

THERMODYNAMIC ANALYSIS OF THERMAL DENATURATION OF HIDE AND LEATHER

Abstract

Both real (based on mole of collagen) and effective (based on mole of independently melting cooperative regions) thermodynamic parameters of collagen denaturation and the number and size of independently melting cooperative regions can be calculated from data obtained using differential scanning calorimetry (DSC). DSC studies of hide collagen and chrome-tanned leather with varied water content were used to validate the following assumptions: (1) the length and number of independently melting cooperative regions are related to the distance between the number of high-charge-density domains known to exist in collagen; (2) enhanced electrostatic interactions in these high-charge density domains stabilize the collagen triple helix leading to a decreased number of domains and higher melting temperatures. For hide collagen, the number of independently melting cooperative regions found was 52, in close agreement with the 57 bands observed by electron microscopy in positively stained native collagen fibrils. Chrome tanning reduced the number of regions found by DSC to 19. Air drying of hide had the same effect as tanning probably due to enhanced electrostatic interactions in the absence of water. Lyophilized hide, adjusted to 20% moisture content, had 15 and chrome-tanned leather at the same moisture content had 10 independently melting regions. Application to leather processing is discussed.

Introduction

The advantages of the chrome tanning process include low cost, higher product quality, good product preservation and, most importantly, a high denaturation temperature. Nevertheless, further improvements in chrome leather manufacture as well as in product utilization appear to be feasible, especially in the drying operation. In 1974 Maire ⁽¹⁾ estimated that 37% of the total energy consumption in a tannery goes into drying, only 1/3 of which is used for desorbing water from leather, the rest being lost due to inefficient drying practices. While many improvements have been introduced in tanneries since that time, the air temperature presently used for drying rarely exceeds 60°C even though the moisture capacity of the drying air would be doubled every time its temperature was raised by 15°C ⁽²⁾. This energy waste is tolerated because leather quality decreases when elevated drying temperatures are used.

There is a paucity of information in the literature on the nature of the modifications in fiber structure and changes in physical properties which occur during drying ⁽³⁾. Drying studies

reported to date for the most part are purely empirical and involve prudent adjustment of process variables until the desired leather properties are obtained. Therefore, many important questions pertaining to leather manufacture remain unanswered such as why an increase in temperature of drying has a deleterious effect on product quality and even why chrome is superior to other tannages.

In 1971 J.H. Sharphouse offered in his "Leather Technicians' Handbook" ⁽⁵⁾, a very simple explanation for the tanning process. According to this explanation, water molecules are attracted to peptide bonds and to charged groups of a wet hide keeping the molecules apart. When the degree of ionization of either the basic or the acidic groups is increased, the charged groups are forced apart, more water is attracted to the charged groups and the hide swells. On the other hand, if water is removed by drying, the long molecules of collagen are made to approach each other. As the water is removed, new bonds are formed: inter- and intramolecular hydrogen bonds and ionic bonds between the acidic and basic groups. According to Sharphouse, chrome tannage involves replacement of intermolecular water, which is hydrogen bonded to the acidic groups, with a positively charged chrome complex that reacts with adjacent acidic groups forming crosslinks. Consequently, upon drying, contact among collagen molecules is impeded, there is less chance of crosslink formation between the remaining charged groups, and the leather stays relatively soft.

According to more recent publications summarized by Bienkiewicz ⁽⁶⁾, there are indeed water molecules hydrogen bonded to the charged groups by a single bond and there might be long chains of doubly hydrogen bonded water molecules connecting the peptide bonds of adjacent molecules. Positively charged chrome complexes reacting with neighboring carboxyl groups and forming crosslinks can really be envisioned. However, this information does not tell us why crosslinking raises the denaturation temperature nor why different tannages give different denaturation temperatures. To answer such questions and to explain area decrease during tanning and drying of leather, Heidemann ^(7,9) examined the primary structure of α -chains of collagen and found that the charged amino groups are not distributed uniformly throughout the length of α -chains and that, to the contrary, they are located in clusters. As a matter of fact, Heidemann pointed out that these clusters overlap in the formation of triple helices and fibrils. Heidemann noted that this fact can be deduced from the well known electron micrographs of collagen fibrils. Staining of a fibril with a negative stain gives 5 part dark and part light bands, the so called D-bands, whereas staining with a positive stain yields 57 dark bands along the length of one collagen molecule, each of the clusters of negative and positive charges giving a dark band ^(7,8).

Based on X-ray diffraction experiments Heidemann ^(10,11) postulated that removal of moisture causes fibrils of tanned hides, which form smooth cylinders in native fibrils, to shrivel up, yielding long, corrugated cylinders containing ridges and grooves which Rougvié and Bear ⁽¹²⁾ named bands and interbands, respectively. Heidemann suggested that the clusters of charged groups are the locations of low heat stability where heat denaturation by unwinding of the triple helix occurs. The ridges, according to him, are formed in the same places that yield the dark bands seen by electron microscopy when using a positive stain. Consequently, the ridges or bands represent the areas which contain the clusters of ionic charges while the grooves or interbands show areas containing predominantly uncharged polar and hydrophobic side-chains.

