Tight-Skin, a New Mutation of the Mouse Causing Excessive Growth of Connective Tissue and Skeleton

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A new dominant mutation, tight-skin (*Tsk*), is located on Chromosome 2, two recombination units distal to pallid (*pa*). Heterozygotes (*Tsk*/+) have tight skins with marked hyperplasia of the subcutaneous loose connective tissue, increased growth of cartilage and bone, and small tendons with hyperplasia of the tendon sheaths. In the loose connective tissue there are large accumulations of microfibrils in the intercellular space. In spite of the increased skeletal size, body weight is not increased. Increase in size of the thoracic skeleton is especially pronounced and leads to pathologic distension of the hollow thoracic viscera. Concentration of growth hormone in the pituitary and plasma is normal. Homozygotes (*Tsk*/*Tsk*) die *in utero* at 7 to 8 days of gestation. We propose the hypothesis that *Tsk* might act by causing defective cell receptors with high affinity for a somatomedin-like factor promoting growth of cartilage, bone, and connective tissue and low affinity for a multiplication-stimulating factor promoting embryonic growth. (Am J Pathol 82:493–512, 1976)

THIS PAPER DESCRIBES a new mutant gene of the mouse that causes excessive growth of connective tissue, including loose connective tissue, cartilage, and endochrondral and membrane bone. The mutation was discovered at the Jackson Laboratory in the inbred B10.D2(58N)/Sn strain in July 1967 by Helen Bunker, who recognized that the mice were abnormal by the tightness of the skin over the shoulders when she attempted to pick them up. The basic defect caused by the gene has not yet been identified, but the fact that its effects are confined to connective tissue and skeleton suggests a possible interference with the growth hormone–somatomedin endocrine pathway.

Materials and Methods

Heterozygous tight-skin (*Tsk*/+) mice of the B10.D2(58N)/Sn strain, in which the mutation arose, were crossed to the C3H/Di strain and subsequent generations maintained by brother–sister matings of the type *Tsk*/+ by normal (+/+). Mice used for the work reported here were from this stock in the F_{10} to F_{20} generations. Except where otherwise noted, comparisons were between like-sexed littermate pairs, and the

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significance of differences in measured characters was assessed by the Student *t* test for paired comparisons.

Tissues from 28 pairs of mice 21 days to 8½ months old were examined. For histologic examination, tissues were fixed in Bouin's or Zenker's fixatives, embedded in paraffin, sectioned at 6 to 10 μ, and stained with hematoxylin and eosin or with a number of connective tissue stains including Halmi's [1] (aldehyde fuchsin, light green, orange G), Gridley's quadruple [2] (aldehyde fuchsin, azocarmine, naphthol green B, hematoxylin), acid orcein and fast green,[3] Van Gieson's [2] (acid fuchsin, picric acid), periodic acid–Schiff reagent (PAS),[4] and alcian blue at pH 2.5.[4] Some pieces of skin were fixed in 10% formalin in Sorensen's buffer at pH 7.4 or in Newcomer's fixative [4] and embedded in glycol methacrylate. One- to 2-μ sections were stained with Weigert's resorcin–fuchsin,[2] acid orcein and fast green, or acid fuchsin and toluidine blue at pH 4.[5]

Skin for electron microscopic examination was fixed in half strength Karnovsky's fluid[6] at room temperature, postfixed in 1% osmium tetroxide in Veronal acetate buffer at 4 C, stained in block in 0.75% or 1% aqueous uranyl acetate, and embedded in Araldite 6005. One-micron sections for light microscope examination were stained with toluidine blue in 1% sodium borate. Thin silver sections were cut with a diamond knife, stained with uranyl acetate and Reynold's lead citrate, and examined in a Hitachi HU-11C electron microscope operated at 75 kV.

Skeletons for study and for bone measurements were prepared by treating the skinned and eviscerated carcasses with 1% KOH, staining with alizarin red, and clearing in glycerin.[7] The bones to be measured were removed from the skeleton, boiled briefly in tap water, cleaned of adhering tissue, and dried at 55 C. All bones except the clavicle and rib were measured with a sliding vernier caliper reading to 0.1 mm. Because the curvature of the clavicle and ribs varied considerably, these bones were measured by placing the bone under a piece of double-sided transparent tape and superimposing a piece of 2-0 surgical silk thread over the bone with a knot at one end and the other end held with fine dissecting forceps. The thread was then removed from the tape and measured with a millimeter ruler. Two measurements were taken and averaged.

The costal and tracheal cartilages were measured without further treatment after removal from the skeletons. The costal cartilages were measured in the same way as the ribs. Because the tracheal cartilages were too small to be measured directly, enlarged projection drawings (45 times) were made and the drawings measured with thread in the same manner as the ribs.

The greatest length of the pelvis and long bones of the limbs was measured. The other dimensions measured on bones were as follows: skull length, occipital protuberance to anterior end of nasal bones; skull width, greatest width just posterior to zygomatic arch; mandible length, articular process to anterior end at ventral border of incisors; mandible width, dorsal to ventral depth at level of first molar tooth; humerus width, distal end, lateral epicondyle to medial epicondyle; ulna width, proximal end, dorsal to ventral at anconal process; femur width, distal end, lateral epicondyle to medial epicondyle; tibia width, proximal end, lateral to medial; scapula length, glenoid process to medial border; scapula width, greatest width, anterior to posterior, at medial border; pelvis width, pubic symphysis to ischial tuberosity; lumbar vertebra length, at centrum; and lumbar vertebra width, at posterior border of centrum.

Cartilage of the ear was prepared according to Boas.[8] The external ear was cut off, immersed in 10% acetic acid at 55 C for about 24 hours, and the skin, connective tissue, and muscle gently cleaned off. The cartilage was then unrolled and mounted flat in glycerin on a microscope slide under a coverglass. Measurement was made with an eyepiece micrometer in a dissecting microscope. Ear length was measured from the tip of the scapha to A4, and width from the medial border of P6 to the lateral border of P4 (See Green and Green [9] for terminology).

Hearts to be weighed were fixed in 10% formalin for at least 24 hours. The blood vessels

were trimmed off, and the auricles separated from the ventricles. The right ventricle was then carefully cut off, leaving the medial wall with the left ventricle. Any clotted blood was washed out, and the tissues were blotted briefly on paper toweling and weighed on a Roller-Smith balance.

