

12

Bulletin of
The Central Leather
Research Institute
Madras

152.
354



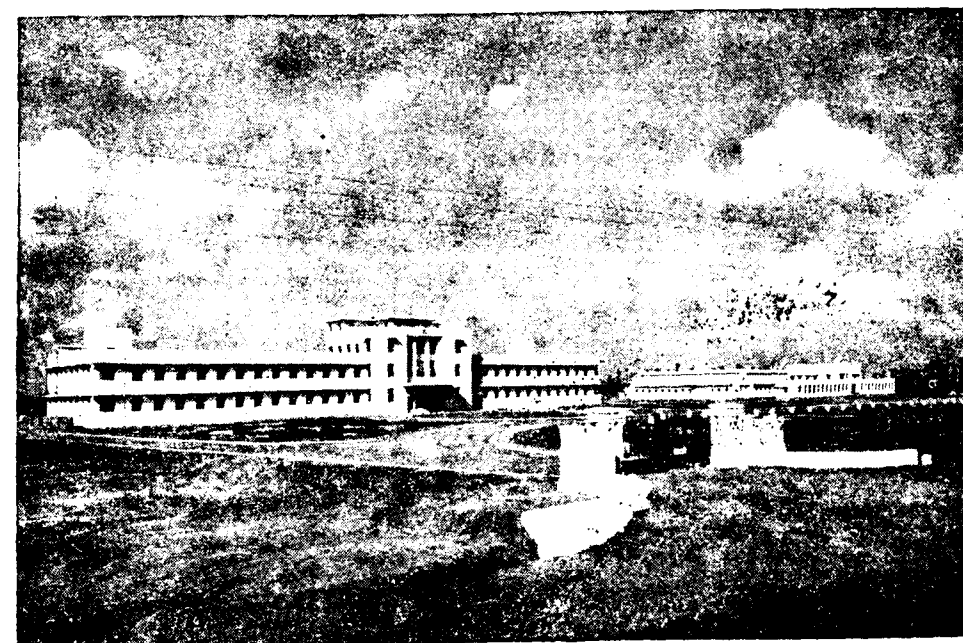
VOL. IV

APRIL, 1958

MADRAS

No. 9

329



COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH
INDIA

Bulletin of The Central Leather Research Institute

Vol. IV

April, 1958

No. 9

CONTENTS

N-Terminal Amino Acids of the Constituent Proteins of Hides and Skins	
—K. Thomas Joseph & S. M. Bose	.. 343
Research Bears Fruit—Practical Demonstration No. 1 (Second Series)—Manufacture of Chrome Retan Heavy Upper Leathers	
—N. R. Sikdar & Y. Nayudamma	.. 361
Technical Seminar—Studies on certain Proteolytic and Amylolytic Enzymes in Leather Manufacture	
—W. Madhava Krishna	.. 367
Classified Abstracts	.. 369
Notes & News	.. 383
Visitors	.. 389

Editorial Board :

Dr. Y. NAYUDAMMA (*Editor*)
Dr. S. K. BARAT
Dr. S. N. SEN
Mr. M. A. GHANI
Mr. N. SUBRAMANIAN (*Secretary*)

Annual Subscription.—Rs. 9/- (inland); Rs. 18/- (foreign); Single Copy 75 nP.

The Bulletin of the Central Leather Research Institute is issued monthly.

Communications regarding contributions for publication in the Bulletin, books for review, subscriptions and advertisements should be made to the Secretary of the Editorial Board, C. L. R. I., Madras-20.

All remittances by cheque or money order should be made payable to the Director, Central Leather Research Institute.

**Bulletin of the
Central Leather Research Institute
MADRAS**

SCALE OF CHARGES FOR ADVERTISEMENTS

Screen 125

Position	Type Area	One insertion	Six insertions	Twelve insertions
Full Page ..	7½" x 4½"	Rs. 60	Rs. 320	Rs. 600
Half Page ..	3½" x 4½"	Rs. 36	Rs. 180	Rs. 320
Second and Third Cover } ..		Rs. 70		
Pages				
Fourth Cover ..		Rs. 80		

*Communications should be addressed to The Secretary,
Editorial Board.*

**CENTRAL LEATHER RESEARCH INSTITUTE,
ADYAR, MADRAS-20.**

**N-TERMINAL AMINO ACIDS OF THE CONSTITUENT
PROTEINS OF HIDES AND SKINS**

BY

K. THOMAS JOSEPH AND S. M. BOSE

Central Leather Research Institute, Madras

Received on 4th February 1958

SUMMARY

Important protein constituents of skins and hides (viz. collagen, procollagen, elastin, reticulin, myosin, albumin, globulin and mucoids) have been individually prepared; each protein was analysed quantitatively for the N-terminal amino acids by the DNP technique. For separation, identification and quantitative estimation of the different DNP amino acids, a two dimensional paper chromatographic technique has been developed. The results obtained by applying this technique, regarding the N-terminal amino acids of the different proteins are critically discussed.

In recent years a good deal of work has been done on the amino end group determination of proteins. The N-terminal amino acid has now become an important point of reference for the characterisation of various proteins. The elegant method of Sanger¹ which depends on the reaction of proteins with flourodinitro benzene (FDNB) resulting in the formation of non-hydrolysable dinitro phenyl derivatives of the N-terminal amino acids has been widely used for this purpose. This method has been mostly applied to the soluble corpuscular proteins but in recent years the amino end groups of a few of the insoluble proteins also have been studied by this method. In wool keratin a comparatively large number of terminal amino acids were detected²⁻⁷ suggesting that the molecule consisted of a relatively large number of polypeptide chains. In myosin and tropomyosin, Bailey⁸ could detect only insignificant amounts of

FOR BETTER LEATHER

... and Improved Processing

Dependable Rohm & Haas chemicals, which improve not only the processes of leather-making, but also the quality of the finished leather, include:

Lutron — The original and standard synthetic bate

Orotan N — Synthetic tan assist responsible for popular acceptance of white leather

Orotan TV — Synthetic tanning material to increase efficiency and speed of natural tanning

Primal — Resin dispersions giving outstanding flexibility in leather finishes

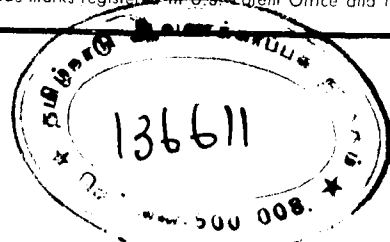
Rohm & Haas leather chemicals are recommended and sold by Amritlal & Company Ltd., 11 Sprott Road, Ballard Estate, P. O. Box 256, Bombay 1.



**ROHM & HAAS COMPANY
PHILADELPHIA**

Washington Square, Philadelphia 5, Pa., U.S.A.
Representatives in principal foreign countries

LUTRON, OROTAN and PRIMAL are trade-marks registered in U.S. Patent Office and in other principal countries.



terminal amino groups and he therefore suggested the possibility of a cyclic structure for these two proteins. Bowes and Moss⁹ and Grassmann and Hörmann¹⁰ have attempted to apply Sanger's method to the determination of the N-terminal amino acids of collagen. Neither group was able to detect any N-terminal residues in native collagen whereas both groups could detect small amounts of several N-terminal amino acids in gelatin. Partridge and Davis¹¹ determined the N-terminal amino acid residues of purified elastin using the same method. About 0.29 moles of amino acid residues were found per 100,000 gms. of elastin. This quantity was composed of smaller amounts of several amino acids.