Heidemann further suggested that the corrugated fibrils must be shorter than the straight fibrils of native collagen and that reduction in area which is observed in tanning is a consequence, at least in part of "ballooning" of the ridges (or bands) caused by the introduction of chrome between molecules and that further area shrinkage during drying is a consequence of further collapse of the corrugated structure.

The above represents but a brief summary of the various observations which have been made to date on collagen tissues. When collagen fibrils undergo heat denaturation, hydrogen bonds are broken and the chains tend to acquire the most random conformation possible. The degree of disorder attained can be measured by calorimetry as a change in entropy. From thermodynamics we know that the melting temperature is equal to $\Delta H/\Delta S$:

$$T_M = \Delta H / \Delta S$$

There is very little difference in enthalpy, ΔH , between soluble and insoluble collagen, but there is a difference in T_M of about 22°C between these two states of collagen. Obviously, the entropy term, ΔS , decreases and hence the melting temperature goes up. The effect of chrome tanning, which introduces additional crosslinks, is similar to the effect of dehydration. Differential scanning calorimetry is ideally suited for studying effects of physical changes because it yields numerical measures of ΔH and T_M and, therefore, permits the calculation of ΔS , the measure of the degree of disorder. While entropy is a very helpful tool in the study of the alterations in hide structure and physical properties and a very sensitive indicator of physical changes, it does not tell us, however, the cause of the change in disorder.

In collagen, each α -chain contains four sites involved in inter- and intramolecular crosslinks. During heat denaturation, an uncoiling of the highly ordered triple helix takes place in the domains between the crosslinks. Using calorimetry, Privalov^(13,14) found that soluble collagen does not denature as one structural unit, as expected, but as 12–13 individual blocks or regions each of which denatures cooperatively at a different temperature. With the realization that chrome tanning thermally stabilizes the molecule by forming additional, stable crosslinks, the question arises how this crosslinking affects the denaturation behavior of collagen, especially with regard to the number of blocks that denature as separate, cooperatively melting structural units.

Differential scanning calorimetry (DSC) can provide an answer to the above question because it yields numerical values not only for the real (calorimetric) enthalpy of the thermal process being studied, but also for the effective (van't Hoff) enthalpy of that process. Privalov⁽¹³⁾ and Jackson and Brandts⁽¹⁵⁾ carried out calorimetric studies on dilute solutions of collagen demonstrating that collagen melting is an extremely cooperative process. Privalov calculated the number of amino acid residues forming a cooperative block that melts as a single structural unit by comparing the effective enthalpy with the calorimetric one. The same approach was taken in this study on hides and leather with the realization that there are interactions in such aggregates that make interpretation of the results more complicated because the reversibility of the denaturation reaction becomes more questionable.

Experimental

Thermograms for leather and hide samples were determined at a scan rate of 5°K/min. on a Perkin-Elmer Model DSC-2 differential scanning calorimeter. The chart speed was 4 cm/min. Preparation of chrome-tanned cattlehide leather and the methods used for calibrating the instruments, for obtaining the experimental data, and for calculating the results have been described earlier⁽¹⁶⁾. However, in this work pre-fleshed fresh hide was used. An approximately 1 lb. sample of hide was soaked, unhaired, relimed, and delimed by a procedure described by Taylor et al.⁽¹⁷⁾. The temperature was kept at below 22°C and after neutralization the hide was washed for 3 hours with 3 changes of water each of approximately 200% float. The leather sample in the blue was also washed the same way to remove excess chemicals.

Results

CALCULATION OF MOLAR ENTHALPY AND ENTROPY

A typical example of a differential scanning calorimetry plot obtained while heating a sample of hide is shown in Fig. 1. The calorimetric enthalpy of the phase transition process is determined from the area of the heat absorption curve, the calorimeter having been calibrated in units of mcal/cm² of the peak area by using appropriate standards (benzoic acid and indium samples were used in this research). The sealed aluminum pan of the calorimeter contained 11.06 mg of hide at 10.5% of moisture. The area under the heat absorption peak above the base line is 42.6 cm² which corresponds to .07304 calories. The melting temperature, T_M, at peak maximum is 406°K. The (denaturation) onset temperature is 397°K, and the temperature at the end of the peak is equal to 423°K. Considering that the dry weight of the sample is 11.06 x 0.895 = 9.9 mg and that its collagen content is 9.9 x 0.7967 = 7.886 mg, the value of the heat of denaturation per gram of collagen, Q_M, is equal to 0.07304/(7.886 x 10⁻³) = 9.262 cal/g. The molar enthalpy of denaturation, ΔH_M, of the whole macromolecule with a molecular weight, M, equal to 300000 is calculated to be

$$\Delta H_M = MQ_M = 300000 \times 9.262 = 2.778 \times 10^6 \text{ cal/mole of collagen} \quad (1)$$

