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THE STABILITY OF LEATHER UNDER TROPICAL STORAGE CONDITIONS

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ABSTRACT

An investigation was conducted on salvaged boots returned from the Southwest Pacific after the war because they were found unfit for issue to troops when the packing cases were opened. In order to determine the cause of the degradation in storage, the Army Retan upper leather was examined by routine physical and chemical test methods. The leather was divided into six lots on the basis of its strength losses; these losses were found to be closely correlated to the quantities of alkali-soluble nitrogen present. The shrinkage temperature declined and the number of amino acids in the hydrolyzed aqueous and 2*N* hydrochloric acid extracts of the leather increased in the same rank order. Before hydrolysis the same extracts were found to be devoid of individual amino acids. The significance of these findings is discussed, particularly as they compare with the results of other workers on the "shelf-life" of combination-tanned bookbinding leather in the temperate zone and on accelerated aging tests under simulated tropical conditions.



INTRODUCTION

During World War II the storage under unfavorable climatic conditions of military items partially or wholly made of leather was unavoidable. Particularly in the Southwest Pacific area (SWP) thousands of pairs of boots in storage were found to be unfit for wear when the packing cases were opened. Not only were the uppers covered with mildew, but also some of the leather was so tender it could be torn apart by hand. This experience led the U. S. Army Quartermaster Corps to initiate studies of the nature of leather degradation and possible means of extending storageability (1-5). An investigation was also conducted on the upper leather of salvaged boots in order to learn more about the nature and causes of their failure.

Despite the many studies on the subject, our knowledge of what causes leather to deteriorate is still fragmentary. Although progress in this field is slow, every bit of information plays its part in advancing our understanding of it. The Quartermaster investigation on salvaged upper leather exemplifies a type of study begun for practical reasons that provides some information on the deterioration process.

EXPERIMENTAL

A shipment of about 200 pairs of boots in unopened cases was returned from the SWP for the purpose of this investigation. The upper leather removed from these boots was considered representative of the combination chrome-vegetable tannage known as Army Retan which was in use at the time. In the examination of this leather, two approaches were taken. In the first place, the usual physical and chemical tests were performed on the leather from each boot in order to rank it according to the seriousness of the damage. Secondly, an attempt was made to link the damage to a breakdown of the collagen by employing chromatographic techniques.

In the first phase of the study, the burst strength of the upper leather parts was taken as the basis for dividing them into six lots, as shown in Table I. A composite sample was then prepared from leather representing each lot for chemical analysis. The following determinations were performed according to Federal Specification KK-L-311 (6): stitch-tearing strength, shrinkage temperature, hide substance, chromic oxide, grease, and pH. For bursting strength, a 1/8" plunger in conjunction with a 3/8" orifice was used. Alkali-soluble nitrogen was extracted with 0.1*N* sodium carbonate (7).

In the second phase of this study, a chromatographic analysis was made of each lot of leather in an attempt to discover what relationship might exist between the degree of physical deterioration and the order of hydrolysis of amino acids. Duplicate samples of each of the six lots were extracted, one with water and the other with 2*N* hydrochloric acid solution. After hydrolysis, the extracted material was identified by means of paper chromatography using the following procedure:

One gram of leather was thoroughly moistened with 20 ml. of the extraction medium. After standing at room temperature for 16-20 hours, the leather was filtered and washed several times with fresh portions of the extraction medium. The extracts and washings were combined and brought to dryness on a steam bath, a process which required approximately 6 hours. The residues were dried overnight in a desiccator, dissolved in 2 ml. of a 10% aqueous solution of *n*-propylalcohol, and analyzed in triplicate by means of two-dimensional ascending chromatography, using 0.02 ml. of the total volume for each determination.

