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The quest for a comprehensive tanning mechanism

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1. Introduction

The conversion of animal hides into leather was one of humankind's first ventures into biomaterial engineering. In the earliest times, the unprocessed animal skins that were used for clothing and shelter became stiff at low temperatures and rotted in the heat. Stone age people in all parts of the world learned to improve the quality and life of their clothing and shelter by soaking the hides in a fatty substance and then kneading the hide until it became soft.[1] The fatty materials chosen for this curing process varied with the local culture; nomadic people used portable materials such as egg yolk, butter or milk, while people in more established settlements used liver or brain tissue. The crucial step was the tumbling or kneading of the hide to assure uniform penetration of the fatty material. If these cured hides were then exposed to smoke, a stabilizing reaction occurred, resulting in what was probably the first leather.

The word 'tanning' now used to describe the conversion of hides and skins into leather is derived from the ancient Celtic word for oak. Bark tanning, based in part on the bark of the oak tree, the precursor of current vegetable tanning, was practiced by several ancient civilizations. Some of the oldest surviving examples date from 5000 to 3000 BC when the Sumerians of southern Mesopotamia used leather to make women's dresses and other items. Other ancient civilizations processed hides into leather for a wide variety of uses. In addition to clothing, the Phoenicians made leather waterpipes, Assyrians made floating devices of leather for rafts, and Egyptians made leather gloves. The Romans became master tanners, as evidenced by the tannery found in the ruins Pompeii containing hide-processing equipment of a type that was still in use several centuries later.[2]

Early bark tanning likely started with the soaking of hides in pits lined with tree bark, and evolved into milling or crushing the bark to increase surface area and make the tannins more available, the remnants of mills for crushing bark prior to 1500 BC have been identified. The crushed bark was placed in a pit with water, and skins were soaked with the tannins released from the bark to produce strong, pliable, durable leather. Similar processes are currently used in several countries. By the Middle Ages, tanning was designated a craft, and tanners guilds (the model for IULTCS and the various local organizations) began to form. The next major period of innovation came with the Industrial Age of the 1800's when the tanning drum largely replaced the open pit, and greatly increased the efficiency of tanning. By 1840, the medical community had adopted sutures that were stabilized by soaking in a Cr(III) solution. A few years later, it was discovered that soaking these chrome tanned sutures in glycerol made them more pliable, or softer. These two advances in medical technology were soon adapted by tanners, and became chrome tanning and fatliquoring. By empirical methods, over millennia, several classes of tanning agents with a variety of properties have been identified.

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[1] Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.
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Fibrous collagen, primarily type I collagen, is the tanner's substrate. The unique structure of fibrous collagen, a long triple helix that self associates into fibers, provides an insoluble matrix or scaffold that gives strength and form to the skin, tendons, bones, cornea and teeth. The function of tanning is to stabilize the structure of the collagen matrix of the hide or skin, increase its hydrothermal stability and protect it from microbial degradation. Current commercial tanning agents, include mineral (mainly Cr(III)), vegetable (polyphenolic tannin), and organic (aldehyde) reagents. The product of each of these tannages is leather, despite the different chemistries. From a 21st century perspective, none of these processes is ideal, and a comprehensive tanning mechanism would provide a basis for the design of more sustainable and eco-friendly tanning processes. The goal of a comprehensive tanning mechanism that can explain the effects of current and past practices, predict requirements for new reagents and processes, and accurately predict the outcomes of proposed new practices is one that leather scientists have pursued at least since the early 20th century. The following paragraphs present a brief survey of the work of a few internationally recognized leather scientists who during the 20th century made major contributions to the understanding of the structures of collagen and tanning materials, and possible interactions between them.

Although the evolution of tanning processes occurred slowly prior to the industrial revolution, technical advances of the 20th century, combined with increasing use of collagen in medical biomaterials, began to provide a basis for understanding the relationship between collagen structure and function in both biology and technology. The early 20th century was a time of rapid development in both basic sciences and applied technologies. This period saw a large increase in scientific publication to document advances in theoretical and applied science. The documented history of the quest for a comprehensive tanning mechanism is a bibliography of great scientists worldwide who applied their talents to this search. H. R. Proctor (UK) started the field with the 1914 publication[3] of "The Making of Leather", a book that detailed the then 'state of the art' for leather technology and science and formed the base for future research.