Assuming, in addition, that collagen denaturation is a phase transition of the melting type for which the change in free energy at the melting point is equal to zero

$$\Delta G_M = \Delta H_M - T_M \Delta S_M = 0 \quad (2)$$

one readily obtains the value of the entropy, ΔS_M, of the process

$$\Delta S_M = \Delta H_M / T_M \quad (3)$$

$$= 2.778 \times 10^6 / 406 = 6843 \text{ cal/mole collagen/}^\circ\text{K.}$$

Since there are approximately 3000 residues per molecule

$$\begin{aligned} \Delta S_M &= (6843 \text{ cal/mole/}^\circ\text{K}) / (3000 \text{ moles residues/mole collagen}) \\ &= 2.3 \text{ calories/}^\circ\text{K/mole residues.} \end{aligned}$$

SIZE OF INDEPENDENTLY MELTING COOPERATIVE REGIONS

Collagen is a very large molecule which does not melt as one block. Instead, it melts as many cooperatively melting regions or blocks. To determine the heat required to melt such a cooperatively melting block, the van't Hoff equation is applicable⁽¹³⁾. The van't Hoff equation states that

$$\Delta H^{\text{eff}} = RT_M^2 (d \ln K^{\text{eff}} / dT) \quad (4)$$

where K^{eff} = equilibrium constant of the two-state denaturation reaction.

It has been repeatedly argued^(14,15) that the denaturation reaction may be considered to be a two-state transition in which there is at all times an equilibrium between the native and the denatured collagen conformation and that equation (4) reduces to the following approximate expression

$$\Delta H^{\text{eff}} = 4RT_M^2 / \Delta T_{M1/2} \quad (5)$$

where ΔT_{M1/2} = half-width of the melting interval (i.e. half-width of the base-line of the heat absorption peak, assumed to be equal to the width of the peak in °K at the midpoint of the height of the peak).

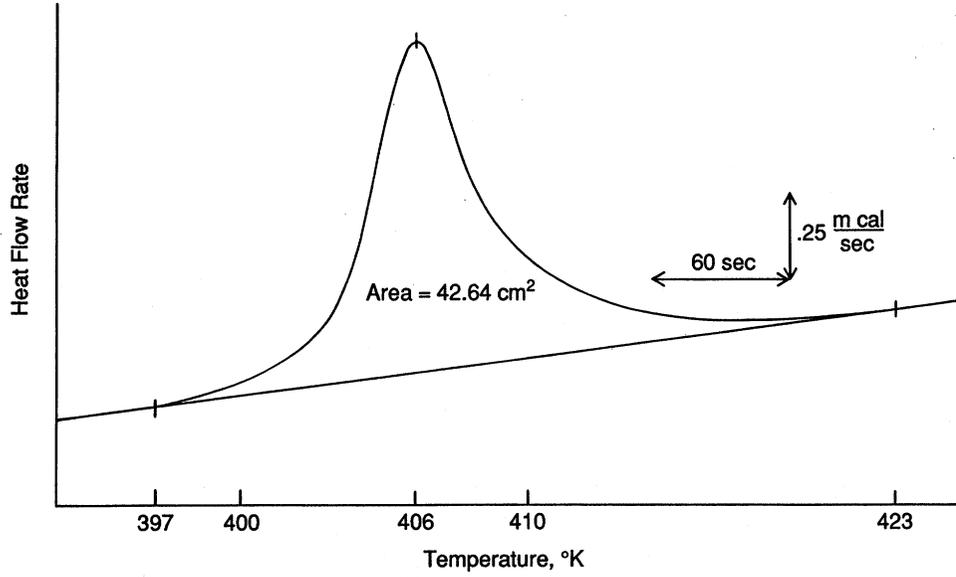


FIG. 1 — Thermal absorption recording of denaturation of limed cattlehide at 10.5% moisture content. The sample tested weighed 9.9 mg on dry solids basis and contained 7.9 mg collagen. Peak area = 42.6 cm² = .07304 calories. The scanning rate was 5°K/minute.

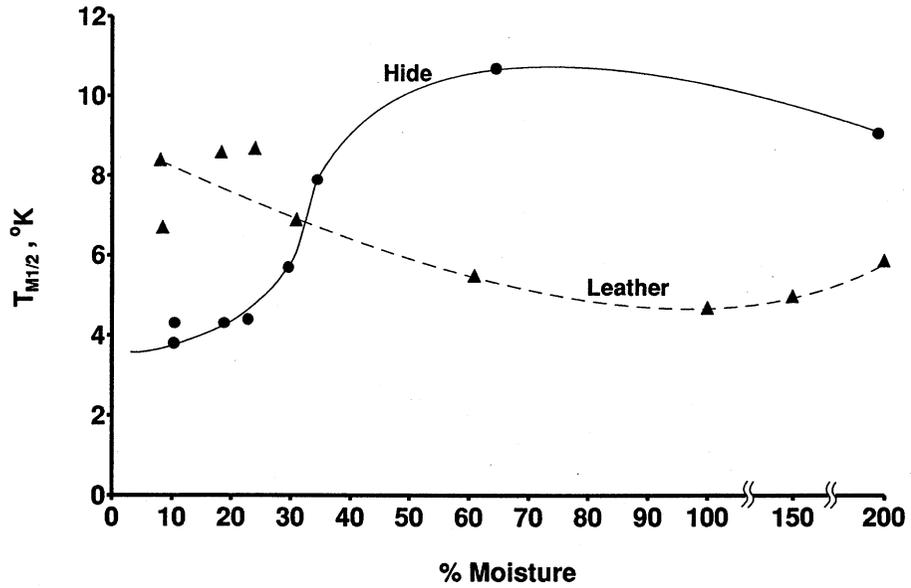


FIG. 2 — Plot of $\Delta T_{M1/2}$ vs. percent moisture content on dry solids basis. $\Delta T_{M1/2}$ = half width of the base-line of the heat absorption peak.
 ● — Line through cattlehide data points
 ▲ — Line through chrome leather data points

