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Effects of Tannery Processes on the Electronoscopic Appearance of Bovine Hide Collagen Fibrils*

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Introduction

Leather manufacture may be defined as a series of processes by which the collagen of skin is converted into leather. In recent years, it has been shown that the collagen fibril is a fundamental structural unit of collagenous tissue [1, 2]. This unit is so small it can be observed directly only in the electron microscope.

Occasional references to the electronoscopic appearance of tanned collagen fibrils, and of collagen treated with some reagents commonly used in the tanning industry, may be found in the literature [3, 4, 5, 6, 7]. However, no investigation has been reported that was specifically designed to determine the effects of those reagents commonly used in beamhouse and tanning operations on the electronoscopic appearance of collagen fibrils. Observations and results of such an investigation will be presented in this paper.

Experimental

With one or two exceptions, the collagen used was derived from fresh bovine corium cubes. The corium cubes were dispersed in a Waring Blender, and the collagen fibrils were isolated by repeated suspension and centrifuging. Dispersions so prepared contained from 10 to 100 milligrams of collagen fibrils per 100 milliliters of dispersion.

Twenty-five milliliter portions of the collagen dispersion were used for each treatment. For brine curing, liming, pickling, and chrome tanning, the dispersions were put in dialysis bags and suspended in 10 volumes of the treatment liquors. For bating and tanning with tan liquors other than chrome, the dispersions were mixed with an equal volume of the treatment solutions. The concentration of reagent in each treatment liquor was adjusted so that an excess of the reagent was assured.

Shrinkage temperature values, determined by the microscopic method [8], were used as an index of tanning.

Samples of the collagen dispersions were prepared for electronoscopic examination before and after each treatment. Specimens were usually examined and photographed at a magnification of about 11,000 diameters.

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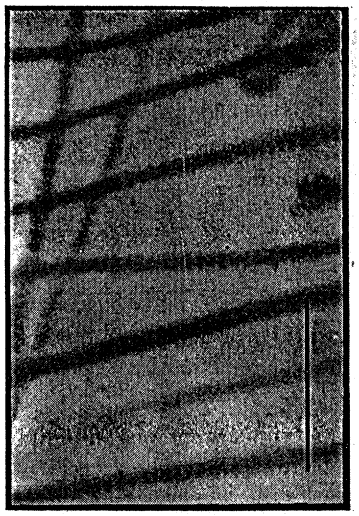
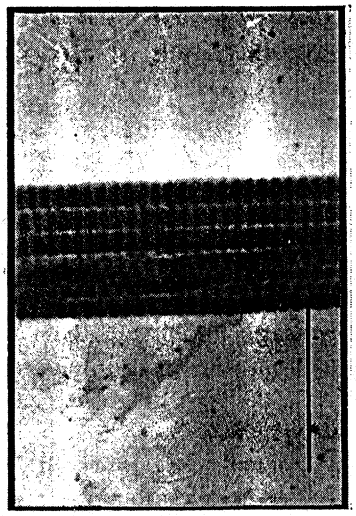
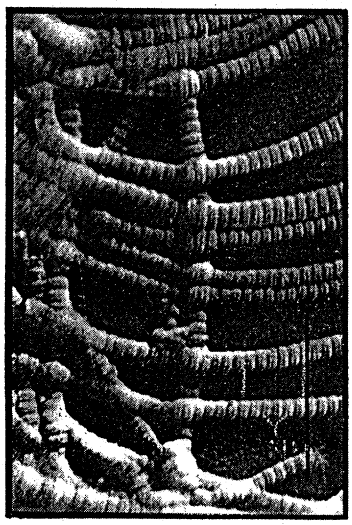


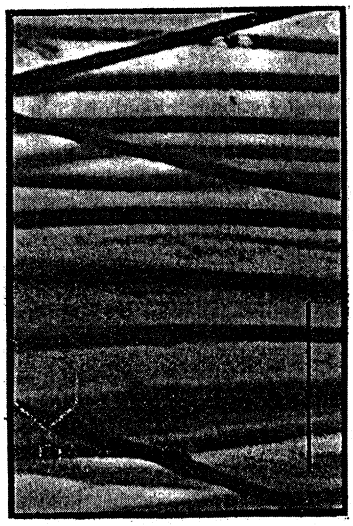
A. 23,000 X



B. 23,000 X



C. 23,000 X



D. 23,000 X

FIGURE I. BOVINE HIDE COLLAGEN FIBRILS.

A. Untreated. Dried on specimen grid.
B. Untreated. Stained with 1% P.T.A.
C. Untreated. Shadowed with chromium.
D. Untreated. Fixed with osmic acid. Dried by Anderson technique.

