

1393

THE SHRINKAGE OF HIDE—A MELTING  
PHENOMENON\*

LEE P. WITNAUER AND JEANNE G. FEE

*Eastern Regional Research Laboratory†  
Philadelphia 18, Pennsylvania*

ABSTRACT

The behavior of hide substance when subjected to heat in the presence of small amounts of compatible diluents such as water, ethylene glycol, formamide, and phenol was shown to be analogous to the depression of the melting point of any crystalline substance by a diluent. Various types of tanning agents interact with the polypeptide chains of the hide in different ways, modifying to some extent the ultimate effect of the diluent on the shrinkage temperature. The results from tanned hide specimens indicated that tanning agents reduce the amount of water that can reach the polypeptide chains and, as a consequence, produce the commonly observed elevation in shrinkage temperature.



INTRODUCTION

It is well known that hide substance when heated in water or other polar substances undergoes a marked dimensional change, namely shrinkage, at a specific temperature. This behavior has been variously described as due to degradation, denaturation, rearrangement, gelation, or various combinations of each of these processes. Recently Garrett and Flory (1) investigated this transformation and observed that tendon collagen containing ethylene glycol abruptly increased in volume at a specific temperature which depended on the glycol content. Is this behavior unique for collagen? No, it is characteristic of the melting of any semicrystalline polymeric substance; melting is always accompanied by dimensional changes. The melting point of such polymers is always lowered by addition of a diluent. The amount of depression of the melting point is proportional to the amount of diluent present. This lowering of the melting point is analogous to that of the molar depression of the freezing point of water or other liquid by the addition of a

\*Presented at the Fifty-fourth Annual Meeting, Swampscott, Massachusetts, May 26, 1958.  
†Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.

solute. For example, if a small amount of ethylene glycol were added to finely crushed ice at a temperature well below the freezing point of water and if the temperature were slowly raised, the melting point of the ice would be lowered well below 0° C. The amount of depression would depend on the ratio of the molar quantities of the glycol and water present. It should be pointed out that the diluent in such a system must be compatible (miscible), that is, be able to form a single phase on fusion. The addition of an inert substance, such as a hydrocarbon, to ice would not alter its melting point.

Flory was able to show that the theory of melting-point depression as applied to semicrystalline polymers was also applicable to the collagen-ethylene glycol system (1); thus, the principal event that occurs in the shrinkage of collagen is a melting phenomenon.

The application of the term "melting" to the shrinkage of collagen is not new. As early as 1932 Wohlisch (2) considered the transformation as such a phase transition. More recently Wiederhorn (3) and coworkers discussed the shrinkage of collagen from this point of view. Additional evidence that lends support to this theory is found in other published works. For example, Astbury (4) observed that the characteristic X-ray-diffraction pattern of crystalline collagen disappeared on shrinkage. Theis (5), Kutyanin (6), and Lenox (7) found that the shrinkage temperature depended on dilution.

Although the exact structure of collagen still is not known, much has been learned about it (8). The polypeptide molecules that make up collagen are found to arrange themselves in the form of a regular coil or helix. This helical structure which is stabilized by hydrogen bonds has the properties of a relatively rigid rod. These rodlike particles are aligned in a parallel array forming an ordered phase. One explanation for shrinkage might be that a disordering of this parallel array takes place. Although the amount and strength of the interhelix forces are not known, experimental evidence indicates they may be relatively small. It is well known that unmodified hide swells when immersed in water or other highly polar substances. Doty (8) recently showed that it is possible under the proper conditions to swell collagen to such an extent that the rodlike particles are separated from one another in a medium. This separation of the helices occurs without loss of the helical structure and implies that interchain order may not play a major part in the shrinkage process of unmodified collagen. It would then appear that the shrinkage process could simply be explained by the transformation that takes place within each helix.

Such a process can still be considered a melting phenomenon analogous to that of the melting of an ice crystal that is held together by hydrogen bonds. Whether the rearrangement is a result of a reduction of order between helices or within a helix does not invalidate the application of principles of first-order transition to collagen.

With these considerations in mind, we decided first to investigate whether the melting-point theory was directly applicable to hide substances (9) and then to determine if it were applicable to tanned specimens. The latter investigation might aid in explaining some of the effects of tanning.

#### EXPERIMENTAL

The hide used was pickled stock obtained from a local tannery. For these experiments it was depickled to pH 4.9, washed in running tap water, and dehydrated in acetone. The vegetable-tanned samples were of commercial upholstery leather which was analyzed for total hide substance by determination of nitrogen. The formaldehyde tanning was carried out in a 1% formaldehyde, 0.1 molar sodium bicarbonate solution to a final pH of 7.5. The leather was then washed free of unreacted tanning agent and air-dried. The dialdehyde starch tanning (10) for all except one sample was carried out with 33, 66, or 96% oxidized dialdehyde starch in a mixed buffer solution at pH 9.8. The exception, a sample tanned with 96% oxidized dialdehyde starch, was tanned in a straight sodium bicarbonate buffer solution at pH 8.8. The samples were subsequently washed in running water and air-dried.

