using the serum from patient 1 (Table 1). The results clearly show that addition of lichen myxedematosus serum to normal skin fibroblast cultures results in an increase in the number of cells at saturation density.

To determine whether the IgG paraprotein found in the serums of the majority of patients with lichen myxedematosus contributed to the in vitro results obtained in this study, the serums of the four patients were subjected to immunoelectrophoresis. Serum from patient 1 contained the IgG paraprotein with lambda-type light chains, serums from patients 2 and 4 contained the IgG paraprotein with kappa-type light chains, and serum from patient 3 did not show the abnormal globulin. Since serum from patient 3 did not contain the IgG paraprotein but stimulated fibroblast DNA synthesis, one might conclude that the abnormal globulin is not responsible for the results obtained in vitro. To further test this conclusion, lichen myxedematosus serum containing the IgG paraprotein was applied to a protein A–Sepharose CL-4B column. The IgG fraction was bound to the column and eluted with 3*M* potassium isothiocyanate. It was then dialyzed against isotonic saline and added to normal fibroblast cultures. The effect on [^{3}H]thymidine uptake was then measured as described in the legend of Table 1. No stimulatory activity was detected. This would seem to eliminate the possibility that the IgG paraprotein was responsible for the increased fibroblast proliferation.

Lichen myxedematosus is one of a number of diseases affecting the connective tissue. Preliminary data from this laboratory indicate that the serums from patients with progressive systemic sclerosis (scleroderma) also stimulate normal fibroblast proliferation in vitro. This finding is especially interesting since the earliest histological findings in scleroderma include endothelial cell hyperplasia and an increase in mucopolysaccharides in the dermis (*7*). Also, Cheung *et al.* (*8*) recently reported a fibroblast-stimulating factor from the serums of patients with pretibial myxedema that stimulated normal human fibroblast mucopolysaccharide biosynthesis. Whether there is a common factor in the etiology of these diseases is unknown. Characterization of the serum factor from lichen myxedematosus should yield valuable information concerning its role in connective tissue diseases.

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Structure of Collagen in Cartilage of Intervertebral Disk

Abstract. *Small-angle x-ray and neutron diffraction patterns have been obtained from the annulus fibrosus of porcine intervertebral disk. These show that the collagen in this tissue is modified compared with that in tendon.*

Small-angle x-ray and neutron diffraction patterns have been obtained from the annulus fibrosus of intervertebral disk. These show that collagen fibrils are wound around the annulus fibrosus at a specific orientation to the axis of the vertebral column and that the one-dimensional structure of these fibrils is different from that of the collagen fibrils in rat tail tendon.

It has been shown by wide-angle x-ray diffraction that in normal articular cartilage, collagen occurs in a disoriented array (*1*) except at the cartilage surface. In certain pathological inclusions of intercostal cartilage, it has been demonstrated by the same techniques, that the collagen fibrils become oriented parallel to each other (*2*). To our knowledge, no small-angle x-ray diffraction patterns have been reported from normal cartilaginous tissue.

Intervertebral disk contains three anatomically distinct regions, the cartilaginous end plates, the hydrated gel-like nucleus pulposus, and the fibrocartilaginous annulus fibrosus. The latter structure connects the neighboring vertebrae and is comprised of 10 to 15 lamellae. When the vertebral column is subjected to axial load, much of this is taken up by deformation of the annulus fibrosus (*3–6*). The lamellae are concentric cylindrical sheets and polarization microscopy has shown that the collagen fibers are oriented at 40° to 70° to the vertebral axis with opposite directions of tilt occurring in adjacent lamellae (*6*, figure 10). The deformation caused by axial load results in the annulus fibrosus bulging in such a way that the angle between the axis of the collagen fibers and the vertebral axis is increased. It has been shown by electron microscopy that the