In the past few years there has been considerable revival of interest in the Biochemistry and Physical Chemistry of the collagen group of proteins. Collagen has now been shown to exist at least in three different forms. One form known as acid soluble collagen can be extracted with dilute acetic acid¹²⁻¹⁴ or by acid buffers.¹⁵ The second form known as 'alkali soluble collagen' or neutral salt soluble collagen was first isolated by Highberger, Gross and Schmitt¹⁶ from skin by extraction with slightly alkaline phosphate buffers but more recently with neutral salt solution.¹⁷⁻¹⁹ These soluble collagens are generally considered as soluble precursors of collagen and hence termed as pro-collagen. The N-terminal amino acids of the acid soluble collagen have been studied recently by Bowes, Elliot and Moss²⁰⁻²¹ but no work has been done on the N-terminal amino acids of neutral salt soluble collagen. No quantitative assay of the N-terminal amino acids of reticulin, albumin, globulin or mucoids prepared from skins and hides has been done yet. In view of the recent controversy²²⁻²³ that reticulin is morphologically identical with collagen, a comparative study of the N-terminal amino acids of these two proteins was of special interest. Burley and de Kock²⁴ in a very recent paper have also reported that there is a difference between

normal and copper deficient wool, in the concentrations of several of the N-terminal amino acids residues. The nutritional status of the Indian sheep is generally considered to be low and in view of the above findings, an investigation of the N-terminal acids of Indian sheep wool was considered to be of great interest. A systematic investigation was therefore, undertaken on the preparation of individual proteins of skins and hides and on the quantitative determination of N-terminal amino acids of each protein.

EXPERIMENTAL

I. Preparation of proteins.

In the present investigation, the individual skin proteins were prepared as far as possible with mild treatments. Treatment with enzymes for the removal of unwanted proteins was avoided owing to the risk of any modification or degradation of the protein to be prepared.

Collagen was prepared by a modification of the method of Bowes and Kenten.²⁵ The back area of a freshly flayed buff-hide was cut out, washed and then cut into 1 cm. cubes. The grain layer containing the hair roots and greater part of the muscle and elastic fibres and a thin layer from the flesh side were split off by a razor blade and the remaining material was kept over-night in 10% sodium chloride solution. It was then washed with water and extracted with 0.15 M. disodium phosphate solution for another day. These two treatments removed most of the albumins, globulins and other connective tissue and possibly collagen precursors. The cubes were then dehydrated in acetone and subsequently degreased with three changes of light petroleum (b. p.—40-60°) at room temperature and then powdered in a Wiley mill.

Citrate soluble procollagen was prepared from a fresh calf skin by the method of Orekhovich *et al.*²⁶ as reported by Bowes, Elliot and Moss.²⁰

Soluble rat tail collagen was prepared by extracting freshly excised tail tendon of albino rats with very dilute acetic acid.²⁷

Neutral salt soluble collagen was prepared by the method of Jackson¹⁹ by extracting a minced rabbit skin with 0.2M. NaCl (pH 7.4) and precipitating by the addition of sodium chloride to a final concentration of 20% (w/v). The product was purified by the method described earlier by Jackson and Fessler.¹⁸

Elastin was prepared from cattle *ligamentum nuchae* which contains about 80% elastin, the remaining being mostly collagen. Conventional procedure for its purification involves autoclaving of the material in water at 120°C for a few hours for the removal of collagen. Hall²⁸ reported that even after such treatments the tissues contained an appreciable amount of amorphous material staining as collagen. It is obvious, therefore, that degradation might occur during purification. As collagen was already reported^{9, 10} to have no N-terminal amino acids, it was thought that for the study of the N-terminal amino acids of elastin the presence of small amounts of collagen would lead to less error than the treatments necessary to remove them. Elastin was therefore prepared as follows:

The ligamentum nuchae was isolated from the neck of an ox and was obtained fresh from slaughter house. The material was freed of any extraneous tissue and cut into thin strips. It was then extracted with 10% NaCl solution, and 0.15 M. Na₂ HPO₄ (each extraction 24 hrs.) and finally with half saturated lime water (1 hr.). It was then thoroughly washed in distilled water, dehydrated in acetone and powdered in a Wiley mill.

The lymph nodes,²⁹ adipose tissue,^{30, 31} spleen of rats³² and the mid cortical zone of human kidney³³ are reported to be rich in reticular tissues. Reticulin was therefore prepared from some of these sources.

Reticulin from lymph node and adipose tissue. The method used was that of Bowes and Kenten.³⁴ The tissues were also given an additional treatment with 0.15M. Na₂ HPO₄ to remove the glycoproteins. Reticulin from rat spleen was prepared by the method of Fell as reported by Ottaway.³²

Wool. The tips were removed and wool was washed in several changes of water and then dried in a forced air draught at 40°C. The dried wool was then extracted with ether for 24 hours followed by a similar extraction with ethanol and dried at room temperature. The moisture content was determined by drying a sample for 24 hours at 103°C.

Myosin. This was prepared by the method of Hasselbach and Schneider³⁵ from rabbit muscle. The protein was further purified by the method of Tsao,³⁶ the 39-50% ammonium sulphate fraction being used.

Albumin, Globulin and Mucoids. These proteins were prepared from a fresh goat skin by the method reported by Bose *et al.*³⁷

Modification of collagen. Extraction of tissues with calcium chloride solution and aqueous formamide have been reported^{38, 39} to remove most of the mucopolysaccharides. Powdered collagen was therefore immersed in a 10% (w/v) CaCl₂ solution adjusted to pH 10.5 with calcium hydroxide. The mixture was heated to 37° for one hour and finally filtered, washed and dried.

II. Dinitro phenylation.

Collagen, elastin, myosin and reticulin were dinitro phenylated by the original method of Sanger;¹ the time of treatment of these proteins was usually five days. After the reaction the protein was thoroughly washed with water and Soxhlet extracted with ethanol for twelve hours.

Wool was dinitrophenylated by the method of Fritze and Zahn.^{40, 6} Wool samples (1 g.) were suspended in 100 ml. of 1.6% sodium-bicarbonate solution and after bringing to 60°C, FDNB (0.35 ml., 50% on weight of the wool) was added and the mixture vigorously stirred. The reaction was allowed to proceed for 7 hours with change of the reaction medium after 2½ and 5 hours. After the reaction the wool was washed and Soxhlet extracted for 6 hours with acetone. The soluble collagens were dinitrophenylated by the method reported by Sanger.¹ Albumin, globulin and mucoids were dinitrophenylated as follows: The proteins were dissolved or suspended in 1.5% sodium bicarbonate solution (1 vol.) and 2 volumes of 2.5% dinitrofluorobenzene in ethanol were added and shaken. The time of the reaction was 2 hours for albumin, 5 hours for globulin and 12 hours for mucoids. The reaction mixture was then brought to pH 2 with dilute hydrochloric acid and extracted with peroxide free ether. The insoluble DNP protein was washed with distilled water, anhydrous ethanol and peroxide free ether.

The amount of protein in each of the DNP protein was determined from the known amino acid composition of the protein assuming that the lysine, tyrosine, histidine and terminal residues have been fully substituted.⁶

III. Hydrolysis of DNP proteins.

Since the breakdown of DNP amino acids on acid hydrolysis is also a function of time, any quantitative study of end group values requires selection of that duration of hydrolysis which gives a maximum for completion of hydrolysis and a minimum for degradation of the N-terminal amino acids to be determined. Partridge and Davis¹¹ found that best results were obtained by hydrolysing DNP protein with 11.4 N hydrochloric acid for 2 hours. In the present study two methods of hydrolysis were used. In the first the DNP protein (0.5 g.) was hydrolysed with 12 N hydro-

chloric acid (50 ml.) under gentle reflux for 3 hours. In the second method the DNP protein (0.5 g.) was hydrolysed completely by heating in a sealed tube at 105°C for 18 hours with 5.7 N. hydrochloric acid (50 ml.). The first method was adopted in the case of collagen, elastin, reticulin, myosin and the globular proteins and the second method in the case of wool. After hydrolysis the reaction mixture was cooled and transferred to a separating funnel with the washings. It was then extracted with 4 × 25 ml. of ether and the combined extracts were washed with 4 × 5 ml. of distilled water each containing one drop of 5.7 N. hydrochloric acid. The ether was evaporated and the residue taken up with acetone and evaporated again to remove traces of water. The dinitrophenols present were then removed by the cold finger sublimation technique of Mills.⁴¹ The aqueous solution after the extraction of the ether soluble DNP amino acids would contain the water soluble DNP amino acids, namely, *o*-DNP- tyrosine, ϵ -DNP- lysine, im- DNP- histidine and DNP- arginine if any present. They were subsequently extracted into normal butanol⁴² and were reserved for chromatography.

