiffer than that of normal siblings [2]. Of particular interest was the observation that the elastic stiffness of tight skin is similar when stretched either in the longitudinal axis of the body or transversely whereas the skin of the +/+ mice shows anisotropy and is substantially stiffer when stretched in the transverse axis than in the longitudinal. These abnormal directional and tensile properties of the skin of Tsk/+ mice were interpreted to be at least in part a result of alterations in the synthesis and fibrous architecture of dermal collagen.

The purpose of this study was to investigate the effects of the Tsk mutation on the fine structure and organization of the fibrillar and cellular elements of the dermis in the hope that this might reveal some morphological basis for the altered rheological properties of Tsk/+ skin. Particular attention was given to the hyalinized areas of the superficial dermis in an effort to determine what structural alterations might be responsible for this homogenous appearance.

MATERIALS AND METHODS

Full thickness dorsal skin was dissected from 10 tight skin (Tsk/+) mice and 10 mice homozygous for the normal allele (+/+) which were raised by inbreeding (Tsk/+) x (+/+) from stock originally obtained from the Jackson Laboratory (courtesy of Dr. Margaret Green). All mice used for microscopical study and comparison were males between 2 and 4 mo old. For light microscopical study, large pieces of skin were fixed in Bouin's fluid, dehydrated, cleared and embedded in paraffin. Following the removal of the paraffin, the sections were stained in Harris' alum hematoxylin or Verhoeff's elastic stain and counterstained with Van Gieson's stain [3]. Photomicrographs were made using a Wratten 58 filter to emphasize the collagen pattern.

Electron microscopical study was performed on pieces of skin from the mid-dorsum which were dissected first in long narrow strips (1 mm wide) parallel to the long axis of the body, and then cut by means of a Cambridge Vibratome into 0.5 mm pieces perpendicular to the long axis of the strip. In this way, thorough infiltration and embedment of the tissue as well as consistent orientation of the specimen was assured. The samples were then fixed for 4 hr at 22°C with 4% paraformaldehyde and 3% glutaraldehyde in 0.1 M cacodylate buffer with 0.01 M CaCl₂ and postfixed for 1 hr at 22°C with 2% osmium tetroxide in the same buffer. Following a distilled water rinse the tissue was stained *en bloc* with aqueous (1%) uranyl acetate for 1 hr at 22°C, dehydrated with 2,2-dimethoxypropane [4] and embedded in Spurr's resin [5].

Selected areas of the dermis were ultrathin sectioned (800-900 Å) with a diamond knife after light microscopical evaluation of 1 μ thick sections. To show general ultrastructural features, these sections were stained with 1% uranyl acetate in 50% ethanol and poststained in Reynold's lead citrate [6]. Some sections were stained only in 1% phosphotungstic acid (PTA) in 95% ethanol to provide more electron-dense staining of collagen. Electron micrographs were taken using a Philips EM 300.

RESULTS

General Light Microscopical Comparison of the Tsk/+ and +/+ Dermis

The reticular dermis of skin from the back of Tsk/+ mice is consistently thicker and often more cellular than that of +/+ siblings (compare Fig 1 and 2). The wickerwork arrangement of collagen that characterizes normal dermis is frequently more

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Abbreviations:

PTA: phosphotungstic acid

RER: rough endoplasmic reticulum

disorganized in the *Tsk*/*+* animal and the fibrous organization is not distinctly visible in the scattered hyalinized areas of the superficial dermis (Fig 1). The hypodermis of *Tsk*/*+* skin is more lamellar in appearance and substantially thicker than that of the *+/+* animal (compare Fig 1 and 2).

*Cytological Comparison of the *Tsk*/*+* and *+/+* Dermis*

Most of the cells in the dermis of both *+/+* and *Tsk*/*+* mice were found on electron microscopical examination to be fibroblasts, identified by their elongate shape, tenuous processes, ovoid nuclei and abundance of rough endoplasmic reticulum (RER). Fibroblasts from *Tsk*/*+* dermis often contain cisternae of RER greatly distended with a moderately electron-dense flocculent material (Fig 3) whereas the RER in fibroblasts of the *+/+* mice show much less distension (Fig 4). The greater cellularity of the *Tsk*/*+* dermis appears to be largely due to exceptionally numerous fibroblasts in both the dermis and hypodermis. Scattered mast cells as well as occasional macrophages, lymphocytes and plasma cells are found throughout the dermis of both *Tsk*/*+* and *+/+* animals but there is no obvious difference in their morphology or number.

Fine Structure and Organization of Collagen Fibrils in the Reticular Dermis

The reticular dermis of *+/+* mice is comprised of irregular fascicles of collagen fibrils of indeterminant length in which all the fibrils of a particular fascicle run in a common direction. The fascicles themselves run at oblique angles to one another but usually are essentially parallel to the free surface of the skin. It is generally assumed that what is resolved as a "fiber" at the light microscope level of observation is in fact one such fascicle.

The collagen fibrils within the fascicles of *Tsk*/*+* dermis are essentially parallel to one another but there appears to be less order among the fascicles of collagen fibrils (fibers) than in the *+/+* mice. The fascicles of *Tsk*/*+* mice for example are thinner and more closely packed and appear to bend and twist more along their course (compare Fig 5 and 6).

The fascicles of collagen fibrils are packed exceptionally close in those areas of the superficial reticular dermis of *Tsk*/*+* mice that correspond with the hyalinized areas observed in the light microscope (Fig 7). Within these fascicles, the fibrils themselves are also packed together very tightly, and, as a result, often assume an angular or hexagonal profile in cross section (Fig 8). Despite this evidence of close packing, the fibrils are typically separated from one another by a rather uniform 3–6 nm wide space filled with a moderately electron-dense material. In some areas the electron density of this material is similar to that of the collagen fibrils making it difficult to discern the boundary between them. No comparable evidence of close packing was observed among collagen fibrils in the dermis of the *+/+* mice.