The number of independently melting cooperative regions or blocks, N , can be calculated by using equation (6):

$$N = \Delta H_M / \Delta H^{\text{eff}} = \text{number of cooperatively melting blocks/molecule.} \quad (6)$$

For this example, $\Delta H^{\text{eff}} = 4(1.987)(406)^2/4.4 = 2.977 \times 10^5$ calories per mole of cooperatively melting blocks, and the number of cooperatively melting blocks per molecule of collagen is equal to $\Delta H_M / \Delta H^{\text{eff}} = 2.778 \times 10^6 / 2.977 \times 10^5 = 9$. Also, $\Delta S^{\text{eff}} = \Delta H^{\text{eff}} / T_M = 2.977 \times 10^5 / 406 = 733$ cal/°K/mole of cooperative blocks.

TREATMENT OF EXPERIMENTAL DATA

The experimental Q_M , T_M , and $T_{M1/2}$ values varied over a wide range from sample to sample because of the small size of samples used in DSC experiments and because of the variable collagen content in the samples. Therefore, following the example of Koop et al.⁽³⁰⁾, the experimental data for Q_M and T_M were fitted to the following equations:

$$Q_M = A(1 - e^{-BW}) + C \text{ and } T_M = Ke^{-LW} + M, \text{ where } W = \text{moisture content} \\ \text{and } A, B, C, K, L \text{ and } M \text{ are constants.}$$

Curve fitting was achieved by use of a non-linear regression program. Table I gives the standard deviation for each parameter and the accuracy of curve fitting at a 95% confidence level in terms of the residual standard deviation of regression. In the absence of a suitable empirical equation, the experimental values of $T_{M1/2}$ for each moisture content were averaged and plotted vs. moisture content in Fig. 2.

Being based on many analyses, the above derived equations yield Q_M and T_M values which are more accurate and are more representative of the whole hide. To improve data interpretation and analysis, Q_M and T_M were calculated for a series of moisture contents considered to be critical by other researchers^(18, 19). Then, the corresponding $T_{M1/2}$ values obtained from Fig. 2 were substituted into equation (5) to calculate ΔH^{eff} , and the appropriate calculated Q_M values were substituted into equation (1) to obtain ΔH_M . Finally, the number of cooperatively melting regions was calculated using equation (6). The results are presented in Table II and in Fig. 3.

Discussion

Hydrogen bonds, charged-pair interactions, and hydrophobic interactions between amino acid side chains on the surface of type I collagen molecules have been demonstrated by model building using known α -chain sequences⁽²⁷⁾. In the absence of direct evidence that these side chain interactions provide structural stability in native collagen tissues, it is assumed that covalent bonds are the principal source of stabilization⁽²⁸⁾ even though it is conceded that charged amino acids appear in clusters much more frequently than would be expected by chance⁽²⁷⁾. Whereas stabilization by charged-pair interactions may be small in the presence of large quantities of water, research data obtained in this study show that heat stabilization of hide is greatly enhanced by formation of strong inter- and intramolecular ionic bonds if water is removed. This can be readily seen from Fig. 3 which gives a plot of the number of cooperatively melting blocks per molecule of collagen, N , as a function of moisture content in cattlehide and chrome leather. In both hide and leather containing between 200% and 50% moisture, the rate of decrease in N is seen to be relatively small. It decreases, however, very sharply in hide in the

TABLE I
Equations Describing Heat and Temperature of Denaturation of Hide
and Leather as a Function of
Moisture Content

$$Q_M = A(1 - e^{-BW}) + C$$

$$T_M = Ke^{-LW} + M$$

Where Q_M = Heat of denaturation per gram of collagen,
 T_M = Temperature at midpoint of denaturation process, °K
 W = Percent moisture content on (dry) collagen basis.
 On moisture free basis, the collagen content of the hide
 and the leather samples was approximately 79.7% and
 71.4%, respectively.

Parameters for Equations:

| Constants | Numerical Values of Constants | |
|-----------|-------------------------------|-----------------------|
| | Cattlehide | Chrome Tanned Leather |
| A | 14.30 ± 2.30 | 11.58 ± 2.05 |
| B | .06237 ± .0176 | .0144 ± .0097 |
| C | 2.83 ± 2.31 | 1.215 ± .859 |
| | n = 23 | n = 18 |
| | s.d. = 2.19 | s.d. = 1.14 |
| K | 75.40 ± 2.27 | 58.6 ± 11.2 |
| L | .0128 ± .0017 | .184 ± .048 |
| M | 334.6 ± 2.2 | 377.9 ± 1.0 |
| | n = 33 | n = 26 |
| | s.d. = 4.4 | s.d. = 4.2 |

n = number of samples.

s.d. = standard deviation of regression.