Following Proctor was J. A. Wilson (USA) who spent his lifetime merging the practice of tanning with the academic science of collagen, and served as an interpreter of basic science to the industrial leather chemists and technologists. Three of Wilson's publications were major reviews of the then current knowledge of the relationship between collagen function and structure. A 1919 publication[4] applied the principles of colloid chemistry to the production of leather, proposing that tanning occurred primarily through electrostatic interactions between tannin molecules and charged sites on the surface of the skin or hide. The following decade was one of rapid advances in most scientific areas, and in the1928 Chandler Lecture at Columbia University, entitled "Chemistry and Leather,"[5] Wilson recognized that leather chemistry was very much concerned with the molecular structure of the protein, collagen, and that the interaction between collagen and chromium was not the simple binding of a Cr^{+3} ion to collagen. In Wilson's final presentation in 1941,[6] structures were beginning to be proposed for vegetable tannin molecules with the potential for multipoint fixation to the fibrous structure of collagen. The effect of these tanning materials on the shrinkage temperature of collagen was recognized as a reliable indicator of degree of tanning.

The Swedish leather chemist, K. H. Gustavson, was active between 1920 and 1969 when details of the shape and composition of collagen molecules became available. He brought to the attention of the leather technologists the unusual amino acid composition of collagen including the presence of 30% glycine, 25% proline plus hydroxyproline and a small amount of the unique hydroxylysine residue as well as a paucity of aromatic and sulfur containing residues.[7] During this time, the molecular architecture of collagen with its periodic pattern of dense and more open areas, and the nonhelical telopeptides to guide the formation of fibers was also becoming apparent. Gustavson's personal research, documented in more than 200 publications, provided a large data base of the reactions of chromium salts, polyphenols and aldehydes with collagen as a start to the molecular understanding of tanning.[8]

G. N. Ramachandran (IN), closely associated with the Central Leather Research Center in what was then called Madras, determined many of the stereochemical characteristics of collagen that contribute to the success of tanning processes. Ramachandran's work in the 1950-1970 period established that to explain the X-ray diffraction pattern, the single chain, left-handed helices must supercoil into a right-handed triple helix. The presence of a glycine residue, lacking sidechain carbons, at every third position in the chain, was the only option for the triple helix. Although nearly any other amino acid could be accommodated in the other positions, the presence of hydroxyproline, with its potential for hydrogen bonding to water, immediately preceding a glycine contributed significantly to the stability of the helical structure.[9]

E. R. Heidemann (DE) of the Darmstadt school was a leading educator on the roles of collagen structure and function as pertaining to tanning, in the latter part of the 20th century. Heidemann's career began with publications of fundamental studies on the nature and reactions of collagen in the 1960's, and culminated with the publication of "Fundamentals of Leather Manufacturing" in 1993. He was among the first to explore the molecular level interactions of collagen peptides with tanning materials of various types. He clearly enunciated the long-term benefits of fundamental research into the structures of hide protein components, tanning and fatliquoring agents and the other materials that are necessary to produce a fine piece of leather. As did his predecessors, he applied the fundamental research results to the practical problems experienced by tanners.[10]

Thus, over the 20th century, innumerable academic and leather scientists, elucidated details of the unique molecular structure of collagen. The basic element is tropocollagen, consisting of three individual chains, each in a left-handed polyproline-type helix, not an α-helix, that coil together to form a right-handed super helix, known as the collagen triple helix. A $(Gly\text{-}Xxx\text{-}Yyy)_n$ amino acid sequence places the glycine residues in the central channel of the triple helix with the X and Y sidechains pointing outward. Although nearly any amino acid can fit into the X and Y positions, about 25% are imino acids, proline in the X position or hydroxyproline in the Y position; the variations Gly-Pro-Hpr, Gly-Pro-Yyy, Gly-Xxx-Hpr and Gly-Xxx-Yyy are all part of the collagen sequence. In type I collagen, the primary skin collagen, two of the three chains are identical (alpha1I) chains while the third is a similar (alpha 2I) chain. Each chain consists of a helical portion about 1000 residues long with short, nonhelical telopeptides extending from either end. The telopeptides direct the assembly of triple helices into fibrils and fibers, and provide anchor points for native crosslinks that are formed as an animal ages.[11]