A comparison of the fine structure of collagen fibrils from the reticular dermis revealed a rather striking structural abnormality common to both the mutant (*Tsk*/*+*) and "normal" (*+/+*) mice. In both animals the diameter of the reticular collagen fibrils varies over an extraordinarily broad range of 30 to 500 nm, with at least 10% of the fibrils being unusually large (300–500 nm) and irregular in shape (Fig 9–12). Those fibrils of *Tsk*/*+* and *+/+* skin which fall in the normal size range (30–170 nm in diameter) are typically circular in cross-sectional profile as expected in normal skin collagen, but most fibrils having a diameter substantially larger than about 200 nm have a highly irregular profile (Fig 11 and 12). Cross-sections of many of these large fibrils, for example, show one or more deep notches in the surface of the fibril which often penetrate tangentially rather than along a radius while still others have a deeply lobed appearance (Fig 11 and 12).

A comparison of the notched and lobed pleomorphic appearance of cross-sections of the large fibrils with their appearance in longitudinal profile, suggests that these fibrils have a helical

or twisted organization. The notches observed in cross sections apparently correspond to grooves which run in a helical fashion along the length of the fibril imparting a helical appearance to the fibril with a pitch of about 850 nm (Fig 13 and 14). In this way a single large fibril might have the superficial appearance of 2 or more helically wrapped fibrils.

Some of the larger fibrils of both *Tsk*/*+* and *+/+* dermis, appear to branch into 2 or more smaller fibrils not necessarily equal in size (Fig 15). Evidence of branching was found in both cross and oblique sections of the fibrils where, for example, one or more small "buds" may be attached to a large "trunk" fibril by contacts that range from a broad confluence to a very tenuous one (Fig 12). These branches, which are typically about the diameter of normal dermal collagen fibrils (100 nm or smaller), occasionally appear to fuse again into 1 or 2 large fibrils (Fig 15). The branches may occasionally twist about one another in a helical fashion giving an appearance similar to that of the helically grooved fibrils previously described.

The banding periodicity of all of the reticular collagen fibrils, regardless of size, is about 55 nm (Fig 13 and 14) and where branching or twisting of the fibrils occurs, the banding of the branches generally appears to be in precise register (Fig 13–15).

Fine Structure and Organization of Collagen Fibrils in the Papillary Dermis

The papillary dermis is about 40 μ thick in both *Tsk*/*+* and *+/+* mice and is rather easily distinguished from the reticular dermis by its smaller collagen fibrils (compare Fig 9 and 16). The collagen fibrils of this layer in both *Tsk*/*+* and *+/+* skin are organized in rather precise orthogonally arranged fascicles (Fig 16 and 17), have a banding periodicity of about 55 nm and range in diameter from 20 to 90 nm. Fibrils measuring about 60 nm in diameter greatly predominate in the *Tsk*/*+* mice (Fig 18) while the fibrils of the *+/+* animals tend to be more variable in diameter (Fig 19). All of the fibrils of the papillary dermis of both *Tsk*/*+* and *+/+* mice are circular in cross-section (Fig 18 and 19) with no evidence of branching or twisting (Fig 16–19).

The principal difference observed between *Tsk*/*+* and *+/+* papillary dermis is the presence of hyalinized areas in the *Tsk*/*+* dermis which are entirely lacking in the *+/+* dermis. In these areas collagen fibrils average about 60 nm in diameter, are less electron-dense and are very tightly packed much as in the hyalinized areas of the superficial reticular dermis (compare Fig 20 with Fig 8).

Fine Structure and Organization of Collagen Fibrils in the Hypodermis

The hypodermis of *Tsk*/*+* mice is very thick compared to that of the *+/+* mice but both consist of what appear to be discrete laminae of collagen fibrils (Fig 21 and 22). The space between the laminae is filled with a material of low electron density, which might represent ground substance though some artifactual separation of the laminae seems likely. Fibroblasts are abundant in these interlaminar spaces and particularly so in those of the *Tsk*/*+* mice. In addition, the interlaminar space of *Tsk*/*+* mice contains numerous disorganized fine filaments, which measure 10–20 nm in diameter and lack an obvious periodicity (Fig 21 and inset).

The collagen fibrils of the hypodermis of both *Tsk*/*+* and *+/+* mice, like those of the papillary dermis, are uniformly circular in cross-section, have a banding period of about 55 nm and show no evidence of branching or twisting (Fig 23–25). The collagen fibrils of the *Tsk*/*+* hypodermis range in size from 35–95 nm in diameter though most are about 70 nm in diameter (Fig 23). The collagen fibrils in the hypodermis of *+/+* mice have a similar range in diameter but generally the smaller fibrils are relatively more abundant (Fig 25). There are scattered areas in the hypodermis of *Tsk*/*+* mice, however, where fascicles of unusually thin fibrils (30–40 nm) are found (Fig 26); these fibrils