TABLE II
Temperature, Heat, and Entropy of Denaturation and the Number of Cooperatively Melting Blocks per Molecule of Collagen of Cattlehide and Chrome Tanned Leather at Different Moisture Contents

| Substance Denatured | Moist. Content | | Melt. Temp. T_M °K | Heat of Denatur. Q_M cal/g of collagen | Van't Hoff Enthalpy ΔH_{eff} cal/mole blocks | Entropy (calorim.) ΔS_M cal/°K/mole | No. of Blocks N | Entropy ΔS_{eff} cal/°K/mole |
|--------------------------|----------------|---------------|-------------------------|---|---|--|-------------------|---|
| | % of Solids | % of Collagen | | | | | | |
| Hide | 200 | 251 | 338 | 17.1 | 98697 | 5.1 | 52 | 292 |
| | 50 | 63 | 374 | 16.8 | 111173 | 4.5 | 45 | 297 |
| | 34.5 | 41 | 378 | 16.2 | 141955 | 4.3 | 34 | 376 |
| | 20 | 25 | 389 | 14.1 | 279697 | 3.6 | 15 | 719 |
| | 10.5 | 13 | 398 | 10.8 | 331314 | 2.7 | 10 | 832 |
| | 2.2 | 2.8 | 407 | 5.1 | 365716 | 1.2 | 4 | 898 |
| Chrome Tanned Cattlehide | 200 | 280 | 378 | 12.6 | 195800 | 3.3 | 19 | 518 |
| | 50 | 70 | 378 | 8.6 | 189274 | 1.9 | 14 | 501 |
| | 34.5 | 48 | 378 | 7.0 | 164586 | 1.8 | 13 | 435 |
| | 20 | 28 | 378 | 5.1 | 145595 | 1.3 | 10 | 385 |
| | 10.5 | 15 | 382 | 3.5 | 138072 | .9 | 8 | 361 |
| | 2.2 | 3.1 | 411 | 1.7 | 149176 | .4 | 3 | 363 |

The moisture content is given on dry solids basis (column 2) and on collagen basis (column 3). The collagen content of hide and leather was 79.67% and 71.40%, respectively. Q_M , in calories per gram of collagen, was calculated using the equation given in Table I. ΔH_M and ΔH_{eff} values were calculated using equations (1) and (5). ΔH_{eff} is given in calories per mole of cooperatively melting blocks, ΔS_M is in calories per °K per mole of amino acid residues in collagen, and ΔS_{eff} is given in calories per °K per mole of cooperatively melting blocks. The melting temperature given in column 3 is the temperature of the peak maximum and not the temperature at the onset of denaturation.

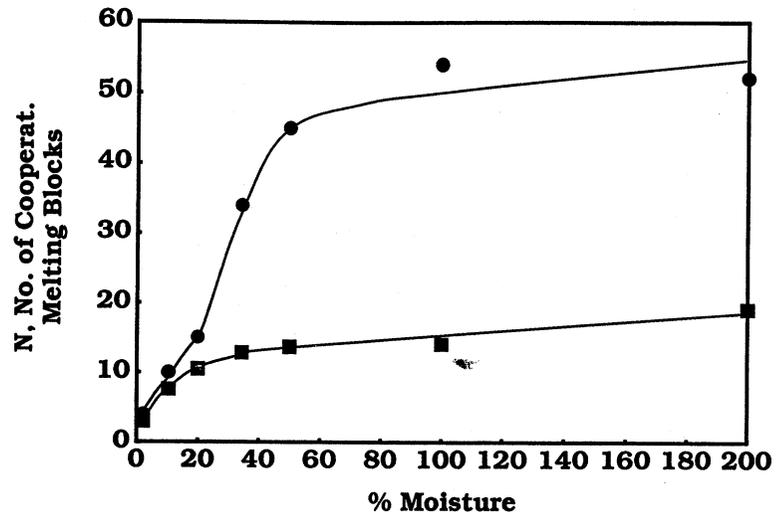


FIG. 3 — Plot of the number of cooperatively melting blocks in a collagen molecule, N , vs. moisture content on dry solids basis.
 —●— Line through cattlehide data points
 —■— Line through chrome leather data points

moisture range from 50% to 20% because removal of water induces formation of ionic bonds, as explained below. In leather, on the other hand, little decrease in N with moisture content is evident in this moisture range simply because its acidic groups are already crosslinked with chrome. Whereas the slopes of the lines for hide and leather are approximately equal at above 50% and at below 20% moisture content, it may be concluded that the same mechanisms may affect the size of the cooperatively melting blocks in both products at these high and low moisture contents.