Blood pressure determinations were performed by Dr. G. Schlager, University of Kansas, Lawrence, Kan. Measurement was made on the tail using the method of Schlager and Weibust.[10] Pressure was determined on three different days, and at least five readings were taken on each day.

Growth hormone assays were performed by Drs. Wesley G. Beamer and Melba E. Wilson of the Jackson Laboratory. Plasma growth hormone was measured by radioimmunoassay [11] with materials supplied by the Rat Pituitary Hormone Program of the National Institute of Arthritis, Metabolic, and Digestive Diseases. Pituitary growth hormone was measured after electrophoresis on 12% acrylamide gels by the method of Cheever *et al.*[12] Relative concentration in *Tsk*/+ and +/+ pituitaries was determined by light densitometry.

Blood glucose measurements were made by Richard Copp of the Jackson Laboratory using the method of Folin and Malmros.[13]

Embryos examined in search for the *Tsk*/*Tsk* phenotype were obtained from timed matings. Time of mating was determined by the presence of a vaginal plug, the day the plug was observed being taken as Day 0. Females were killed with chloroform, and the embryos removed from the uterus, as much as possible with the membranes intact. In some cases at 7 and 8 days of gestation, the membranes were accidentally punctured before removal from the uterus, and the embryo was extruded and lost. These embryos were counted in the total number of implantations but not in the number classified. Seven- and 8-day embryos were fixed and sectioned with the membranes intact. Later stages were dissected out and the membranes and placenta removed. Fixation was in Bouin's fluid, and serial sections were stained with hematoxylin and eosin.

Results

Genetics

Table 1 gives the results of crosses to determine the mode of inheritance and linkage of *Tsk*. *Tsk* behaves as a dominant (Crosses 1, 2, and 3). Heterozygotes are easily classifiable, and there is no evidence of incomplete penetrance. Viability of heterozygotes may be reduced in some matings (Crosses 1 and 3). The low frequency of tight-skin mice from matings between heterozygotes (Cross 3) suggests that *Tsk* is lethal in homozygotes. This conclusion was confirmed by progeny tests of 18 of the tight-skin offspring of Cross 3, all of which produced some +/+ offspring and were therefore shown to be *Tsk*/+.

Both male and female *Tsk*/+ mice are fertile, but their breeding performance is often somewhat less than normal.

Crosses 4, 5, and 6 (Table 1) show that *Tsk* is linked to nonagouti (*a*) in Chromosome 2, in the order *Tsk*, *a*, *Ra* (ragged). Crosses 7, 8, 9, and 10 with pallid (*pa*) show that *Tsk* is very close to *pa* in the order *pa*, *Tsk*, *a*. Recombination with *pa*, estimated from the combined data of Crosses 7, 8, 9, and 10, is 2.00 ± 0.81%. Recombination with *a*, estimated from

Table 1—Segregation and Linkage of *Tsk*

Cross	Parents		Offspring								
			Tsk/+	+/+	Total	χ^2					
1	*Tsk*/+	+/+ Outcross	29	53	82	7.02					
2	*Tsk*/+	+/+ Sib matings	477	529	1006	2.69					
3	*Tsk*/+	*Tsk*/+	42	39	81	8.00					
			Tsk +	+ *a*	*Tsk a*	+ +	Total				
4	♀*Tsk* +/+ *a*	♂ + *a*/+ *a*	5	1	0	1	7				
5	♂*Tsk* +/+ *a*	♀ + *a*/+ *a*	28	26	5	5	64				
			Tsk a +	+ + *Ra*	*Tsk* + *Ra*	+ *a* +	*Tsk a Ra*	+ + +	*Tsk* + +	+ *a Ra*	Total
6	♂*Tsk a* +/+ + *Ra*	♀ + *a* +/+ *a* +	72	74	18	14	38	41	0	0	257
			+ *Tsk* +	*pa* + *a*	*pa Tsk* +	+ + *a*	*pa* ++	+ *Tsk a*	+ + +	*pa Tsk a*	Total
7	♀+ *Tsk* +/*pa* + *a*	♂*pa* + *a*/*pa* + *a*	68	66	3	0	10	9	0	0	156
8	♂+ *Tsk* +/*pa* + *a*	♀ *pa* + *a*/*pa* + *a*	56	50	1	0	4	7	0	0	118
9	♀+ *Tsk a*/*pa* + *a*	♂ *pa* + *a*/*pa* + *a*	0	7	0	0	0	9	0	0	16
10	♂+ *Tsk a*/*pa* + *a*	♀ *pa* + *a*/*pa* + *a*	0	4	0	2	0	4	0	0	10

Recombination—*pa*-*Tsk*: ♀ (Crosses 7 and 9) 3/172 = 1.74 ± 1.00%; ♂ (Crosses 8 and 10) 3/128 = 2.34 ± 1.34%; total 6/300 = 2.00 ± 0.81%. *Tsk*-*a*: ♀ (Crosses 4 and 7) 20/163 = 12.27 ± 2.63%; ♂ (Crosses 5, 6 and 8) 53/439 = 12.07 ± 1.55%; total 73/602 = 12.13 ± 1.33%

Crosses 4, 5, 6, 7, and 8, is 12.13 ± 1.33%. There are no significant differences in recombination values between the sexes.

The close linkage with *pa* has allowed construction of a stock that uses *pa* as a marker for early recognition of *Tsk*/+ and +/+ mice from matings of the type *Tsk* +/+ *pa* by + *pa*/+ *pa*. Pallid mice are recognizable at 11 days of gestation by absence of pigment in the eye. Ninety-eight percent of the pallid embryos are expected to be non-tight-skin and 98 percent of nonpallid embryos to be tight-skin. The stock was established by crossing a C57BL/6By-*Tsk*/+ (N13) male to a C57BL/6J-*pa*/*pa* (N35) female. Since the two C57BL/6 sublines are closely related, the new subline, C57BL/6-*Tsk* +/+ *pa*, is highly inbred and congenic with C57BL/6.

