

tion of relative electron density along  $a$  using experimental ( $0kl$ ) amplitude data is in satisfactory agreement with the parameter values given above.

Rubidium fluogermanate is an aggregate of  $K^+$  and practically regular octahedral  $GeF_6^-$  ions (for diagrams of the structural type see ref. 1). The lattice constants and parameter values are only very slightly different from those found for ammonium fluogermanate, so that corresponding interatomic separations are virtually identical

in the two cases. The near identity in the effective radii of rubidium and ammonium ions appearing in corresponding compounds has been repeatedly observed excepting in cases where ammonium ion is restricted to a small coordination number (usually four) through the formation of strong hydrogen bonds. We may conclude again<sup>1</sup> that hydrogen bonding plays a relatively minor role in ammonium fluogermanate.

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RECEIVED FEBRUARY 20, 1942

## COMMUNICATIONS TO THE EDITOR

### ELECTRON MICROSCOPE OBSERVATIONS OF COLLAGEN

Sir:

Electron micrographs have been made of collagen fibers from a variety of sources, including rat tail tendon, beef tendons and ligaments, and human skin. Fibers were obtained either by teasing small bits of tendon in water or by dissolving the material in acetic acid and reprecipitating the fibers by neutralization.

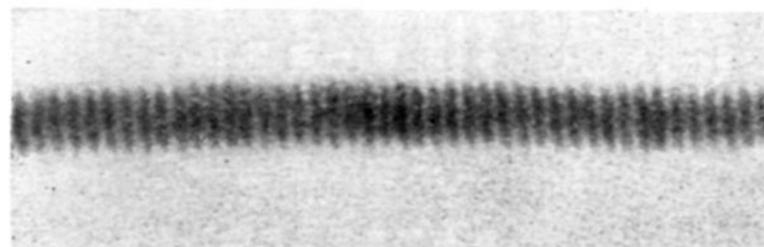


Fig. 1.—Electron micrograph of collagen fibers from beef tendon, magnification 25,000  $\times$ .

Under appropriate conditions the fibers appear characteristically cross-striated, the relatively opaque and transparent bands extending uniformly across the fiber (see Fig. 1). The average distance between like bands can be measured with an accuracy of about 3%. The interband distance is independent of fiber width and varies considerably from one fiber to another; the extremes thus far measured are 902 and 522  $\text{\AA}$ ., though the range shown in the fibers of any single preparation is more restricted.

Recent X-ray diffraction investigations in this

Laboratory [R. S. Bear, *THIS JOURNAL*, **64**, 727 (1942)] have demonstrated the presence in collagen of a fiber-axis periodicity of approximately 640  $\text{\AA}$ . This spacing was obtained from all the types of collagen mentioned above and appears to be characteristic of this protein in intact tissues. There seems little doubt that the periodicities observed in the electron micrographs represent a manifestation of the X-ray diffraction periodicity in intact tendon and that the phenomenon is a consequence of the structure and arrangement of the collagen molecules in the fibers. The range of spacings observed in the electron micrographs is doubtless due to the special conditions required for the preparation of the material, chief among which are the isolation and vacuum drying of individual fibers. It is reasonable to expect individual fibers to behave differently when isolated than when present in compact bundles as in normal tendon where lateral restraints, possibly by enclosing membranes and cement substance, restrict their behavior mechanically. This interpretation is being tested by an X-ray diffraction study of teased fibers similar to those observed with the electron microscope. In addition, the effect of various physical and chemical conditions on the appearance of the fibers in the electron micrographs is being further investigated in an effort to get more information concerning the molecular architecture of collagen.

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RECEIVED MARCH 17, 1942

### THE OCCURRENCE AND INTERCONVERSION OF VARIOUS FUCOXANTHINS

Sir:

Many polyene compounds occur as two or more spatial or *cis-trans* forms, some of which are interconvertible.<sup>1,2,3</sup> Among the carotenoid pigments of green leaves, only the high-melting, stable polyene isomers have been observed.<sup>3</sup> Now, however, additional observations on algal carotenoids indicate that fucoxanthin, which participates in photosynthesis,<sup>4</sup> occurs in at least three interconvertible modifications.