EFFECT OF CROSSLINKING

For hide at 200% moisture content there are 52 blocks per collagen molecule which melt cooperatively (see Table II). This means that the amino acid residues comprising each of these blocks melt simultaneously if enough activation energy is provided, but each block melts at a different temperature. This number of 52 cooperatively melting blocks agrees well with the number of 57 charged clusters per molecule which, according to Heidemann's postulate, are the sites of low thermal stability at which unwinding of the triple helix is expected to be initiated.

A decrease in moisture content from 200% to 50% represents removal of 75% of the original moisture; nevertheless, the number of cooperatively melting blocks drops only to 45 (i.e. only 22%) because in that moisture range most of the water is removed from the spaces between fibrils, fibers and fiber bundles and not from between molecules^(11, 12, 18, 19). A further drop in moisture content to 20% shows the largest decrease in the number of cooperatively melting blocks: a decrease in 30 blocks (about 52% of the blocks) due to the removal of merely 15% of the water initially present. According to numerous researchers^(18, 19) this is the moisture range in which water hydrogen bonded to charged groups depicted by Bienkiewicz⁽⁶⁾ is removed during desorption causing the charged particles to react presumably forming ionic bonds which cross link different molecules as well as parts of the same molecule of collagen. This is also the moisture range in which most of the shrinkage in area has been reported⁽²⁰⁾ and in which firming up of the hide material occurs⁽⁴⁾.

As can be seen from Table II and from Fig. 3., in chrome tanned hide at 200% moisture, there are only 19 cooperatively melting blocks, most of the 52 (or possibly 57) clusters having been crosslinked in this wet condition by chrome molecules. In the presence of these chrome crosslinks, a drop in moisture content to 50% reduces the number of cooperatively melting blocks by only five. Just as in the hide, there is a further decrease in the number of cooperatively melting blocks as the moisture content is reduced to 10.5%. But, unlike in the hide, the decrease is rather gradual (as expected) even in the 50% – 20% moisture range. For both hide and leather, there is a considerable decrease in the number of cooperatively melting blocks in the 10.55–2.2% moisture range which is obvious also from the sharp increase in T_M in that moisture range.

EFFECT OF MOISTURE

It may be readily argued that results obtained by calorimetry on dry samples are of questionable validity because in compact structures, such as dehydrated collagen tissues, different intermolecular interactions are likely to exist unlike in a very dilute solution of soluble collagen where only minor hydrophobic, van der Waals, or electrostatic interactions are observed. However, it is precisely the ability to pick up the effect of these interactions that renders calorimetry a useful tool for comparison of π results obtained on soluble collagen with results obtained on hides and leather at lower moisture contents and, thereby, helps to deduce the reasons for the observed change.

This study examines the effects of crosslinking and drying on thermal stability of hide and leather. Thermal stability of insoluble collagen in a fully wetted state has been studied by Flandin et al. ⁽²¹⁾ who used ratskins, and by Kronick et al. ⁽²²⁾, who used hide and leather. Kronick et al. concluded that there are two discrete collagen populations with different stabilities as evidenced by the presence of a shoulder on the higher temperature side of the thermogram peak. Flandin et al. attributed the shoulder to the presence of heat-labile crosslinks. Such a shoulder was also observed in this study, but only in the specimens of hide containing 200% moisture. The drier hide samples had only one peak similar to the peak shown in Fig. 1, whose shape, in turn, is similar to the peaks reported in the literature for soluble collagen. This is probably due to the fact that in the absence of water the heat-labile bonds break less readily as well as due to the fact that after additional, more numerous crosslinking by ionic bonds or a tanning agent the cooperatively melting blocks become stronger and the heat-labile bonds break cooperatively at a higher temperature. Because of the symmetric, bell-like shape of the peaks, equation (5) was considered to be applicable for calculating ΔH^{eff} . Since equation (5) was also used in calculating the ΔH^{eff} of the wet hide which had 200% moisture and gave a thermogram with an asymmetric peak, as explained above, the number of 52 cooperatively melting blocks which was obtained for wet hide may be an approximate value.

COMPARISON OF CALORIMETRIC AND KINETIC DATA.