Specimens for the shrinkage measurements were cut from these samples in strips 2.5'' long, 0.25'' wide, and about 0.15'' thick. The specimens were subsequently dried under vacuum at 50° C. Approximately seven hours was required for the sample to attain constant weight. The dried specimens were equilibrated in an evacuated desiccator over the diluent in the cases of ethylene glycol and water. The glycol-containing desiccator was heated to 60° C. to facilitate attainment of equilibrium, which required as long as six days in some cases. Although water vapor was quickly absorbed by the hide specimens at room temperature and 100% relative humidity, water contents of above 40% (based on total weight of specimen plus water) could not be obtained by this technique. For the higher water contents, the specimens were soaked three minutes in distilled water and then allowed to dry slowly in an atmosphere of 100% relative humidity until the desired water content was attained. In preparing samples of varying phenol or formamide content, it was necessary to soak the dry hide samples in absolute alcohol solutions of phenol or formamide. Varying amounts of these diluents were obtained in the specimens by changing the concentration or time of soaking. After soaking, the samples were conditioned in a vacuum desiccator containing desiccant for at least 24 hours. The diluent content of the cowhide samples reported here is based on total weight of sample and diluent.

The instrument used in measuring the shrinkage temperatures of the samples has been described in a previous paper (11) and simply provides a means of measuring changes in length with an accuracy of  $\pm 0.001''$ . In determining the shrinkage temperature a sample of known diluent content was immersed in a mercury bath to prevent loss of diluent during the test.

The mercury bath was surrounded by a glycol bath in which the heater and stirrer were immersed. The temperature was measured by placing a thermometer in the mercury bath. As a result of numerous trial runs the following rate of heating was chosen as most suitable and expedient. The sample was heated at a rate of 3° C. per minute up to approximately 10° C. below the expected shrinkage temperature (on the basis of a trial run). The rate of heating was then decreased to ½° C. per minute through the shrinkage range. The shrinkage temperature was considered to be that at which a sharp change in slope occurred in the temperature versus length plot. This temperature was reproducible to ±1° C. in separate runs.

#### RESULTS AND DISCUSSION

In order to determine whether shrinkage is associated with a melting phenomenon in hide substance, the apparent melting (shrinkage) temperatures of unmodified hide specimens containing varying amounts of ethylene glycol were measured. The results are shown in Fig. 1 along with the data

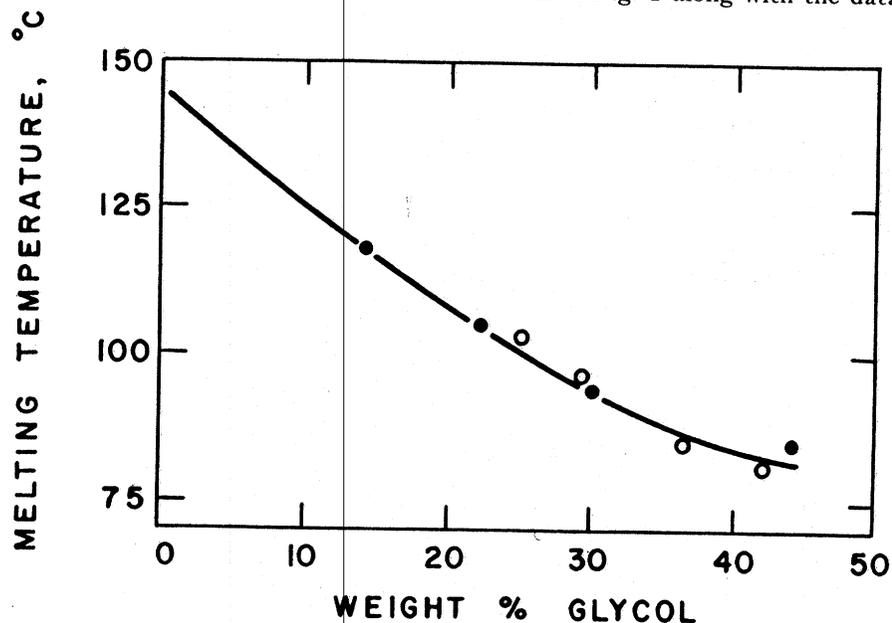


FIGURE 1.—Plot of apparent melting (shrinkage) temperature of hide vs. weight percent glycol. Dilatometric melting-point data of Garrett and Flory (●); data obtained by shrinkage measurements (○).

reported by Garrett and Flory (1) for ethylene glycol-tendon obtained using a dilatometer. The agreement between the two sets of data is remarkable considering the fact that both the origin and treatment of the samples studied were quite different.