TABLE I
STRENGTH AND SHRINKAGE MEASUREMENTS ON
ARMY RETAN LEATHERS

Lot	Load lb.	Bursting* Strength lb./in.	Stitch-Tear* Strength lb./in.	Ts °C.
<i>Southwest Pacific Storage</i>				
1. (Strongest)	101	1140	75	85
2.	85	880	60	83
3.	67	690	49	77
4.	50	530	35	74
5.	39	400	20	74
6. (Weakest)	26	230	10	73
Average	61	640	42	78
<i>Tropical Chamber Storage of Ten Production Lots†</i>				
Before storage	112	1320	—	108
After 3 years	63	690	—	81
<i>Tropical Chamber Storage and Accelerated Aging Test of Five Production Lots (4)‡</i>				
Before storage	74	—	78	100**
After one year in chamber	61	—	65	97**
After 10 months at 35°C. and 100% rel. humidity in sealed jars	52	—	42	90**

*Each figure represents the mean value of at least 16 measurements.

†Each figure represents the mean value of at least 160 measurements.

‡Each figure represents the mean value of 60 measurements.

**In water.

The remainder of the alcoholic solution of the water-extracted residue was treated with 8 ml. of 6*N* hydrochloric acid, again brought to dryness on the steam bath, dried overnight in a desiccator, and dissolved as described before for a second chromatographic analysis.

All leather extractions were made in duplicate. Additional extractions with 2*N* hydrochloric acid on the two weakest lots were evaporated under vacuum at room temperature instead of being heated on the steam bath. The residues were dissolved in 2 ml. of a 10% solution of *n*-propylalcohol and analyzed chromatographically as follows.

Chromatographic analysis.—Two-dimensional ascending chromatographic analysis was run in an airtight cylindrical apparatus; 0.02 ml. of the alcoholic solution was applied to a piece of Whatman #1 filter paper (6½" x 6" or 12" x 12½"), ½" from the side of the paper and ¼" up from the

bottom. The paper was formed into a cylinder and placed in the chromatographic apparatus containing a butanol-acetic acid-water solvent (8). When the solvent had reached the top of the paper (in 3 to 4 hours for the small paper and overnight for the large one), the cylinder was removed and air-dried at room temperature. The two-dimensional chromatogram was made by rolling the paper at right angles to the first direction of flow, placing it in another cylindrical apparatus containing a 1-to-1 m-cresol-phenol mixture at pH 9.3 (9). After the second solvent came within $\frac{1}{2}$ " of the top of the paper (advancing at the same speed as the first), the cylinder was removed and allowed to stand at room temperature overnight.

The chromatograms were then treated with a 0.4% solution of ninhydrin* and developed at 100°C. for three minutes. The reaction areas were compared with those obtained by chromatographing a synthetic amino acid mixture and a hide powder hydrolyzate. In the case of the hide powder it was possible to identify all the known amino acids in collagen with the exception of isoleucine and hydroxylysine.

RESULTS

Physical properties.—The average strength properties and shrinkage temperatures of the six lots of SWP leather are shown in Table I. For comparison the table also includes results obtained in the tropical chamber of the QM Research and Engineering Center (10), as well as data extracted from the report of Lollar (4). The climatization chamber results are averages of ten production lots of Army Retan upper leather in the so-called USXL series (11), representing a desired chrome level of 6.5% on hide-substance basis, but with variations in grease and retan levels. The specimens were treated for mildew resistance by swabbing with p-nitrophenol before they were placed in the chamber. The figures taken from Lollar's report allow a direct comparison between storage of almost equal duration in a tropical chamber and in an accelerated laboratory test. All figures are mean values of measurements comprising specimens treated and not treated against mildew. Lollar showed that the fungicidal treatment had no effect on leather stability.

Chemical composition.—The chemical analysis of the SWP leather is presented in Table II. This table also contains unpublished figures on the chemical composition of the Army Retan leather in the tropical chamber test cited in Table I.

Chromatographic results.—The aqueous and the 2*N* hydrochloric acid extractions of newly tanned Army Retan leather contained only 0.17 and 0.48% respectively of its total nitrogen. After acid hydrolysis these extractions showed no ninhydrin reaction spots on their chromatograms.