During this same period, tanning baths were being analyzed to determine the composition of tanning mixtures and the structures of probable tanning agents. Early attention was given to vegetable tannins, where the process is still closely related to the bark tanning practiced 3000 – 4000 years ago, although polyphenolic tannin extracts from tannin rich plants including mimosa, quebracho, and chestnut are now in common use. Lollar's comprehensive review[12] at the mid 20th century concluded that the previous 50 years of research had revealed many facets of the chemistry of tannins, but the conclusions drawn by different investigators were inconsistent. Lollar was optimistic that the tools then available would shortly produce correct structures and chemistries. Considerable research since that time has confirmed the complexity and nonhomogenity of vegetable tanning agents, and has led most researchers in this area to develop model systems to gather data from a few polyphenolic compounds and extrapolate the results to the tanning process. Na's 1988 study[13] using monomeric polyphenols showed that only those polyphenols with acid functionality could bind directly to collagen. However, when tannin solutions were allowed to stand, autopolymerization led to the formation of much larger species that could compete for hydrogen-bonding sites on collagen, thus stabilizing the fiber structure. Haslam in 1997 first proposed that these larger tannin complexes contribute to collagen stabilization by filling gap regions of the fibril.[14]

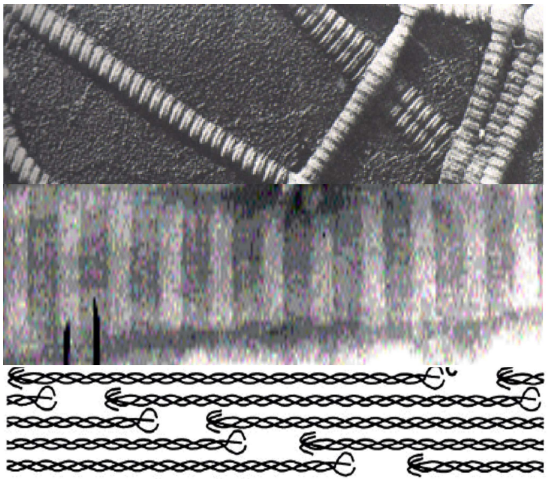


Figure 1. Top: micrograph of skin. Middle: higher resolution micrograph of a collagen fibril showing gap and overlap regions; Bottom: schematic of gap and overlap with telopeptides.

Aldehyde tanning originated in the reaction of smoke with cured hides, and the use of formaldehyde as a tanning agent was patented before 1900. Theis and co-workers, in the late 1930's, published extensively on the chemistry of the reaction between formaldehyde and collagen, noting that even when the substrate was soluble collagen, formaldehyde could react with only a fraction of the amino side chains.[15,16] More active research on the structures and mechanisms involved in aldehyde tanning dates from the 1960's, when glutaraldehyde tanning to produce washable leathers was developed at ERRC. Two questions arise from this work: is the actual tanning agent glutaraldehyde or glutaraldehyde condensation polymers, and why has glutaraldehyde superiority as a tanning agent to other dialdehydes not yet been fully resolved.[17] Modifications of glutaraldehyde tanning are currently the most usual commercial process for the production of wet-white leathers.

Little more than hundred years ago, a short time in the history of leather, the question was whether the stabilization of hides by treatment with mineral salts could properly be called tanning.[18] The nomenclature question has clearly been resolved, and chrome tanning accounts for about 90% of the production of high quality leathers today. Less clear still are the natures of the tanning species and the tanning reaction, and the final product. Basic chrome sulfate (BCS), the usual tanning salt is most likely a mixture of mono and polynuclear chromium species bridged with oxygen, hydroxyl and possibly sulfate groups.[19] The composition is fluid, and with addition of masking agents even less well defined. The chrome tanning reaction has traditionally been described as crosslinking of carboxyl groups on collagen by multinuclear chromium complexes.[20] The formation of both intra- and interfibillar crosslinks in collagen when treated with different chrome tanning formulations has been demonstrated.[21]

Leather researchers at the Eastern Regional Research Center (ERRC), USDA over the past 70 years, have pursued research that contributes to the search for a comprehensive tanning theory. One of the earliest publications from the Center dealt with the stabilization of vegetable tanned leathers.[22] In fundamental studies of collagen structure, ERRC leather scientists of the 1940's and 50's used electron microscopy, a then emerging technique, to obtain micrographs of cattle hide collagen in its native state[23] and after the various steps of beamhouse processing.[24] In the 1970's, they examined the effects of hydration on collagen structure[25] and the flexibility of the nonhelical regions.[26] The fundamental understanding of collagen structure was advanced in the 1980's through studies of fibril assembly.[27,28]