IV. Separation and identification of DNP- amino acids.

Different paper chromatographic systems have been proposed for the separation of DNP amino acids but most of them are not entirely satisfactory because they achieve only a partial separation of the DNP amino acids. Levy,⁴³ however, found that all the ether soluble DNP amino acids could be separated by a two dimensional system on a single piece of paper.

After trying a large number of solvents commonly used in chromatography in various combinations it has now been found that satisfactory separation of DNP amino acids can be achieved by using three different solvent systems. For the first dimension the organic phase of a mixture of tertiary butyl alcohol and benzyl alcohol (1 : 1) saturated with an

equal volume of phthalate buffer (pH 6) was used. Whatman No. 1 paper (22.5" × 18.25" was used). The paper was uniformly sprayed with the pH 6 phthalate buffer (50 ml. of 0.1 molar potassium hydrogen phthalate plus 45.45 ml. of 0.1 N sodium hydroxide) and dried in a current of air.

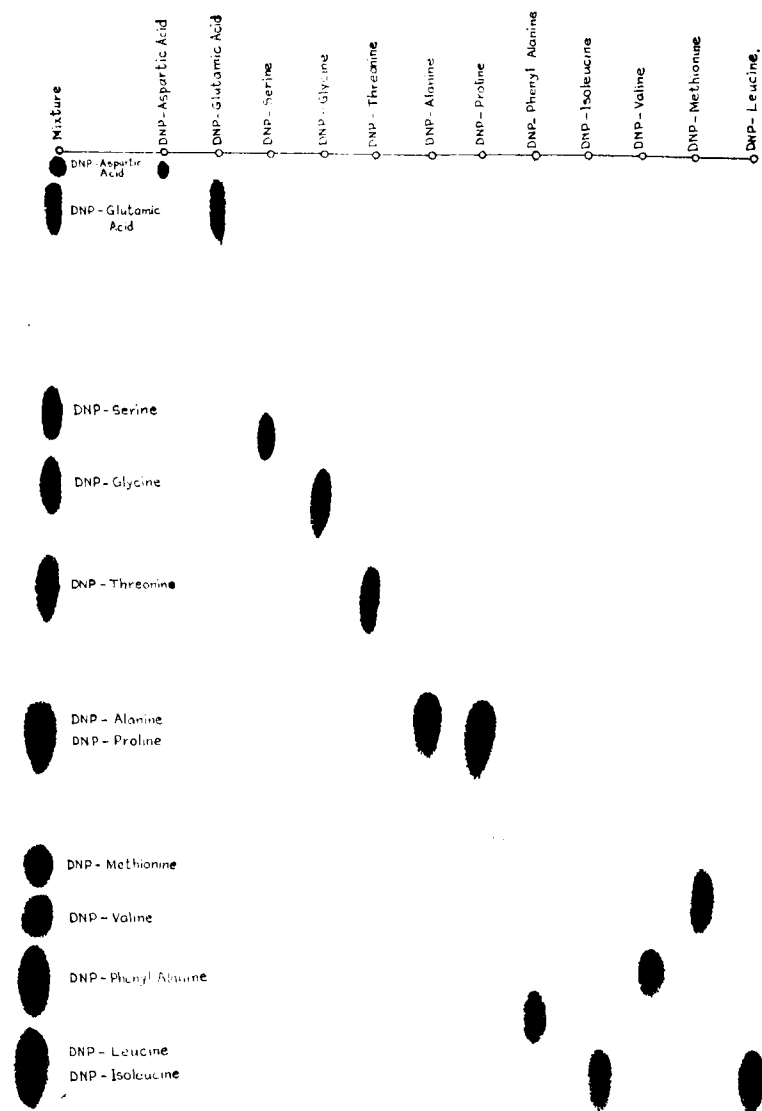


Fig. 1. Single dimensional chromatogram of a synthetic mixture of DNP Amino acids in the system n-butanol-isoamyl alcohol-ethanol-phthalate buffer. Time of running 40 hours.

The DNP amino acids were dissolved in acetone and applied to the paper by means of a micropipette. The chromatogram was then allowed to run overnight (18 hours) with the above solvent mixture, the aqueous phase being used to saturate the chamber. The paper was then removed, dried completely and then run in the second dimension with the upper phase of a mixture of normal butanol, isoamyl alcohol (2 : 1) plus 10% (v/v) of ethanol saturated with an equal volume of pH 6 phthalate buffer. As the DNP amino acids moved rather slowly in this solvent system better results could be obtained by allowing the solvents to run off the paper. At 30-32°C running for about 40-42 hours was found to give very good results. Using this solvent mixture in single dimension in the above manner about ten DNP amino acids could be separated as shown in Fig. 1. Alternately 1.5 Molar phosphate buffer (pH 6)⁴³ could also be used in the second dimension. By using these solvent systems almost all the ether soluble DNP amino acids could be separated. The two new solvent systems mentioned above have the general advantage over that of Levy's in that they are less unpleasant to handle. Further no equilibration with the solvent system prior to the running of the chromatogram with the solvents was necessary. The DNP derivatives of the various amino acids were prepared by methods reviewed by Fraenkel-Conrat, Harris and Levy.⁴⁴

V. Estimation of DNP amino acids.

For the quantitative estimation of the DNP amino acids the method described by Fraenkel-Conrat, Harris and Levy (*loc. cit*) was followed. The end group spots after separation were cut out and eluted with 4 ml. of 1% sodium bicarbonate solution at 55—60° C; the optical density at 360 m μ was taken in a Beckmann DU-model spectrophotometer against the extract of a blank portion of the paper. The amount of DNP amino acids was calculated by the method of Shore⁴⁵ assuming the molar extinction coefficients of DNP amino acids at 360 m μ to be 17,000.

As the DNP derivatives of proline and hydroxy proline are highly unstable under the conditions of hydrolysis adopted, the presence of these amino acids as terminal residue could not be established by the above method. The spectral method of Phyllips⁴⁶ was therefore followed for detecting the presence of these amino acids as terminal residues in collagen and some of the other proteins studied. For this the DNP protein was dissolved in 10N hydrochloric acid and

TABLE I

N-Terminal amino acids of skin proteins

Values are uncorrected for losses and are expressed as millimoles per 100 g. of protein. Time of hydrolysis 3 hours with 12N hydrochloric acid.

Protein	N-terminal amino acids				
	Aspartic acid	Serine	Glycine	Alanine	Threonine
Collagen	..	trace	trace	trace	nil
CaCl ₂ treated collagen	..	trace	trace	trace	nil
Citrate soluble collagen	..	0.11	0.05	nil	0.04
Soluble rat tail collagen	..	0.07	trace	nil	0.03
Neutral salt soluble collagen	..	0.03	0.035	nil	nil
Elastin (ligamentum nuchae)	..	0.04	0.05	0.05	0.09
Reticulin (rat spleen)	..	0.04	0.18	0.04	0.05
Reticulin (lymph nodes)	..	0.206	0.20	0.064	0.18
Reticulin (adipose tissue)	..	trace	trace	trace	nil
Mvosin	..	0.045*	0.02	nil	0.06
Skin albumin	..	0.20	0.16	0.04	0.20
Skin globulin	..	0.32	0.43	0.05	0.34
Skin mucoids	..	0.33	0.35	0.05	0.20

*Includes a trace of glutamic acid also.

TABLE II

N-Terminal amino acids of different keratin fibres.

Values are corrected for losses and expressed in millimoles 100 g. of wool. Time of hydrolysis 18 hours with 6N hydrochloric acid in sealed tube.