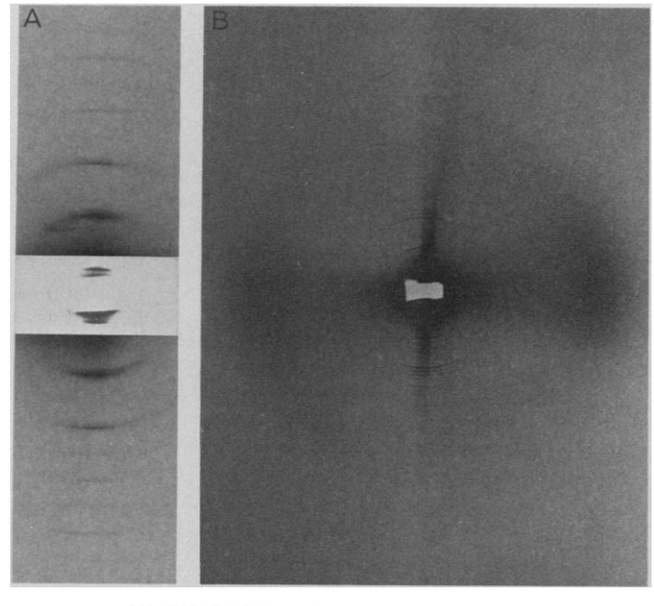

Fig. 1. (A) Low-angle x-ray diffraction pattern from structural fibers of porcine annulus fibrosus. X-rays were collimated by a mirror-monochromator camera (wavelength, 1.5405 Å). The specimen-film distance was 80 cm. Orders 1, 3, 5, and 7 to 10 of the 670 Å period from collagen are visible. Note the low exposure to show first order. (B) Medium-angle x-ray diffraction pattern from the same specimen. The specimen-film distance was 17.6 cm. Reflections up to order 22 from the 670 Å period of collagen may be seen.

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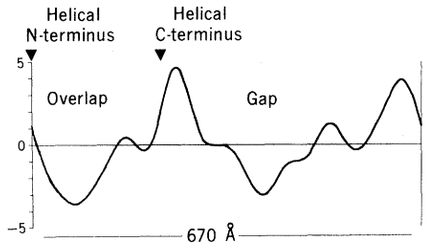

Fig. 2. Difference Fourier synthesis, using the annulus fibrosus and the rat tail tendon moduli (square root of intensities) and the phases from model E in (*8*). In scaling, the first-order intensities were assumed to be equal (*8*). The ordinate is in net electrons per unit axial residue translation. The peaks near the C and N termini of the molecule indicate that there is more matter at those locations in the annulus fibrosus collagen than in the tendon collagen.

collagen fibrils are 200 to 500 Å in diameter, separated from each other by a slightly electron-dense material (*6*). Chemical studies reveal that the annulus fibrosus consists of more noncollagenous material than tendon and also contains both type I and type II collagen. The distribution of these two types is not uniform; type I predominates at larger and type II at smaller radial locations (*7*).

It has been shown that the low-angle x-ray (*8*) and neutron (*9*) diffraction patterns from tendon can be related to the molecular structure and arrangement of the collagen. On this basis the molecular structures of different collagenous tissues may be compared and the location of noncollagen components in more complex connective tissues determined. For example, the axially projected location of the apatite in mineralizing turkey leg tendon has been established (*10*). We report here the use of low-angle x-ray and neutron diffraction from cartilaginous tissues to investigate the collagen and the possible interaction between collagen and proteoglycans or glycoaminoglycans.

Specimens for x-ray diffraction were obtained by cutting sections about 0.5 mm thick from the annulus fibrosus of a bovine intervertebral disk. The tissue was not homogeneous but was made up of long fibers about 0.3 mm in diameter embedded in a matrix. The x-ray specimen was made by isolating a single fiber and cutting away the matrix with a razor blade. Dissection was carried out with the tissue immersed in 0.15*M* NaCl solution. The fiber was finally mounted in a sealed cell above 0.15*M* NaCl and stretched between two clamps. The cell had Mylar windows to allow the passage of x-rays through the fiber. The x-ray camera was a mirror-monochromator (*11*) which isolated the Cu $K\alpha_1$ wavelength with the best focusing in the meridional direction. The x-ray sources were Elliott rotating-anode generators GX 13 (used with a camera with a specimen-film distance of 80 cm) and GX 20 (used with a camera with a specimen-film distance of 17.6 cm). Two diffraction patterns were obtained (Fig. 1, A and B) which allowed an estimate of the relative intensities of the first 22 orders of the 670 Å (*D*) spacing parallel to the fibril axis. In particular, the long camera permitted good resolution of the first order. It is clear that these intensities are similar to those from the collagen in rat tail tendon (*8*), particularly in the alternation of strong odd and weak even reflections in the first few orders and the strong group of order 20 to 22. This observation demonstrates the existence of a well-ordered staggered molecular collagen array in a cartilaginous tissue. However, a detailed examination shows clear differences in the relative intensities of the reflections from those for the collagen in rat tail tendon.