*In normal butanol containing 4 g. glacial acetic acid/100 ml.

TABLE II
CHEMICAL COMPOSITION OF DETERIORATED
ARMY RETAN LEATHERS

Lot	Percent on Dry Basis			Alkali-soluble Nitrogen in % of Total Nitrogen	pH
	Hide Substance	Chromic Oxide	Grease		
<i>Southwest Pacific</i>					
Lot 1 (Strongest)	56.6	2.8	13.2	2.8	3.0
Lot 2	56.8	2.8	13.0	3.4	2.9
Lot 3	55.3	2.3	12.9	4.3	2.9
Lot 4	58.2	2.1	10.0	6.2	2.8
Lot 5	59.1	2.2	7.1	8.6	2.8
Lot 6 (Weakest)	57.3	2.6	5.3	11.1	2.9
<i>Tropical Chamber*</i>					
Before storage	49.6	3.1	30.1	—	3.0
After 3 years	—	—	27.6	—	3.1

*Leather was treated with paranitrophenol.

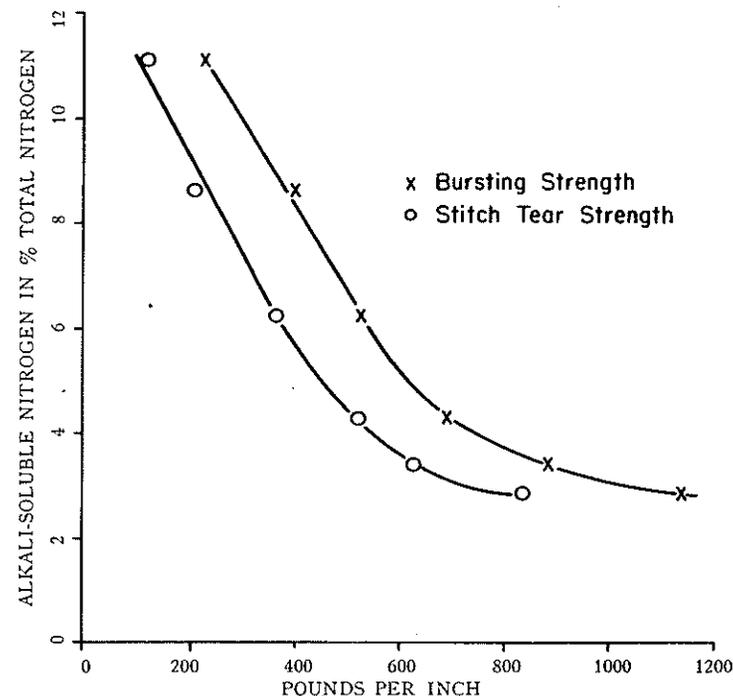


FIGURE 1.—Strength versus alkali-soluble nitrogen in Army Retan after S.W. Pacific storage.

The aqueous extract of the strongest SWP leathers contained about nine times as much soluble nitrogen as the freshly tanned Army Retan. As the strength losses increased, so did the soluble nitrogen. In the hydrochloric acid extracts, the soluble nitrogen rose more steeply from lot to lot than in the aqueous extracts, presenting a curve similar to that depicted in Fig. 1 for alkali-soluble nitrogen.

TABLE III
AMINO ACIDS IN THE HYDROLYZATES OF EXTRACTS
OBTAINED FROM DETERIORATED ARMY RETAN LEATHER

Amino Acid	Collagen, g/100 g (12)	Lot					
		1	2	3	4	5	6
Glycine	26.1	○	○	○	●	●	●
Alanine	10.1	○	○	○	●	●	●
Leucines	5.6		○	○	○	○	○
Valine	3.0				○	○	○
Proline	14.8			○	○	○	●
Hydroxyproline	12.4				○	○	●
Phenylalanine	2.9			○	○	●	●
Tyrosine	1.1					○	○
Arginine	8.9				○	○	○
Lysines	5.0					○	○
Aspartic Acid	6.6			●	●	●	●
Glutamic Acid	11.3			●	●	●	●
Frequency of amino acid identification							
(a) hydrochloric acid extractions		2	3	7	10	12	12
(b) aqueous extractions				2	4	6	7