Results and Discussion

The collagen fibrils used in this investigation were of colloidal dimensions and were unprotected by association with other fibrils or with other skin constituents, such as elastin. Therefore, the collagen was many times more reactive than the collagen of intact skin. For this reason, the effects of reagents such as acids, alkalies and enzymes may be exaggerated, because the reaction of the collagen with the reagent proceeds at a faster rate or goes further than that with undispersed hide. This factor and the inherent limitations of electron microscopy [3–6], were constantly borne in mind, and due allowances were made for them in interpreting the observations and results.

"Native" or Untreated Collagen

The first series of electron micrographs (Figure I) illustrates the characteristic properties of native or untreated collagen fibrils. These micrographs also represent fibrils cured by drying in air.

Figure I shows four representative micrographs of untreated or "native" bovine hide collagen fibrils. The vertical line in the lower right-hand corner of each micrograph represents 1 micron, or 1/25,000 of an inch. The fibrils in Figures I A, I B, and I C, in general, are wider than the fibrils in Figure I D. In all instances, the fibrils are infinitely long, as compared with their width. The collagen in Figure I A has low electron scattering power, and the characteristic periodic striations are just barely discernible. Axial periodicity is distinct in the fibrils of Figure I B. The average length of the axial period is 650 Å. Note the sub-period bands. The shrinkage temperature (Ts) of the phosphotungstic acid (P.T.A.) stained collagen was 60°C., as compared with 63°C. for untreated collagen. This indicates that P.T.A.-stained collagen is not tanned. The chromium-shadowed collagen in Figure I C has the same over-all dimensions as the fibrils in Figures I A and I B. The width-to-height ratio of the fibrils in this preparation is approximately 2.75:1. The hills and valleys (bright and dark bands) in the fibrils correspond to the dark and light bands of the previous pictures.

It is of special interest to note how the fibrils flatten where they cross one another. When biological materials are dried in air or in the evaporator previous to shadowing, they are subjected to great surface tension forces, which flatten the specimens and press them into the supporting film [9]. This makes for poor definition of the specimen edges and sometimes distorted images. The drying technique devised by Dr. T. F. Anderson eliminates surface tension forces by the use of liquid CO_2 at the critical temperature and pressure [9]. Figure I D shows cowhide collagen dried by the Anderson technique*. Compared with the fibrils in Figure I A, these fibrils have noticeably greater electron scattering power. The increased electron scattering power is ascribed to the osmic acid fixation and also to the fact that these

*The authors are indebted to Dr. T. F. Anderson, of the Johnson Foundation, University of Pennsylvania, for preparing the specimens.

fibrils are more nearly round than the fibrils in Figures I A, I B, and I C. This view is supported by the fact that the average width of these fibrils is less than the average width of fibrils dried by the usual methods.

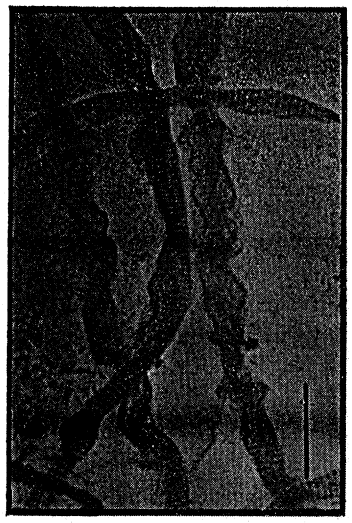
Only dry fibrils can be observed in the electron microscope. From their electronoscopic appearance, however, it is possible to gain some knowledge concerning the state of hydration of the fibrils before drying. By replica techniques, Gross has shown that the edges of moist fibrils are smooth, whereas the edges of dried fibrils are serrated[10]. The fibrils in Figures I A, and I B have the appearance of hydrated fibrils because their edges are smooth. In shadowed preparations, such as shown in Figure I C, collagen fibrils invariably have serrated edges. Serrated or beaded fibril edges are frequently found in preparations not shadowed, but they are by no means characteristic[3], [4]. The higher electron scattering portions of the collagen fibril may represent loci of slightly higher concentration of protein, or high concentrations of positively charged water-binding end groups such as free amino groups of lysine[11]. In shadowed preparations, the high scattering portions are thicker than the low scattering portions of the fibrils. The low scattering parts of the fibrils (light bands of stained fibrils or valleys of shadowed fibrils) may represent loci of slightly lower protein concentration. This evidence suggests that the serrated fibril edges are formed on drying by the loss of water from low electron-scattering portions (the light bands).

Briefly, "native" or untreated collagen fibrils are smooth cylinders that are infinitely long as compared with their diameters. The fibrils are plastic and tend to flatten when dried by ordinary methods. The edges become serrated because of loss of water from the low electron-scattering portions of the fibril. Collagen fibrils have characteristic periodic striations, the average length of the period being 650 Å.