By means of chromatographic adsorption,<sup>5</sup> we have prepared these isomeric fucoxanthins, in addition to smaller quantities of other xanthophylls, from brown algae (*Phaeophyceae*) and the diatom *Nitzschia closterium*. The principal fucoxanthin, here called *fucoxanthin a*, comprises about nine-tenths of the total fucoxanthins and forms an orange band on adsorption columns of powdered sugar. Above this, *fucoxanthin b* forms a smaller, slightly yellow zone. Immediately above fucoxanthin *b*, there occurs another pale orange zone containing *fucoxanthin c*. Traces of similar pigments appear higher on the column.

When pigments were extracted rapidly from *Nitzschia* and adsorbed under mild conditions, the same mixture of fucoxanthins was always obtained. Fucoxanthin *a* treated similarly gave only traces of its isomers. Unless a rapid conversion takes place immediately upon death of the cells, fucoxanthins *b* and *c*, as well as *a*, probably represent normal constituents of brown algae and diatoms.

Interconversion of the fucoxanthins occurs

(1) Strain, *THIS JOURNAL*, **63**, 3448 (1941); and included refs.

(2) Zechmeister and Schroeder, *Science*, **94**, 609 (1941); Zechmeister, Le Rosen, Went and Pauling, *Proc. Nat. Acad. Sci.*, **27**, 468 (1941).

(3) Strain, "Leaf Xanthophylls," Carnegie Inst. of Wash., Publication No. 490, 1938, p. 29.

(4) Dutton and Manning, *Am. J. Bot.*, **28**, 516 (1941).

(5) Strain, "Chromatographic Adsorption Analysis," Interscience Publishers, Inc., New York, N. Y., 1942.

very slowly in ethanol at 20°, rapidly at 76°. It appears to be catalyzed by substances upon which the pigments are strongly adsorbed. Iodine dissolved in petroleum ether containing dimethylaniline<sup>1</sup> causes rapid interconversion of fucoxanthins *a*, *b* and *c*, yielding a mixture similar to that found in the algae. Acids, or iodine with little or no dimethylaniline,<sup>1,2,3</sup> form additional pigments (adsorbed above fucoxanthin *c* and below fucoxanthin *a*).

Isomeric fucoxanthins exhibit similar spectral absorption curves. In alcohols, each pigment has a single definite absorption maximum:  $\lambda$  max. (ethanol), fucoxanthin *a*, 452 m $\mu$ ; *b*, 445 m $\mu$ ; *c*, 446 m $\mu$ . Each isomer is decomposed by alkalis and is converted into water-soluble, blue products by concentrated hydrochloric acid. Fucoxanthin *a* represents the bulk of the fucoxanthin ordinarily prepared by crystallization<sup>3</sup> (p. 27) and probably corresponds to the fucoxanthin  $\alpha$  detected by adsorption on filter paper.<sup>6</sup> A second more strongly adsorbed pigment (fucoxanthin  $\beta$ ),<sup>6</sup> also observed by adsorption,<sup>7</sup> was likely a mixture of our fucoxanthins *b* and *c*.

By analogy with other xanthophyll pigments,<sup>1,2,3</sup> fucoxanthin *a* should be the stable, *trans* form. Isomers *b* and *c* presumably contain an unknown arrangement of hydroxyl groups and *cis* double bonds. If this be true, the original appellation of alpha and beta given to fucoxanthin isomers<sup>6</sup> is the inverse of that employed with isomeric polyene acids. Moreover, there is no rational system of nomenclature for various *cis* and *trans* carotenoid isomers; hence, use of Latin rather than of Greek letters to designate the various fucoxanthins seems desirable<sup>3</sup> (pp. 68-73).

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RECEIVED APRIL 1, 1942

(6) Kylin, *Z. physiol. Chem.*, **166**, 39 (1927).

(7) Pace, *J. Biol. Chem.*, **140**, 483 (1941); and included refs.