Description

Tsk/+ mice look normal up to about 2 months of age but can be recognized, as the name implies, by tightness of the skin. The amount of skin one can readily gather into a skin fold is considerably less than normal (Figure 1). At autopsy, the skin is much more difficult to separate from the underlying muscle than normal. The skin tightness is not recognizable at birth but develops during the first postnatal week, so that by 7 days of age it is usually possible to distinguish affected and normal littermates. As the mice age they develop a pronounced hump in the shoulder region and hunched posture, and their fur looks slightly rough (Figure 2).

Loose Connective Tissue

Histologic examination of the skin and subcutaneous loose connective tissue reveals a marked hyperplasia of the loose connective tissue in *Tsk*/+ mice. Sections perpendicular to the surface of the skin show a much thicker layer in affected mice than in +/+ sibs, with increased number of cells and amount of intercellular material (Figure 3). The loose connective tissue appears to be affected rather generally. We have examined the dorsal, lateral, and ventral thoracic and abdominal regions, and the fore and hind limbs, and found extensive subcutaneous hyperplasia in all of them, especially over the knee and elbow. In the subcutaneous layer of the trunk, hyperplasia occurs both external and internal to the panniculus carnosus. The connective tissues between muscles and between and within bundles of muscle fibers may be affected. Hyperplasia of the connective tissue is pronounced around the mammary glands, around the brown fat of the scapular region, and between the muscles of the thorax that lie between the external intercostals and the skin. It is marked in the connective tissue along the ventral side of the

cartilaginous xiphisternum, both distally where the xiphisternum lies free in the abdominal cavity and proximally between the xiphisternum and the adjacent layer of muscle. Hyperplasia is also found in some other parts of the subperitoneal connective tissue. It occurs at the medial border of the kidney and between the kidney and adrenal. Between the lobes and lobules of the pancreas more connective tissue is found in *Tsk*/+ than in +/+ sibs. The difference is most easily seen in sections stained with aldehyde fuchsin, in which the elastic fibers stand out as intense purple strands. In the mesentery of the duodenum, however, no difference in amount of connective tissue between mutant and normal was seen. Hyperplasia of the peritoneum of the ventral abdominal wall is slight, if it occurs at all. The peritoneum of the spleen and liver is unaffected.

We have examined the subcutaneous loose connective tissue of the dorsolateral abdominal area by light microscopy using several connective tissue stains and by electron microscopy. The most striking difference from normal is the presence of large amounts of pale-staining acidophilic material (Figures 3B and 4B). No such material is found in similar sections of +/+ mice (Figures 3A and 4A). In the electron microscope the pale-staining material is seen to consist of masses of microfibrils (Figure 5B). They usually occur in thick layers adjacent to the fibroblasts. Such regions in +/+ mice possess only a few scattered microfibrils or are devoid of them (Figure 5A). Within the mass of microfibrils in *Tsk*/+ mice are found scattered collagen fibrils, usually single (Figures 5B and 6), and individual or small groups of elastic fibers (Figure 7). The microfibrils lack periodicity and are about 100 Å in diameter.

In +/+ mice the most abundant intercellular material is collagen (Figure 4A). Elastic fibers are also numerous in a narrow layer of dense tissue immediately internal to the panniculus but are rarer in the underlying looser tissue (Figure 3A). In *Tsk*/+ mice, collagen is also abundant but does not appear to be greatly increased in proportion to the number of fibroblasts (Figure 4B). Elastic fibers are distributed throughout the subpannicular layer (Figure 3B). They appear to be relatively more numerous than in +/+ mice, but we do not have quantitative evidence to substantiate this conclusion.

Staining with alcian blue at pH 2.5 showed the presence of acid mucopolysaccharides in the ground substance of the connective tissue of both +/+ and *Tsk*/+ mice. There was considerably more stained material in the mutant mice, but the increase was related to the increase in other intercellular elements. In an 8½-month-old *Tsk*/+ mouse in which a very marked increase was found in the pale-staining acidophilic

material (probably microfibrils), this material also stained brightly with alcian blue.

Other Connective Tissue

Several other kinds of connective tissue have been examined histologically in adult tight-skin mice. No abnormalities were detected in the dermis, the perichondrium, or the periosteum. Cartilage and bone in general appeared normal, and there was little if any difference in width of the proximal epiphyseal plate of the tibia of the three pairs examined (38 to 78 days old). The costochondral junctions appeared somewhat enlarged, as would be expected from the marked effect of *Tsk* on rib length (see below), but we have not made a quantitative histologic study of this region.

One additional type of connective tissue noticeably affected by *Tsk* is tendon. This can be seen in the tail, where there is marked reduction in size of the tendons that is often associated with hyperplasia of the tendon sheath or with accumulation of fluid within the sheath (Figure 8A and B). Hyperplasia of the sheaths is not enough to account for all the reduction in size of the tail tendons, however, since the total diameter of the tendons and their sheaths is smaller in *Tsk*/+ mice than in normal sibs. We have also examined tendons of the ankle and found similar reduction in size of the tendons and marked hyperplasia of the sheaths, as well as marked hyperplasia of the loose connective tissue between the tendons. In older *Tsk*/+ animals, some tendons may show severe degenerative changes with increased cellularity and decreased amount of collagen.

Skeletal Size

Although *Tsk*/+ mice do not appear grossly larger than their +/+ littermates and rarely weigh more after about 4 weeks of age (see below), they regularly have larger skeletons. Table 2 shows the effect of *Tsk* on the size of a number of bones and cartilages. In every case, the *Tsk*/+ measurements exceeded those of controls, and in most cases the differences were significant. In this group of mice, which were 8 to 11 weeks old, the skull was longer and the mandible was longer and wider than normal. The length of the skull is probably determined by growth of both endochondral and membrane bone, but the mandible, while it forms around Meckel's cartilage, is composed entirely of membrane bone. Among the endochondral bones, the long bones and girdles were approximately 5% larger than controls with the exception of the pelvic bone which was 10% larger. Most of this difference was in the length of