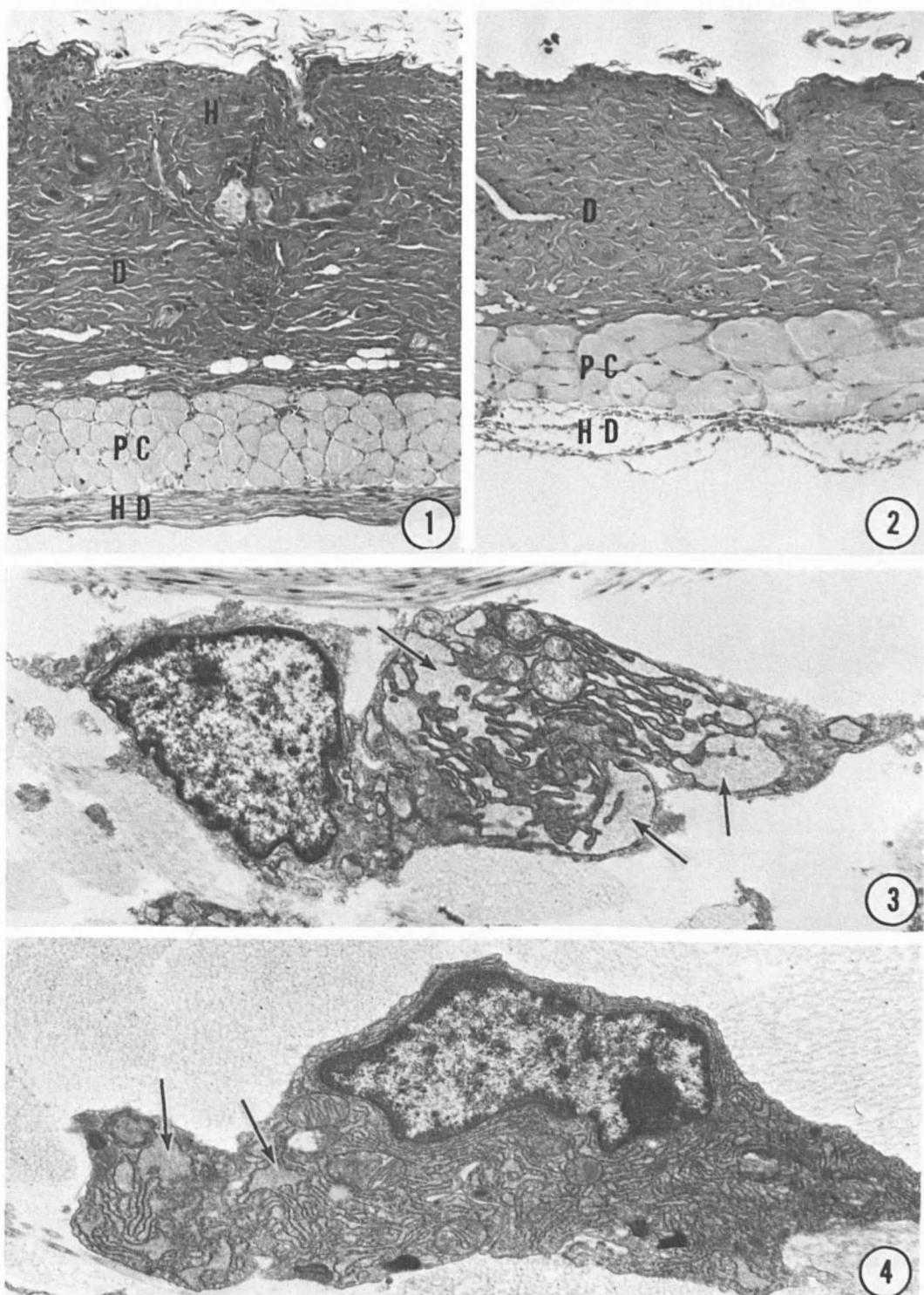


Plate 1

FIG 1. Light micrograph of back skin from a tight-skin heterozygous mouse (Tsk/+) showing irregular fibrous architecture of hypertrophic dermis (D). Note swirls of collagen around hyalinized (H) areas of superficial dermis. There are several laminae of collagen fibers in the hypodermis (HD) deep to the paniculus carnosus (PC) ($\times 130$).

FIG 2. Light micrograph of back skin from a normal sibling (+/+/). The dermis (D) is thinner than in the Tsk/+ animal and consists of more regularly woven collagen fibers. The hypodermis (HD) just deep to the paniculus carnosus (PC) consists of only a few strands of loosely arranged collagen fibers ($\times 130$).

FIG 3. Electron micrograph showing a typical fibroblast from the dermis of Tsk/+ mice. Note the distended cisternae of rough endoplasmic reticulum containing a flocculent material (arrows) ($\times 7,300$).

FIG 4. Electron micrograph of a typical fibroblast from the dermis of a +/+ mouse showing abundant rough endoplasmic reticulum with occasional small distensions (arrows) ($\times 9,750$).