○—amino acid found in hydrochloric acid extracts

●—amino acid found in aqueous and hydrochloric acid extracts

Chromatograms obtained from these extractions showed only negative ninhydrin reactions. Evidently the extracted nitrogenous constituents still are of high molecular weight. Negative ninhydrin reactions were obtained even on those acidic extracts which, as noted above, were evaporated to dryness under vacuum before being tested chromatographically. This observation is comparable to that of Kanagy that alkali-soluble nitrogen fractions of deteriorated leather could still be precipitated by phosphotungstic acid or tannin (7).

After hydrolysis, the aqueous extracts of the SWP leather showed strong, positive ninhydrin chromatograms, the number of amino acids identified increasing as the strength of the lots decreased. The hydrolyzed hydrochloric

acid extractions showed the same trend beginning with the strongest lot. The amino acids identified in these hydrolyzates are shown in Table III, arranged in four groups of which the first consists of the nonpolar amino acids; the second, the aromatic ring compounds; the third, the two basic amino acids; and the fourth, the two acidic amino acids.

Of the 12 amino acids found, seven appeared in the hydrolyzates of the water extracts and all 12 in the hydrolyzates of the hydrochloric acid extractions. Three amino acids (serine, threonine, and methionine) could not be identified in any hydrolyzate. The presence of histidine in the hydrolyzate of the acid extraction of the three weakest lots was doubtful; therefore it was not included in the table. In no case, however, did the hydrolyzate of the aqueous extractions contain an amino acid that had not been identified in the hydrolyzates of the hydrochloric acid extractions. The arrangement of Table III not only shows at a glance which amino acids were present in the hydrolyzates of the different lots, but also the frequency of their occurrence.

DISCUSSION

Physical properties.—The figures of Table I give a measure of the breakdown of SWP leather, compared with aging effects observed on Army Retan in the tropical QM climatization chamber and in an accelerated aging test under hot-humid conditions. In all three instances strength and shrinkage resistance were drastically lowered. The average strength of the SWP leather is approximately equal to that of ten production lots after storage for 3 years in the tropical chamber. However, the weakest lots of the SWP leathers rank far below any of the leathers aged artificially. The strongest lot, on the other hand, is about as strong as new leather, but its T_s is well below normal for Army Retan. The initial strength of Lollar's five production lots is low for new Army Retan, and after accelerated aging, it is of the same magnitude as the strength of the SWP leather and of the USXL lots after 3 years of tropical storage. The T_s decrease in sealed jars is rather small, indicating that chemically the breakdown is not the same in the sealed jars as in the tropical chamber or in the SWP.

Chemical composition.—The analyses figures presented in Table II show that alkali-soluble nitrogen rises sharply from Lot 1 to Lot 6 as the strength decreases. The grease content is low in all lots, as compared to new leather, and gradually decreases further in the weaker lots. The other chemical properties shown, hide substance, chrome, and pH, do not seem to be related to the ranking by strength.

Grease.—A minimum of 18% grease was required in the specification BQD No. 132, valid at the time when the shoes were manufactured. A review of test reports indicated that leather generally met this requirement; therefore

it is apparent that much of the grease has been lost, probably because of the growth of mildew. The very severe grease losses of the weaker lots certainly are caused by mildew. The role of grease in leather as a nutrient for fungi has been clarified in earlier studies (4, 13). They also showed that grease losses caused by mildew are not responsible for the strength losses which occur with treated and untreated specimens alike (4).