Table 2—Effect of *Tsk* on Size of Bones and Cartilages

Structure	No. of Pairs	Mean (mm)		Difference (mm)			Significance (*P* value)
		Tsk/+	+/+	Mean	SE	Percent of +/+	
Skull							
Length	7	20.9	20.4	0.5	0.15	2.5	<0.05
Width	7	10.6	10.4	0.2	0.12	2.2	NS
Mandible							
Length	8	11.1	10.5	0.6	0.11	5.6	<0.01
Width	8	3.1	3.0	0.1	0.04	4.2	<0.05
Humerus							
Length	8	11.8	11.4	0.3	0.09	3.0	<0.05
Width	8	2.8	2.7	0.1	0.06	4.6	NS
Ulna							
Length	8	13.9	13.4	0.5	0.10	3.8	<0.05
Width	8	1.7	1.5	0.1	0.03	8.3	<0.05
Femur							
Length	8	15.5	14.6	0.9	0.06	6.3	<0.01
Width	8	2.8	2.7	0.1	0.03	3.7	<0.05
Tibia							
Length	8	17.9	17.1	0.8	0.12	4.7	<0.01
Width	8	3.0	2.9	0.1	0.03	3.9	<0.05
Scapula							
Length	8	10.0	9.7	0.3	0.11	3.5	<0.05
Width	8	8.0	7.5	0.5	0.13	6.0	<0.05
Clavicle							
Length	8	8.9	8.5	0.4	0.11	4.6	<0.01
Pelvis							
Length	8	19.3	17.5	1.8	0.15	10.4	<0.01
Width	8	7.0	6.7	0.3	0.10	4.3	<0.05
Third lumbar vertebra							
Length	8	3.8	3.3	0.5	0.09	15.2	<0.01
Width	8	2.2	2.1	0.1	0.02	7.7	<0.01
Seventh rib							
Length	8	15.9	14.2	1.6	0.21	11.5	<0.01
Seventh costal cartilage							
Length	6	15.2	12.7	2.5	0.30	19.8	<0.01
Fourth tracheal cartilage							
Length	8	3.4	3.2	0.2	0.04	5.8	<0.01
Ear cartilage							
Length	6	8.3	7.9	0.4	0.05	4.7	<0.01
Width		4.6	4.3	0.3	0.05	7.3	<0.01

the ilium. The lumbar vertebrae were markedly enlarged in both length and width. In cleared and stained skeletons, the thoracic cavity was noticeably enlarged and somewhat deformed. Viewed from the posterior end, it appeared squarish rather than oval. The costal cartilages were obviously elongated and much more bowed than normal. Measurements of the seventh rib and costal cartilage showed the rib to be 11% and the costal cartilage 20% longer than controls. To see if the effect on cartilage was a general one, the length of the fourth tracheal ring and the length and width of the ear cartilage were determined. They were found to be about 5 to 7% greater in *Tsk*/+ than in +/+ littermates.

Body Weight

In spite of their larger skeletons, adult *Tsk*/+ mice usually do not weigh more than normal littermates. This may be due to a general unthriftiness and lower amount of body fat. In 8 pairs of females that were 37 to 106 days old, the *Tsk*/+ mice averaged 21.75 g and the +/+ mice, 22.78 g. Corresponding values for 11 pairs of males 37 to 121 days old were 25.40 g and 27.37 g. The differences—1.02 ± 1.56 g for females and 1.97 ± 1.17 g for males—are not significant.

Thoracic Viscera

The lungs of *Tsk*/+ mice become abnormally distended in the enlarged thorax, and vesicular emphysema results (Figure 9A and B). When removed from the thorax, the lungs remain much more distended than those of normal sibs. We have observed slight distension of the air spaces as early as 21 days of age.

The hearts of *Tsk*/+ mice are usually enlarged, particularly the auricles (Table 3). At autopsy the auricles often appear enormously distended. The auricular tissue of both auricles weighed about 25% more than that of normal sibs. The right ventricle was also significantly enlarged but less so than the auricles. The left ventricle was not significantly enlarged. In one pair of 21-day-old mice not included in the data of Table 3, the auricles were noticeably more distended in the *Tsk*/+ mouse than in its +/+ sib.

When the enlarged hearts were first discovered, we thought they might be the result of increased blood pressure caused possibly by inelasticity of the arteries related to the connective tissue defect. However, blood pressure determinations of four pairs of 5-month-old males gave mean values of 98.8 ± 1.25 for *Tsk*/+ and 106.3 ± 4.33 for +/+. Although the difference is not significant, in this small sample the blood pressure was actually lower in *Tsk*/+ mice than in +/+.

Endocrine Studies

The symptoms of tight-skin mice show considerable resemblance to those of human acromegaly, a condition associated with excess growth

Table 3—Effect of *Tsk* on the Size of the Heart

Part	No. of pairs	Mean (mg)		Difference (mg)			Significance (*P* value)
		Tsk/+	+/+	Mean	SE	Percent of +/+	
Left ventricle	7	81.42	76.67	4.75	2.21	6.2	NS
Right ventricle	7	27.04	23.91	3.13	1.26	13.1	<0.05
Left auricle	7	4.79	3.75	1.04	0.34	27.8	<0.05
Right auricle	7	6.38	5.08	1.30	0.28	25.6	<0.01

hormone usually produced by tumors of the anterior pituitary gland. We therefore examined sections of pituitaries stained with PAS from one pair of male and one pair of female mice 3½ months old. The pituitaries of *Tsk*/+ and +/+ mice were alike in size and indistinguishable histologically.

As a further test of the hypothesis that excess growth hormone might be the cause of the tight-skin symptoms, growth hormone was assayed in the pituitary by polyacrylamide gel electrophoresis.[10] The pituitaries were obtained from four pairs of males and four pairs of females at 30 days and equal numbers at 100 days of age. There was no difference between the genotypes in size or intensity of staining of the growth hormone band at either age or in either sex.

Radioimmunoassays of growth hormone in plasma gave extremely variable results, with values ranging from 0 to over 200 ng/ml. Variation was large in both *Tsk*/+ and +/+ mice, and there was no consistent difference between the two genotypes.

Growth hormone has a diabetogenic effect in man and some other animals.[14] Blood sugar determinations on one pair of 14-month-old females and on three pairs of males and three pairs of females that were 2 to 2½ months old gave a mean of 98.4 mg/100 ml for *Tsk*/+ and 101.7 mg/100 ml for +/+. The difference, 3.3 ± 11.5, is not significant.