As mentioned previously, scanning calorimetry has the advantage of yielding not only the real (calorimetric) enthalpy, ΔH_M , which gives the energy needed to denature a gram or a mole of collagen and is calculated by using equation (1), but also the effective, van't Hoff, enthalpy of denaturation, ΔH^{eff} , which gives the energy absorbed in denaturing a mole of cooperatively melting regions and is calculated using equation (5). Weir ⁽²⁴⁾, on the other hand, used the theory of absolute reaction rates to calculate the thermodynamic parameters such as the enthalpy of activation, ΔH_A , for denaturation of wet rat tendon collagen. The derivation of ΔH_A is based on statistical mechanics; nevertheless, it represents the activation energy for the same cooperatively melting regions or blocks. Therefore, the number of cooperatively melting regions calculated by that kinetic approach should be the same as that obtained by the calorimetric method used in this paper. Weir found that for kangaroo tail tendon ΔH_A equals 140 kcal/mole of cooperative blocks and that ΔS_A equals 350 cal/°K/mole of cooperatively melting blocks. These values are higher than the ΔH^{eff} and ΔS^{eff} values in Table 2 for hide at 200% moisture content. This is to be expected: the activation energy and the activation entropy are always larger than the corresponding values for the reaction. Using a value of 17.1 cal/g obtained in this work for the heat of denaturation of cattlehide, the number of cooperative blocks per molecule is seen to be equal to $(17.1 \times 300,000)/140,000 = 37$. But 17.1 cal/g is the calorimetric value for the heat absorbed during the reaction. The heat of activation per gram should be higher and has been estimated to be equal to 25 cal/g ⁽²³⁾. Using this value, the number of cooperatively melting regions = $(25 \times 300,000)/140,000 = 54$, which is in excellent agreement with the number of bands seen by electron microscopy and the number of cooperatively melting blocks obtained by calorimetry in this work. For chrome tanned cattlehide Weir found that $\Delta H_A = 390$ kcal/mole and $\Delta S_A = 1040$ cal/mole/°K. Thus there is a drastic increase in both ΔH_A and ΔS_A due to tanning which needs to be clarified. Using the experimental value of 12.6 cal/g the number of cooperatively melting blocks = $(12.6 \times 3000,000)/390,000 = 10$. Again for lack of an experimental value for ΔH_A a small value for the number of blocks is obtained, but it is obvious that a much higher value, a value closer numerically to the 19 blocks determined by calorimetry,

would be obtained if the appropriate energy of activation were known. Thus the entropy and enthalpy of activation reported by Weir increase in T_M after different chemical treatments because they are calculated on a variable basis: for example, the ΔH_A for leather is higher than for hide because the former melts in larger sized cooperative blocks. This, of course, is also true for the effective thermodynamic parameters because they are calculated on a per-mole-of-cooperative-regions basis.

CHANGE IN STRUCTURE DUE TO DEHYDRATION

While amino acid side chains can form four types of bonds, hydrogen, ionic, hydrophobic and van der Waals, it is primarily hydrogen bonding that plays the major role affecting physical properties and area of hide and leather during water removal. Water has been shown to have a high structuring ability ⁽¹³⁾ which stabilizes collagen tissues. During thermal denaturation this water structure collapses with a large absorption of heat and a large change in entropy.

Entropy is a thermodynamic quantity whose value increases as the degree of disorder decreases, or conversely, whose value decreases as the degree of order increases. The numerical value of the free energy change, ΔG , being equal to zero at the denaturation point ⁽¹³⁾, the change in entropy, ΔS , due to denaturation can be calculated from the expression

$$T_M = \Delta H_M / \Delta S_M = \Delta H_M / (S_f - S_i)$$

where S_i = entropy before denaturation
 S_f = entropy after denaturation

Collagen which has been crosslinked either by tanning or by drying cannot assume the same random conformation upon denaturation as that which has not been crosslinked. Therefore, crosslinked collagen has a lower entropy, S_f . In the presence of a large quantity of water the degree of structuring of the collagen chains by water is large, S_i is numerically small (smaller than S_f) and therefore ΔS_M is numerically relatively large. As more water is removed the degree of structuring of the chains by water decreases, S_i becomes numerically larger and consequently, the ΔS_M term in the denominator becomes progressively smaller; hence, T_M goes up. This can be readily seen from Table II.

Since under neutral conditions some amino acid side chains are hydrogen bonded to water, removal of water has a profound affect not only on ΔH_M but also on area and firmness of hide and leather. As described by Komanowsky ⁽²⁰⁾, at air/water interfaces in capillaries, hydrogen bonds exert an inward pull or tension. This hydrogen-bond mediated surface tension force, which is often referred to as capillary attraction, pulls the particles comprising the tissue together and causes it to aggregate and to contract. This effect brings together primarily the polar groups that can interact with water to form hydrogen bonds. Since the side chains which contain charged groups are relatively long, it is these charged groups that contact each other first to form ionic bonds. According to the results shown above, this occurs primarily in the moisture range between 50% and 20%. As is well documented in the leather literature ^(2,4), this is also the moisture range in which the texture of leather becomes firmer.

All the changes in the properties of hide and leather so far have been attributed to changes in hydrogen and ionic bonding in the intermolecular regions of the fibrils (the band regions) that desorb at between 50% and 20% moisture content because these changes involve crosslinking by dehydration of the same ionic amino acid residues that are crosslinked by tanning. Support for this view is found in research studies carried out by shrinkage rate experiments ⁽²⁴⁾, dynamic mechanical testing ^(18,19), calorimetry ⁽¹⁹⁾ and X-ray diffraction ⁽¹²⁾. The last of these testing methods shows reductions in intermolecular and interhelical distances with decrease in moisture content and an enhanced loss in structural order in the intermolecular regions containing

predominantly charged groups. It has been demonstrated ^(18, 19) in recent years that the structure of hide is also held together by additional hydrogen bonds involving capillary water that desorbs at moisture contents above 50% and by water that desorbs at moisture ranges of 20% to 10% and 10% to 2% between peptide bonds in fibrils and between α -chains in triple helices, respectively. In these moisture ranges, unlike in the 50% to 20% moisture range, desorption of water has the same effect on heat stability of both hide and leather. This can be deduced from the fact that the plots of N, the number of cooperatively melting blocks, vs. moisture content in Figure 3 have almost the same slopes in these moisture ranges for both materials.