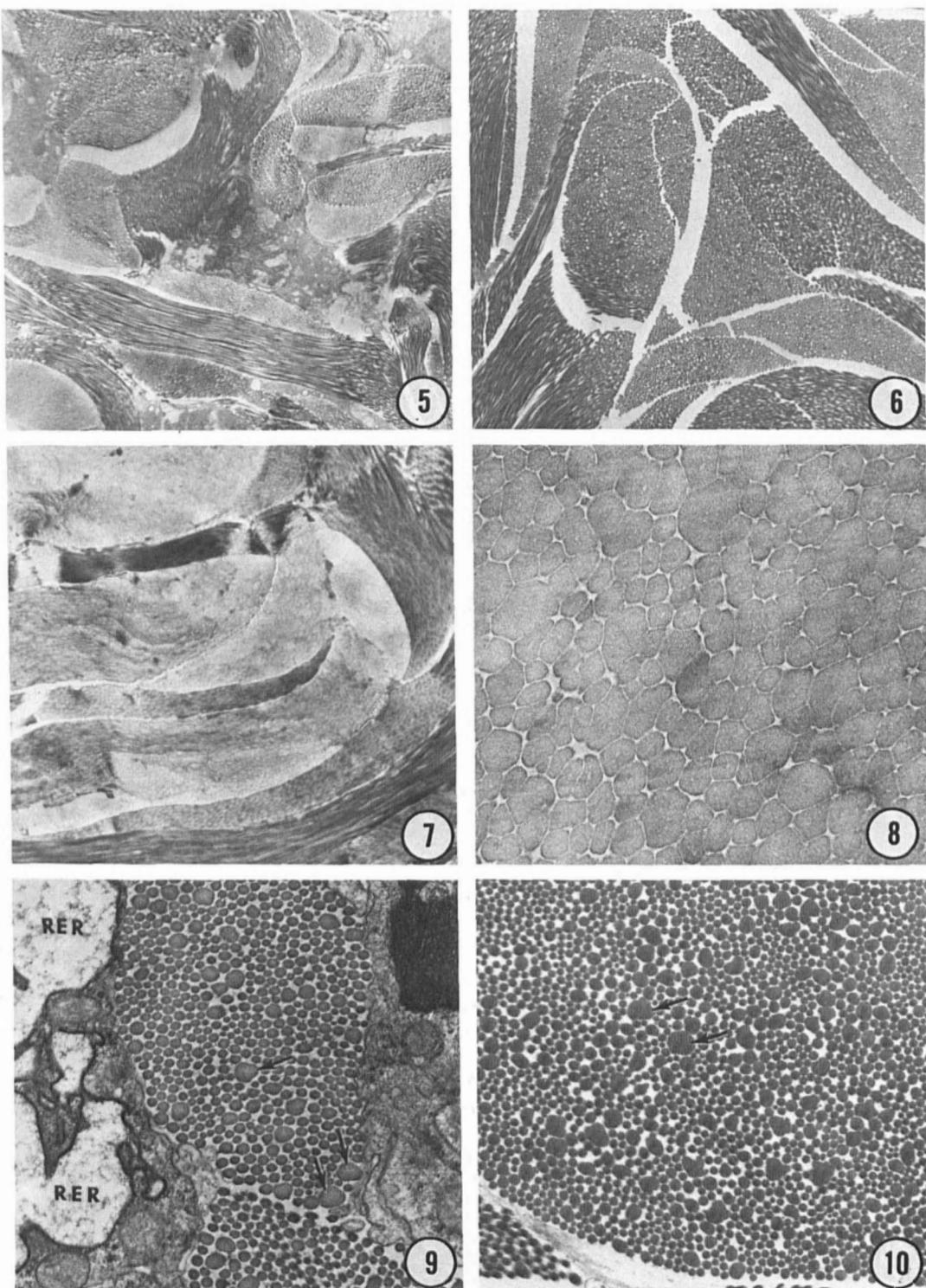


Plate 2

FIG 5. This low magnification electron micrograph of Tsk/+ reticular dermis displays smaller, closely-packed and disorganized collagen bundles compared to those of a +/+ mouse (Fig 6) ($\times 3,360$).

FIG 6. Low magnification electron micrograph of +/+ reticular dermis having more discretely organized collagen bundles which are larger and more loosely packed than those of Tsk/+ mice ($\times 3,360$).

FIG 7. Electron micrograph of an area in the reticular dermis (Tsk+) which appeared hyalinized in the light microscope. The collagen fascicles and their fibrils are packed very tightly and appear nearly homogenous. Difficulty was encountered in ultrathin sectioning and staining of these areas resulting in scratches, compression and electronlucent areas ($\times 5,600$).

FIG 8. Higher magnification of the hyalinized collagen shown in Fig 7. The fibrils exhibit evidence of hexagonal close packing though the individual fibrils are consistently separated by a space of about 3–6 nm. The ground substance occupying this space is more electron dense than in other nonhyalinized areas ($\times 38,750$).

FIG 9. Cross-section of collagen fibrils from the reticular dermis of a Tsk/+ mouse. There is an unusually broad range in the diameter of the fibrils with scattered fibrils substantially exceeding the diameter of normal skin collagen fibrils (arrows). Note the greatly distended rough endoplasmic reticulum (RER) in the fibroblast. ($\times 13,300$).

FIG 10. Cross-sections of collagen fibrils from reticular dermis of a +/+ mouse. Surprisingly, the dermis of this presumably normal sibling shows a scattered population of extraordinarily large fibrils (arrows) similar to those of the Tsk/+ mouse ($\times 13,300$).