Terminal amino acid	Recovery assumed %	Indian sheep wool (present study)	Lincoln fleece wool ²	New Zealand Romney wool ¹	S. African merino wool ²⁴	Virgin merino wools	Human hair ⁴
Aspartic acid	..	0.078	0.062	0.06	0.03	0.05	0.05
Glutamic acid	..	0.13	0.124	0.12	0.044	0.07	0.10
Serine	..	0.097	0.124	0.12	0.087	0.12	0.10
Threonine	..	0.067	0.480	0.49	0.24	0.41	0.40
Glycine	..	0.35	0.52	0.45	0.60	0.86	0.39
Alanine	..	0.16	0.12	0.12	0.047	0.13	0.10
Valine	..	0.15	0.24	0.24	0.14	0.17	0.40
Total	..	1.03	1.67	1.6	1.19	1.81	1.54

the absorption readings were taken in the spectrophotometer at different wavelengths. It was then corrected for the protein contribution to yield the absorption spectrum of the substituted amino acids. The characteristic peak at 385 m μ for DNP proline and hydroxyproline could not be observed in any of the proteins in which these two amino acids were present.

The normal butanol extract of the water soluble DNP amino acids was examined by the paper chromatographic system of Blackburn and Lowther.⁴⁷ No DNP arginine could be detected in any of the skin proteins.

The results obtained on the analysis of all the proteins excepting wool are given in Table I. The N-terminal amino acids of wool obtained in the present study are compared with those of other workers and are presented in Table II.

DISCUSSION

Only traces of α -DNP amino acids could be detected in hydrolysate of collagen treated with FDNB. The only other spots obtained in the case of DNP—collagen were those of dinitrophenol which disappeared when exposed to hydrochloric acid vapours and dinitroaniline which moved along with the solvent front. Bowes and Moss⁹ and Grassman and Hörmann¹⁰ were also unable to detect any N-terminal amino acids in native collagen. In the case of elastin small amounts of aspartic acid, serine, glycine and alanine have been found to occur as N-terminal amino acids. Partridge and Davis¹¹ detected aspartic acid, serine, glycine, alanine and traces of valine, leucine and iso leucine as N-terminal amino acids of their purified elastin. As their method of preparation of elastin involved certain drastic treatments such as autoclaving with acetic acid for the removal of collagen, it was thought that the N-terminal amino acids which they detected might have originated from collagen breakdown pro-

ducts. But in the present study even when such a drastic treatment was avoided small amounts of aspartic acid, serine, glycine and alanine were detected as the amino end groups of elastin. No trace of valine, leucine or iso leucine were detected. In the case of acid soluble rat tail collagen and citrate soluble collagen, aspartic acid, serine and alanine were detected as N-terminal amino acids in both cases, whereas aspartic acid and serine were detected as the amino end groups of neutral salt soluble collagen. Bowes, Elliot and Moss could detect only aspartic acid and alanine in citrate soluble collagen.

The essential features of the backbone of the collagen structure are now fairly well established.⁴⁸ The structure consists of three polypeptide chains each coiled into a helix and linked to one another by means of interchain hydrogen bonds between the imino-hydrogen and carboxyl oxygen atoms. Elastin is also shown to be built up on a similar structure. The relationship between collagen and elastin is given in a recent paper by Ramachandran and Santhanam.⁴⁹ Elastin is now supposed to be the precursor of collagen, the ordered structure of collagen being produced from elastin by the incorporation of residues which serve to cross link the protofibrils.⁵⁰ Some of the N-terminal amino acids detected in elastin and soluble collagen are common to both the proteins. The small amounts of these N-terminal amino acids in these two proteins may be attributed to the different polypeptide chains of these proteins or alternately they might have arisen from extraneous matter and do not represent terminal amino groups at all. If the protein studied contains impurities of approximately the same molecular weight as the protein which are present only in small amounts the N-terminal amino acids contributed by the impurities will be practically negligible. Whereas if the impurity is of much lower molecular weight than the protein, then its contribution will be relatively more by way of N-terminal amino acids. In the present study special treatments were given

to free the proteins from low molecular weight impurities like the globular and plasma proteins.

The absence of detectable terminal residue in insoluble collagen can be explained on the basis of a very large molecule or by masking of the terminal group by association with some other group possibly a mucopolysaccharide. A considerable body of evidence concerning the biogenesis of collagen is now available⁵¹ from histological, electron optical and biochemical studies. The fibro-blast is first assumed to synthesise a collagen subunit or molecule which may correspond to tropocollagen. This is secreted into the extracellular space where it begins to aggregate into a fibrous structure forming first the neutral salt soluble collagen. As development proceeds, the neutral salt soluble collagen accumulates further tropocollagen molecules increasing in length and thickness until they become visible histologically as argyrophil fibres which probably correspond to the citrate soluble collagen. As the visible fibres increase in thickness and length, closer packing of the constituent collagen units will lead to increasing insolubility. Bowes, Elliot and Moss^{52, 53} have also suggested that insoluble collagen is a complex of acid soluble collagens and a mucoprotein of high tyrosine content. Even though it has been reported^{38, 39} that calcium chloride and formamide will remove most of the mucopolysaccharides from tissues it has been found that even after treating collagen with calcium chloride only traces of N-terminal amino acids could be detected. Smith, Gross and Highberger⁵⁴ have recently reported that their collagen preparation still contained an appreciable amount of polysaccharides even after treating with half saturated lime water for 62 times. Calcium chloride or hydroxide may not therefore remove the mucopolysaccharides that are intimately associated with the collagen and which in some manner may be responsible for the blocking of the terminal amino groups. Partridge,³⁹ Jackson^{55, 56} and others have advocated the view that mucopolysaccharides such as

chondroitin sulphate are intimately associated with the collagen in such a way as to stabilise its fibrous structure. Another possibility for the absence of terminal amino groups in insoluble collagen may be that steric and solubility factors might have prevented the complete reaction of FDNB with this protein.

In the case of reticulin the N-terminal amino acids varied depending up on the source from which reticulin was prepared. While no N-terminal amino acids could be detected in reticulin prepared from adipose tissue small amounts of aspartic acid, serine, glycine and alanine were detected in the samples prepared from rat spleen and the lymph nodes. Ottaway³² has reported that the connective tissues of rat spleen and certain other organs are associated with other proteins like insulin. The N-terminal amino acids detected in some of these tissues might have therefore arisen from such associated impurities. In fact the investigation of the N-terminal amino acids of reticulin has been greatly handicapped by the difficulty of obtaining a material which was acceptable reticulin histologically, unmixed with other proteins and unaltered by chemical treatments.

In the case of wool the data agreed qualitatively with those of other workers, but quantitatively the results were lower than those reported by Thompson,⁶ Middlebrook,² and Speakman.⁴ Such species differences in the terminal amino acids have also been observed in some other proteins.

In myosin, Bailey⁸ detected small amounts of lysine, alanine, valine and an unidentified slow moving derivative as N-terminal amino acids. In the present study small amounts of aspartic acid, serine, threonine and alanine have been detected in myosin.

In the case of albumin, globulin and mucoids prepared from skin, aspartic acid, serine, glycine and alanine were detected in different quantities in all the three proteins.

Paper electrophoretic examination of these protein fractions by the methods of Durrum⁵⁷ have shown that all these protein fractions contain more than one component. Hence the different N-terminal amino acids detected in these cases may be due to the different protein components, present in each fraction. Bowes, Elliot and Moss⁵⁸ have also recently shown that in addition to the plasma proteins, skin contains appreciable amounts of non-collageous proteins which can be extracted by dilute alkaline solutions. By electrophoretic study they have shown that alkaline extracts of skin contain two or more non-collagenous protein components. The mucoids were prepared in the present study by extracting the skin with half saturated lime water.

It is also possible that some of the N-terminal amino acids detected in these proteins might have arisen by a slight degradation of the protein during dinitrophenylation. Other investigators have shown that bicarbonate solutions of DNFB may cause extensive degradation of insoluble proteins such as ichthylepidine,⁵⁹ while with soluble proteins such as old yellow enzyme⁶⁰ and carboxypeptidase⁶¹ certain very labile peptide bonds undergo partial cleavage resulting in slight degradation. On the other hand Middlebrook² could not observe any chain hydrolysis in wool keratin even at an elevated temperature.