Time of Death of Homozygote

The results of a preliminary search for *Tsk/Tsk* embryos were not encouraging. Embryos were examined from matings of *Tsk*/+ females by *Tsk*/+ males (expected to produce one-fourth *Tsk/Tsk* offspring) and from matings of *Tsk*/+ females by +/+ males (expected to produce no *Tsk/Tsk* offspring) (Table 4). In the *Tsk*/+ by *Tsk*/+ matings, on Day 8 there was a higher frequency of abnormal and retarded embryos than at earlier or later stages or than in controls, but no characteristic phenotype

Table 4—Frequency of Abnormal Embryos From Matings Expected to Produce *Tsk/Tsk* Embryos and From Control Matings

Parents ♀	Parents ♂	Age (days)	No. of litters	Average No. of implantations	No. classified	No. abnormal	No. resorbed	Percent abnormal and resorbed
Tsk/+	*Tsk*/+	7	3	9.3	28	3	1	14.3
		8	3	11.0	28	9	1	35.7
		9	1	11.0	11	—	2	18.2
		12	1	11.0	11	—	2	18.2
		18	1	10.0	10	—	1	10.0
Tsk/+	+/+	7	1	9.0	9	1	—	11.1
		8	4	11.0	43	3	3	14.0

different from those found in control matings was identified. It seems likely that homozygotes die or degenerate at about 8 days of gestation, but the specific phenotype of the homozygotes is as yet unrecognized.

Discussion

The tight-skin mutation in heterozygotes (*Tsk*/+) causes a general hyperplasia of the loose connective tissue, cartilage, and bone. Most of the loose connective tissue is hyperplastic, including especially that in subcutaneous regions and between and within skeletal muscles; all bones and cartilages examined were larger than normal. In contrast, the tendons are smaller than normal, with hyperplasia of the tendon sheaths. The hollow thoracic viscera are pathologically distended, but other tissues and organs appear to be unaffected.

The effects of *Tsk* on loose connective tissue and on the skeleton are consistent with each other in that both involve excessive growth. It is not clear how the decreased growth of tendons is related to the other effects. Hyperplasia of the tendon sheaths does not appear to be sufficient to account for the small size of the tendons. Hyperplasia of the loose connective tissue is accompanied by change in the relative amounts of the extracellular fibers. There is a massive increase in number of microfibrils, and probably some increase in elastin and about the same amount of collagen relative to the number of fibroblasts. Tendons are composed chiefly of collagen, and it may be that they suffer some competitive disadvantage in *Tsk*/+ mice in relation to the other two types of connective tissue fibers.

Greenlee, Ross, and Hartman [15] have shown that in the development of the calf ligamentum nuchae, which is composed largely of thick fibers of elastin, the formation of elastin is preceded by the presence of numerous microfibrils. When elastin fibers appear, the homogenous substance of the fiber is bordered by a layer of microfibrils on the surface. However, Ross and Bornstein [16] have also shown that microfibrils have a different amino acid composition from either collagen or elastin, and it is therefore unlikely that they are precursors of either of these substances. The developmental sequence suggests that microfibrils may play a role in determining the shape of elastic fibers.[16] The present observations on the tight-skin mutation shed little light on the developmental interrelations of connective tissue fibers, but determination of the cause of the massive increase in microfibrils in this mutant might answer questions about their developmental role.

Distension of the thoracic viscera in *Tsk*/+ mice is almost certainly a secondary effect of the enlarged thorax. Increase in skeletal size is

especially pronounced in the ribs and costal cartilages, and the increased size of the thoracic cavity thus produced causes distension of the thin-walled viscera. Evidence in favor of this conclusion is the marked distension of the lungs and the thin-walled auricles and right ventricle and the normal size of the thick-walled left ventricle. The pathologic condition of the thoracic viscera makes it clear that growth of the viscera in *Tsk*/+ mice is not proportional to growth of the skeleton. The normal body weight of *Tsk*/+ mice supports this conclusion.

The skeleton of *Tsk*/+ mice, while larger than normal, is not noticeably misshapen, except for the thorax. It seems likely that the cause of the misshapen thorax is lack of growth of the thoracic viscera proportionate to growth of the skeleton. The enlarged thoracic skeleton exerts a distending effect on the thoracic viscera, and at the same time the small viscera exert a constricting effect on the growing skeleton, causing the costal cartilages to bow.

The symptoms of *Tsk*/+ mice resemble some of the effects of growth hormone administered to rats and mice,[17-19] and some of the symptoms of human acromegaly.[20] Acromegaly is usually associated with tumors involving the somatotropic cells of the anterior pituitary, and the characteristic symptoms are caused by the excessive amount of growth hormone produced by the tumor.[20] When growth hormone excess begins in early life, pituitary giantism, with a more or less proportional increase in body size, results.

Like *Tsk*/+ mice, growth hormone–treated rats and human acromegalics have enlarged skeletons. Acromegalics also have thickened subcutaneous connective tissue. Unlike *Tsk*/+ mice, growth hormone–treated animals and acromegalics have enlarged viscera and a general increase in body size. The growth hormone–like effects of *Tsk* thus appear to be confined to effects on the skeleton and connective tissue, rather than being a general effect on body size. Furthermore, we were unable to demonstrate an increase in growth hormone in either the pituitary or in plasma. Since the effect of *Tsk* is present at the latest by 1 week of age, a proportionate giantism would be expected if the effect were mediated by an excess of growth hormone. These observations suggest that the abnormality of tight-skin mice might involve one of the somatomedins, hormones produced by the liver in response to growth hormone. These hormones are thought to be the direct stimulators of growth of cartilage and connective tissue.[21]

Information that would allow comparison of the alterations in loose connective tissue and in tendons seen in *Tsk*/+ mice with the connective tissue changes induced by growth hormone or by somatomedin is only

partially available. We are not aware of any description of alterations of the tendons in acromegalics or in growth hormone–treated animals. The thick skin of acromegalics is particularly pronounced in the hands and feet and in the lips, giving these areas a swollen, pudgy appearance.[20] In *Tsk*/+ mice these areas are not appreciably swollen. On the contrary, the hind feet and ankles appear thinner than normal, no doubt as a result of the decrease in size of the numerous tendons in this area. However, our finding of increased staining with alcian blue in *Tsk*/+ mice is in accord with an increase in mucopolysaccharides in the skin of acromegalics found by Asboe-Hansen.[23] We do not know of any electron microscopic studies of the subcutaneous connective tissue of acromegalics or of growth hormone–treated animals such as would be necessary to determine whether microfibrils are increased.