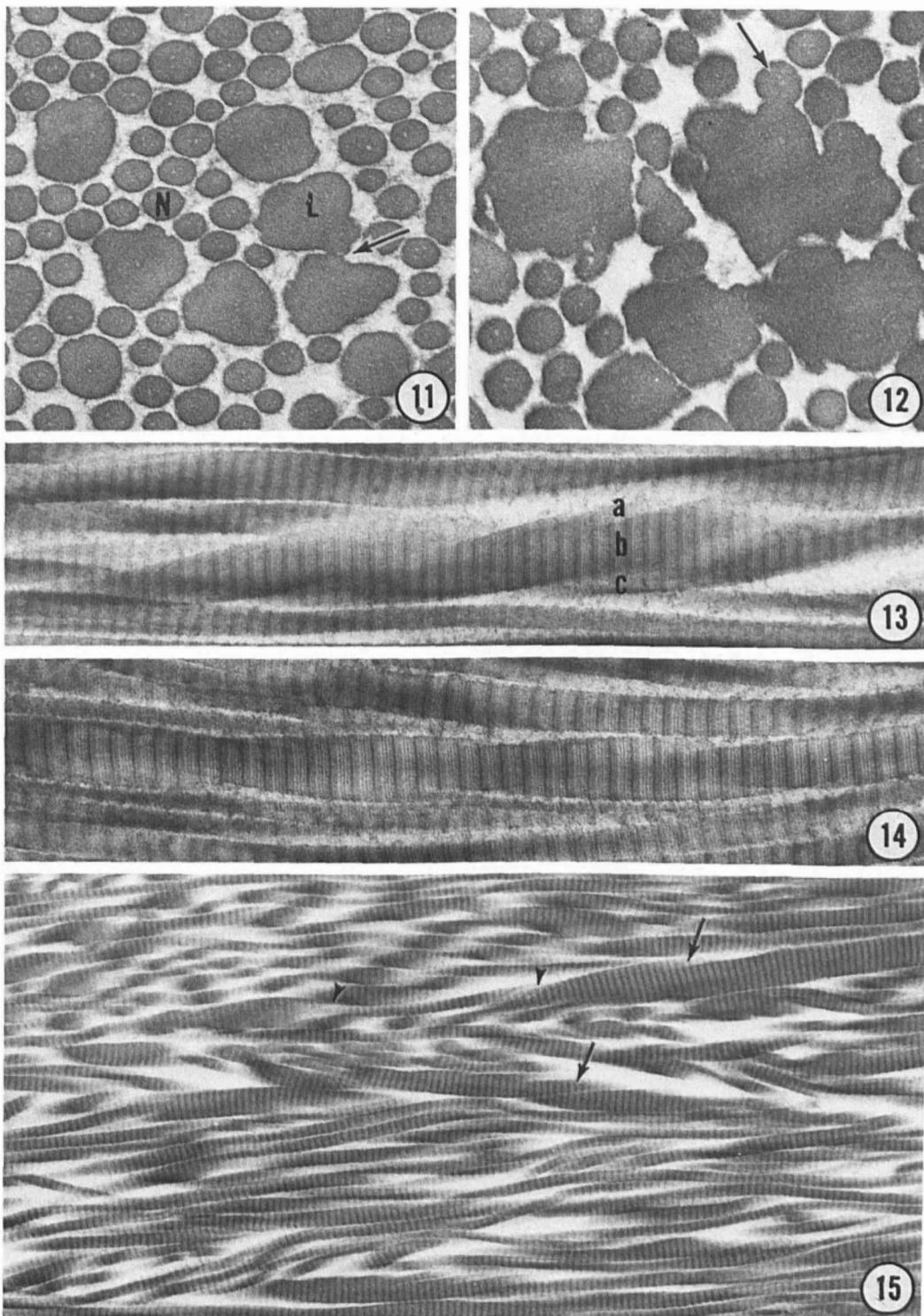


Plate 3

FIG. 11. Cross-section of collagen from Tsk/+ reticular dermis at higher magnification showing fibrils having a normal diameter and circular profile (N) and other extraordinarily large fibrils having a highly irregular profile (L). "Two" of the large fibrils are connected at a small isthmus (arrow) ($\times 51,340$).

FIG. 12. This cross-section of collagen from +/+ reticular dermis shows a broad range in fibril diameters and morphology including some of the most irregular fibrils observed in this study. One of the larger fibrils has a small circular "bud" (arrow) which is comparable in diameter to a normal collagen fibril ($\times 52,700$).

FIG. 13. Longitudinal section of collagen from the reticular dermis of a Tsk/+ mouse. The large fibril in the center has a twisted rope-like appearance with at least 3 components (a, b, c). The pitch (one complete twist) is about 850 nm in length and the periodicity of the banding is 52.4 nm. The twisted appearance in this case is believed to be a result of helical grooves running the length of the fibril ($\times 49,600$).

FIG. 14. Longitudinal section through collagen from the +/+ reticular dermis displaying the twisted appearance of a large fibril similar to that of Tsk/+ skin. The pitch is about 850 nm and the banding periodicity is 50 nm ($\times 62,000$).

FIG. 15. Reticular dermis of a +/+ mouse showing twisted and branched collagen fibrils of varying diameters. The twisted appearance may be a result of either helical grooves on the large fibrils (arrows) or actual branches which give rise to smaller fibrils which may in turn twist about one another (arrow heads). Occasionally branched fibrils appear to fuse again into a large collagen fibril (arrowheads) ($\times 17,250$).