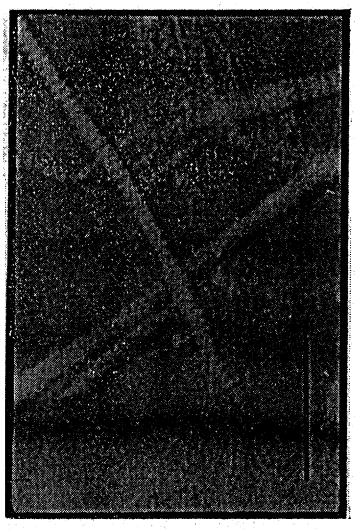
Effects of Curing and Beamhouse Processes

In general, curing and beamhouse operations preserve, purify and prepare the collagen of skin for the reaction with tanning agents. These processes, if properly conducted, should not produce any permanent deleterious effects on the collagen. The electronoscopic appearance of collagen fibrils treated with saturated salt (NaCl) solutions is identical with that of untreated native, or fresh, collagen fibrils. Similarly, limed, bated, or pickled collagen fibrils present the normal electronoscopic appearance when the treatment reagent is completely removed. However, if the collagen fibrils are derived from improperly cured hides, if liming and bating are unduly prolonged, or if the pickle liquor does not contain enough salt, there are marked changes in the electronoscopic appearance of the collagen fibrils.

Figure II A shows cowhide collagen which was soaked in a 0.4 per cent acetic acid solution (pH 3.5) overnight. The collagen was stained with P.T.A. to enhance periodic structure. The shrinkage temperature (Ts) of this collagen was less than 40°C.



A. 12,500 X



B. 19,000 X

C. 19,000 X

FIGURE II. FILAMENTED BOVINE HIDE COLLAGEN FIBRILS.

A. Treated with 0.4% acetic acid; pH 3.5. Stained with P.T.A.
B. Obtained from improperly cured cowhide; pH 5.5. Shadowed with palladium.
C. Treated with sodium silicofluoride; pH 3.3. Shadowed with uranium.

Figure II B is a palladium-shadowed preparation of collagen fibrils obtained from a salt-cured cowhide which had been stored in a damp, cold room for more than 6 months. The Ts of this collagen was 55°C. The pH of the collagen dispersion was 5.5. Figure II C is a uranium-shadowed preparation of collagen which was soaked in a saturated (0.3%) solution of sodium silicofluoride. The pH of the solution was 3.3, and the Ts of the collagen was 46-47°C.

The splitting of collagen fibrils into their component filaments as shown in Figure II may be regarded as the first stage in the degradation of collagen. Filamentation results from prolonged soaking in water, as shown in Figure II B; by soaking in solutions with high hydrogen ion concentrations, as shown in Figures II A and II C; by soaking in highly alkaline solutions [1 2] (Figure III B); and by soaking in solutions of certain salts such as $BaCl_2$ [3]. In all these instances, adequate concentration of sodium chloride prevented longitudinal splitting into filaments. Thus collagen soaked in a saturated NaCl brine for months had the same electronoscopic appearance and shrinkage temperature as fresh collagen. The same was true of collagen soaked in a pickle liquor (10 per cent NaCl solution adjusted to pH 2.0 with HCl) or in a saturated sodium silicofluoride solution containing 10 per cent NaCl (pH 3.3).

From these observations, we may conclude that sodium chloride is effective in preventing collagen fibrils from splitting into their component filaments. Collagen fibrils derived from properly cured and stored hides rarely are filamented. The collagen shown in Figure II B came from a salt-cured hide from which some of the salt was leached out during storage in the damp, cold room. Consequently, the concentration of salt was not high enough to prevent the onset of degradation, as evidenced by the filamentation. This type of degradation (filamentation) may also take place in soaking-back operations. As will be shown later, the collagen is digested by enzymes only after the fibrils are filamented. These results are in accordance with the results obtained in practice when salt is added to the soak liquors for dried skins, which usually require long soaking periods.

Sodium silicofluoride is added to curing salt to function as a rodenticide, insecticide and mothproofing agent. Used alone, sodium silicofluoride damages the collagen. This is the effect of the pH [8]. When used in conjunction with sodium chloride, it does not affect the collagen and affords the cured hides protection against damage by rodents, insects and moths.

Acid is used in pickle liquors to adjust the pH of the collagen so that it will be compatible with the acid pH of the chrome-tan liquors. Acid alone causes considerable damage, as shown in Figure II A, but when used with sufficient salt it does not affect the collagen.

Briefly, sodium chloride prevents collagen filamentation, but does not interfere with the functioning of other reagents, such as acids in pickle

A. 23,000 X

B. 23,000 X

FIGURE III. LIMED BOVINE HIDE COLLAGEN FIBRILS.