The application of the end group methods to fibrous proteins is also met with special difficulties, mainly due to their insolubility. It is very difficult in many cases to know whether they are homogeneous proteins. In fact the preparation of collagen, elastin and reticulin free from impurities is rather very difficult. No methods are available for the removal of all the associated impurities without the risk of simultaneous modification of the protein itself. These difficulties make the unequivocal interpretation of the results difficult and in certain instances impossible.

ACKNOWLEDGEMENT

Our thanks are due to Dr. Nayudamma, Director, Central Leather Research Institute, for his keen interest in this work and for permission to publish these results.

REFERENCES

1. Sanger, *Biochem. J.*, **39**, 507 (1945).
2. Middlebrook, *Biochim. et Biophys. Acta*, **7**, 547 (1951).
3. Blackburn, *Biochem. J.*, **45**, 579 (1949).
4. Speakman, "Fibrous proteins and their biological significance", Cambridge University Press, 1955, page, 169.
5. Alexander and Smith, *Proc. Int. Wool. Text. Res. Conf. Austr.*, vol. B, 1955, page, B-56.
6. Thompson, *Austr. J. Biol. Science*, **10**, 225 (1957).
7. Blackburn, *Biochem. J.*, **47**, 443 (1950).
8. Bailey, *ibid.*, **49**, 23 (1951).
9. Bowes and Moss, *ibid.*, **55**, 735 (1953).
10. Grassmann and Hörmann, *Z. Physiol. Chem.*, **292**, 24 (1953).
11. Partridge and Davis, *Biochem. J.*, **61**, 22 (1955).
12. Zachariades, *C. R. Soc. Biol. Paris*, **52**, 182 (1900).
13. Nageotte, *ibid.*, **96**, 172, 464, 828 (1927).
14. Leplat, *ibid.*, **112**, 1256 (1933).
15. Tustanovskii, *Biochem. Leningra.*, **12**, 285 (1947).
16. Highberger, Gross and Schmitt, *Proc. Nat. Acad. Sci., Washington*, **37**, 286 (1951).
17. Gross, Highberger and Schmitt, *ibid.*, **41**, 1 (1955).
18. Jackson and Fessler, *Nature, London*, **176**, 69 (1955).
19. Jackson, *Biochem. J.*, **65**, 277 (1957).
20. Bowes, Elliot and Moss, *ibid.*, **61**, 143 (1955).
21. Idem, *J. Soc. Leath. Trad. Chemists*, **41**, 249 (1957).
22. Tomlin, *Nature*, **171**, 302 (1953).
23. Kuntzel, *Das Leder*, **8**, 73 (1957).
24. Burley and deKock, *Arch. Biochem. Biophys.*, **68**, 21 (1957).
25. Bowes and Kenton, *Biochem. J.*, **43**, 358 (1948).
26. Orekhovich, Tustanovskii, Orekhovich and Plotnikova, *Biochem. Leningra.*, **13**, 55 (1948).
27. Noda, *Biochim. Biophys. Acta*, **17**, 92 (1955).
28. Hall, *Biochem. J.*, **59**, 459 (1955).
29. Batesmith, *J. Soc. Leath. Trade Chem.*, **31**, 161 (1947).
30. Maximow and Bloom, "Text-book of Histology", 6th Ed., Saunders and Co., London, 1953, p. 49.
31. Dempsy, "Progress in Leather Science," 1920-45, BLMRA, London, 1946, page, 3.
32. Ottaway, *Biochem. J.*, **58**, viii, (1954).
33. Windrum, Kent and Eastoe, *Brit. J. Exptl. Pathol.*, **36**, 49 (1955).
34. Bowes and Kenton, *Biochem. J.*, **45**, 281 (1949).

35. Hasselbach and Schneider, *Biochem. Z.*, **321**, 462 (1951).
36. Tsao, *Biochim. Biophys. Acta*, **11**, 368 (1953).
37. Bose, Madhavakrishna and Das, *J.A.L.C.A.*, **50**, 192 (1955).
38. Woodin, *Biochem. J.*, **51**, 319 (1952).
39. Partridge, *ibid*, **43**, 387 (1948).
40. Fritze and Zahn, *Proc. Int. Wool text. Res. Conf. Austr.*, Vol. C.P.C. 1955, page 119.
41. Mills, *Biochem. J.*, **50**, 707 (1952).
42. Bromer et al. *J. Amer. chem. Soc.*, **97**, 2794 (1957).
43. Levy, *Nature*, **174**, 126 (1954).
44. Fraenkel Conrat, Harris and Levy in "Methods of Biochemical analysis" Vol. II, Interscience Publishers, N.Y., 1955, Page, 359.
45. Shore, *Arch. Biochem. Biophys.*, **71**, 1 (1957).
46. Phyllips, *Biochem. J.*, **60**, 403 (1955).
47. Blackburn and Lowther, *ibid*, **48**, 126 (1951).
48. Ramachandran, *Nature*, **177**, 710 (1956).
49. Ramachandran and Santhanam, *Proc. Ind. Acad. Sci.*, **45A**, 124 (1957).
50. Ramachandran in "Recent advances in gelatin and glue research", Pergamon Press, Lond. 1958. p. 32.
51. Jackson in *ibid*. p. 50.
52. Bowes, Elliot and Moss, "Nature and structure of collagen", Butterworth, London, 1953, page, 199.
53. Bowes, Elliot and Moss, *Biochem. J.*, **63**, ip, (1956).
54. Smith, Gross and Highberger, in "Fibrous proteins and their Biological significance", University Press, Cambridge, 1955, page, 148.
55. Jackson *Biochem. J.*, **43**, 387 (1948).
56. Jackson, *ibid*, **56**, 699 (1954).
57. Durrum, *J. Am. Chem. Soc.*, **72**, 2943 (1950).
58. Bowes, Elliot and Moss, "Recent advances in gelatin and glue research", Pergamon Press, Lond. 1958, p. 71.
59. Burley and Solomons, *Biochim. Biophys. Acta*, **18**, 637 (1955).
60. Weigand and Junk, *Naturwiss*, **38**, 432 (1951).
61. Thompson, *Biochim. Biophys. Acta*, **10**, 633 (1953).

RESEARCH BEARS FRUIT

Practical Demonstration No. 1*

(Second Series)

Manufacture of Chrome Retan Heavy Upper Leathers

BY

N. R. SIKDAR & Y. NAYUDAMMA

Raw Materials. Wet salted cow hides of inferior quality weighing 20 to 25 lbs.

Soaking. Raw hides are weighed and soaked overnight in the following solution.

Commercial Caustic Soda: 2 oz. % on raw weight.

Common Salt: 3% on raw weight.

Water (total): 500% on raw weight.

The soaked hides are fleshed next morning and this soaked weight is recorded. The hides are now washed in pit with cold water for 15 minutes.

Liming. Chemicals for liming: Slaked shell lime: 3% on soaked weight.

Sodium sulphide (fused): 3% on soaked weight.

Water (cold): 300% on soaked weight.

For liming the hides are put into a drum containing the required amount of lime and water and the drum run for 1½ hours, the speed of the drum being very slow preferably 2 to 5 RPM. The lime bath is now drained off and then sodium sulphide bath prepared with the above men-

* Process demonstrated during April 1958.

tioned amount of sulphide and 300% water is put in. The drum is run for 2½ to 3 hours and the goods are left in the drum overnight.

Next day the drum is run for about an hour or alternatively till the removal of hair is complete. The sides are then washed in running water in a drum for 15 to 20 minutes and then unhaired, fleshed and weighed. This weight is recorded as limed pelt weight. The unhaired and fleshed pelt is now washed in running water for 30 minutes in another drum. Alternatively, it may be washed in a drum with 3 changes of cold water.

Deliming.

Chemicals required:

Amonium sulphate or Boric acid: 2% on limed pelt weight.

Water: 200% on limed pelt weight.

Deliming is carried out with the above quantities of chemicals, the drum being run for 1½ to 2 hours. The delimed sides are then scudded and washed for 15 to 20 minutes in running water. (Deliming should be nearly complete.)

Pickling.

Chemical required:

Sulphuric acid (sp. gr. 1.84): 1½ to 1¾% on limed pelt weight.