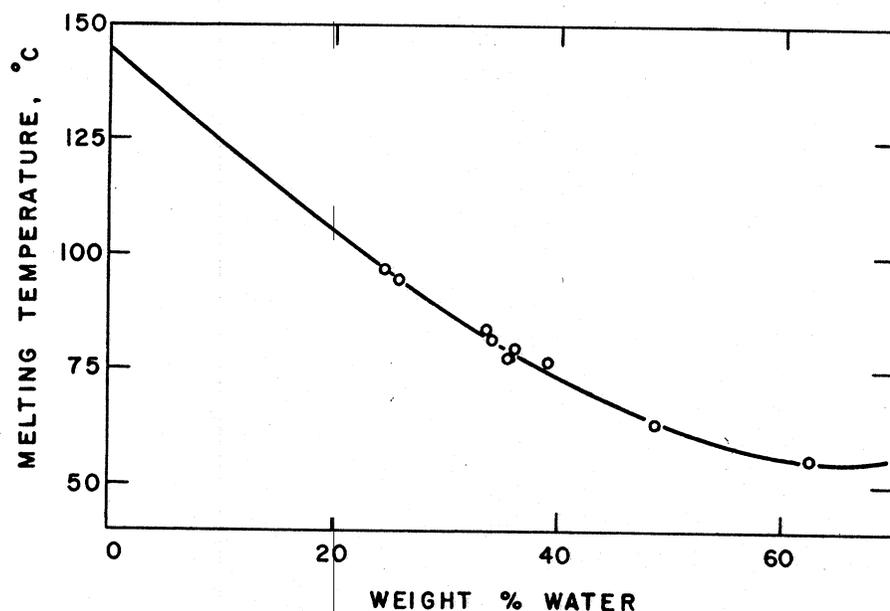


FIGURE 2.—Plot of apparent melting (shrinkage) temperature of hide vs. weight percent water.

The variation in the apparent melting (shrinkage) temperature for unmodified hide containing 25 to 62% by weight of water is shown in Fig. 2. Like the ethylene glycol system, the apparent melting temperature decreased as the diluent content increased. For example, the hide specimen containing 24.5% water contracted at 97° C., while the one containing 62% water contracted at 56° C. The latter temperature was the same as that obtained when the specimen was completely immersed in water. Apparently the maximum amount of water that is effective in lowering the melting temperature of the polypeptide helix of bovine collagen is about 62%. Specimens taken from different portions of the same hide and from other hides (including calfskin) in general gave the same melting temperature (56° C.) when completely solvated with water. The temperature 56° C. is therefore the fundamental characteristic apparent melting (shrinkage) temperature of limed bovine hide substances. The corresponding temperature obtained on specimens of unlimed fresh hide (immersed in water) is about 4° higher or 60° C. This value is still below the value, 65° C., reported throughout the literature for untreated hide. However, the heating rates generally employed are much larger, about 3° to 5° per minute, than those employed in the present work, 1° per minute. In addition, the instruments usually employed do not permit detection of extremely small changes in length as was possible in the present case (11).

If the melting (shrinkage) of hide substance is an outward manifestation of uncoiling of protein molecule or molecules, then the temperature at which shrinkage takes place should be very nearly that observed for the breakup of collagen helices suspended in aqueous solutions. Recently Doty and Nishihara (12) reported the temperature at which the structure of intact collagen helices isolated from calfskins disappeared. For the calfskin collagen helix this temperature was  $37^{\circ}\text{C}$ . and was denoted as a denaturation temperature, as distinct from the thermal shrinkage temperature. This temperature is  $23^{\circ}$  lower than observed in our study on untreated fresh hide. They presumed the difference between the so-called denaturation temperature and thermal shrinkage temperature to be a measure of crystal energy of the collagen fiber. Careful consideration of their data, however, showed that the so-called denaturation temperature was obtained on calfskin collagen helices which were suspended in a citrate buffer medium (pH 3.7), while the thermal shrinkage temperature used for comparison was measured in distilled water.

It is well known that the shrinkage temperature of hide substance varies with the pH of the solution (13). Therefore, it was thought that the large difference,  $23^{\circ}\text{C}$ ., between the reported denaturation temperature and the shrinkage temperature might be due in part to this fact. We then measured the shrinkage temperature of fresh hide substance in citrate buffer (pH 3.7) and found a shrinkage temperature of  $40^{\circ}\text{C}$ ., much lower than the value of  $60^{\circ}\text{C}$ . found in distilled water and only  $4^{\circ}\text{C}$ . higher than the reported denaturation temperature. (Hide which had been previously limed shrank at a slightly higher temperature,  $43^{\circ}\text{C}$ ., in citrate buffer.) Thus it appears that there is only a slight difference in temperature between the two. It is not unexpected that the hide substance gave a slightly higher apparent melting (shrinkage) temperature than that observed for collagen helices suspended in solution. Flory and coworkers (14) have recently shown thermodynamically that the apparent melting (shrinkage) temperature may be  $5^{\circ}$  to  $10^{\circ}$  higher than the equilibrium melting temperature. Thus, we have referred to our values as *apparent* melting temperatures. It appears, therefore, that the shrinkage of intact hide substance is a melting phenomenon and an outward manifestation of the collapse of the helical structure of collagen.