A. Limed for 1 hour. pH of washed dispersion, 10.5. Shadowed with chromium.
B. Limed for 24 hours. pH of washed dispersion, 6.5. Stained with P.T.A.

liquors, or pesticides, such as sodium silicofluoride, in curing mixtures or brines.

Figure III A shows a chromium-shadowed preparation of collagen, which was treated with a saturated lime solution for 1 hour. The dispersion was washed by dialysis but was not completely free of lime, as shown by the fact that the washed disperions had a pH of 10.5. Many individual fibrils vary in width. They are flattened, as evidenced by the relatively short shadow. Their fibril edges are smoother, and the periodic structure is less pronounced than in the fibrils of Figure I C. These characteristics indicate that the fibrils were in a thoroughly hydrated form before they were prepared for electron microscopic observation. If the lime is completely removed, as shown by a dispersion pH of 5.5 to 6.0, the fibrils have the appearance of normal untreated fibrils in every respect.

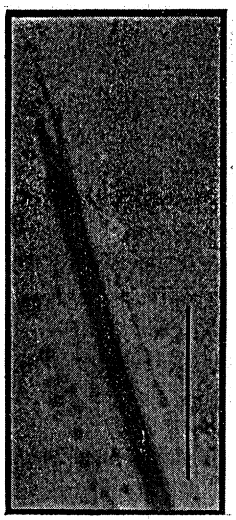
Figure III B shows collagen which was treated with saturated lime solution for 24 hours. The lime was completely removed by dialysis against water. The pH of the washed dispersion was 6.5. This preparation was stained with P.T.A. Small tufts of filaments along the edges give the fibrils a frayed, ravelled appearance. The fibril ends are frayed. Numerous collagen filaments may be seen in the background. Several are of finite length. Fibrils of finite length, which are invariably found in preparations of degraded collagen, are a good indication of irretrievable damage. The periodic structure is obscured by excess stain. This micrograph of limed collagen was selected because it shows degradation due to excess liming and illustrates a case in which the reaction proceeded at a faster rate than desired. However, it is not typical. We have examined many disperions of collagen limed for 24 hours, whose electronoscopic appearance after washing was identical with that of fresh collagen. (The tanned collagen fibrils shown in Figure V A were limed for 24 hours and bated for 1 hour before tanning.)

From these observations, we conclude that when collagen swells in lime liquor, the swelling is temporary and reversible, in that upon removal of the lime, the collagen recovers its normal appearance. Excess liming, however, caused filamentation and breaking of the collagen fibrils into short lengths, signifying irretrievable collagen degradation.

There has been, and still is, much controversy on the effects of bating on hides and skins, especially on the effect of the bates on the collagen. Electron microscope observations suggest that the intact collagen fibril is not subject to enzymatic attack. It is only after the fibril is broken down into filaments or is degraded into soluble products, such as gelatin, that the enzymes can attack the collagen [1 2].

Figure IV A shows collagen treated with Oropon A* for 2½ hours at 37°C. The fibril was stained with P.T.A. Figure IV B shows collagen

*Mention of commercial products does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.



A. 23,000 X

B. 23,500 X

C. 19,000 X

FIGURE IV. ENZYME-TREATED COLLAGEN FIBRILS.

A. Bullhide collagen treated with Oropon A for 2½ hours. Stained with P.T.A.
B. Cowhide collagen treated with papain. Stained with P.T.A.
C. Sharkskin collagen treated with trypsin.

treated with 0.1 per cent papain in acetate buffer (pH 4.8) and activated with 0.01 M cysteine. This preparation was also stained with P.T.A. Figure IV C shows sharkskin collagen, which was digested by trypsin (pH 7.8) at 37°C. for 2 hours.

The fibril in Figure IV A is normal in every respect except the fibril end, which is tapered. The pH of the dispersion was 6.5. Figure IV A is not a typical micrograph. Most of the collagen fibrils from the same preparation had the same appearance and average dimensions as the fibrils shown in Figure I.

Figure IV B shows more clearly the characteristics of the tapered fibril ends of enzyme-treated collagen. The main shaft of this particular fibril is about 0.2 micron wide and tapers down to about 0.04 micron at the tip. The fairly well defined period striations in the shaft measure about 600 Å. The width and shortened spacings indicate that the shaft of the fibril is swollen. The sharkskin collagen in Figure IV C is obviously much degraded. The debris in the background is probably degradation products of the collagen.

