Table 1. Composition of grain membrane.

Preparative method	Total N (%)	Hydroxyproline (%)
White hide (steer)		
10% Cl_3CCOOH	11.6	0.51
98% H_2SO_4	13.5	1.46
10% lactic acid	16.8	1.55
5% lactic acid	12.0	1.20
Calfskin		
5% lactic acid	—	1.07
10% tartaric acid	11.6	1.01
10% citric acid	12.6	1.04
Autoclaved	10.2	1.05
Autoclaved	13.3	1.54

carbohydrate was confirmed by the anthrone method. The low hydroxyproline content shows that the membrane cannot be considered to be collagen, for collagen contains about 13 percent of hydroxyproline. This is supported by an x-ray examination of the isolated grain membrane by L. P. Witnauer, which revealed an amorphous scattering completely lacking in the characteristic collagen pattern.

The isolated grain membrane was also found to be low in cystine content (0.8 percent Sullivan method) and, therefore, cannot be considered to be a keratin. The membrane was also found to be readily solubilized by trypsin.

The properties of the isolated grain membrane of cattle hide appear to be very close to those of elastin. Elastin is resistant to autoclaving and the action of acid and alkaline solutions. It contains small amounts of cystine and hydroxyproline and is digested by proteolytic enzymes. Since the filmy (reticular) material removed from the flesh side of the grain membrane seems to have properties similar to those of the grain membrane, the membrane probably has the same composition as the amorphous-matrix protein of reticular tissue, and this protein is probably elastin or a very similar protein. The presence of a dense layer of elastin on the surface of a hide seems to be contrary to histological findings. The preparative procedure would remove collagen and some other constituents so the grain tissue of the animal may be much more complex than the grain membrane isolated here.

Further work is in progress, and a complete report will be submitted to the *Journal of the American Leather Chemists Association.*

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* A laboratory of the Eastern Utilization Research Branch, Agricultural Research Service, U.S. Department of Agriculture.

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30 March 1955.

Tubular Structure of Collagen Fibrils

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Previous electron microscope studies of connective tissue have dealt almost entirely with fibrils teased from tendon or reconstituted preparations of relatively *intact* collagen fibrils. In this investigation, both transversely and longitudinally *sectioned* normal collagen fibrils have been examined.

The most interesting observation to date concerns the distinctly tubular appearance of collagen fibrils when they are viewed in section. The possibility of these fibrils being hollow structures was discussed by R. W. G. Wyckoff [*Connective Tissues* (Josiah Macy, Jr., Foundation, New York, 1952), p. 60], but a definite conclusion could not be drawn from the evidence then available.

Sections containing collagen fibrils were prepared from specimens of periodontal membrane (human and monkey), tail tendon (rat), Achilles tendon (rabbit), cartilage (rat and rabbit), bone (rat, rabbit, and human), and skin (rat and rabbit). Small cubes of tissue were fixed in neutral formalin, dehydrated in cellosolve, and imbedded in methacrylate. Sections were made with both a Minot international microtome and a Spencer rotary microtome equipped with a thermal expansion adapter. Following removal of the imbedding material, sections were mounted on Formvar substrate films and shadowed with palladium.

A transverse section of three collagenous principal fibers of human periodontal membrane is shown at low magnification in Fig. 1. It is evident that the component fibrils of these fibers are imbedded in an amorphous ground substance that imparts a cloudy appearance to the interfibrillar areas. The clarity with which the fine detail of individual fibrils can be seen at higher magnifications depends to a large extent on the successful removal of this investing material during the processing of the specimens. Part of a cross section of a single periodontal membrane fiber from which most of the ground substance was removed by prolonged washing in water immediately after fixation is shown in Figs. 2 and 3. The tubular character of the collagen fibrils is clearly evident.

In most cases enough ground substance remains on the surfaces of the fibrils to obscure the cross striations characteristic of collagen. A typical 640-A periodicity can often be seen on the interior surface of the wall when a fibril has been opened by oblique or longitudinal sectioning, as is shown in Figs. 4 and 5. A few fibrils small enough to escape longitudinal sec-