The observed differences between the effects of *Tsk* and of growth hormone do not rule out an involvement of somatomedin in the *Tsk* lesion, since growth hormone could have other effects on connective tissue in addition to those mediated by somatomedin. There is very little information on changes in connective tissue morphology induced by somatomedin. Uthne (in discussion following paper by VanWyk *et al.*[22]) briefly mentions that hypophysectomized rats treated with somatomedin A show no weight gain but an increase in tibial width and thickening of the skin. These effects grossly resemble those of *Tsk*, but the description does not allow comparison of the pathologic changes in connective tissue.

Any theory to explain the effect of *Tsk* must take into account the fact that the homozygotes die *in utero*, probably between 7 and 8 days of gestation. It therefore seems unlikely that the gene causes either a deficiency or overproduction of a hormone, since there is no evidence that a 7-day embryo is yet producing any hormones. Ignoring for the present the lack of evidence for similarity of effect on loose connective tissue and tendon of *Tsk* and somatomedin, we propose the following tentative hypothesis as a basis for further experimentation.

The somatomedins belong to a family of peptides that have insulin-like and growth-promoting activity or are necessary for the proliferation of cells in tissue culture.[22] One of these, possibly the multiplication stimulating factor of Pierson and Temin,[24] may be necessary for the growth of embryos *in utero*, particularly during the early stages. Our hypothesis proposes that the *Tsk* locus is responsible for a receptor protein necessary for binding the active factor to the target cells. The *Tsk* mutation might produce an altered protein that binds with great affinity to a factor promoting growth of cartilage and connective tissue and that no longer binds to the factor producing growth of embryo cells. *Tsk*/*Tsk*

mice would therefore die because of inability to bind the cell multiplication factor during embryogenesis. *Tsk/+* mice would have enough of the normal binding protein to grow normally as embryos, but in postnatal life would bind an excessive amount of factor promoting growth of cartilage and connective tissue. It should be possible to design experiments to test this hypothesis.

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[*Illustrations follow*]

Figure 1—Normal (*left*) and tight-skin (*right*) females, 5½ months old, showing the amount of skin that can be readily gathered into a skin fold.

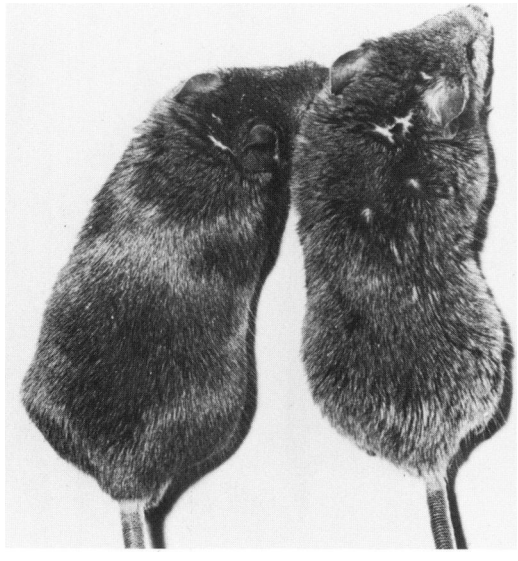
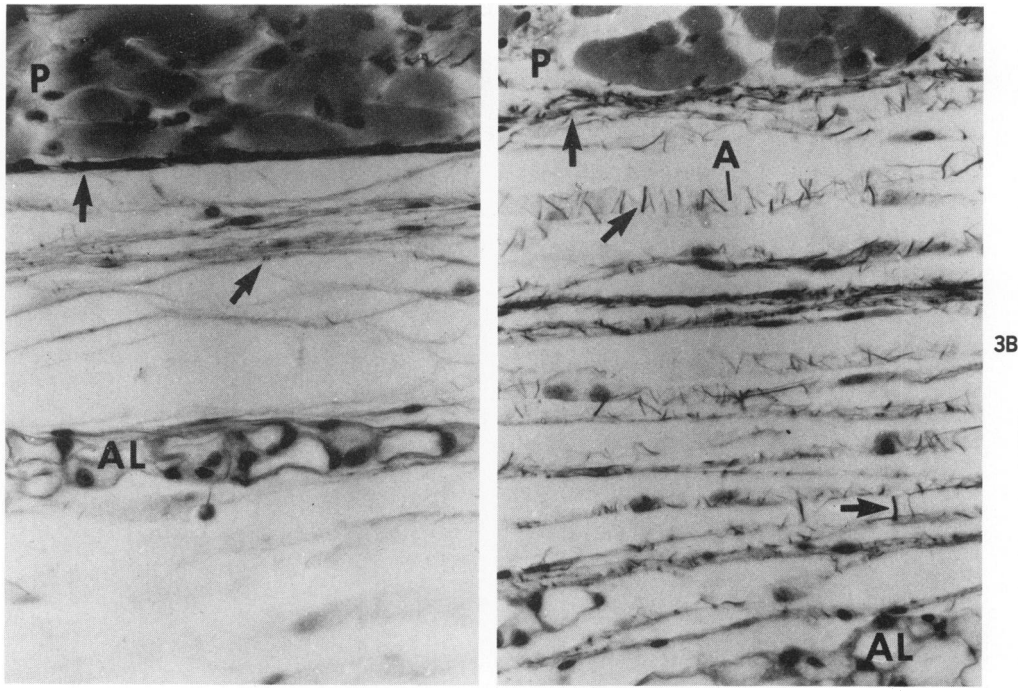
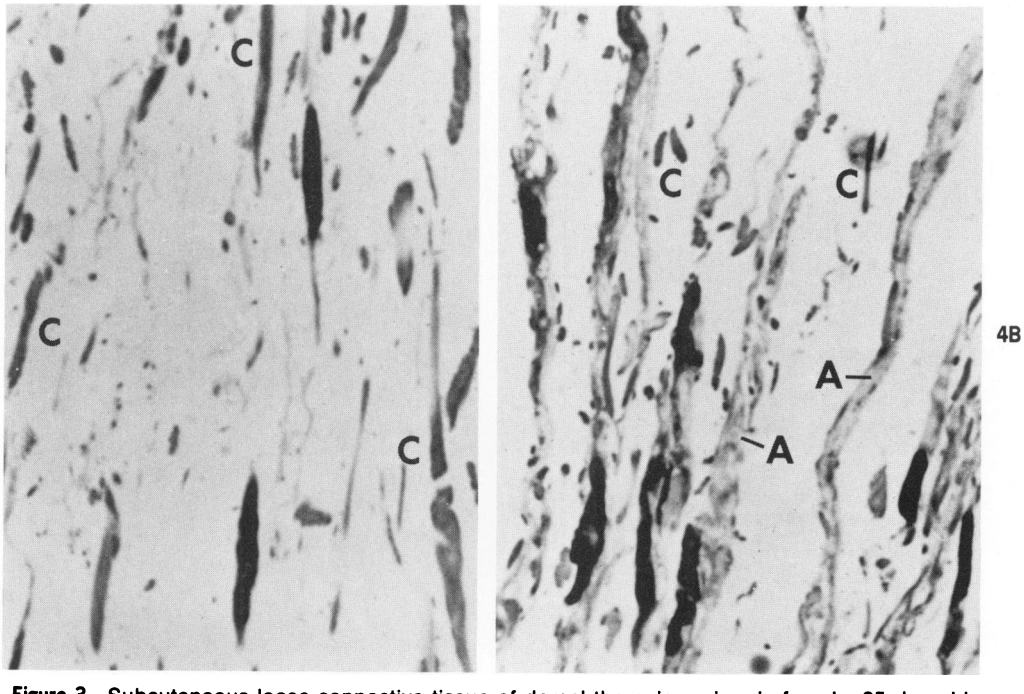


