X-RAY DIFFRACTION STUDIES OF HUMAN CHORDAE TENDINEÆ*

by

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When an X-ray beam is passed through material having a periodic structure, it is diffracted in a characteristic fashion according to the dimensions of the crystalline lattice of the material, the degree of crystallinity, and the amount of orientation of the component molecules. Diffraction studies have yielded knowledge of the intimate structure of many organic and inorganic compounds and varieties of biological material.

The diffraction pattern of collagen is well established. The three characteristic spacings obtained in large-angle diffraction studies are of approximately 2.8, 4 and 10 Ångstrom units, and are considered[1] to represent respectively the average amino acid residue length, the average backbone spacing, and the average side-chain distance of the collagen molecules. The presence of these three spacings is sufficient to identify the material as collagen, and no matter what its source—tunica albuginea, tendon, scar tissue, chorda tendinea, etc.—there is little variation in the pattern. X-ray diffraction studies can also furnish information as to the degree of orientation of the molecules; complete circles or discrete arcs represent lesser or greater orientation respectively. The degree of crystallinity is indicated by the width of the rings themselves, a thicker ring appearing with a specimen of low crystallinity.

Studies have been undertaken in this laboratory to determine whether alteration of the X-ray diffraction pattern of human chordae tendineae accompanies aging. This tissue was selected because it is easy to obtain and to prepare, consists almost entirely of connective tissue, and is under continual stress during life.

Method: At routine autopsies, groups of chordae tendineae were removed from the mitral valve, together with contiguous portions of valve and papillary muscle. In the presence of valvular disease or deformation of the chordae, the specimen was discarded. Following 20% formalin fixation, the tissue was washed in water, and a single chorda approximately 100 to 150 microns in thickness removed, dried on glass without tension, and mounted over the collimating hole in the microcamera so that the beam would pass through the center of its axis. In the microcamera used in these investigations***, the collimated beam was about 60 by 80 microns in width, the film to specimen distance 13.4 mm, the film diameter about 43 mm. Copper K-alpha radiation (1.54 Ångstrom

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*** The camera was built in this laboratory, and is identical in all essential features to the microcamera designed by FANNKUCHEN. A similar camera has been recently described by F. G. CHESLEY (X-ray Diffraction Camera for Microtechniques, *Rev. Sci. Instr.*, 18 (June, 1947) 422–424.

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units) was used, with Eastman Kodak No-Screen film and an exposure time of about 10 hours.

Preliminary studies revealed that the degree of orientation of the diffraction pattern was unaltered whether the specimen was fixed or unfixed, the exposure time long or short. When the beam passed through the periphery of the chorda, the pattern was less distinct but otherwise identical with that formed when the beam passed through the center of the axis. Repeated examinations along the same chorda, exposures of several chordae from mitral and tricuspid valves of the same subject and of chordae of various thicknesses from the same subject, all failed to show significant variations. The classical collagen X-ray diffraction pattern is illustrated schematically in Fig. 1.

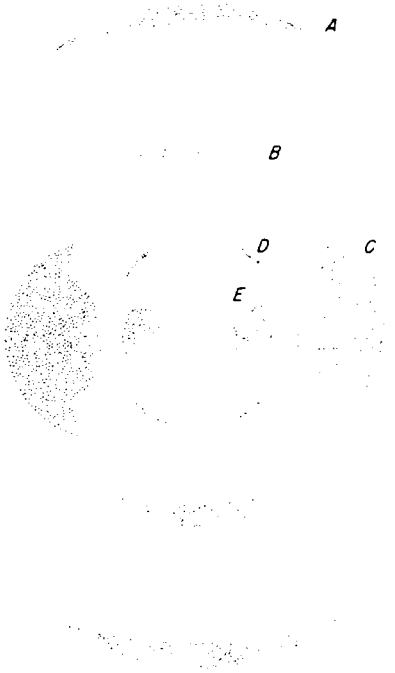

Fig. 1. Schematic X-ray diffraction pattern of oriented collagen.