Two other diluents which were investigated with hide substance were phenol and formamide. The results are shown in Fig. 3. The formamide-hide data are represented by the lower curve, the phenol-hide data by the upper curve. Like ethylene glycol and water systems, these show a decrease in the melting temperature with increase in diluent concentration. Although the melting temperatures for the four systems investigated show the same dependence on the diluent concentration, the individual values obtained at the same diluent concentration (weight percent) are different. It should be emphasized that the diluents employed must be able to associate in some manner with the polypeptide chain or chains. They must contain a group

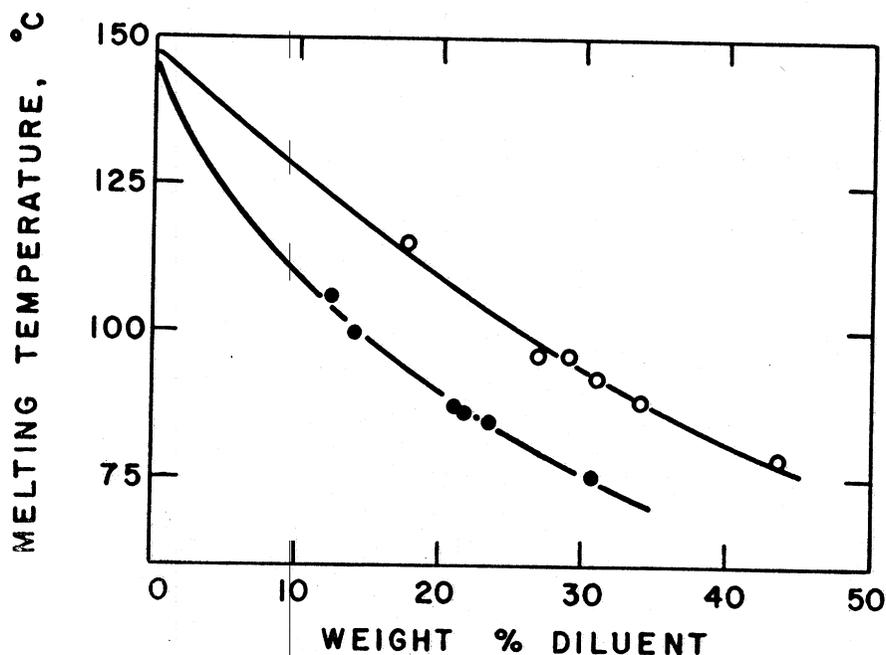


FIGURE 3.—Plot of apparent melting (shrinkage) temperature of hide vs. weight percent formamide (●); phenol (○).

or groups that can interact with a group or groups contained on or within the collagen molecule. For example, phenol is able to act as a diluent, while toluene is completely inert. Extrapolation of the melting (shrinkage) temperature to zero diluent content for all the above-mentioned diluents yielded a value of 145° C. for the theoretical melting temperature of vacuum-dried bovine collagen. Vacuum-dried unmodified hide showed no appreciable contraction even at temperatures up to 150° C.

These results indicate that the shrinkage temperature can be elevated quite simply by limiting the amount of diluent that can solvate (associate with) the helical polypeptide coils. One of the well-known effects that a tanning agent has on hide substance is to elevate the shrinkage temperature as measured immersed in water or other diluent systems. Thus, a tanning agent might be considered as a substance that reduces the availability of the polypeptide chains to the shrinkage medium. This will, in general, be true regardless of the types of tanning agents employed. It should be emphasized again that the tanning agent must itself become associated with the polypeptide, either through hydrogen bonds, covalent bonds, or electrostatic bonds. A completely unassociated hydrophobic material incorporated in unmodified hide substance would only exude out or sweat out, and any blocking action toward a diluent would be of very short duration.

A preliminary investigation of the melting-point depression in hide specimens that had been tanned with a vegetable tannin, formaldehyde, and dialdehyde starch was undertaken. Water was the diluent used. Like the untanned hide, the shrinkage temperature of the tanned specimens decreased with increasing water content. Diluents other than water would be expected to exhibit a similar behavior.

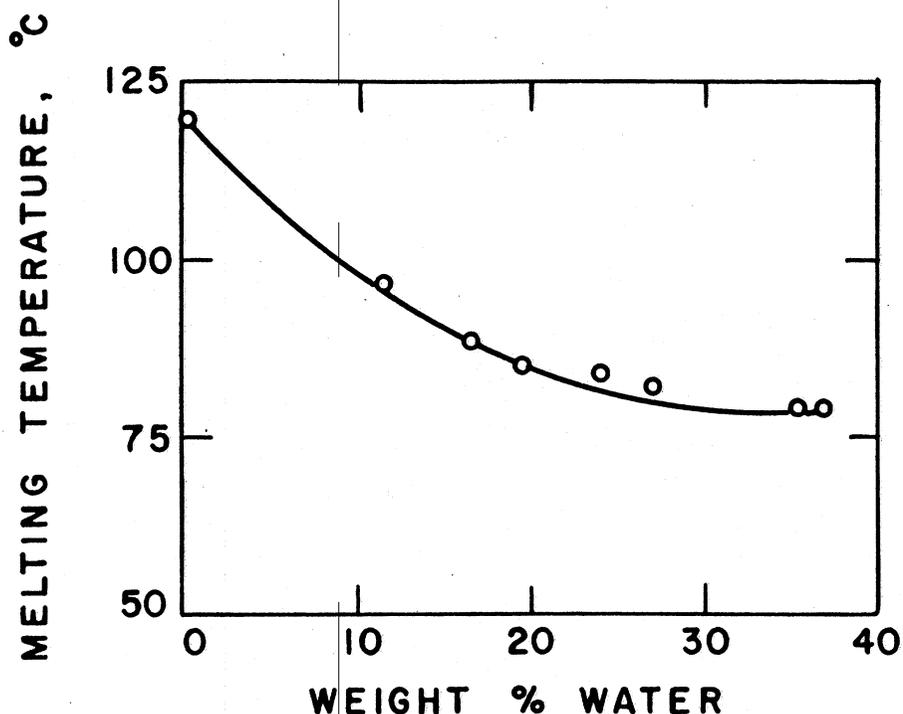


FIGURE 4.—Plot of apparent melting (shrinkage) temperature of vegetable-tanned hide vs. weight percent water.