Alkali-soluble nitrogen.—A relationship between strength losses and alkali-soluble nitrogen has been postulated in the past by workers who studied the deterioration of bookbinding leather (14, 15). When vegetable leather was degraded by the absorption of mineral acid, it was possible to obtain a fairly good correlation between strength losses and extractable nitrogen. Combinations of vegetable and mineral tannages substantially reduced the quantities of alkali-soluble nitrogen formed on aging (16). However, no correlation could be established for many leathers after shelf exposure over periods of 12 to 18 years, as less than 0.5% of the total nitrogen was soluble, whereas strength losses ranged from 6 to 70%. On the other hand, the SWP leather in Table II showed between 2.8 and 11% alkali-soluble nitrogen. In Fig. 1 alkali-soluble nitrogen is plotted against strength in order to illustrate the close relationship between them. For the weaker lots the relationship appears nearly linear, but the two strongest lots, although about as strong as Army Retan leather is expected to be, still yielded substantial quantities of alkali-soluble nitrogen. The results indicated, therefore, that deterioration by exposure to a hot humid environment is fundamentally different from shelf exposure in the temperate zone.

Chromic oxide.—The six lots into which the SWP leathers were separated show different chrome levels but no trend with decreasing strength. This finding is in agreement with results of other studies. For example, it has been reported (10) that the rate of strength losses in the tropical chamber is not influenced by variations in the chrome level of Army Retan. Nor is this the case in the other climatization chambers.

Hide substance.—The values for hide substance in Table II include "alkali-soluble nitrogen" and, therefore, vary by only a few percent from one lot to another. Not shown in Table II are constituents comprising the chrome complex, the vegetable tannin and the water-soluble fraction which for each group was about 0.5% on the dry basis.

pH (acidity).—The pH of the strongest lot of SWP leather and the initial pH of the USXL leathers shown in Table II were the same. The slightly lower pH values of the other SWP lots may indicate that this leather originally had a lower pH and, therefore, lost relatively more strength in storage, or that it supported a more prolific growth of mold. The latter is the more probable interpretation. Mildew is known to cause a rise in pH

of leather (17). In the tropical chamber where the leather is aged without being attacked by mildew, the pH actually rose slightly from 3.0 to 3.1.

For the purpose of securing more information on this point, the pH figures in Lollar's paper (4) were averaged separately for untreated and treated leathers, before and after his aging tests. Under Lollar's experimental conditions the changes in pH were as follows:

(a) in the QM tropical chamber	
in the untreated leather	+ 0.06
in treated leather	+ 0.08
(b) in the accelerated aging test	
in untreated leather	- 0.28 (mold grew profusely)
in treated leather	- 0.16

Here, too, the pH rose in the QM climatization chamber, while it dropped in the laboratory test; this decrease, moreover, was almost twice as great with untreated than with treated leather. In the tropical chamber the pH increase of treated and untreated leathers was about the same; this could be expected, since Lollar remarks that he did not find mold growing on many specimens. The drop in pH of treated leather while being aged in the laboratory was confirmed by Roddy and Jansing (5). Although their test extended only over 16 weeks, the decreases were about the same as found by Lollar.

One characteristic of accelerated aging tests which may be of importance in connection with pH changes during aging is that in sealed jars the atmosphere is stagnant, while in the chambers fresh air constantly circulates around the leather, carrying off any gases and volatile acids formed during aging. Army Retan containing vegetable tannins most certainly gives off some carbon dioxide, as straight vegetable leather does, according to Kanagy (18). Moreover, masking compounds like formic acid, if present, may gradually leave the chrome complex and escape from the surface of the leather. Both reactions would result in a trend toward higher pH values during prolonged storage of mildew-resistant leather in climatization chambers in agreement with the actual observations.

Chromatographic analysis.—The chromatographic procedures used on the SWP leathers represent an attempt to augment other results on the breakdown of these leathers and, within this limited scope, produced some interesting data, although of a purely qualitative nature. These results are shown in Table III.