Patterns were arbitrarily placed in three categories, "poor", "medium", and "good", depending on the degree of orientation. With "good" orientation, A, B, C, D and E (Fig. 1), are distinct, as in Fig. 2. In "poor" orientation (Fig. 4), A appears as a complete circle, B and C form the uniform middle ring, and D and E comprise the inner circle, with D usually merging with the background. In "medium" orientation (Fig. 3), A appears as a larger arc, C appears as meridional accentuation, E appears as arcs, and D is not discernible*.

Results: Data are summarized in Table I, which is a listing, by chronological age of the subjects, of the degree of orientation of the chordae tendineae examined. It is to be noted that with very few exceptions the best orientation was found in the older subjects. Higher orientation indicates a higher degree of alignment of collagen molecules in the axis of the chordae. Whether this is a result of constant tension, reorientation or reorganization of connective tissue as part of the aging process, or some process not heretofore recognized, remains to be determined. It appears that during adolescence the maximum degree of orientation is achieved, as far as can be inferred from the arrangement of data in our three groups. A quantitative measure of orientation by densitometric measurements is under consideration. Preliminary results, however, do not appear promising. It may be pertinent that CLARK AND ZIEGLER[2], studying the X-ray diffraction patterns of catgut ligatures, correlated increased tensile strength with higher orientation. Studies are now being undertaken to correlate the histological appearance of the chordae with the diffraction pattern. In addition, it is planned to investigate other collagenous structures of the body from the standpoint of the X-ray diffraction pattern at various ages during health and disease.

* The outer arc A in Fig. 3 and the corresponding ring in Fig. 4 is not visible in the half tone reproductions; they are quite distinct in the original films.

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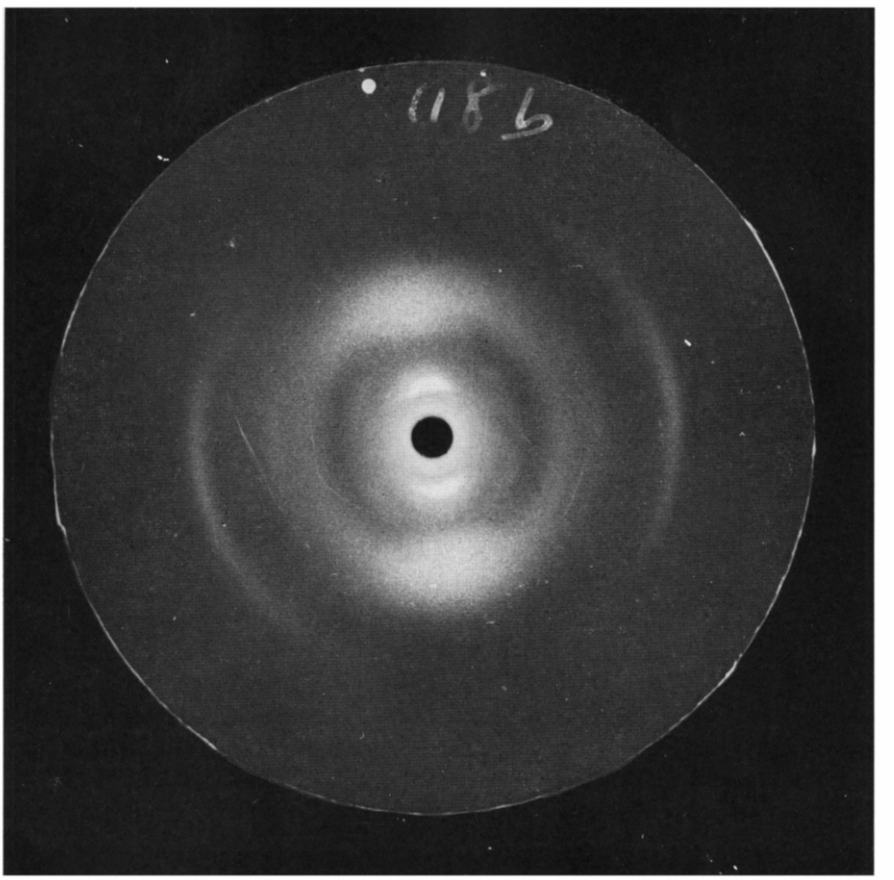