Shown in Fig. 4 are the data obtained on vegetable-tanned samples of commercial upholstery leather. The shrinkage temperatures ranged from 97° C. with 11.5% water to 79° C. with 37% water. The shrinkage temperatures of vegetable-tanned hide specimens at low water contents are lower than those found for the unmodified hide-water system. For example, a tanned specimen containing 25% water was 13° lower than that of unmodified hide of similar water content. When specimens were immersed in water, these shrinkage temperatures were reversed: the vegetable-tanned specimens shrank 22° higher. The reason for this reversal might be that vegetable tannin itself could act as a diluent, probably because of a loose association of its polar groups with the polypeptide chains. This diluent action of

vegetable tannin was verified experimentally. It was found that vacuum-dried vegetable-tanned hide on heating underwent an unmistakable shrinkage at about 120° C. The shrinkage temperature was not nearly as well defined or reproducible as the other shrinkage measurements. It appeared to have occurred over a temperature range, indicating that in the tanned specimens used, the tanning agent was not distributed uniformly throughout the hide substance.

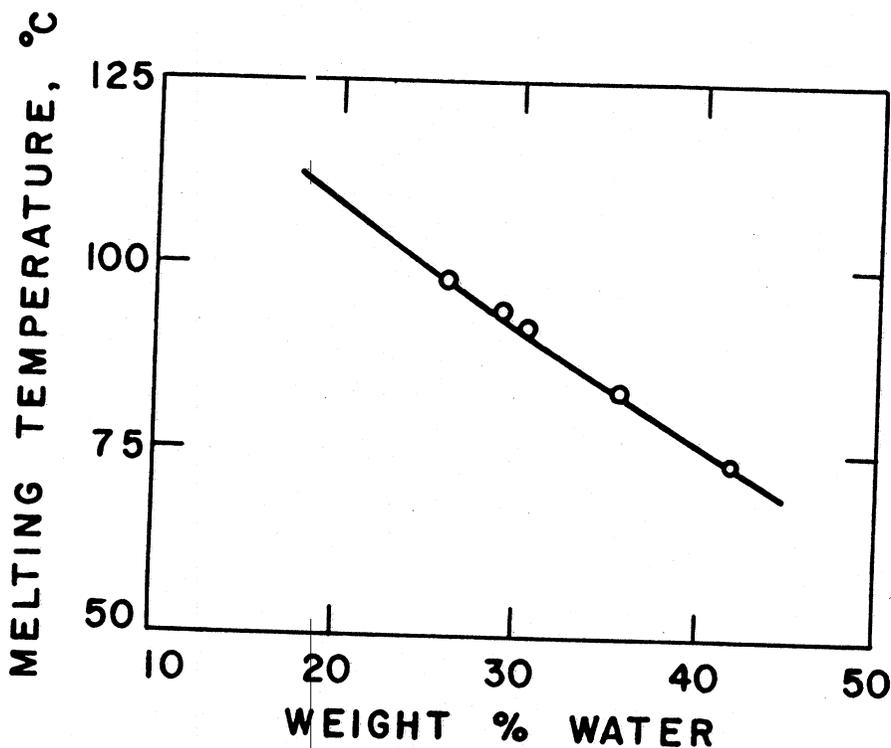


FIGURE 5.—Plot of apparent melting (shrinkage) temperature of formaldehyde-tanned hide vs. weight percent water.

Shown in Fig. 5 are similar data for formaldehyde-tanned hide. The melting temperatures ranged from 90° C. for a specimen containing 26% water to 73° for 40% water. When immersed in water, its shrinkage temperature was also 73° C. The effective water content of the immersed formaldehyde-tanned hide was about 23% less than that obtained with unmodified hide. Dry formaldehyde-tanned specimens of hide, when heated at temperatures as high as 170° C., showed no appreciable shrinkage. This behavior was quite unlike that of the vegetable-tanned samples. Apparently the mode of attachment of the formaldehyde to the polypeptide chains is different from that of vegetable tannin. This is not unexpected, as formalde-

hyde is known to form covalent cross links. Since such cross links are relatively short and fixed compared with those of a vegetable tannin, the formaldehyde cannot act as a diluent.

Figure 6 shows the variation in melting temperature with water content for various types of the new tanning agent, dialdehyde starch (10), that has been under investigation at our laboratories. Starches subjected to different degrees of oxidation were employed. In all cases the shrinkage temperature decreased with increased water content. Below 25% water content, the dialdehyde starch specimens all showed essentially the same shrinkage behavior; above 25%, the shrinkage temperature appeared to depend on the amount of oxidation and the type of medium that was employed to tan the hides. Further investigation is required to explain this behavior.