2. Approach

By 1990, after half a century of studies, the fibrillar collagens that are triple-helical over essentially their entire length, give structure to tissues and organs, and are technologically important as molecular frameworks in the leather, food and medical industries, were well described at both the monomer and fiber level, but less well described at the microfibril or intermediate range.[29] When protein modeling software became available, ERRC scientists decided to explore the collagen microfibril structure by constructing a computational model to facilitate simulated tanning studies. From among several proposed models, we selected the 'Smith' microfibril, comprised of five triple helices in a left-handed super coil for the ERRC model.[30] An initial $(Gly\text{-}Pro\text{-}Hpr)_n$ template was mutated stepwise to produce a model that when colored to represent a negative microscopy stain, showed a banding pattern typical of those seen in collagen micrographs, and because the individual chains are staggered, the entire tropocollagen sequence of bovine type I collagen could be represented, without making the model overly long. This 15-chain model, encompasses a single gap region, 159 residues long, and with a 78- residue-long overlap region at either end.[31] As data became available on the function and *in situ* conformation of the telopepetides became available, these nonhelical domains that serve as anchors for native collagen crosslinks were inserted into the gap region, to complete the model.[32,33]

Our first attempt at using this model, was to explain the effects on thermal stability when dicarboxylic acids with varying chain lengths were bound to collagen. In tanning experiments, C-7 to C-12 chains had a much greater effect on collagen thermal stability than did either shorter or longer chains. Computer simulation of this reaction with the microfibril showed that short chain dicarboxylic acids could crosslink only within a triple helix, medium length chains could form inter-helical crosslinks, and longer chain molecules might bind to the surface, but be unable to fit within the microfibril.[34] Most crosslink-based theories of tanning have focused on the triple helical domains of collagen, because these regions are well described, and specific crosslinks can be identified. Our early attempts at modeling chrome tanning were restricted to identifying potential binding sites on the helical structure with suitably spaced acidic residues.[35] More recent studies[36-38] suggest that tanning may better be described in terms of protein modification than as simple crosslinking.

To complement the theoretical model, experimental models ranging from bench to pilot scale were developed. At the bench scale, models using soluble collagen, insoluble fibrous collagen, and powdered hide are subjected to treatments characteristic of isolated steps in a tanning process, and the effect on collagen structure is evaluated. For each of these model systems a variety of methods are available with which to analyze the effects of tanning processes on collagen. For soluble collagen, the amount of collagen present can be accurately determined from the UV absorbance spectrum (Figure 2) with the extinction coefficients ($E_{218nm} = 9.43_{cm^{-1}ml\text{-}mg^{-1}}$) or ($\varepsilon = 883129$) on a molar basis.[39] The helical content of the collagen is then calculated from the circular dichroism (CD) spectrum (Figure 2) by Equation 1.

$$\%\ \text{helix} = ((\text{mdeg}_{222nm})\times(1/\text{path length in mm})\times(1/[C])\times(1/1023))/6680 \qquad (1)$$

Where

[C] is the molar concentration determined from the UV spectrum
1023 is the length in amino acid residues of the helical portion of bovine type 1 collagen
6680 represents 100% helix.

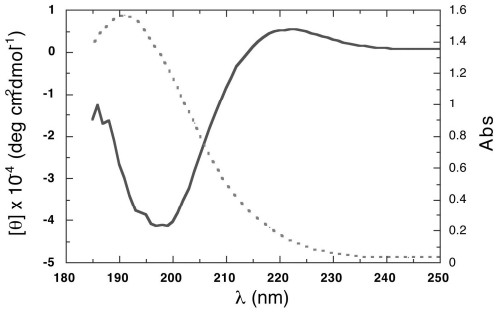


Figure 2. Collagen spectra, (---) is the UV absorbance, (—) is circular dichroism (CD) spectrum.

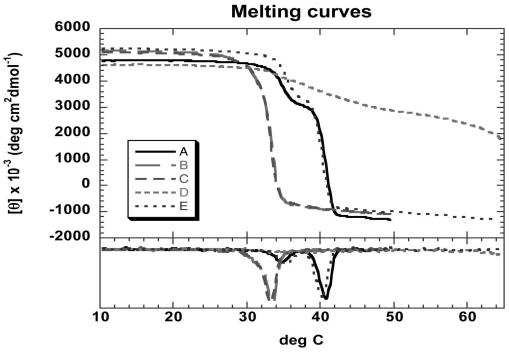


Table 1

	Tp	Td	sample	pH
A	34.8	40.8	collagen	4.5
B	33.6			2.0
C	33.4		+ Al	2.0
D	34.2	38.1	+ Al	4.5
E	35.4	40.4	control	4.5

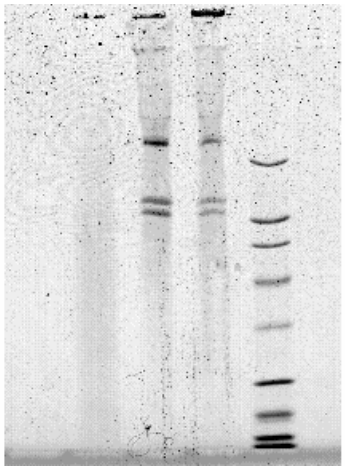
Samples and conditions for Figure 3.