Table 1. Uncorrected relative intensities of the first ten orders in the x-ray diffraction patterns from annulus fibrosus and rat tail tendon. The intensities were measured with a position-sensitive detector and scaled so that the first-order intensities were equal.

Order	Intensity	
	Annulus fibrosus	Rat tail tendon
1	1000.0	1000.0
2	1.0	19.5
3	94.3	71.0
4	0.4	4.1
5	34.8	20.2
6	3.6	4.7
7	9.20	2.5
8	3.7	1.2
9	8.9	8.6
10	3.3	1.4

Table 1 shows a comparison of the intensities obtained on a linear detector. The alternation of strong odd and weak even reflections is more pronounced in the intensities from annulus fibrosus. This indicates a gap/overlap ratio very close to 1/1 for the cartilage compared to 0.53/0.47 for rat tail tendon. A difference Fourier synthesis, using the annulus fibrosus and the rat tail tendon intensities and the phases from model E in (*8*), confirms this interpretation (see Fig. 2). There are two major peaks at positions corresponding to the N and C termini of the collagen molecule. Since the period *D* is the same in both tissues, this indicates that either the collagen molecules in annulus fibrosus are longer than in rat tail tendon or that there is extra material bound to each end of the molecule in annulus fibrosus.

The intensity distribution along the equator of the x-ray diffraction pattern was also recorded on the 17.6-cm camera. There was a clear intensity maximum at a position corresponding to 16 Å, in contrast to a position of 13 Å for the intensity maximum from rat tail tendon measured under the same conditions (over 0.15*M* NaCl). This suggests that the water content of the fibrils is modified by either the proteoglycan-mucopolysaccharide matrix in which the collagen fibrils are embedded or the di-

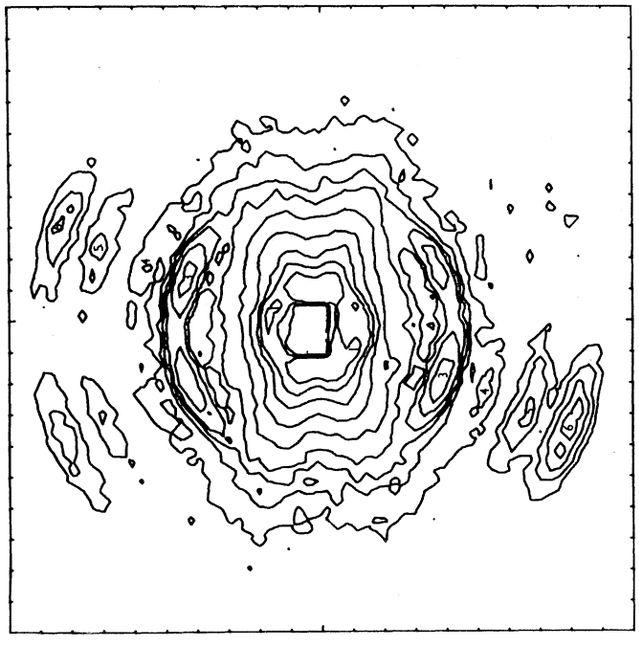

Fig. 3. Low-angle neutron diffraction pattern from a dissected sheet of annulus fibrosus immersed in a D_2O solution of 0.15*M* NaCl. The specimen-detector distance was 2.82 m and the wavelength of incident neutrons 11.1 Å.

saccharide moieties bound to the collagen.