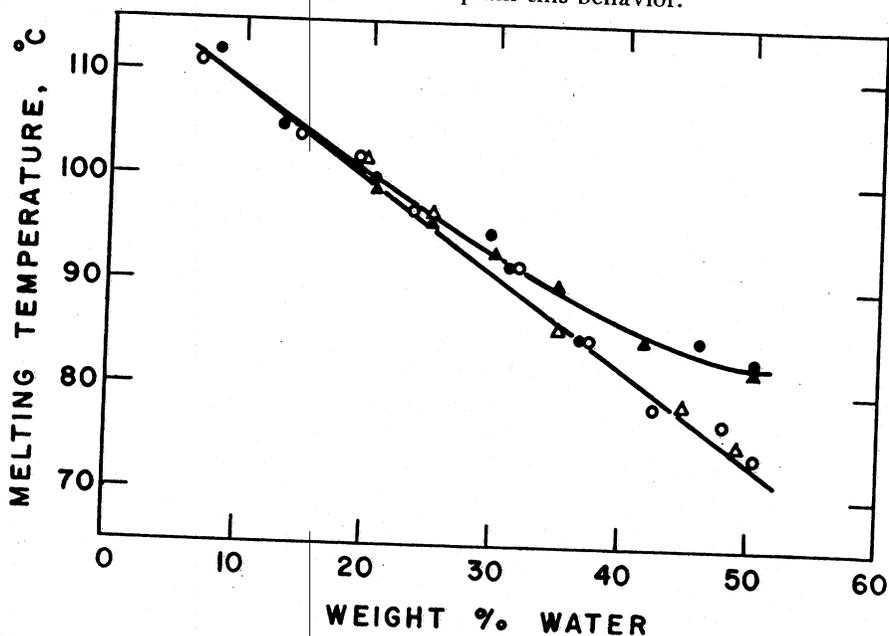


FIGURE 6.—Plot of apparent melting (shrinkage) temperature of hide tanned with 96% oxidized dialdehyde starch, mixed buffer (●); 96% oxidized dialdehyde starch, straight buffer (○); 66% oxidized dialdehyde, mixed buffer starch, (▲); and 33% oxidized dialdehyde starch, mixed buffer (△) vs. weight percent water.

#### CONCLUSION

The behavior of untanned hide substance when subjected to heat in the presence of small amounts of compatible diluents such as water, ethylene glycol, formamide, and phenol was shown to be analogous to the depression of the melting point of any crystalline substance by a diluent. The temperature at which shrinkage takes place is a fundamental characteristic of bovine

collagen but is dependent on previous treatment. Fresh hide, when heated in citrate buffer at pH 3.7, shrank at 40° C., only 4° C. higher than that observed by Doty *et al.* (12) for collagen helices dispersed in the same medium. This indicated that the macroscopic shrinkage of hide was an outward manifestation of the melting of the collagen helices.

The results obtained from tanned hide indicated that tanning agents reduce the amount of water that can reach the polypeptide chains, which results in an elevation of the commonly measured shrinkage temperature. Various types of tanning agents interact with the polypeptide chains in different ways, modifying to some extent the ultimate effect of the diluent on the shrinkage temperature. Although the discussion presented here has been restricted to the effects of tanning on the shrinkage temperature, the importance of its role in producing the mechanical and other properties desired in quality leather is recognized.

#### ACKNOWLEDGMENT

The authors wish to thank D. R. Killen for the melting-temperature measurements and Dr. J. Naghski and associates of this Laboratory for the hide and leather samples.

#### REFERENCES

1. Garrett, R. R., and Flory, P. J. *Nature*, **177**, 176 (1956).
2. Wohlisch, E. *Biochem. Z.*, **247**, 329 (1932).
3. Wright, B. A., and Wiederhorn, N.M. *J. Polymer Sci.*, **7**, 105 (1951).
4. Astbury, W. T. *J. Intern. Soc. Leather Trades' Chem.*, **24**, 69 (1940).
5. Theis, E. R. *Trans. Farad. Soc.*, **42B**, 244 (1946).
6. Kutyanin, G. I. *Kolloid. Zhur.*, **15**, 36 (1953).
7. Lennox, F. G., *Biochem. et Biophys. Acta*, **3**, 170 (1949).
8. a. Schmitt, F. O., Gross, J., and Highberger, J. H. *Proc. Natl. Acad. Sci., U.S.*, **39**, 459 (1953).  
b. Gross, J., Highberger, J. H., and Schmitt, F. O. *Ibid.*, **40**, 679 (1954).  
c. Boedtker, H., and Doty, P. *J. Amer. Chem. Soc.*, **78**, 4267 (1956).  
d. Pauling, L., and Corey, R. B. *Proc. Natl. Acad. Sci., U.S.*, **37**, 272 (1951).  
e. Rich, A., and Crick, F. H. C. *Nature*, **176**, 915 (1955).  
f. Ramachandran, G. N. *Ibid.*, **177**, 710 (1956).
9. Witnauer, L. P., and Fee, J. G. *J. Polymer Sci.*, **26**, 141 (1957).
10. Filachione, E. M., Harris, E. H., Fein, M. L., Korn, A. H., Naghski, J., and Wells, P. A. *JALCA*, **53**, 77 (1958).
11. Fee, J. G., Calhoun, R. R., and Witnauer, L. P. *JALCA*, **51**, 530 (1956).
12. Doty, P., and Nishihara, T. *Recent Advances in Gelatin and Glue Research* (London: Pergamon Press, 1957), p. 92.
13. McLaughlin, G. D., and Theis, E. R. *The Chemistry of Leather Manufacture* (New York: Reinhold Publishing Corporation, 1954).
14. Oth, J. F. M., Dumitru, E. I., Spurr, O. K., Jr., and Flory, P. J. *J. Amer. Chem. Soc.*, **79**, 3288 (1957).