Figure 3. Top: Melting curves for collagen under tanning conditions, obtained by following the CD signal at 223 nm as a function of temperature. Bottom: Derivative curve shows the two-phase denaturation of collagen except under acid conditions.

The effects of tanning processes on the melting of the helical structure in soluble collagen can be observed by monitoring the peak helix signal at 223 nm as the temperature of the collagen solution is slowly raised (Figure 3), while the derivative of this curve provides the pretransition and denaturation temperatures (Table 1). The pretransition is a characteristic of collagen that disappears at low pH, but is not affected by tanning. Figure 3 was obtained with aluminum tanning, the results for chromium are similar, although the blue color obscures some details. Other protein analytical tools can provide insights for both soluble and insoluble collagen. Determination of available amino groups (Table 2) combined with SDS PAGE (Figure 4) gives clues to the interactions of tanning or crosslinking agents with lysine residues on fibrous collagen.

Reaction of glutaraldehyde with collagen appears to form intermolecular crosslinks that produce collagen complexes too large to enter the gel; the 75% decrease in available amino groups and the known chemistry of glutaraldhyde suggest that lys-GA-lys crosslinks are formed, but the size of the glutaraldehyde oligomer cannot be determined from this data. The dehydrothermal reaction, not a tanning process, but of interest to the biomaterial industries, suggests considerable aggregation of collagen to produce a very high molecular weight band and an unresolved smear in the electrophoretic pattern; the 19% decrease in available amino groups suggests that these groups are a minor factor in this reaction. The transglutaminase (mTGase) data is more interesting, the polymerization of gelatin via mTgase-catalyzed epsilon(gamma-glutamyl)lysine crosslinks is well documented,[40] and the 38% decrease in available amino groups, considering one amino group per crosslink, suggests a similar number of crosslinks to those formed by glutaraldehyde treatment. Such crosslinks are formed in intact collagen but have no tanning effect,[41] and the similarity of the electrophoretic patterns for mTgase treated collagen and the control is evidence that these crosslinks are

intra-molecular. Collagenase sensitivity (Figure 5) an independent measure of the intact nature of the collagen chains provided data compatible with the other protein analyses.



Sample	available amino groups / 1000 AA	st dev
Control	35.4	1.8
GA	8.98	0.7
DHT	28.68	0.47
mTgase	22.04	4.75

Table 2: For sample code, see Figure 4. All values are averages of 3 determinations.

Figure 4. SDS PAGE gel, 4-15% gradient. Shown from left to right: GA cross-linked collagen, DHT treated collagen, mTgase cross-linked collagen, control, and broad range molecular weight standard.

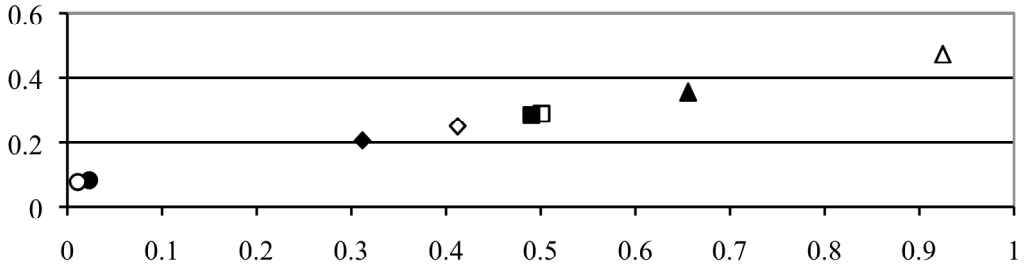


Figure 5: Availability of free amino groups after collagenase digestion of fibrous collagen treated with crosslinking or tanning agents. Collagen treatments were: circles = glutaraldehyde, diamonds – mTgase, triangles – DHT, and squares are controls. The ratio of collagenase to collagen represented by open markers is double that represented by solid markers. The vertical axis is in μmols available amino groups, on the horizontal axis A_{600} is obtained from a ninhydrin assay.