The three micrographs in Figure IV illustrate enzymatic-digested collagen fibrils. As pointed out previously, limited exposure to enzymes does not alter the characteristic appearance of collagen fibrils. Enzymatic digestion of collagen may be considered a two-stage process. The first stage is the breakdown of the fibrils into filaments, or into water-soluble products such as gelatin. In this degraded form, the collagen is available to the enzyme for digestion, which is the second phase. The initial breakdown of the fibrils to smaller components or soluble degradation products is caused by one or more factors. As illustrated below, for optimal activity, a particular enzyme requires specific conditions of pH, temperature, and frequently specific activators. When collagen is placed in a medium devoid of enzyme, but adjusted for optimal activity of a specific enzyme, the collagen splits into its component filaments, swells, or is actually degraded into soluble products such as gelatin. Thus for peptic digestion, a pH of 2.0 – 2.5 is required. In media from pH 0.5 – 3.5, collagen fibrils split into their component filaments (Figure II A and II C). Collagen fibrils break up into their component filaments when allowed to stand in phosphate buffers at pH 7.0 or in citrate buffers at pH levels from 4 to 7 for extended periods of time at 30 – 37°C.[2] The start of the shrinking reaction when collagen is gradually heated in water is characterized by swelling and gelatinization of the fibril ends[3]. In each instance noted above, the collagen fibril is broken down into filaments or hydrolyzed to a soluble degradation product.

Sharkskin collagen (Ts 45°C.) is much more easily attacked by trypsin than is cowhide collagen (Ts 63°C.), principally because it is more sensitive to warm water than is the cowhide collagen. A possible explanation for the fact that pepsin digests collagen much more rapidly and more completely than does trypsin is that at pH 2 the collagen fibril is in the form of filaments (Ts below

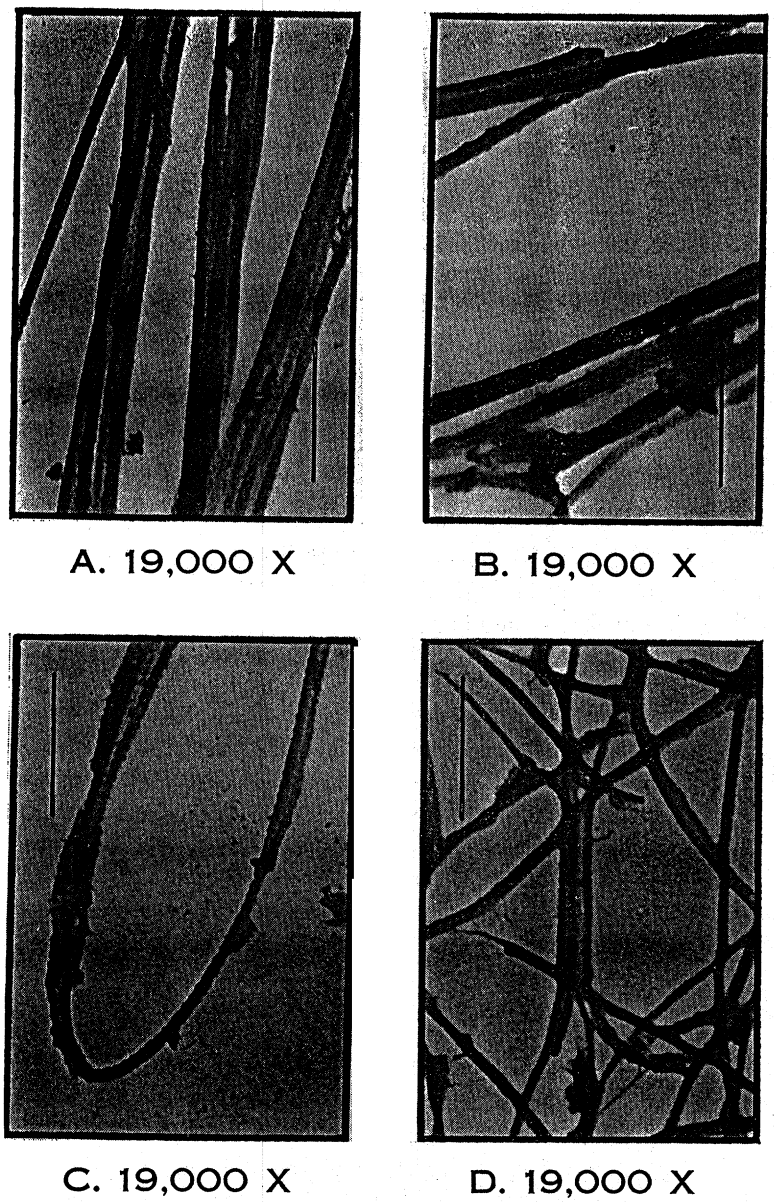


FIGURE V. TANNED BOVINE COLLAGEN FIBRILS.