Received January 2, 1959.

## DISCUSSION

PRESIDENT THORSTENSEN: I want to thank Dr. Witnauer for this very fine paper.

My first introduction to this subject was in 1946 when Dr. Theis gave a dissertation on swelling and shrinking before the Faraday Society in London. I never realized at that time what a terrific subject it was and what an honor it was for Dr. Theis to be asked to address that very august body.

Now, in order that we might clarify some of these things, I will ask Dr. Stubbings to come up here and lead the discussion.

DR. ROBERT STUBBINGS (Division of Leather Technology, Lehigh University): We would like to thank Dr. Witnauer for presenting this paper to us on shrinkage. It certainly is an area of shrinkage which I do not think many of us have considered—the area between fully soaked and dry hide.

I was particularly interested in the reduction of shrinkage for untanned specimens between the area of the wet state and the dry state. We had never thought about it much in our laboratory, and yet we knew that dry specimens certainly could be taken to extremely high temperatures, almost to decomposition, without shrinkage. This fills in an area of data, I think, and explains in a much more logical fashion the shrinkage phenomenon.

I would like to ask Dr. Witnauer, before we ask for general questions from the floor, how he feels about the rate process approach to shrinkage which was presented by the Bureau of Standards about five or six years ago by Dr. Weir. Would you like to comment on that?

DR. WITNAUER: All melting phenomena are rate processes. The rate at which melting takes place depends on the particular temperature employed. This is not only true for collagen or semicrystalline polymers; it is also characteristic of crystalline materials. The rate of melting becomes increasingly slower the further the temperature (selected) is below the normally observed melting point.

DR. STUBBINGS: Dr. Witnauer talked about single-intact helices being able to be separated by swelling phenomena and yet retain their helical structure without shrinkage. Dr. Highberger has done work in this field for several years. Will he comment? Can we get single helices?

DR. JOHN H. HIGHBERGER (United Shoe Machinery Corporation, Beverly, Massachusetts): The collagen structure is not a single helix. It is a triple helix. I don't know what the effect would be on what you are talking about. I wondered about that, myself. Does it have any effect?

DR. WITNAUER: No, the melting phenomenon described is generally applicable even if the exact nature of the melting process is unknown. Doty

measured the size, shape, and weight of collagen that was obtained by extraction of carp swim bladder tunics. It was found that the extract consisted of rigid rod-shaped particles or coils. On heating, these coils broke down into three separate molecules. Over-all molecular weight of the new particles formed was slightly greater than one-third that of the original particles. This indicated that the three particles that make up the single coil do not have the same amino acid composition.

DR. HIGHBERGER: I might add that after Doty had gone through that, it was also done with calfskin and codskin collagen.

The Gustavson rule is followed in this particular case, in that the hydroxyproline content of each of these collagens is different, the highest content being at the highest massive shrinkage temperature and the highest denaturation temperature, and the lowest content at the lowest massive shrinkage temperature and the lowest denaturation temperature of the soluble collagen.

There are two possibilities in the structure for the collagen molecule, which are called structures 1 and 2. In one of these, the hydroxyproline bonds are on the outside, and in the other case they are on the inside and take part in holding the three chains together.

Doty believes his work indicates that the latter case is the correct one, although the crystal structure people believe that the first possibility is correct.

DR. WITNAUER: Flory at Cornell University has recently shown that highly oriented rubber undergoes a shrinkage or melting which closely resembles the shrinkage of collagen. The temperature at which melting of the crystallites takes place was found to increase with the amount of tension that the chains have on them. Thus, the observed shrinkage temperature of intact hide substance might be expected to be slightly higher than the equilibrium melting temperature.

DR. HIGHBERGER: That is true, but I would like to point out that the amount of stretch possible in the collagen helix is very small, as you know.

DR. WITNAUER: I meant to imply that as laid down by nature in the fibril, it is under tension.

DR. STUBBINGS: This discussion in terms of the Doty three-helix model, of course, leads to some interesting questions for Dr. Witnauer and perhaps Dr. Highberger.

Following out this method that Dr. Witnauer has shown us this morning, in the region from soaking wet to perfectly dry specimens, it would seem that maybe they could get at some of this internal helix bonding as against bonding between helices by some ionic studies in this area.