Models that approach more closely a true tanning system are powdered hide and intact hide pieces. Powdered hide for model tanning studies was initially prepared by cutting fresh hide into strips, subjecting it to pretanning steps, extracting with acetone, air-drying and grinding in a Wiley mill.[42] More recently, in response to queries from tannery byproduct users about changes in the materials they receive, the powdered hide model has been broadened to examine the effects of newer pretanning processes on collagen structure and stability. Similar steps without grinding were used to prepare hide pieces. The primary analytical tools at this level are differential scanning calorimetry (DSC) for comparisons of hydrothermal stability (Table 3), scanning electron microscopy (SEM) for examining fiber structure (Figure 6), collagenase sensitivity, and extraction of collagen for evaluation by general protein methods.

The data in Table 3 illustrate the similarity in the degree of collagen thermal stability imparted by the treatment of powdered[43] or intact hide[44] with potential tanning agents. More importantly, from a

mechanistic view point, is the difference in effectiveness of the aluminum-genipin combination, depending on the order in which the tannages are applied. Although the probable tanning structures of Al,[45] and genipin may be of similar size, genipin can block Al interaction sites on collagen, but not the reverse.

Table 3

Sample	Powdered Hide (°C)	Intact Hide (°C)
Genipin alone	79[a]	79
Genipin - Al	83	87
Al-genipin	97	104
Al-alone	87	NA

[a]Temperatures are the average of three determinations.

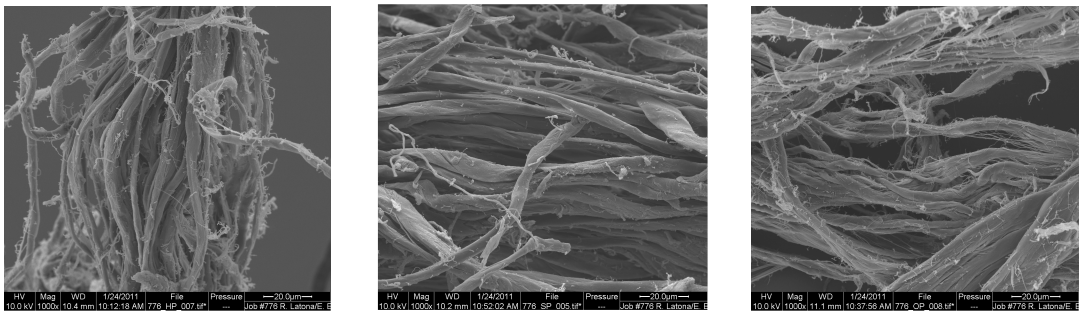


Figure 6: Scanning electron microscopy images from left to right, of a commercial hide powder, sulfide dehaired powdered hide and oxidatively dehaired powdered hide.

The SEM images (Figure 6) show a greater separation of fibrils in the oxidatively dehaired powdered hide, than in the sulfide or commercial materials, suggesting that there may be differences in its reaction with tanning agents.

3. Summary

The quest for a comprehensive tanning mechanism has been on going for more than a century. The salient characteristics of this mechanism are that it will explain effects of current and past practice, predict requirements for new reactions, and accurately predict the outcomes of new practices. Leather is, simply put, animal skin that has been tanned by any of several methods to prevent putrefaction and increase it hydrothermal stability. The diversity and complexity of methods by which leather can be produced significantly complicates the quest, and has led many practical leather scientists to the conclusion that no such mechanism exists.

During much of the 20th century, crosslinking of acidic groups on collagen by chromium ions, crosslinking of basic groups by organic aldehydes, and hydrophobic interactions and hydrogen bonds between vegetable tannins and collagen were assumed to be the important tanning mechanisms. Recently, the role of collagen modification and fibril coating in tanning has increased interest in tanning agents other than chromium, and a better understanding of the role of telopeptides in fibril stabilization has led to a closer examination of the gap region. A successful tanning agent is one that interacts with the collagen

matrix of the hide in a way that provides stability. Under the conditions of tanning, most tanning agents are in oligomeric form and effective interactions with collagen are intermolecular. Thus one of the requirements for tanning may be adequate open space within the fiber structure to accommodate a moderately sized oligomer without major distortion of the collagen ultrastructure.

ERRC researchers have focused on the development of both theoretical, and experimental tools for investigating methods of stabilizing the collagen matrix of a hide or skin. Matrix stabilization can be described broadly as modification of the matrix, by one or more of the following paths: the addition of covalent crosslinks, hydrophobic interactions, electrostatic interactions (salt bridges), altered water structure (reduced water activity), and hydrogen bonds. In this quest, the ERRC molecular model has been shared with scientists worldwide who have used it in studies of beamhouse and tanning processes. The model is modified as new data becomes available, and used to predict proposed interactions between tanning agents and collagen. Experimental modeling helps to explain some tanning requirements, gives a preliminary evaluation of potential new tannages, and insight into the effects of changes in tannery operations on collagen structure/function.