The specimens for neutron diffraction were much larger than those for x-ray diffraction. Porcine intervertebral disks were used once more and sections of about 0.5 mm were cut so that the plane of the section was parallel to the vertebral column axis and the section itself was from a fixed radius. Sections were thus segments of thin cylinders, which were then flattened out and held between clamps in a cell. The specimens were immersed in a D_2O solution of 0.15*M* NaCl and the cell had quartz windows to allow the passage of neutrons through the specimen. Neutron diffraction was carried out on the D17 camera at the Institut Laue-Langevin. The diffraction pattern was recorded on multidetector of 128 × 128 elements, each element being in a 0.5 by 0.5 cm area. A contoured diffraction pattern is shown in Fig. 3, where the direction of the vertebral column is vertical. In Fig. 3 the first and the third through the sixth orders of the 670 Å collagen period may be clearly seen and are labeled by numbers. The second order is too weak to appear on the contour map. The direction of the line of reflections indicates that the collagen fibrils are oriented at about 70° to the direction of the vertebral column. A second, less distinct line of reflections in Fig. 3 reveals another population of collagen fibrils oriented again at 70° to the vertebral column but tilted in the opposite direction to the first set. Since the reflections in the first set are of similar intensity on both sides of the origin of the diffraction pattern, the collagen fibrils from which they originate must lie in the plane of the specimen. Since the specimen was cut at a fixed radius, these two sets of reflections originate from the radial lamellae of collagen fibers seen by optical polarization microscopy. Adjacent lamellae were seen by that method to be alternatively tilted in opposite senses from the vertebral column direction (*3*). The neutron diffraction pattern confirms that the collagen fibrils follow the direction of the optically visible fibers. The intensities of the reflections in Fig. 3 are strikingly different from those in the neutron diffraction pattern from rat tail tendon in a D_2O solution of 0.15*M* NaCl (*9*), particularly in the fifth order, which is considerably stronger in the pattern from annulus fibrosus.

These preliminary neutron diffraction results are consistent with the conclusion based on the x-ray diffraction pattern, namely that the differences between the diffraction patterns from tendon and annulus fibrosus either reflect a difference in molecular length or indicate that noncollagenous materials in the fibrocartilaginous tissue are regularly attached to the collagen. The latter possibility is being tested by the contrast variation method for neutron diffraction data [for example, see (*10*)], and we are also testing whether the dissected fibers used as specimens for x-ray diffraction are markedly different in chemistry and structure from the matrix in which they are embedded and whether differences are observable between fibers at different radial positions in the annulus fibrosus.

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Coexistence of Clones in a Heterogeneous Environment

Abstract. *Two genetically distinct clones of the asexual triploid fish* Poeciliopsis 2 monacha-lucida *inhabit the Rio del Fuerte of northwestern Mexico. Their coexistence apparently depends on feeding specializations that result in partitioning of the limited food resources in the desert streams. The findings suggest that these asexual organisms have sufficient clonal diversity to occupy a broad, heterogeneous, adaptive zone.*

The absence of genetic recombination in asexual organisms is generally thought to result in a slow rate of evolution and a high rate of extinction (*1*). Presumably, the genetic variability contained in sexual populations allows them to respond to diverse environmental conditions, to expand their adaptive zone, and thereby to decrease their likelihood of extinction (*2*). Recent genetic studies have revealed that naturally occurring asexual populations of weevils (*3*), fishes (*4–6*), amphibians (*7*), and lizards (*8*) contain considerable variation in the form of multiple sympatric clones. Clonal diversity in an asexual population reflects the balance between those forces that generate new clones from other asexual or sexual ancestors and those factors that cause clonal extinction (*5*). In this report I describe two clones of all-female fish in the genus *Poeciliopsis* (Poeciliidae) and provide evidence that coexistence of clones within an asexual population is associated with partitioning of the natural resources.

The triploid "species," *P. 2 monacha-lucida*, arose by hybridization between the sexual species, *P. monacha* and *P. lucida*; its name reflects the fact that it contains two sets of *monacha* chromosomes and one set from *lucida* (*9*). The all-female populations reproduce gynogenetically; sperm from males of *P. monacha* are required to stimulate development of the triploid eggs, but the sperm contribute nothing to the genotypes or phenotypes of the progeny (*10*). Cytogenetic, immunogenetic, and electrophoretic studies revealed that the all-female progeny are identical to their mothers (*6, 11*). Their lineage constitutes a true clone. Three distinct clones were identified by electrophoretic studies in which the products of 23 gene loci were used (*12*). Clones 1 and 2 coexist in the Rio del Fuerte, Sonora, Mexico, and clone 3 occurs alone in the Rio Mayo, a river system north of the Rio del Fuerte. Tissue-grafting studies confirmed the genetic distinctiveness of clones 1 and 2; clone 3 remains to be tested (*13*).

The sperm-dependent, all-female clones and their sexual host, *P. monacha*, inhabit the unpredictable headwater streams that drain into the Sonoran desert. During the annual dry season, streams often dry up and local extinctions occur (*14*). Surviving populations are densely crowded into small

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