

ticular vitamin G-deficient diet is completely cured by lacto-flavin, as contrasted with the negative results of flavin-administration for the cure of so-called rat-pellagra by György,⁸ Chick, Copping, and Edgar,⁹ and others.

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X-Ray Diffraction Patterns from Reprecipitated Connective Tissue.

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For a number of years¹ it has been known that tendon gives excellent crystalline reflections. Recently² large spacings have been observed which provide strong evidence for the view that collagenous structures may be fibrous arrays of true crystals composed of very large molecules.

Most forms of tendon from adult animals cannot be put into solution without obvious chemical disintegration. The work of Nageotte and of others³ has, however, demonstrated that optically active fibrous precipitates can be obtained from weakly acid solutions made from the tail tendon of adult rats and from many connective tissues of foetal animals. The optical appearance of these precipitates suggests that they are at least similar to the tissue from which they were dissolved. We have carried out a series of experiments to determine: (a) whether reprecipitated tendon is really crystalline and (b) whether its X-ray diffraction pattern is like that of the original tissue.

Reprecipitated connective tissue was obtained according to the procedures outlined by Nageotte. Pieces of freshly excised tail tendon from adult rats were placed in dilute (1 to 25,000) acetic

⁸ György, P., *Biochem. J.*, 1935, **29**, 741.

⁹ Chick, H. J., Copping, A. M., and Edgar, C. E., *Biochem. J.*, 1935, **29**, 722.

¹ See for example, Katz, J., *Die Röntgenspektrographie als Untersuchungsmethode*, Berlin, 1934, p. 189.

² Wyckoff, R. W. G., Corey, R. B., and Biscoe, J., *Science*, 1935, **82**, 175; Clark, G. L., Parker, E. A., Schaad, J. A., and Warren, W. J., *J. Am. Chem. Soc.*, 1935, **57**, 1509

³ Nageotte, J., and Guyon, L., *Compt. rend. Assoc. Anatomistes, Bruxelles*, 1934, 25-28 Mars.

acid. In such acid the tendon swells and a portion of it dissolves to form a clear but highly viscous solution. The reprecipitated tissue is obtained from this solution by salting out with 5% NaCl. Series of X-ray diffraction studies have been made of a number of these preparations. Some of these diffraction photographs were made with the usual short crystal-to-film distances; others were made with very small slit systems and with the large camera radii needed to record spacings of the order of 100 Å. Diffraction patterns have been photographed and measured from (1) the original tail tendon after drying over P_2O_5 , (2) reprecipitated tissue dried under tension, (3) the extracted tendon dried under tension. These photographs and typical measurements made upon them (Table I) prove that reprecipitated tissue shows exactly the same X-ray diffraction lines as the initial tendon. Furthermore if the precipitate is carefully dried under stretch it shows the same type of preferred orientation as the original tendon. Drawings of the most conspicuous features of patterns of initial and reprecipitated tendon are reproduced in Fig. 1. Except that the orientation is

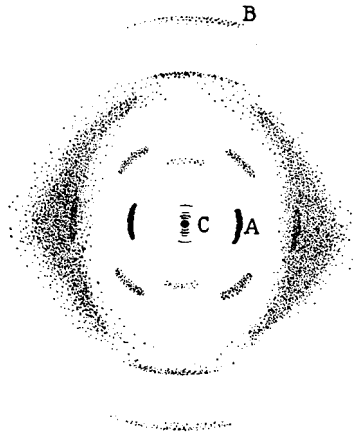


FIG. 1a.

A drawing showing essential features of the X-ray diagram of dried rat tail tendon.

poorer in the reprecipitated sample, as shown by greater length of the reflection arcs, the diagrams are the same. Photographs from carefully dried bits of extracted tendon are essentially the same as those of the original dried tissue.

These experiments demonstrate that reprecipitated connective tissue prepared according to the procedures of Nageotte is crystalline with the same X-ray diffraction pattern as the original tendon. Such a reprecipitated tissue thus can differ only slightly if at all

from the original either in chemical composition or in crystalline array. This conclusion is important because it means that suitably made collagen solutions may be expected to contain substan-

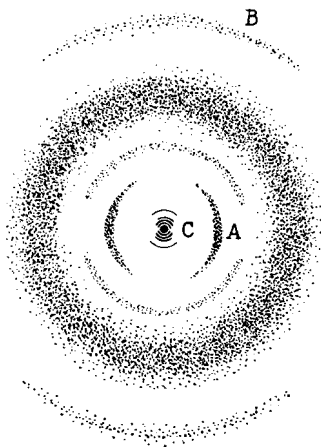


FIG. 1b.

A similar drawing of reprecipitated tendon dried under stretch. The groups of lines lettered A, B, and C in both drawings and in Table I can be more accurately measured than most other reflections in the original photographs.

tially the same large molecules that have gone to make up the original connective tissue. A study of such solutions together with a further examination of X-ray crystal diagrams can hardly fail to throw light upon the nature of these molecules.

TABLE I.

Reflection*	Intensity	(1) Original tendon	(2) Reprecipitated tissue	(3) Extracted tendon
B	medium	2.88 A	2.88 A	2.84 A
A	strong	10.66	10.75	10.71
C	very faint	33.3	32.7	†
	faint	54.6	53.1	55.3
	strong	70.1	71.4	71.4
	medium	109	103	109

*See Fig. 1.

†Too faint for measurement.