Technical Papers

Protein of the Grain Membrane of Cattle Hide

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The grain membrane is the skin tissue that becomes the outer layer of finished leather; therefore many of the excellent surface properties of leather depend upon it. The preservation of the grain surface without damage during the liming and bating of skins is a major problem in tanning technology. It is perhaps surprising that the chemical structure and properties of the grain membrane have not been established. Some investigators have considered it to be a specialized tissue similar to the reticular tissue of various organs, but in recent years it has been generally thought to be collagen in a special netlike layer.

Turley (*1*) carried out a careful chemical-histological study of cattle hide in an attempt to correlate the processes of tanning with the classical histological description of skin. He concluded that below the epidermis, which is removed during liming, is a hyaline layer overlying the grain membrane. He was not able to distinguish this hyaline layer in fresh steer skin but believed it could be demonstrated in limed hide. The next layer described was the grain membrane. The latter was stated to be a "thin band of fine felted connective tissue which forms the actual surface of the skin as seen in tanned leathers."

Kaye concluded that there was a specialized grain membrane of reticular tissue composed of the protein "reticulin" (*2*). Dempsey, in 1946, stated that the hyaline layer could be demonstrated in suitably prepared sections of delimed ox hide (*3*). She continued:

> It is not altogether clear if the reticular network and the hyaline layer are separate entities, but this seems most likely in view of the apparent structurelessness of the layer in the delimed skin.

Küntzel rejected these views and stated that the grain membrane is collagen, which differs from the underlying collagen of the corium only through its close netlike coalescence of the fibrils and does not differ in chemical constitution (*4*). Stather accepts this explanation in his recent book (*5*).

Kramer and Little recently studied the reticulin of the renal cortex and concluded that it consists of an amorphous protein matrix, rich in carbohydrate, in which the collagen fibrils lie (*6*). They boiled this tissue for 15 min and found that both the soluble and insoluble portions were high in hydroxyproline and low in phosphorus and sulfur. On the basis of these data, they concluded that both the fibrous and amorphous protein components of reticulin are closely related to collagen. It is quite probable that the collagen present was incompletely converted to gelatin by their procedure, and that the amorphous matrix remaining was highly contaminated with fibrous collagen, as their electron micrographs of boiled preparations indicate.

When the collagen of hide is completely removed by autoclaving or treatment with acid, the membrane of the grain surface remains, along with a filmy network of tissue derived from the lower layers of the skin. This filmy (reticular) material can be teased off, leaving a continuous sheet of grain membrane (Fig. 1).

Even though there is a great difference in thickness between calfskin and cattle hide, the yield of grain membrane is essentially the same per unit area of the skin. The yield of grain membrane varies slightly with position on the hide and also probably for each animal, but in general 1.0 ± 0.1 mg dry weight is obtained per square centimeter of the original skin area. The membrane shrinks about 40 to 50 percent in area during the isolation process.

The total nitrogen and hydroxyproline (*7*) content determined on a number of samples prepared from the skins by autoclaving or treatment with various acids is shown in Table 1. The autoclaving was done in water at neutrality for two 3-hr periods at 22 lb pressure. The acid preparations were heated for 1 hr on a steam bath, except that the sulfuric acid preparation was for 24 hr at room temperature. All loose (reticular) tissue was removed and the samples were washed free of acid and gelatin before they were dried for analysis.

The membrane is predominantly protein, but the low nitrogen is indicative of the presence of a fair amount of carbohydrate material. The presence of

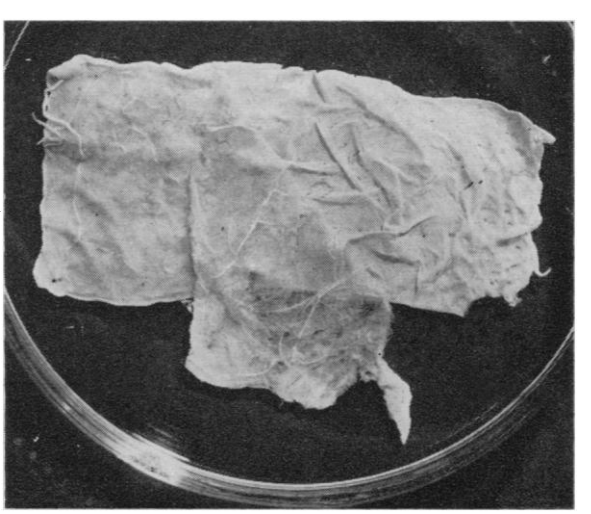

Fig. 1. Grain membrane of calfskin with filamentous material partially teased off. The blood vessels of the corium, which are clearly visible in the filamentous material, can be completely removed from the grain membrane.

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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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-