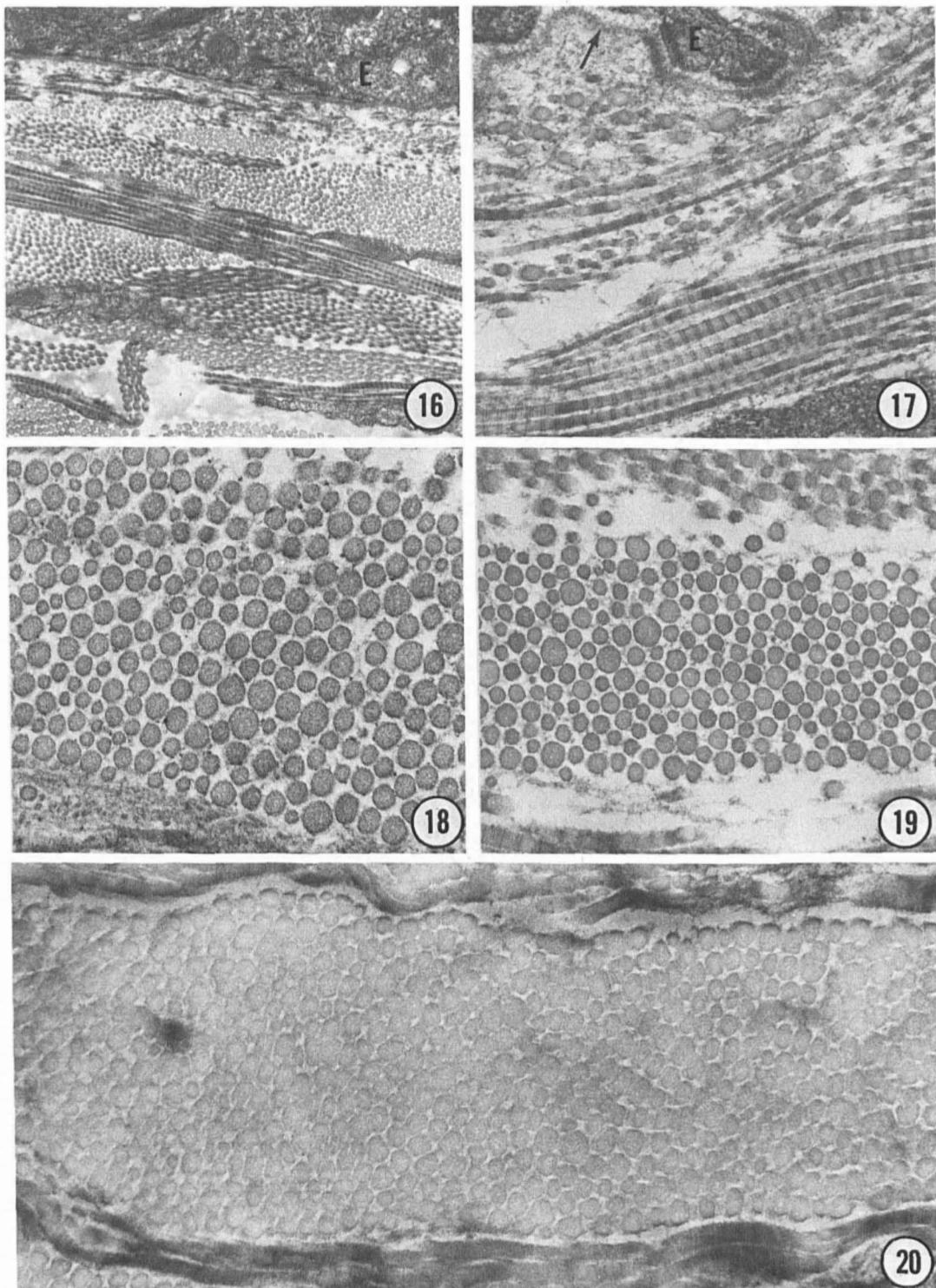


Plate 4

FIG 16. Papillary dermis of a Tsk/+ mouse showing apparently normal collagen fibrils just beneath the epidermis (E) and basal lamina ($\times 14,500$).

FIG 17. Papillary dermis of a +/+ mouse showing apparently normal collagen fibrils beneath the epidermis (E) and basal lamina (arrow) ($\times 40,000$).

FIG 18. Cross-sections of Tsk/+ papillary collagen fibrils having a circular profile and an upper limit in diameter of about 90 nm ($\times 48,000$).

FIG 19. Cross-section of +/+ papillary collagen fibrils similar in shape and range in size to those of Tsk/+ mice ($\times 48,000$).

FIG 20. Papillary dermis of a Tsk/+ animal in a region of hyalinization. Notice the tightly packed collagen fibrils and the increased density of ground substance ($\times 51,000$).

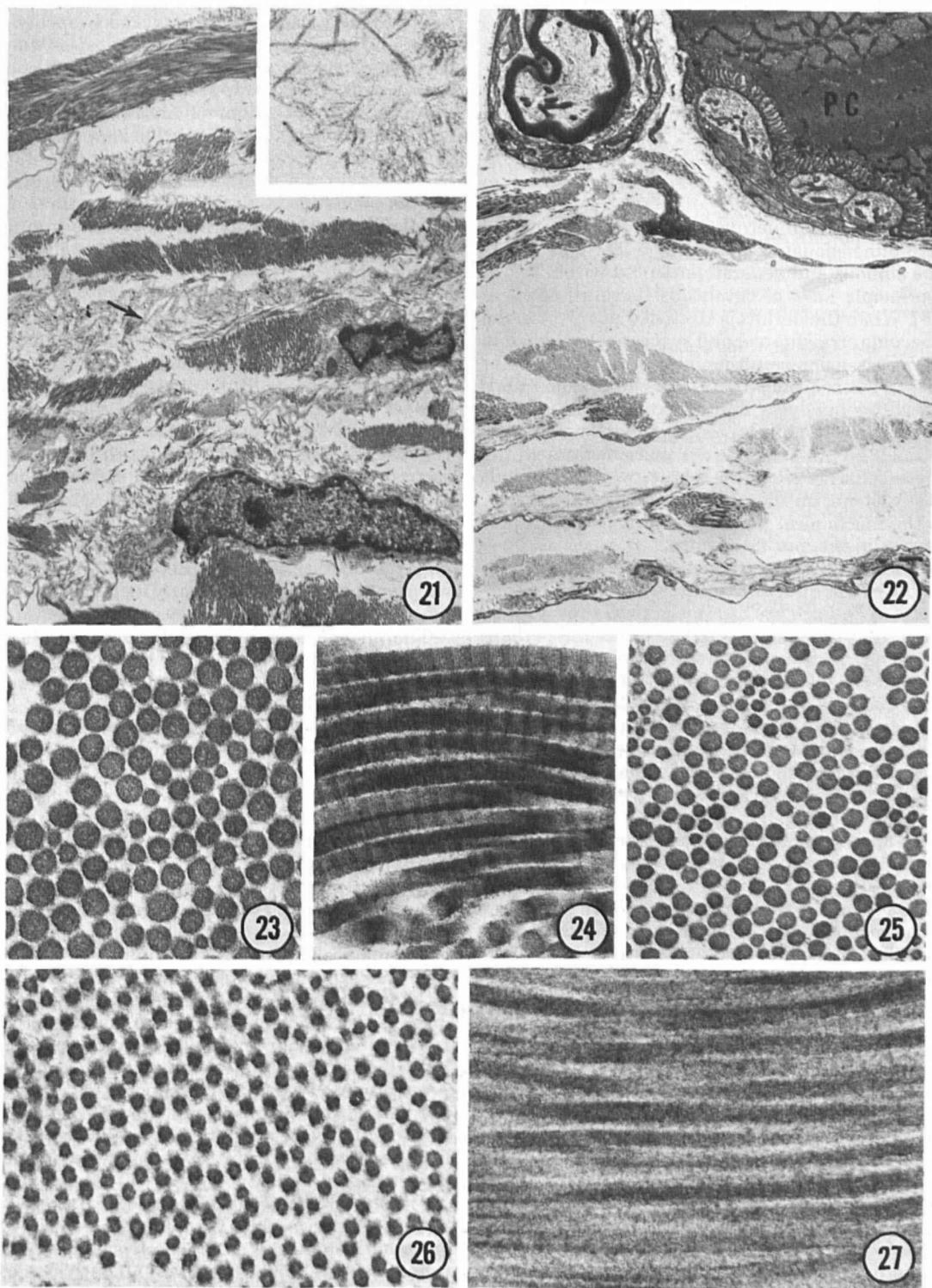


Plate 5

FIG. 21. A portion of the thickened hypodermis of a *Tsk*/*+* mouse demonstrating the laminar arrangement of the collagen fibrils. Fine filaments 10-20 nm thick are found between the laminae ($\times 3,950$). Insert: Higher magnification of the fine filaments between the laminae ($\times 15,300$).