Common salt: 7 to 8 % on pelt weight.

Water (total): 100% on pelt weight.

The delimed pelt is pickled with the above pickle, the drum being run for one hour and then the goods are left in the drum overnight. Next day, the drum is run for 50 to 60 minutes. The pH of the exhaust bath is tested with the pH meter and that of the pelt with Methyl Red indicator. Exhaust pH should be in the range of 3.20 to 3.4 and the

colour of the cut edge of the pelt should be red through and through with Methyl Red.

The pickled pelt is next chrome tanned in separate drum.

Chrome tanning.

Stock chrome liquor is prepared with the following chemicals:—

Sodium Bichromate: 100 lbs.

Sulphuric Acid (sp. gr. 1.84): 95 lbs.

Molasses: 40 lbs.

Water: 250 lbs.

After preparation of the liquor, the volume of the liquor is made up so as to correspond to 300 lbs. or 30 gallons with water. The chrome liquor bath is prepared as follows:

Common Salt: 3% on pelt weight.

Cold Water: 75% on pelt weight.

Chrome liquor: equivalent to 3.5% sodium bichromate on pelt weight.

Salt and water are put into the drum and the drum rotated for 10 minutes. The pickled pelt is now put in and the drum run for 10 minutes. The chrome liquor is now added in three equal instalments every 30 minutes. The drum is run for one more hour. When the penetration is complete, pH of the chrome liquor bath is checked. The pH is now adjusted to be between 3.2 and 3.6, with 10% solution of sodium bicarbonate (8 to 10 ozs. per cent of sodium bicarbonate should normally be enough for basification). The goods are left overnight in the drum. Next day, the drum is run for 30 minutes. The pH is again adjusted, if necessary, with a little more sodium bicarbonate. The leathers are now piled grain to grain. Next day, they are sammed, split and finally shaved down to 2.2 mm. thickness. The shaved weight of the chromed leather is recorded

and the leathers are washed in running water in a drum for 15 minutes.

Neutralisation.

Chemicals required:

Sodium Bicarbonate: $1\frac{1}{2}$ to 2% on shaved weight.

Water: 250 to 300% „

The chromed leathers are drummed with the above mentioned chemicals for 45 minutes to 60 minutes, water drained off, and the neutralised leathers washed in running water for 15 minutes. The bath is drained off and the leathers are now retanned in a clean drum as follows:—

Retanning.

Chemicals for Retanning:

Cutch extract (Indian): 20 to 25% on shaved weight of sides.

Water (total): 200 to 225% on the shaved weight of sides.

The extract is dissolved in hot water first and then when the solution is cooled down, it is diluted so as to bring the retanning liquor to 200 to 225% on the shaved weight of the sides. $\frac{3}{4}$ quantity of this liquor is added into the drum and the leathers are put into the drum in running condition. The remaining liquor is added into the drum after 30 minutes interval. The drumming is continued for 3 to 4 hours depending upon the penetration of the liquor into the inner layers of the leathers and on the exhaustion desired. The retanned sides are piled up overnight. Next morning they are rinsed with cold water and fat-liquored as follows.

Fat-liquoring.

Chemicals required:

Sulpholin Oil P.: $2\frac{1}{2}$ to 3% on the shaved weight.

Fish oil: 1 to $1\frac{1}{4}$ % on the shaved weight.

Hot-water (50 to 55° C): 100% on the shaved weight.

After drumming with the quantities gives above for 1 to $1\frac{1}{4}$ hrs., the bath is drained.

Refatliquoring.

Chemicals required:

Fish Oil: $\frac{3}{4}$ to 1% on the shaved weight of the sides.

Fixanol V. R. (Cationic dispersing agent): 20% on the weight of oil used.

Water (50 to 55°C): 100% on the shaved weight.

About 90% of the total water is taken in the drum and the fish oil is dispersed separately in it with the remaining amount of hot water and the dispersing agent. The leathers are now put into the drum which is run for about 5 minutes. The emulsified oil is now added into the drum, which is then run for 25 to 30 minutes.

The sides are next horsed up overnight. Next day they are lightly oiled on the grain and set out and hung up for full drying. They are then put into saw dust overnight and staked next day. The sides are then dried in air trimmed, buffed and snuffed on grain if required.

Finishing—Light Brown.

Staining.—Chemicals required:

Bismarck Brown: 4 parts.

Crysodine YN Dye: 6 parts.

Acetic Acid: $2\frac{1}{2}$ parts.

Neats Foot Oil: 15 parts.

Cationic dispersing agent: $3\frac{1}{2}$ parts.

(Total) water: 1000 parts.

The stain prepared with the above given substances is filtered and applied to the leathers with a brush and dried in air. The stained leathers are finished as follows:—

Finishing.

(A) *Pigment Coat*—Chemicals required:

Yellow Pigment: 6 ozs.

Tan Pigment: 6 ozs.

Liquor Ammonia: 1 oz.

Turkey Red Oil: 1 oz.

15% Casein: 6 oz.

Suitable resin emulsion: 1 lb.

Water: To make up to 1 gallon.

The finish prepared as above is applied on the leather twice with pad and then one or two spray coats are given to cover. The leathers are well dried before the next coat of finish is given.

(B) *Fixing coat.* Composition of the second coat.

Casein (15% solution): 40 parts.

Formaldehyde: 20 parts.

Water: 100 parts.

Two cross spray coats of the above solution is sprayed and the leathers are then dried and piled up overnight. Next morning the areas are measured and then they are hot-pressed in hydraulic press at 60-65°C and 250 kg. pressure. The sides are now ready for grading.

TECHNICAL SEMINAR

STUDIES ON CERTAIN PROTEOLYTIC AND
AMYLOLYTIC ENZYMES IN LEATHER MANUFACTURE

BY

W. MADHAVA KRISHNA

Proteolytic enzymes have found a wide application in leather manufacture in the form of bates and depilants. Certain amylases also have been used for unhairing of skins and hides. Papain is the only plant enzyme hitherto studied in this direction and so it was thought interesting to examine a few of the indigenous latex bearing plants like fig, madar, banyan and prickly pear as sources of supply of a proteolytic enzyme which could be used for unhairing and bating of skins and hides. From these investigations, madar latex (latex of *Calotropis gigantea*) was found to have the highest proteolytic activity and an enzymic unhairing process was developed using this. It is also known that certain cereals develop amylases on germination which in turn could be used for unhairing skins and hides. Of the many cereals tried, 'ragi' (*Eleusine coracana*) was found to be cheap and at the same time yielding amylases of high activity. Making use of the enzyme extract from germinated 'ragi', another new process of unhairing skins and hides was developed.

The keeping quality of the madar latex was found to be the maximum at a temperature of 4°C and at a pH of 7.0 as against the natural pH which is about 5.0. The protease from madar latex was isolated by the standard methods of

*Summary of the Seminar talk delivered on 8-11-1957.

isolation and the acetone precipitated sample was found to have the maximum keeping quality when stored at 37°C. The protease thus isolated was further purified by a combination of steps like supercentrifuging, ultra-filtration adsorption on gels, elution, dialysis, solvent precipitation, etc., which resulted in a 196-fold purification of the enzyme. The purified enzyme was later subjected to the activation and inhibition studies which revealed that the enzyme was dependent more or less on the essential—SH group for its proteolytic activity.

Since a study on the mechanism of unhairing of skins or hides by enzymes revealed that it is essentially the hydrolysis and removal of mucoids it was thought necessary to investigate further and standardise the optimum conditions for the effective removal of these mucoids. The mucoids were isolated from a fresh goatskin; the amylase was obtained from the water extract of germinated 'ragi' by solvent precipitation. The optimum pH for the hydrolysis of mucoids by total amylase was 4.5 while those for α - and β -amylases were 5.0 and 4.5 respectively. When the period of digestion was kept at 30 minutes, the optimum temperature was found to be 55°C for the digestion of 5 cc. of 5% suspension of mucoids by 2 cc. of 4% enzyme solution.