A. Tanned with a 50–50 blend of chestnut-sulfited quebracho liquor. Ts=89°C.
B. Tanned with Orotan. Ts=86°C.
C. Tanned with canaigre extract. Ts=98°C.
D. Tanned with sumac extract. Ts=80°C. Shadowed with palladium. Positive print.

40°C.), which are much more sensitive to warm water than intact fibrils (Ts about 63°C.) at pH 7.5 – 8. In all instances, the ends are more subject to degradation than the main body of the fibril; thus, the degraded ends are digested by the enzyme and become tapered.

From these observations, we may conclude that bating, if properly conducted in the beamhouse, will not destroy the collagen of skin. Bating solubilizes non-collagenous proteins such as elastin and whatever collagen may have been degraded in prior beamhouse processes. If the bating is allowed to go on for too long a period at the optimum temperature for bating, the collagen is attacked, and there is a definite loss of hide substance.

Effects of Tanning Agents on the Electronoscopic Appearance of Collagen Fibrils.

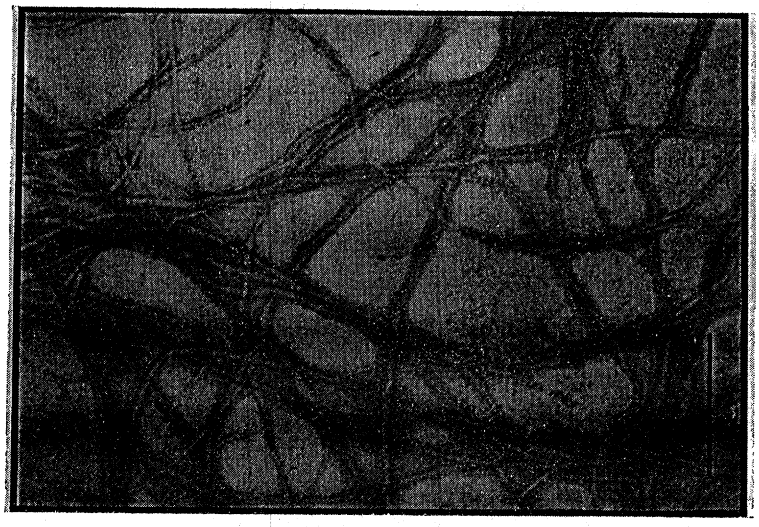
The electronoscopic appearance of tanned collagen fibrils is somewhat different from that of untanned fibrils. These differences will become apparent as we examine and discuss the subsequent electron micrographs. When tan liquor was added to a collagen dispersion, the collagen fibrils aggregated and formed a fibrous clot. After being washed, the fibrous clots were dispersed in water by teasing or in a Waring Blendor.

Figure V A shows collagen tanned with a 50–50 blend of chestnut-sulfited quebracho tan liquor. The Ts of the washed and redispersed vegetable-tanned collagen was 89°C. The fibrils are more nearly round than those of untanned collagen, but not quite so round as fibrils of chrome-tanned collagen (Figure VI B). The fibril edges are comparatively smooth. Period and intra-period structures are obscured. In a few places where the macro periodicity may be measured, the average length per period is within the normal range (600–700 Å). Periodicity is more clearly defined in shadowed preparations (Figure V D). Contrast between the tanned fibrils and the background is better than between untreated fibrils and background (Figure I A). The increase in electron scattering power is ascribed in part to the circular form of the tanned fibrils and in part to the fact that there is more material (collagen plus tannin) to scatter electrons.

Figure V B shows collagen tanned with Orotan. The Ts of the washed redispersed tanned collagen was 86°C. The contrast of the fibrils against the background is good and, in general, the electronoscopic features are the same as for the chestnut-quebracho tanned fibrils. Figure V C shows canaigre-tanned collagen fibrils. The Ts of this preparation was 98°C. Periodic structure is fairly well defined, more so than in the Orotan-tanned collagen.

Figure V D is a positive print of lightly palladium-shadowed sumac-tanned collagen fibrils. The general dimensions of these fibrils are the same as in Figures V A, V B, and V C, and fine period structure is more or less obscured.

The tendency for the tanned fibrils to aggregate and form small bundles is apparent in all micrographs of tanned collagen (Figure VI B). This



A. 19,000 X

B. 19,000 X

FIGURE VI. CHROME TANNING.

A. In 3% chrome alum liquor, plus 10% NaCl; pH 2.8.
B. The same collagen after neutralization; pH 4.5. Ts 99°C.

aggregation may be due to the formation by the tanning material of the same type of chemical bonds between the fibrils as are formed between the collagen molecules in the fibrils.

Figure I A shows that where two collagen fibrils cross, the electron-scattering power is not materially increased. In Figure I C, the fibrils are flattened where they cross. These observations denote an elliptical cross section and a fair degree of plasticity in untreated collagen fibrils. In tanned collagen fibrils, points at which fibrils cross show a marked increase in electron-scattering power and little tendency to flatten, signifying that the tanned fibrils are more nearly circular and less plastic.