FIG. 22. This shows the full thickness of the *+/+* hypodermis containing thin laminae of collagen fibrils beneath the panniculus carnosus (PC) with motor end plate. There is no obvious accumulation of fine filaments between laminae ($\times 3,600$).

FIG. 23. Cross-section of hypodermal collagen in a *Tsk*/*+* mouse. The fibrils have a considerable range in diameter but are consistently circular in profile. Note that these fibrils have an average diameter which is greater than those in Fig 26 ($\times 51,300$).

FIG. 24. Longitudinal section of *Tsk*/*+* hypodermal collagen showing a banding periodicity of about 50 nm ($\times 51,300$).

FIG. 25. Hypodermal collagen fibrils from a *+/+* mouse. These fibrils also show a wide range in diameter but have an average diameter smaller than those of *Tsk*/*+* mice ($\times 52,700$).

FIG. 26. Unusual type of hypodermal collagen from *Tsk*/*+* mouse; the fibrils are small in diameter, with a maximum of about 41 nm, and rather uniform in profile ($\times 52,700$).

FIG. 27. Longitudinal section of the thinner type *Tsk*/*+* hypodermal collagen fibrils showing indistinct banding ($\times 58,900$).

appear to be collagen but they have a very poorly defined banding pattern (Fig 27).

DISCUSSION

The precise fibrous organization of the dermis has never been fully characterized and its complex architecture presents a formidable problem to the morphologist. In a recent review Szirmai [7] has stated that the architecture of the dermis "is usually treated in what appears to be the shortest paragraph in most textbooks on the subject."

The bulk of the mammalian dermis appears to be composed of an intricate 3-dimensional wickerwork of collagen fibers which tend to be sinuous and generally oriented within 45° of an axis of the principle lines of cutaneous tension known as Langer's lines [8]. When the dermis is stretched in any axis, the collagen fibers become straightened and successively aligned in the direction of the extension resulting in progressively greater dermal stiffness [8,9]. Since the structure of the collagen fibril itself apparently allows only very limited extension, the extensibility of the dermis must be due largely to a rearrangement of fibers as they straighten and move into alignment through the gel-like mucopolysaccharide ground substance which surrounds them [7-9]. Elastic fibers, on the other hand, apparently play a minor role in the mechanical properties of mouse skin since they are very sparse in the murine dermis.

The results of this study suggest that the greater stiffness, induration and loss of the directional properties which characterize the skin of Tsk/+ mice are at least in part a result of an alteration in the fibrous architecture of the dermis and perhaps an alteration in the quantity or quality of the ground substance. It is well known that the physical or rheological characteristics of any connective tissue is in part determined by the arrangement of its fibrous elements and specifically there is evidence that the fibrous arrangement of the dermis greatly influences its anisotropic elastic stiffness [7-9]. The 2-dimensional nature of electron micrographs makes an analysis of the 3-dimensional configuration of Tsk/+ collagen fascicles difficult, but it does appear that they are smaller in diameter than those of +/+ skin and are both more tightly packed and more irregular in their spatial organization.

The most obvious structural differences between Tsk/+ and +/+ skin are the greater dermal thickness and denser packing of the fibrils in Tsk/+ skin. The greater thickness of the Tsk/+ dermis presumably reflects a greater quantity of collagen fibrils than +/+ dermis and the hyalin appearance of scattered portions of the superficial dermis of Tsk/+ mice is apparently a consequence of close packing of collagen fibrils. It is reasonable to assume that both of these changes would affect the physical properties of Tsk/+ skin since rheological studies have shown that the tensile strength and plastic stiffness of skin increase directly with its cross-sectional area [10] and total insoluble collagen content [11,12]. Moreover, the close packing of the collagen fibers and fibrils would minimize the amount of mucopolysaccharide ground substance between the fibers which in turn might impede fibrous rearrangement in the hypertrophic Tsk/+ dermis during extension. Alcian blue-PAS staining at an acid pH has been reported to be more intense in the Tsk/+ dermis than the +/+ dermis [1,2], but this might be accounted for by the observation of Szirmai ('56) [13] that PAS reactive glycosaminoglycans are largely associated with the fibrils rather than the inter-fibrillar material.

An important objective of this study was to investigate the affects of the Tsk mutation on the fine structure of collagen fibrils themselves, but the results of these observations are rather difficult to interpret in terms of the tight skin mutation. Electron microscopical study revealed that many dermal collagen fibrils of the Tsk/+ animals are indeed abnormal in both size and shape but surprisingly those of the +/+ animals show this same abnormality. In both animals, for example, scattered fibrils in the reticular dermis are extraordinarily large in diam-

eter, highly irregular in cross-section and, in longitudinal section, appear helically organized and branched. The branched nature of many of the helical fibrils raises the possibility that these fibrils are actually a composite strand constructed of 3 or more smaller fibrils wrapped about one another. It is clear from comparing cross-sections of fibrils with longitudinal sections, however, that the twisted or helical appearance of the fibril can also result from rather deep helical grooves in what appears to be a single extraordinarily thick fibril. Apparently these grooves are occasionally deep enough to result in 2 or more discrete smaller fibrils which may twist about one another and even fuse again to form a large fibril. Some of the fibrils in the reticular dermis which appear normal in cross-section may in fact be branches from large abnormal fibrils.