Figure 2—Male normal (*left*) and tight-skin (*right*) littermates of mice in Figure 1, showing rough fur and unthrifty appearance of *Tsk/+* mouse.

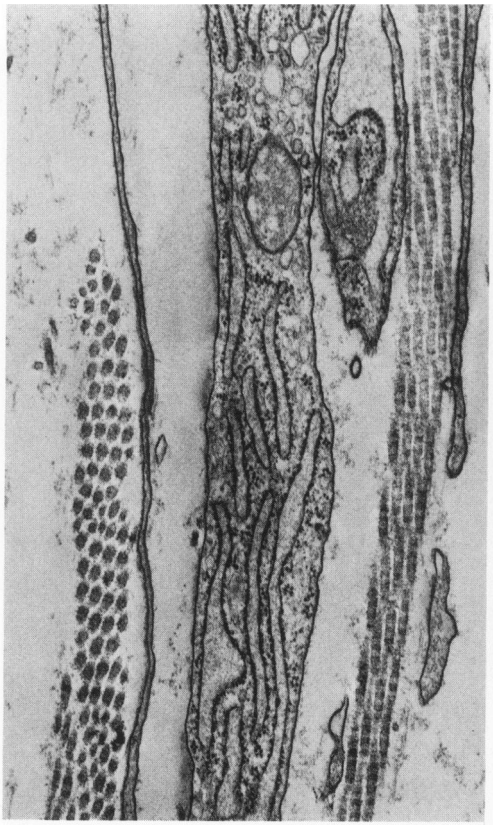


3A 3B

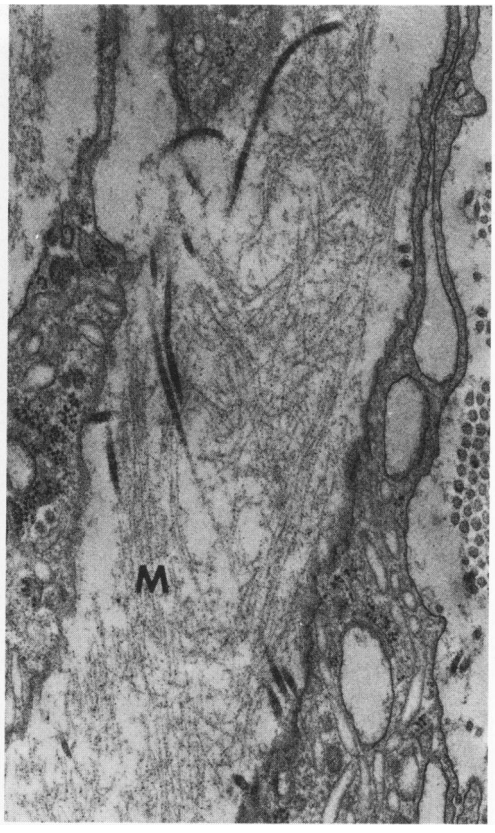


4A 4B

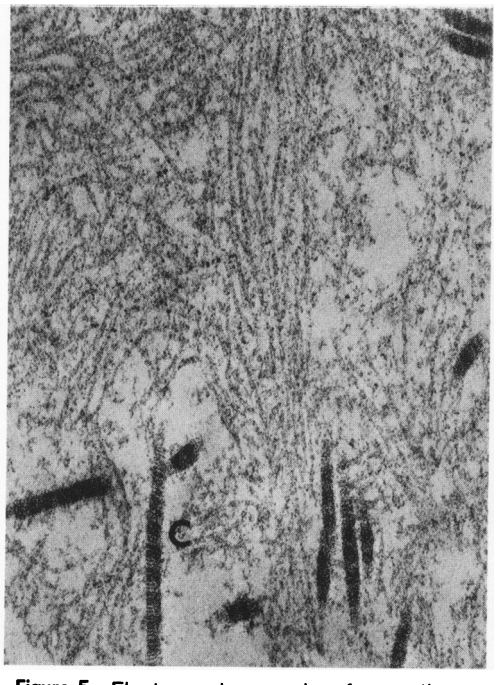
Figure 3—Subcutaneous loose connective tissue of dorsal thoracic region, in female, 25-day-old, +/+ mouse (**A**) and *Tsk*/+ mouse (**B**). Most of the elastin (*arrows*) is concentrated in a layer under the panniculus carnosus muscle (*P*) in +/+ animals. It is more abundant and evenly distributed throughout the tissue between the panniculus carnosus and the subcutaneous adipose layer (*AL*) in the *Tsk*/+ mouse. There is abundant pale-staining acidophilic material (*A*) in *Tsk*/+ animals. (Halmi's aldehyde fuchsin stain, × 350) **Figure 4**—Subcutaneous connective tissue of dorsal lumbar region of male, 37-day-old +/+ mouse (**A**) and *Tsk*/+ mouse. (**B**). Collagen (*C*) is present in both tissues, but pale-staining intercellular material (*A*) is present only in *Tsk*/+ mouse. (1-μ Araldite sections stained with toluidine blue, × 1400)