Figure VI A shows collagen fibrils from a dispersion suspended in a chrome-alum liquor (pH 2.8) to which 10 per cent sodium chloride was added to prevent swelling. The salt was added a few minutes after the dispersion was suspended in the chrome liquor. Figure VI B shows the same collagen after the pH of the liquor was adjusted to 4.5 with sodium bicarbonate solution. The collagen in Figure VI A appears to be tubular. This illusion is created by the fact that there is excess chromium along the edges of the fibrils and the fibrils appear to be devoid of periodicity. Close inspection of the original prints, however, reveals fine cross-banding in limited portions of many of the fibrils.

The chromium does not seem to have penetrated into the fibrils at this pH. Similar observations were obtained when properly reduced commercial chrome tan liquors were used instead of the 3 per cent potassium chrome alum liquor. The chrome-tanned collagen fibrils shown in Figure VI B have the same features outlined for the vegetable- and syntan-tanned fibrils shown in Figure V.

TABLE I

Some Physical and Electronoscopic Properties of Collagen Fibrils

Property	Untanned	Tanned	
		Chestnut-Quebracho	Chrome Alum
Shrinkage temperature	63°C.	89°C.	99°C.
Reaggregation of dispersed fibrils	Very little, if any	Pronounced	Pronounced
Electron scattering power (contrast)	Poor	Good	Good
Shape of cross section	Pronounced ellipse	Slightly elliptical	Circular
Plasticity (flattening of fibrils where they cross each other)	Pronounced	Slight	Slight
Period structure	Fairly distinct	Mostly obscured	Mostly obscured

Table I shows some physical properties and the electronoscopic features of untanned (Figure I A), vegetable-tanned (Figure V A), and chrome-tanned (Figure VI B) collagen fibrils.

Summary and Conclusions

The effects of pretanning and tanning processes on collagen fibrils were studied by examining the fibrils in the electron microscope before and after treatment.

The fundamental fibrous units of skin collagen are so small they can be seen only in the electron microscope. The fibrils are smooth cylinders, which are infinitely long as compared with their diameters. The fibrils are plastic and when dried have an elliptical cross section.

The initial stage of collagen degradation is the longitudinal splitting of collagen fibrils into their component filaments. This filamentation occurs when collagen fibrils are allowed to soak in water for prolonged periods of time, or when soaked in a highly acid or highly alkaline medium or in solutions of salts such as $BaCl_2$.

Adequate concentrations of sodium chloride in green salted skins, in curing mixtures and in pickle liquors prevent collagen fibril filamentation.

The swelling of collagen fibrils in lime liquors is reversible. Excess liming causes irreversible collagen degradation.

Bating is an enzymatic digestion of the non-collagenous proteins and of the degraded collagen left in skin after curing and liming. The enzymatic digestion of collagen is a two-stage process. The initial stage, the degradation of the collagen into filaments or water-soluble products, is produced by environmental conditions (pH, temperature, specific activators) essential for optimal enzyme activity. The second stage is the digestion of the degraded collagen by the enzyme. Prolonged bating causes a loss in hide substance.

In general, pretanning or beamhouse processes do not alter the characteristic appearance of collagen fibrils.

Collagen may be degraded when salt cured skins are stored under highly humid conditions, which results in reducing the salt content to less than that required, when skins are subjected to prolonged soaking in lime liquors or prolonged bating, or when there is an inadequate concentration of salt in pickle liquors.

Tanned collagen fibrils are different from untanned fibrils in the following respects. Tanned fibrils have higher shrinkage temperatures. Tanned fibrils tend to appear as aggregates rather than separate individual fibrils. They have good electron-scattering power. The cross section of dried tanned fibrils is more nearly round than that of untanned fibrils. They are less plastic then untanned fibrils.

This electron microscope study of collagen at different processing stages from fresh hide or skin to tanned leather provides a better understanding of

the effects of the various processing treatments. The illustrations make it possible to visualize more clearly the changes that take place in the fibrils during processing and indicate some extreme treatments which might result in degradation of the collagen fibrils.

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Discussion

J. H. Highberger: The electron miscroscopic results of collagen research of the last few years have yielded a characteristic picture of collagen fibrils which Dr. Borasky has described to you this morning. I think the most characteristic thing about this picture is the repetition of the period of 640 to 650 Angstom units which Dr. Borasky mentioned, which might be spoken of as the molecular fingerprint of collagen. This characteristic fine structure has enabled the ready identification of collagen fibrils by the electron microscope, in the presence of other types of fibrous materials.