CLASSIFIED ABSTRACTS

RAW MATERIALS

THE USE OF DISINFECTANTS IN BRINING FRIGORIFICO HIDES. *M. Dempsey, J.S.L.T.C.* 42, 39 (1958). Hides were cured in brine containing sodium pentachlorophenate (0.1% or 0.2% on the salt) or sodium silicofluoride (0.5% or 1% on the salt) or no added disinfectant, and then packed in clean, unused salt. Some were stored 3 months and some 6 months in Uruguay before being sent to England where they were stored for a further period. Hides which had not been disinfected were the poorest after storage. Those treated with sodium silicofluoride were better, but 0.5% was definitely too little silicofluoride, and even 1% was not enough to allow prolonged storage once 'red heat' had started to grow. The best results were obtained with sodium pentachlorophenate, 0.2% on the salt being rather better than 0.1%, and the hides kept very well for the period tested, namely, 14 months in all. The hides were tanned at two sole leather tanneries and their condition through the two yards and as finished leather is reported. The disinfectants had no adverse effect on the leather although the flesh side of the hides brined with pentachlorophenate had been rather dingy in colour.

Deterioration of hide where 'red heat' is allowed to develop has been confirmed chemically and microscopically.

THE INFLUENCE OF TEMPERATURE UPON THE UPTAKE OF SALT BY CATTLE-HIDE DURING BRINING. *Raymond Hauck and Robert M. Lollar, J.A.L.C.A.*, 53, 149 (1958). The influence of elevated temperatures for short periods of time during brine curing of cattle-hide was studied by means of a Latin-square split-plot experimental design. Pieces of cattlehide were brined for periods of 1½, 3, 4½ and 24 hours at 73°, 98° and 120°F. The ratio of ash to moisture secured from the pieces brined for short periods and held for 30 days was in the range of 5.02% to 12.12% while the ratio for pieces brined 24 hours and held for 30 days was in the range of 21.20% to 27.42%. Thus, it is evident

that elevated temperatures for short periods of time do not give adequate brining to the hide.

PROCESSES PRIOR TO TANNING

THE RELATIONSHIP OF VARIOUS BEAMHOUSE PROCEDURES TO THE INCIDENCE OF "LEOPARD GRAIN" DAMAGE IN SHEEPSKIN LEATHER. *Eldon J. Strandine J.A.L.C.A., 52, 667 (1957)*. The cause of a type of abrasion damage in sheepskin leather known as "leopard grain" damage was investigated. Total amount of abrasion as influenced by length of paddling periods and clearance under paddles was shown to be an important factor in the incidence of leopard grain damage. A higher percentage of skins was abraded during bating than during the liming or sulfiding operations. Experiments demonstrated that those parts of the alkaline skin which were subjected to abnormally high temperatures were weakened and rendered susceptible to this type of grain damage. The importance of equipment for uniformly and gradually heating the bating solution is emphasized. If heating is carefully controlled, concentrations of sodium sulfide, lime and bate can be varied over a rather wide range without the danger of abrasion damage known as leopard grain.

VEGETABLE TANNING

RATIO OF LIGNOSULFONATE TO VEGETABLE TANNIN TAKE-UP FROM BLENDS IN SOLE LEATHER TANNING. *John Davis, Richard Benning, and Martin Baum, J.A.L.C.A. 53, 24 (1958)*. Fractionated sodium lignosulfinate was mixed with vegetable tans—wattle and/or quebracho—to give blends of approximately 10.75% total tannin, of which 35% was lignosulfonate. Bated heavy hides were immersed for 15 days in these blends at a temperature of 95°F. Liquor pH was adjusted between 3.0 and 3.7 at the start and after the first five days of each cycle.

The liquor was refortified to approximately 10.75% tannin as based on sap liquor analyses, and the tanning cycle was repeated until six cycles were run on each blend.

Analysis of new and refortified liquors showed that ratio of tannin methoxyl to tannin content and ratio of lignosulfonate to vegetable tannin as determined by paper chromatography remained constant throughout the six cycles. These results therefore indicate that fractionated sodium lignosulfonate is taken up by hide

in the same ratio that it is present in the original lignosulfonate-vegetable blend.

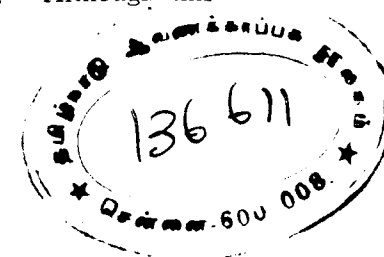
CHROME TANNING

VERTICAL FIBRE IN RELATION TO THE PROPERTIES OF CHROME SIDE LEATHER. *G. L. Amos, J.S.L.T.C., 42, 79 (1958)*. A small percentage of hides entering tanneries have a layer of vertically oriented corium fibres below the thermostat layer in the butt region. The depth and extent of vertical orientation varies between and within hides showing this structure and, in severe cases, may cause trouble in lasting. Sections of vertical fibre hides show very few bundles cut in cross section. Vertical orientation persists throughout the tanning process, and is not subject to modification by changes in tanning procedure. Mechanical treatments which apply pressure to vertical fibre leather produce a microscopically visible undulation in the fibres; dyes and fatliquors penetrate rapidly and extensively. Slicking out during pasting extends to grain and causes more separation of fibre bundles than normal during drying. Vertical fibred hides yield soft leathers which lack fullness of feel, with a very fine break and poor lasting properties, notably low pressures at grain crack and, if pasted for drying, low distensions when tested by lastometer.

OTHER TANNAGES

TANNING STUDIES WITH DIALDEHYDE STARCH. *E. M. Filachione, E. H. Harris, M. L. Fein, A. H. Korn, J. Naghski and P. A. Wells, J.A.L.C.A., 53, 77 (1958)*. Dialdehyde starch, a reactive derivative potentially available at low cost by oxidation of starch, has been shown to have tanning action. Studies were made to determine the effect of such factors as degree of oxidation, method of solubilization, time of treatment, and pH of medium on the rates and degree of tannage.

THE PREPARATION AND TANNING PROPERTIES OF PHENOLIC DERIVATIVES OF LIGNINSULFONIC ACID PART IV. TANNING WITH PREPARATIONS OF LOW MOLECULAR WEIGHT. *W. R. Kenzie, P. A. Deutsch, and J. Benko, J.A.L.C.A., 52, 652 (1957)*. The importance of counter-current evaluation of tanning materials to be used for making heavy leather is illustrated by an experiment in which a blend of phenol condensate and quebracho was used to tan pieces of steerhide in a laboratory-scale rocker section. Although this blend



had given good results when tanning was performed in fresh liquors, the countercurrent experiment showed that the tannin in the blend was not completely utilized. The unreactive part of the tannin was found to consist of fraction of the condensate of high molecular weight.

Removal of the ligninsulfonates of high molecular weight before condensation with phenol resulted in a superior tanning agent. A large batch of this low molecular condensate was prepared and evaluated as solo tanning agent in a countercurrent tanning test. By experiment suitable values were worked out for pH and tanning concentration of the tanning liquors. The resulting leather specimens were compared with paired vegetable-tanned controls. Chemical analyses were very similar, as were thickness, compressibility, and apparent density. The condensate leather had lower shrink temperature, higher water absorption, and a greater tendency to lose water solubles, but these properties could be improved by retannage with formaldehyde. The similarity of the condensate leather to commercial sole leather in stiffness and feel properties indicated its commercial potentialities, although its light gray color might be objectionable.

A number of sole leather bends were tanned with the condensate in the laboratory with satisfactory results. The low purity of the tanning agent was a disadvantage which could be corrected through the use of raw material of higher ligninsulfonate content.

UNCOMMON INORGANIC TANNAGES. H. P. Chakravorthy and H. E. Nursten, *J.S.L.T.C.* **42**, 2 (1958). Chemical inertness, radioactivity, and cost reduce to forty-nine the elements to be considered for inorganic tannage and the literature yields information about the tanning power of compounds of forty-one of these. Thus, most elements have received some attention, but, if the methods used for assessing tanning power are examined and compared, the need for further work becomes apparent.