Covington's theory[46] based on the link-lock concept that requires an initial reaction to link the collagen into the surrounding matrix of water and a second reaction component to lock the linked structure together, creating a macromolecular structure around the triple helices, is the most interesting current proposal. The application of our theoretical and experimental tools to the exploration of this concept should provide valuable insights to the mechanism.

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5. References

1. O'Flaherty, F. (1956) in *The Chemistry and Technology of Leather,* ACS Monograph No. 134, Reinhold Publishing Corporation, New York.
2. Anon. History of Leather; *Simply Leather* http://www.simplyleather.co.uk/acatalog/History_of_Leather.html
3. Proctor, H. R. (1914) in *The Making of Leather*, Cambridge University Press.
4. Wilson, J. A. (1919) The application of colloid chemistry to the leather industry. *J. Am. Leather Chem. Assoc*. **14**, 450-468.
5. Wilson, J. A. (1928) Chemistry and leather. *J. Amer Leather Chem. Assn.* **24**, 157-190.
6. Wilson, J. A. and Porth, I. H. (1943) Protein structure and the mechanism of tanning. *J. Am. Leather Chem. Assoc*. **38**, 20-30.
7. Gustavson, K. H. (1964) On the chemistry of collagen. *Federation Proceed*. **23**, 613-617.
8. Tapernoux, A., Mitton, R. and Baumann, E. (1966) A tribute to Dr. K. H. Gustavson. *J. Am. Leather Chem. Assoc.* **61**, 307-308.
9. Ramachandran, G. N. (1988) Stereochemistry of collagen. *Int. J. Peptide Protein Res*. **31**, 1-16.
10. Heidemann E, (1983) How can we help solve the problems of practical tanners using fundamental research? *J. Am. Leather Chem. Assoc*. **78**, 251-266.
11. Orgel J. P., Wess, T. J. and Miller, A. (2000) The in situ conformation and axial location of the intermolecular cross-linked non-helical telopeptides of type I collagen. *Structure with Folding & Design* **8**, 137-142.

12. Lollar, R. M. (1953) The organic chemistry of the vegetable tannins – A half century in retrospect. *J. Am. Leather Chem. Assoc*. **48**, 656-672.
13. Na, G. C. (1988) Interactions of polyphenols and their derivatives with collagen: A model study of vegetable tannins. *J. Am. Leather Chem. Assoc*. **83**, 337-349.
14. Haslam, E. (1997) Vegetable tannage – Where do the tannins go? *J. Soc. Leather Tech. Chem*. **81**, 45-51.
15. Theis, E. R. and Schaffer, E. J. (1936) Studies in aldehyde tannage. *J. Am. Leather Chem. Assoc*. **31**, 515-529.
16. Theis, E. R. and Esterly, A. R. (1940) Studies in aldehyde tannage. IV The effect of hydrogen-ion concentration during tannage upon the shrink temperature of the leather. *J. Am. Leather Chem. Assoc*. **35**, 563-576.
17. Feairheller, S. H. (1979) The chemistry of glutaraldehyde tanning and the properties of glutaraldehyde tanned leather. A review after 20 years practice. *Proceed. 16th Congress IULTCS* Versailles, France, pp. 79-87.
18. Watt, A. (1906) in *Leather Manufacture: A Practical Handbook of Tanning, Currying, and Chrome Leather Dressing*, 5th edition Van Nostrand, Chapter XXI Chemical Tanning
19. Imer S. and Varnali, T. (2000) Modeling chromium sulfate complexes in relation to chromium tannage in leather technology: a computational study. *Appl. Organometallic Chem.* **14**, 660-669.
20. Chagne, V., Silvestre, F. and Gaset, A. (1996) Review of chrome tanning. Part 1. *Leather* **47**, March 17-26.
21. Cuq, M. H., Palevody, C. and Delmas, M. (1999) Fundamental study of cross-linking of collagen with chrome tanning agents in traditional and Cr.A.B. processes. *J. Soc. Leather Tech. Chem*. **83**, 233-238.
22. Frey, R. W. and Beebe, C. W. (1940) The permanence to acid deterioration of vegetable leather retanned with alum. *J. Am. Leather Chem. Assoc*. **35**, 440-443.
23. Nutting, G. C. and Borasky, R. (1948) Electron microscopy of collagen *J. Am. Leather Chem. Assoc*. **43**, 96-110.
24. Borasky, R. and Rogers, J. S. (1952) Effects of tannery processes on the electronoscopic appearance of bovine hide collagen fibrils. *J. Am. Leather Chem. Assoc*. **47**, 312-329.
25. Susi, H., Ard, J. S. and Carroll, R. J. (1971) Hydration and denaturation of collagen as observed by infrared spectroscopy *J. Am. Leather Chem. Assoc*. **66**, 508-520.
26. Quimby, D. and Witnauer, L. P. (1975) Fluorescence polarization of dye-labeled collagen: local flexibility in the nonhelical region. *J. Am. Leather Chem. Assoc*. **70**, 510-518.
27. Na, G. C., Butz, L. J., Bailey, D. G. and Carroll R. J. (1986) In vitro collagen fibril assembly in glycerol solution: Evidence for a helical cooperative mechanism involving microfibrils. *Biochemistry* **25**, 958-966.
28. Na, G. C. (1989) Monomer and oligomer of type I collagen: Molecular properties and fibril assembly. *Biochemistry* **28**, 7161-7167.
29. Bailey, A. J. (1992) Collagen--Natures's framework in the medical, food and leather industries. *J. Soc. Leather Tech. Chem*. **76**, 111-128.
30. Chen, J. M., Kung, C. E., Feairheller, S. H. and Brown, E. M. (1991) An energetic evaluation of a "Smith" collagen microfibril model. *J. Prot. Chem*. **10**, 535-552.
31. King, G., Brown, E. M. and Chen, J. M. (1996) Computer model of a bovine type I collagen microfibril. *Protein Engineering* **9**, 43-49.
32. Qi, P. X. and Brown, E. M. (2002) Molecular modeling of N-terminal telopeptides of bovine type I collagen. *J. Am. Leather Chem. Assoc*. **97**, 235-242.
33. Brown, E. M. (2004) Potential interactions of the C-telopeptides of bovine type I collagen. *J. Am. Leather Chem. Assoc.* **99**, 376-385.