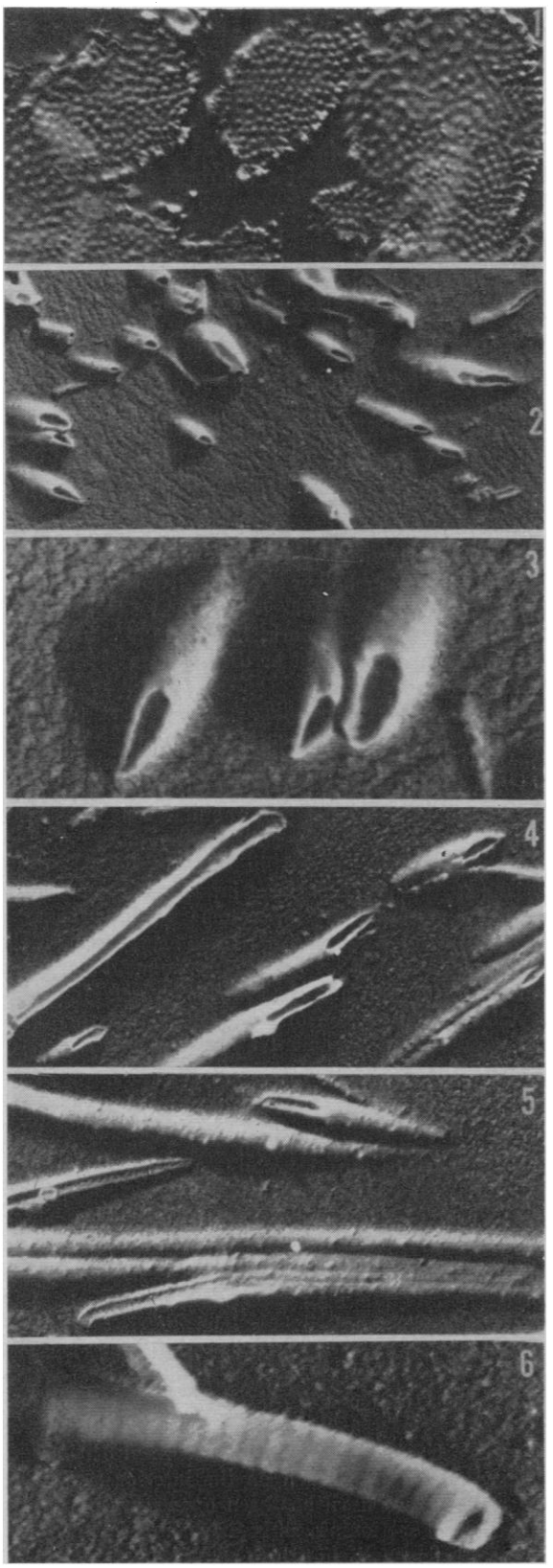

Fig. 1. Cross section of three collagenous principal fibers of human periodontal membrane showing component fibrils partially masked by amorphous ground substance. (× 3000) Fig. 2. Section of human periodontal membrane fibrils with ground substance partially removed. (× 18,000) Fig. 3. Higher magnification of three fibrils from Fig. 2. (× 55,000) Fig. 4. Oblique section of periodontal membrane fibrils. (×16,500) Fig. 5. Human periodontal collagen fibrils, sectioned longitudinally, exhibit striations on the interior surface of the wall. Uncut fibrils appear cylindrical and show ground substance remaining on the exterior surface. (× 21,000) Fig. 6 Sectioned collagen fibril from rat tail tendon. Cut end at right shows tubular character, and 640-A striations are visible on the external surface. (×45,000.)

tioning can also be seen. A fibril exhibiting both periodicity and tubularity is shown in Fig. 6. Because of the masking effect of residual ground substance, the number of instances in which these two characteristics have been seen simultaneously has been relatively small.

On the basis of the purely morphological evidence presented, the physiological significance of the tubular structure is not yet clear. The tubular character, however, has been common to fibrils of all tissues so far examined in this experiment.

10 January 1955.

Method for Counting Tritium in Tritiated Water

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Many experimentalists overcome the difficulty of detecting the weak beta rays of tritium by counting tritium hydride as a component of the Geiger filling (*1*). This technique can be used for measuring the tritium in tritiated water by converting to tritium hydride, usually with zinc, at elevated temperatures (*2*). Although this procedure is satisfactory, it involves handling hydrogen at about 400°C and either mixing a three-component Geiger filling or using electronic quenching.

An alternative technique is the incorporation of THO in the detector of a liquid scintillation spectrometer (*3*). Although this procedure gives excellent measurements, it suffers from the high cost of the instrumentation (one excellent commercially available liquid scintillator costs about $7500).

This paper establishes a simple, inexpensive method for measuring THO by a one-step conversion to acetylene with calcium carbide and Geiger counting of a self-quenching mixture of acetylene plus argon (*4*).

The reproducibility of this method is indicated in Table 1, which shows the sample converted, the partial pressure of the acetylene used, the net counts per minute, and the measured specific activity of the acetylene. All conversions were made with a large excess of