It is not known if the large-branched and twisted collagen fibrils contribute to the alterations in mechanical properties of Tsk/+ skin but this seems unlikely in view of the comparable occurrence of these fibrils in the physically normal +/+ skin. Similar large and irregular dermal collagen fibrils of helical construction have recently been reported in type I Ehlers-Danlos syndrome in man which the authors suggest is the structural correlate of the altered cutaneous mechanical properties which characterize this disorder [14]. This interpretation seems unlikely in view of the fact that the hyperelastic and fragile nature of skin in type I Ehlers-Danlos is quite the opposite of the cutaneous mechanical properties of tight skin mice, yet both have these nearly identical abnormalities in fibril morphology.

The unexpected presence of the large helically twisted and branched collagen fibrils in the control +/+ animals as well as in the mutant Tsk/+ animals raises some interesting questions. In what sense is a collagen fibril abnormal if it is consistently observed in presumably normal controls? Do such fibrils perhaps occur commonly in murine skin but have somehow been overlooked? Since the mutation for tight skin arose in the inbred B10.D2 (58N)/Sn line which is a substrain of the C57BL strain, it may be that at least some of the substrains of the C57 line are similarly abnormal. This possibility is now under investigation. Branched and twisted collagen fibrils of unusually large diameter have not been previously reported in normal dermis of any animal using conventional electron microscopical techniques, however, a helical organization has been observed in normal collagen fibrils of human and guinea pig dermis following exposure to the denaturing agents urea or guanidine-HCl [15]. Apparently the twisted or helical appearance of dissociated collagen fibrils reflects a helical organization that is intrinsic to the supramolecular organization of the normal fibril but which is usually masked in routine electron microscopical preparations. It has been proposed that the reason certain preparative procedures demonstrate this helical organization in fibrils is that they disrupt hydrogen bonds between the microfibrils making up the fibril, whereas more conventional methods do not [15].

Finally, the physical properties of the skin of Tsk/+ mice invites comparison with that of the human disorder scleroderma since both share the common physical properties of stiffness, rigidity and relative inelasticity. The histological changes in scleroderma are inconsistent but they may include dermal hypertrophy by fibrosis, hyalinization of the connective tissue and replacement of the subcutaneous fat by abnormal connective tissue [16], all of which are similar to changes found in Tsk/+ mice. Hyalinization, however, has been reported only in the hypodermis in scleroderma whereas it occurs principally in the superficial dermis of Tsk/+ mice.

Electron microscopical studies of generalized scleroderma have shown that the collagen fibrils of the reticular dermis are slightly smaller in diameter than normal but show no other structural abnormalities [17-19]. Although this observation contrasts with the appearance of Tsk/+ reticular dermis where some unusually large fibrils with a twisted and branched mor-

phology are found, these abnormalities are apparently not a specific feature of the *Tsk* mutation. Indeed, the fibrils of the *Tsk*/*+* animals actually are smaller on the average than those of the "normal" *+/+* animals. The hypodermis in scleroderma, on the other hand, has been reported to have several alterations in fine structure which appear similar to *Tsk*/*+* skin; these include fine and presumably immature collagen fibrils (200–400 Å in diameter) [17–19], dense packing of fibrils [20] and numerous fibroblasts with dilated endoplasmic reticulum [18]. These changes have been interpreted to indicate an increase in fibrilogenesis in scleroderma [17,18] and LeRoy [21] has reported that cultured scleroderma fibroblasts do in fact synthesize more collagen than normal fibroblasts.

The greater dermal thickness and close packing of collagen fibrils in the skin of *Tsk*/*+* mice also suggests that fibrilogenesis of collagen is increased or perhaps its degradation is decreased. The abundance of fibroblasts with dilated endoplasmic reticulum in the dermis of *Tsk*/*+* mice is consistent with an increase in protein synthesis and the thin fibrils and filaments (less than 40 nm) in the hypodermis of *Tsk*/*+* mice resemble the recently deposited collagen in embryonic and infant skin [18,22]. It would appear that in both the *Tsk* mutation and scleroderma, there is an alteration in collagen synthesis, metabolism and fibrous architecture resulting in hypertrophy of the dermis and significant alterations in the mechanical properties of the skin but the biochemical mechanisms responsible for these changes remain to be elucidated.

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Pacific Dermatologic Association Essay Contest

The Nelson Paul Anderson Essay Prize will be awarded at the 32nd Annual Meeting of the Pacific Dermatological Association in Palm Springs, California, October 23–28, 1980. The contest for essays on original work is open to all physicians in graduate dermatologic training or those who are not more than 5 yr out of residency training, residing in the geographical area of the Pacific Dermatologic Association: The Western United States (California, Oregon, Alaska, Nevada, Washington, Idaho, Utah, Arizona, Hawaii, Montana, Wyoming, Colorado, New Mexico), British Columbia and Alberta, Canada, Mexico, Australia, New Zealand, Japan, and the Philippines. The winning essayist will receive a cash prize of \$500 and his expenses will be paid to the next Annual Meeting. The sponsoring department will receive \$250 for educational materials.

Essays will be judged on the following considerations: A. Originality, B. Potential importance of work, C. Evaluation of results, D. Experimental methods and use of control, E. Clarity of presentation. Six copies of the essay should be submitted under a *nom-de-plume*, with no information in the paper which will lead to recognition by the judges of the institution or clinic where the work was done. The essay with *nom-de-plume* should be accompanied by a plain sealed envelope enclosing the name and address and *nom-de-plume* of the author. Entries must be received by the Secretary-Treasurer, Gerald A. Gellin, M.D., 3838 California St., San Francisco, California 94118, no later than August 31, 1980.