Dr. Witnauer has used hydrogen bonding agents as his diluents to study the phenomenon. I am wondering if he has also used any ionic agents which

would tend to disrupt or mask ionic charges perhaps between helices, to see if the breakup then occurs at different temperatures.

DR. WITNAUER: No, we haven't studied ionic systems. I don't know whether you are referring to chrome tanning or not. If you are, our data on this system have not been reproducible. The temperatures required to produce shrinkage in chrome-tanned specimens were 100°C. and higher. At these temperatures bubbling was observed in our immersion medium, and we didn't know whether air or water or water vapor was being driven out of the specimens.

DR. STUBBINGS: I was not specifically referring to chrome tanning, although that too is an interesting phase. I was thinking of salt solutions which would tend to mask the ionic charges but not disrupt the hydrogen-bonded type of structure, to see if there was a difference between the two.

DR. WITNAUER: This is a good point. We have made no investigations along this line.

DR. J. R. KANAGY (National Bureau of Standards, Leather Section): If I understood you right, I believe your work indicates that when you tan with vegetable tanning material, you actually are starting to break down the structure which would be expected with a large molecule. It actually tends to separate the lattices.

Now, the postulation has always been made that in chrome tanning you get cross links. Does your theory still include the fact that you get cross-linking when you have chrome tanning?

DR. WITNAUER: No, vegetable tannins do not appear to break down structure. X-ray studies do not show any effect of the tannin on structure. The only feature that X-ray studies have demonstrated so far is that small molecules, like water, can get between the helices and produce a change in certain lattice dimensions. I don't know whether I have answered your question.

DR. KANAGY: Well, it appears to me from one of the curves you showed in vegetable tanning that when you extrapolated, you had a lower shrinkage temperature than if you extrapolated the curve for the hide.

DR. WITNAUER: Yes, this is due to the fact that vegetable tannin can act in a manner similar to that of water. In other words, the vegetable tannin itself acts as a diluent because of its loose association with the polypeptide chain, just as water or phenol does. Formaldehyde cannot.

DR. KANAGY: I thought possibly the large vegetable tannin molecules might get into the lattices and separate the polypeptide chains. That, of course,

would begin the shrinkage phenomenon, and for that reason you would get a lower shrinkage temperature for tanned hide than for the original hide.

DR. WITNAUER: We tried to take unmodified hide and introduce 10, 20, 30, 40, and 50% of vegetable tannin in it and then apply this theory to it. Because, if it acted as a diluent, then it must obey the theory of melting point depression, just as water did. We were unable to prepare suitable specimens.

DR. ROBERT M. LOLLAR (Tanners' Council Research Laboratory, University of Cincinnati): Did you consider using quebracho in acetone to get known levels of tannin, as Gustavson has done?

DR. WITNAUER: That is the procedure we tried to follow, but we were unsuccessful.

DR. HIGHBERGER: I would just like to point out that with the newer knowledge of collagen structure we know a great deal more than we ever did before about exactly where these cross links do take place in connection with chrome tanning and other types. They take place between groups of three chains and not, as postulated many times before, between individual chains.

DR. PETER R. BUECHLER (Rohm & Haas Company, Philadelphia, Pa.): You have expressed your results here on rate percent of diluent which is added. Have you tried calculating some of these diluents on a molar basis? Does one mole of one material lower the shrinkage temperature as much as one mole of another?

DR. WITNAUER: I did not go into that. If the reciprocal of the shrinkage temperature is plotted against volume fraction of diluent for unmodified hide and the curve extrapolated to zero concentration, the melting point of pure collagen should be obtained. The melting point should be independent of diluent. The same temperature was found for the four diluents investigated. In general the application of thermodynamic equilibrium to phase transitions in the hide systems was satisfactory.

DR. STUBBINGS: In concluding the discussion I would like to say that this approach to tanning by shrinkage of hide in the range from wet to dry might be an interesting way to get at things we have been arguing for years—differences between cross-linked tannage, and materials which we know don't cross-link but which we also know raise the shrinkage temperature in the normal accepted sense of shrinkage temperature.

This business of blocking the sites so that the diluent or solvent medium cannot reach the necessary places may be the answer to a lot of the tannages that have been very vaguely described in the past. I hope this work will be continued at the Eastern Regional Laboratories in this general area.

MR. SALAMATOV: I am interested in what would happen to the structure of the collagen when you remove water completely. That is, to have zero content of water in the molecule. Then electrolytic forces will be dead. Then where and how will the components of the collagen be held together without the electrolytic forces? Is it going to be an amorphous entity, or does it decompose, or what happens to the structure?

DR. WITNAUER: Specimens that were vacuum-dried (constant weight) and studied by X-ray defraction still showed a crystalline type of pattern. The hydrogen bonds in this system are so strong that decomposition takes place before melting. Many other simpler systems on heating will not melt if they are strongly hydrogen-bonded. They decompose.

---

*Reprinted from*  
THE JOURNAL of the AMERICAN LEATHER CHEMISTS ASSOCIATION  
Vol. LIV, No. 7, July, 1959  
*Copyright 1959*