Fig. 2. X-ray diffraction pattern of chordae tendineæ of a 70 year old adult, showing "good" orientation

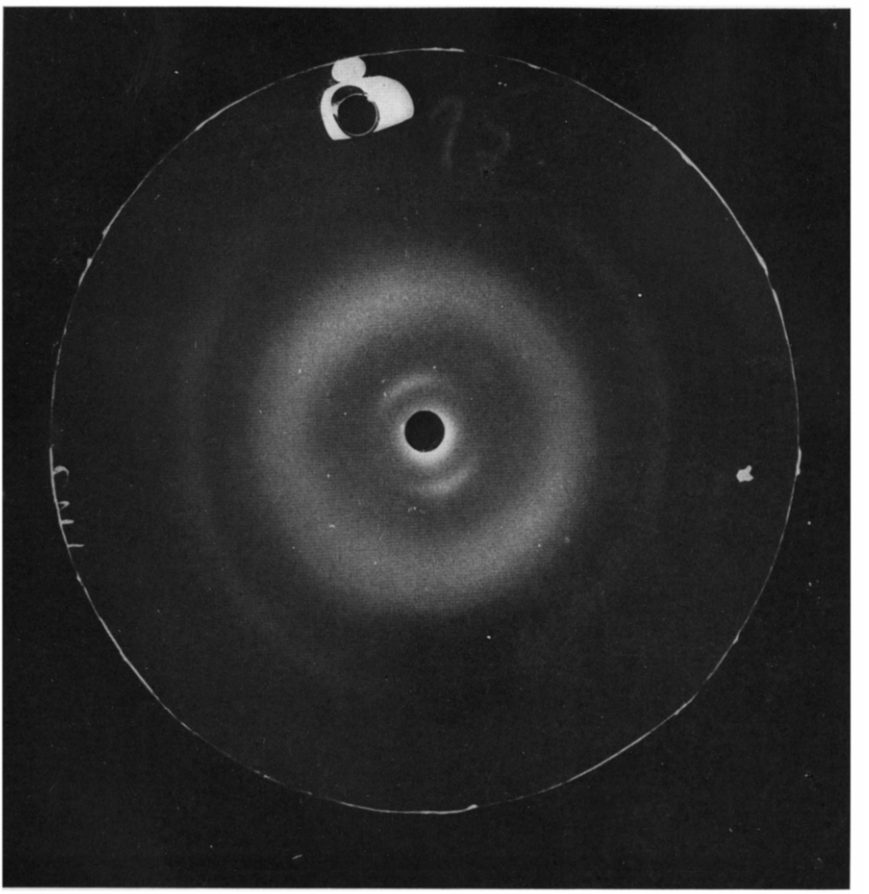

Fig. 3. X-ray diffraction pattern of chordae tendineæ of a 45 year old adult, showing "medium" orientation