5A



5B



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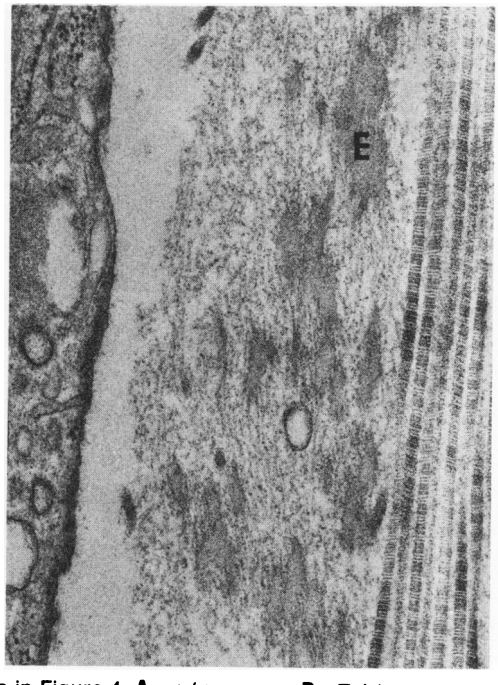

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Figure 5—Electron micrographs of same tissue as in Figure 4. **A**—+/+ mouse. **B**—*Tsk*/+ mouse. Microfibrils (*M*) are abundant in *Tsk*/+ and very sparse in +/+ mice. (× 27,000) **Figure 6**—Higher magnification of mass of microfibrils containing individual collagen fibrils (*C*). The microfibrils lack periodicity. (× 54,000) **Figure 7**—Microfibrils containing oblique sections of amorphous elastic fibers (*E*) (× 40,000)

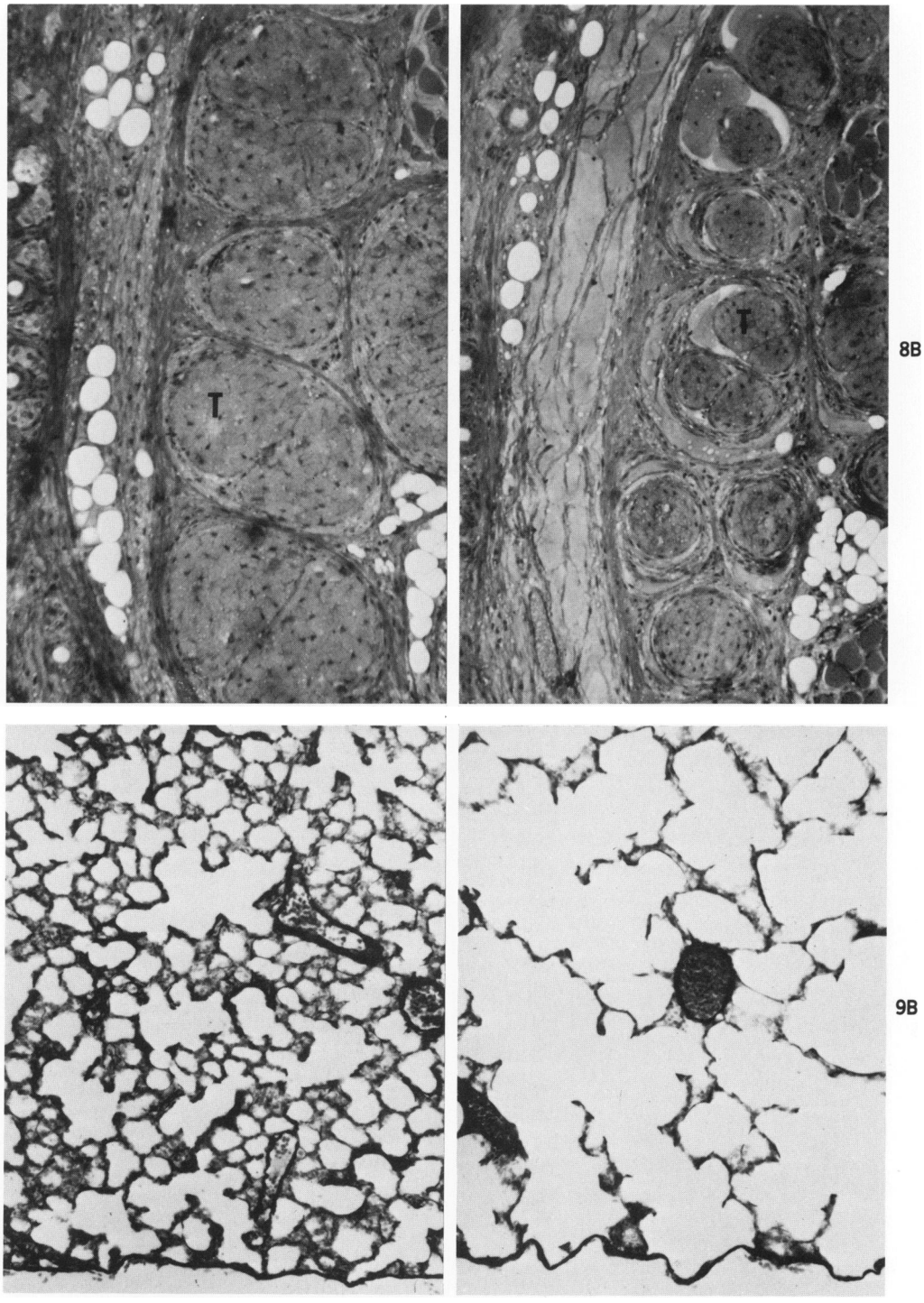

Figure 8—Transverse sections of tail from female, 21-day-old +/+ mouse (**A**) and *Tsk*/+ mouse (**B**). Tendons (*T*) of *Tsk*/+ mice are smaller and have thicker sheaths, in some cases with accumulation of fluid around the tendon. (Zenker fixation, acid orcein–fast green stain, × 150) **Figure 9**—Sections of lung of male, 57-day-old +/+ mouse (**A**) and *Tsk*/+ mouse, (**B**). Vesicular emphysema is found in *Tsk*/+. (Gridley's quadruple stain, × 150)