The identification of the amino acids listed in this table was not hampered by streaking or lack of separation, as may have been expected because of the improvisations used in obtaining the hydrolyzates. In this respect, the technique may, therefore, be considered an improvement over the *modus*

operandi used recently by Bjorksten and Gottlieb in a somewhat related field (19). These authors, partially hydrolyzing chrome- and formaldehyde-tanned gelatin, could separate 12 or more amino acids by chromatographic analysis. Indirect evidence concerning the degree of hydrolysis reached in the chromatographic work on SWP leather may be cited from a paper by Greenberg and Burk (20). With gelatin, according to the authors, the rate of hydrolysis, in presence of 3*N* hydrochloric acid, is so rapid that 84.5% are hydrolyzed after 5 hours at 96°-98°C., e.g., under conditions approaching those in the chromatographic study of the SWP leathers. It is not unlikely that the aqueous and hydrochloric acid extractions of these leathers would be hydrolyzed even faster and more completely than gelatin itself and that, for this reason, excellent separation was obtained in all chromatograms.

As Table III shows, the number of amino acids appearing in the hydrolyzed aqueous and hydrochloric acid extractions closely follows the degree of deterioration. Once an amino acid was identified in the hydrolyzates from one of the six lots, it was also picked up in the extractions from any of the lots ranking lower in strength.

The strongest lot yielded, upon extracting with hydrochloric acid and hydrolysis, only the two most common nonpolar amino acids, glycine and alanine. The leucines appear in the second strongest lot. In the third lot proline and phenylalanine appear for the first time in the hydrochloric acid fraction, and aspartic and glutamic acids in both the aqueous and hydrochloric acid extractions. These two dicarboxylic acids are the only ones appearing simultaneously in both fractions. In Lot 4, three more amino acids, valine, hydroxyproline, and arginine, appear in the acid extraction; glycine and alanine make their first appearance in the aqueous extract. In Lot 5 tyrosine and the lysines completed the identification of the amino acids in the hydrolyzates. Lots 5 and 6 differed in that hydroxyproline was extracted only by hydrochloric acid extract from the fifth lot but could be extracted by both water and hydrochloric acid from the weakest lot.

A question still to be answered is why serine and threonine sequences were absent from the SWP leather extracts. In experiments on heat degradation of collagen Cassel (21) found that these two acids were among those most susceptible to oxidation. In the SWP leathers, perhaps the serine and threonine sequences in the water and hydrochloric acid-soluble fractions also degraded and, therefore, remained unidentified.

In this connection it is of interest that internal cross-linking through hydrogen bonds is, according to Gustavson, unfavorable to the fixation of vegetable tannins (22). In proteins like collagen that are rich in hydroxyamino acids, the OH-side chains form hydrogen bonds with other side chains containing -COO^- and ≡NH^+ groups (23). In the relatively rigid structure thus obtained, fewer sites are available for interaction with neutral molecules, such as vegetable tannins. It may well be that this non-participation

of serine and threonine in the binding of vegetable tannins is another reason why they do not appear in polypeptide fragments of deteriorated SWP leather.

Whichever explanation of their absence is correct, the usefulness of chromatography for investigating the breakdown of leather has been demonstrated in this preliminary study. Work on a larger scale is needed in order to develop more satisfying theories about the breakdown of leather stored and aged in various climates.

CONCLUSION

The degree of deterioration of Army Retan upper leather in SWP has been measured by various physical and chemical means. The leather was divided into six lots on the basis of declining strength measurements. It was observed that T_s decreased and alkali-soluble nitrogen increased as the strength of the leathers decreased. A fair correlation between physical breakdown and the number of amino acids in soluble polypeptide fragments was also found. This phenomenon of physical and chemical deterioration remaining parallel to each other has not as yet been matched fully by accelerated aging tests simulating a tropical climate on the same type of leather. Nor do combination-tanned bookbinding leathers after 12 to 18 years of shelf exposure in the temperate zone show a similar correlation between strength losses and soluble nitrogen.

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