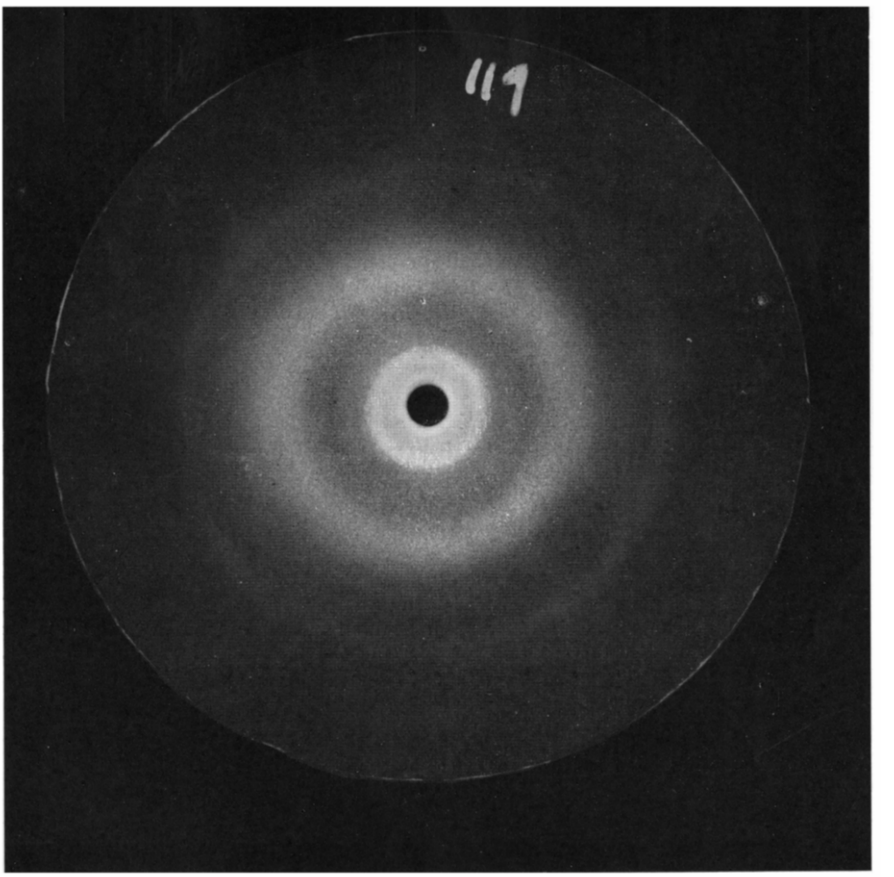

Fig. 4. X-ray diffraction pattern of chordae tendineæ of a year old infant, showing "poor" orientation

Table I

ORIENTATION OF THE X RAY DIFFRACTION PATTERN OF THE CHORDAL TENDINEAE OF HUMANS AT VARIOUS AGES, ARRANGED IN CHRONOLOGICAL ORDER

Age	Orientation	Age	Orientation	Age	Orientation	Age	Orientation
Premature	Poor	5 months	Poor	42 years	Good	70 years	Good
Premature	..	5 months	..	44 years	Medium	70 years	..
Premature	..	2 years	Medium	45 years	Good	72 years	..
Newborn	..	1 years	Poor	61 years	..	72 years	..
Newborn	..	1 years	Medium	62 years	..	72 years	..
Newborn	..	4 years	Poor	65 years	..	73 years	..
3 weeks	..	35 years	Good	65 years	..	74 years	..
4 weeks	..	35 years	..	69 years	..	71 years	..

SUMMARY

X-Ray diffraction patterns of chordae tendineæ in 32 autopsy cases in subjects of varying age show a poor orientation of collagen molecules in the younger age groups (below four years) and an increasing degree of orientation in older age groups (all subjects over 45 years show a high degree of orientation).

RÉSUMÉ

Les diagrammes de diffraction des rayons X des chordae tendineæ prélevées dans 32 cas d'autopsie chez des sujets d'âges différents, montrent que l'orientation des molécules de collagène est peu marquée chez les individus les plus jeunes (en-dessous de 4 ans) et que cette orientation s'accroît avec l'âge (tous les individus âgés de plus de 45 ans présentant un degré élevé d'orientation).

ZUSAMMENFASSUNG

Röntgenstrahlinterferenzbilder von chordae tendineæ in 32 Autopsien zeigen geringe Gleichrichtung der Kollagenmoleküle bei Individuen unter 4 Jahren und ein Zunehmen dieser Gleichrichtung mit dem Alter (in allen Fällen über 45 Jahre wurde ein hoher Grad der Gleichrichtung gefunden).

REFERENCES

1 W. T. ASTBURY AND F. O. BELL, *Nature*, 145 (16 March, 1940) 421.
2 P. F. ZIEGLER AND G. L. CLARK, *Surg. Gynecol. Obstet.*, 58 (1930) 578.

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