34. Scholnick, F., Liao, L. L., Brown, E. M. and Feairheller, S. H. (1992) Crosslinking of collagen with dicarboxylic acids. *J. Am. Leather Chem. Assoc*. **87**, 333-338.
35. Brown, E. M., Chen, J. M. and Feairheller, S. H. (1993) Predicted interactions of ionizable sidechains in a fragment of the 3D model for calf skin Type I collagen microfibril. *J. Am. Leather Chem. Assoc*. **88**, 2-11.
36. Covington, A. D. and Song, L. (2003) Crosslinking - what crosslinking? Studies on the general mechanism of tanning. *Proceedings 27th Congress IULTCS* Cancun, Mexico, pp. 148-152.
37. Ramasami, T. (2001) Approach towards a unified theory for tanning: Wilson's dream. *J. Am. Leather Chem. Assoc*. **96**, 290-304.
38. Brown, E. M. and Taylor, M. M. (2003) Essential chromium? *J. Am. Leather Chem. Assoc*. **98**, 408-414.
39. Na, G. C. (1988) UV spectroscopic characterization of type I collagen. *Collagen Rel. Res.* 8, 315-330.
40. Taylor, M. M., Marmer, W. N. and Brown, E. M. (2004) Molecular weight distribution and functional properties of enzymatically modified commercial and experimental gelatins. *J. Am. Leather Chem. Assoc*. **99**, 129-141.
41. Collighan, R. J., Li, X., Parry, J., Griffin, M. and Clara, S. (2004) Transglutaminases as tanning agents for the leather industry. *J. Am. Leather Chem. Assoc*. **99**, 293-302.
42. Brown, E. M., Latona, R. J. and Taylor, M. M. (2010) Powdered hide for research on tanning mechanisms. *J. Am. Leather Chem. Assoc*. **105**, 116-120.
43. Ding, K., Taylor, M. M. and Brown, E. M. (2007) Genipin -aluminum or -vegetable tannin combinations on hide powder. *J. Am. Leather Chem. Assoc*. **102**, 164-170.
44. Ding, K., Taylor, M. M. and Brown, E. M. (2008) Tanning effects of aluminum-genipin or -vegetable tannin combinations. *J. Am. Leather Chem. Assoc*. **103**, 377-382.
45. Ding, K., Liu, J. and Zhang, T. (2006) The composition analysis of organic acid masked aluminum tanning solutions by ferron assay and GFC. *J. Am. Leather Chem. Assoc*. **101**, 260-265.
46. Covington, A. D., Song, L., Suparno, O., Koon, H. E. C. and Collins, M. J. (2008) Link-lock: An explanation of the chemical stabilisation of collagen. *J. Soc. Leather Tech. Chem*. **92**, 1-7.