In regard to the effects of tannery processes, Dr. Borasky's electron micrographs would appear to indicate that these, with the exception of drastic liming, produce no marked effect on this characteristic fine structure picture of collagen. I might say that the fragmentary results which we have obtained in our own laboratories would tend to confirm this.

I should like to mention the following fact which, of course, Dr. Borasky is quite aware of, for the information of those who are not familiar with electron miscroscopic techniques. One has to be extremely cautious in the interpretation of the results obtained, because of the fact that one deals with extremely minute samples of material. One must be quite certain, through repeated observation and by other means, that what one sees is really representative of the whole. I do not mean this to be any criticism of what Dr. Borasky has described and has shown. I merely present it for the information of those who are not familiar with the field.

I have mentioned the characteristic electron miscroscopic appearance of collagen fibrils and I thought it would be appropriate in connection with the discussion of Dr. Borasky's paper to draw your attention to some very recent results connected with the fine structure of collagen which my associates, Dr. Schmitt and Dr. Gross, of the Massachusetts Institute of Technology, and I have obtained. These have to do with reconstituted collagen. By reconstituted collagen I mean that the collagen is precipitated from a very fine dispersion of collagen fibrils which have been passed through a fine filter paper or other filtration medium. From such filtrates it is possible by various means to obtain collagen fibrils which present all of the fine structural characteristics which are to be seen in the native skin and other types of connective tissue.

About two years or more ago, a group of Russian workers began the study of buffer extracts of mammalian skin. They showed that it was possible to obtain a material from filtrates of such extracts, which they called procollagen, because they assumed this material to represent a biological precursor of collagen in the skin.

If, for example, a filtrate of a pH 4 citrate buffer extract of skin is dialyzed a precipitate is formed. On examining such precipitates in the electron microscope we found them to consist of material such as is shown in this slide. (Slide). You will observe the presence of normal collagen fibrils, and in addition, fibrils of a new type which show a much larger axial periodicity than ordinary collagen. We have called these "long spacing fibrils".

For a long time we have been puzzled by what this new type of fibrous structure might represent. I would like to show in the second slide (Slide) another example which was obtained from rat skin, and which shows the detailed fine structure found in these forms. A statistical study of the length of the period in the long spacing fibrils shows it to have a rather broad distribution, but with a well marked peak at about 2000 Angstroms.

We have found that we can produce these new types of fibrous structures at will by the addition of mucoprotein, obtained from bovine or human serum, to a soluble collagen filtrate, and dialysis of the mixture.

If, for example, a filtrate of a dilute acetic acid extract of ichthyocol, obtained from the collagenous tunics of the swim bladders of certain fish, is dialyzed against water, a loose gel is obtained which has the appearance in the electron microscope shown in the next slide. (Slide). As you see, it consistes merely of very fine amorphous filaments which show no discernible structure. If, however, a small percentage of mucoprotein has been added to such a filtrate, dialysis produces masses of large, well-formed long-spacing fibrils, as shown in the next slide. (Slide). With smaller proportions of mucoprotein, a mixture of long-spacing and normal fibrils is obtained, and with still smaller amounts the precipitate consists predominantly of normal fibrils.

It is our belief at the present time that the long-spacing fibrils represent a modification of collagen which does not exist in the native tissue—so far it has never been seen there—but which is formed under rather abnormal conditions. The role of mucoprotein in its production, however, and the possible involvement of mucoprotein in the formation of normal collagen fibrils under these conditions of reconstitution, raise large questions as to the nature of the normal collagen structure. We hope that further work of this kind will throw additional light on this subject.

A. N. KAY: In the slide which Dr. Borasky showed, the normal collagen showed the characteristic "fingerprint" of collagen. However, in the special technique, the CO_2 and pretanned fibers did not show the characteristic "fingerprint". Does that imply that you would change the surface by these tannages or by liquid CO_2?

R. BORASKY: The fibrils dried by the liquid CO_2 method have the characterisitic striations but could not be seen on the particular slide shown.

KAY: You do not suppose that fine structure is, in a sense, a symptom of stress caused during the drying?

BORASKY: The serrated edges characteristic of shadowed collagen fibrils may be due to a preferential loss of water from parts of the fibrils where the protein concentration is low and the water concentration is high. I believe Dr. Schmitt expressed the same idea several years ago. I do not believe that the fine structure, that is the period and intra-period bands are a symptom of stress caused during drying. X-ray diffraction data obtained from studies of moist collagen fibers show the same periodicity as shown by electron microscopic observations.

HIGHBERGER: It is possible to make wet replicas of fully hydrated fibrils and then examine the replica in the electron microscope. By this means Dr. Schmitt showed that collagen fibrils are essentially smooth cylinders, acquiring the serrated edges only on drying.