As in many other natural products, the breaking of these hydrogen bonds by dehydration is not reversible. In the experience of tanners there is an irreversible change in physical properties which is due to water removal and simultaneous segmental chain movements which, in this work, are measurable calorimetrically as a decrease in entropy. Because these changes occur in conjunction with hysteresis effects, it is possible that contraction of the fiber structure during the last stages of water removal may be causing a decrease in the number of sites which are able to form hydrogen bonds ⁽⁶⁾ upon rehydration, but could equally likely be a consequence of some hitherto unexplained interactions such as bringing together hydrophobic parts of polypeptide or side chains which remain associated upon rehydration because clustering of weakly soluble molecules is thermodynamically favored. It is also probable that the shapes of some regions of molecules would be complementary enough to form weak van der Waals interactions. It is equally quite likely that an increase in drying temperature further enhances interfacial contact and adhesion preventing tanners from using elevated drying temperatures. The presence of such interactions could be readily seen by examining a sample of the comminuted hide which had been prepared for the study. The sample had been dried by lyophilization from a pasty consistency to a firm, tough sheet containing 2% moisture. When placed into a huge amount of water it rehydrated very slowly and remained aggregated for several days. Only by considerable mechanical action could it be redispersed to its original particulate condition.

Summary and Conclusion

Water removal allows oppositely charged groups of collagen to come together to form electrovalent bonds (or crosslinks) which are almost as strong and heat stable as covalent bonds. This crosslinking occurs in the clusters rich in charged groups of which there are 57 per molecule. In native collagen tissues unraveling of the triple-helices is initiated in these clusters during the denaturation process. A sufficient degree of crosslinking prevents unravelling of the triple-helices of collagen molecules which is initiated in the clusters. Consequently, as more water is removed and the crosslinks in the clusters become more numerous, the molecules unravel in progressively fewer locations, which means that the molecules melt in progressively fewer cooperatively melting blocks at progressively higher temperatures because it takes more energy to unravel a longer block of molecules. Of course, if all clusters were identical, all of them would melt as one unit at some elevated temperature. But the clusters are not identical and, therefore, the increase in block size is gradual and so is the increase in shrink temperature.

Hydrogen bonding (both intermolecular and intramolecular) is responsible for much of the structural order observed in collagen fibers in their native, fully hydrated environment. Introduction of tanning materials disrupts that order and so does drying. Heidemann and co-workers ^(10, 11) showed that chrome enters the intermolecular spaces and explained how steric changes in the ordered and the disordered regions are responsible for loss in area due to tanning and drying.

It is apparent from the results of this study that the denaturation temperature of hide and leather is a function of moisture content. From the literature it is well known that these materials decrease in area and thickness rather slowly at first as water is removed from them to about 50% moisture content (on solids basis); and fiber bundles, fibers and fibrils are made to approach each other by surface tension. It has also been reported that there is a drastic increase in firmness and decrease in area as the moisture content is reduced further, especially through the 50% – 20% moisture range. It follows from these facts that firmness, area yield, as well as denaturation temperature are all interrelated phenomena which are dependent on water content.

At high moisture content, little change in entropy was observed as moisture was removed. Crosslinking of molecules by ionic bonds was observed to occur mostly in the moisture range between 50 and 20% as gauged by the large decrease in entropy and in the number of cooperatively melting blocks present in a molecule of collagen. However, further drying to 2.2 % moisture content revealed an additional progressive decrease in entropy indicating a continued alteration of the structure toward a less orderly arrangement. This disordering of the structure has been observed repeatedly by X-ray diffraction studies ⁽²⁵⁾.

There are several conclusions which can be drawn from the results obtained in this study which have practical significance. The experimental results support the hypothesis that collagen denaturation occurs in large cooperatively melting blocks and that collagen denaturation is initiated in clusters occupied by charged groups. The results imply that heat stabilization by tanning is most successful if it involves crosslinking between charged groups, especially those occupying the clusters of charged groups which yield bands observed in electron microscopy.

Also, since the distribution of amino acids along the α -chains of collagen is becoming more known, this experimental approach may eventually be useful to pinpoint weak spots in the collagen structure from the standpoint of thermal stability. Furthermore, this study helps to understand why other tanning agents are inferior to chrome and what type of tanning agent could possibly replace chrome. And finally, while the additional drop in the number of cooperatively melting blocks observed upon drying of leather to lower moisture is small, the experimental data show that it has a significant effect on heat stability. A better understanding of hydrogen bonding in leather is essential because it may help to explain such important commercial processing steps as the lasting of shoes in which tensile stresses are released and the shoe is made to conform to the size and shape of the last by the use of high temperature and low moisture probably to break and reform hydrogen bonds ⁽²⁹⁾ as well as ionic bonds.

It is believed that measurement of mechanical properties at different moisture contents may substantiate the significance of the observed drop in entropy and provide additional insight into the alterations of structure as affected by moisture and heat.

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