The action of thirty-one inorganic compounds on bated calf-skin has now been studied, the effect of pH having been examined in every case, in some, the influence of the time of treatment also. The shrinkage temperature and the appearance after drying of the pelt were employed as criteria of tannage. Occasionally, the amount of metal fixed and the effect of washing on the shrinkage temperature were measured as well. Three examples show that

for each metal the pH of maximum absorption by pelt is near that which gives the highest shrinkage temperature. The titration curves and precipitation points of the majority of the compounds studied were determined in aqueous solution. Almost without exception, the precipitation point of a compound lies near the pH at which its solutions yield samples of maximum shrinkage temperature, but for high tanning power this pH need not be near the isoelectric point of collagen. As expected, none of the six complex salts used gave a product of shrinkage temperature greater than that obtained with the same metal ion not complexed.

Of the forty-nine elements considered, real tanning power can be associated only with compounds of mercury, aluminium, zirconium, carbon, silicon, phosphorus, chromium, iron, and possibly vanadium, tungsten and cobalt. It does not seem profitable to search for high tanning potency amongst the compounds of other elements. If metallic ions are arranged in order according to the maximum shrinkage temperature which they impart to collagen, the series obtained is similar to that constructed from the stability constants of the metallic complexes of aminoacids.

THEORIES

THE SOLUBLE PROTEINS OF BOVINE HIDE: EXTRACTION BY AQUEOUS SODIUM CHLORIDE. D. R. Cooper and P. Johnson, *Biochim. et Biophys. Acta* **26**, 317 (1957). Fresh bovine hide was extracted with 10% aqueous sodium chloride. Only one-fifth of the extracted nitrogen was non-dialysable. The non-dialysable material prepared from cow hide was electrophoretically and ultracentrifugally complex and hydroxy proline was totally absent. Amino acid analysis showed much more resemblance to serum albumin than to collagen. Partial fractionation of the whole extract by ammonium sulphate was performed and the material soluble at 40% saturation was studied in some detail. Three main components occurred of which A showed great similarity to bovine serum albumin and component C was comparable with β globulin. Evidence that carbohydrate was associated with a very slow moving electrophoretic component (E) was also obtained.

K.T.J.

ON THE AGGREGATION OF BOVINE SERUM ALBUMIN IN ACID SOLUTIONS. P. Bro, S. J. Singer and J. M. Sturtevant, *J. Amer. Chem. Soc.* **80**, 389 (1958). Bovine Serum albumin forms aggregates in 0.1 M. NaCl-HCl solutions below pH 3.4. This aggregation reaction appears to be reversible with respect to pH but the rate of the reaction in both directions is subject to erratic fluctuations. The reaction is inhibited by excess mercuric ion. The aggregates can be converted to monomer on an ion exchange column in the presence of thioglycollate. The experimental evidence available is consistent with the hypotheses that the reaction is a thiol disulphide exchange.

COMPARISON OF COLLAGEN PREPARATIONS FROM BEEF CORNEA AND SCLERA. Jerome Polatnick, A. J. Latessa and H. M. Katzin, *Biochim. et Biophys. Acta* **26**, 365 (1957). Bovine Corneal and Scleral tissues were fractionated to yield acid soluble, alkali soluble and insoluble collagen preparations. Very small differences in analytical composition were found. The most significant observations were the increased amounts of both acid soluble collagen and non-collagenous soluble proteins found in the cornea, the presence of the latter indicating a larger amount of corneal ground substance. Collagen preparations from both tissues showed comparable susceptibility to digestion by collagenase, and resistance to the action of trypsin.

THE AMINO ACID COMPOSITION OF THE COLLAGEN FRACTIONS OF RABBIT SKIN. D. S. Jackson, A. A. Leach and S. Jacobs, *Biochim. et Biophys. Acta* **27**, 418 (1958). A method for the isolation and purification of neutral salt soluble collagen is described. The amino acid compositions of all the three types of collagen namely citrate soluble collagen, neutral salt soluble collagen, and insoluble collagen isolated from the skins of young growing rabbits are presented. The amino acid analyses were carried out on columns of Dowex-50. The results showed that the amino acid compositions of the three fractions were essentially the same although there were some differences, notably the lower value for proline and hydroxy proline and the higher value for tyrosine found for neutral salt soluble collagen. About 0.43% ornithine was found in neutral salt soluble collagen in an experiment using Zeocarb 225. Hexosamine was absent in neutral salt soluble collagen.

K.T.J.

ELECTROPHORESIS STUDIES OF TENDON COLLAGEN SOLUTIONS. S. G. Tomlin and K. J. Turner, *Biochim. et Biophys. Acta* **26**, 170 (1957). The electrophoretic behaviour of tendon collagen dissolved in acetate buffers of pH 3.2 has been studied. The effects of raising the ionic strength of the buffer solvent have been studied by viscometry and electrophoresis and it was found that in 0.3M acetate buffer satisfactory migration patterns could be obtained. Some observations made on thermally denatured collagen are also reported.

K.T.J.

DETERMINATION OF N-TERMINAL AMINO ACIDS OF COLLAGEN BY THE 2, 4-DINITROFLUOROBENZENE METHOD. C. Deasy *J.A.L.C.A.* **53**, 203 (1958). Serine, aspartic acid, and glycine were found by a modified 2, 4-dinitrofluorobenzene method to be N-terminal amino acids of steerhide collagen.

STUDIES ON THE REACTIVE GROUPS OF COLLAGEN I ACYLATION AND GUANIDINO GROUP DESTRUCTION. S. M. Bose and K. Thomas Joseph, *Arch. Biochem. and Biophys.* **74**, 46 (1958). Collagen has been acylated totally and selectively under different conditions of reaction and the trans esterification method for quantitative estimation of total acetyl groups standardised. The benzoylation of collagen has been achieved by treatment with benzoyl chloride and benzoic anhydride and it has been further shown that the benzoyl groups are attached to the amino and guanidino groups. About 70% arginine and about 17% histidine in collagen were destroyed by sodium hypobromite treatment.

THE REVERSAL OF BIREFRINGENCE OF COLLAGEN FIBRES BY TANNING WITH SUMACH EXTRACT. A. Kuntzel and E. Grataeos, *Das Leder*, **9**, 25 (1958). A few tanstuffs like sumach leaves and myrobalan nuts, when used in tanning, change, the sign of birefringence of collagen fibres from positive to negative. Mariott has drawn attention to the role of water content and water solubles content in this reversal. The question, therefore, arises, whether this inversion of sign is due to any particular component of these tanstuffs. The following experiments were carried out to verify this.

Hide powder was tanned with sumach extract solution. After washing and drying part of the tanned hide powder (A) was subjected to extraction with methanol. The water solubles free hide

powder (B) thus obtained was kept apart. The methanol extract was converted into aqueous extract and then used for tanning a fresh batch of hide powder. The tanned powder (A) thus obtained was treated exactly as above. This repetition was done a number of times. The tan, nontan, and insolubles values and the Lowenthal number were determined for the extracts successively obtained by methanol extraction. The various tanned hide powder samples obtained, with and without the water solubles, were brought to equilibrium with atmospheric humidity and then their moisture contents determined. The percentage tan content (Ratio of tan content to tan plus nontan content, $\times 100$) and the Lowenthal number increase with successive stages of extraction possibly due to condensation during extraction. The B series hide powders (i.e., those free from water solubles) had higher moisture contents than those of A series. This would mean that removal of watersolubles followed by drying created capillary spaces wherein a capillary condensation of water vapour occurs leading to higher moisture content. The free surface area of the watersolubles free hide powder was double that of the other. The A series of hide powders showed negative birefringence while the B series had positive birefringence.

However much sumach extract is fractioned, these fractions are able to effect reversal of birefringence as long as the tannin is present as watersolubles in sufficient quantity.

In further experiments, part of the 'fixed' tannins also were extracted with acetone-water mixture and this extract was used for tanning. The hide powder, doubly extracted was positively birefringent while the hide powder tanned with the extract obtained as above was negatively birefringent. Experiments have shown that the type of tanstuff figuring as 'fixed' tannin has no influence on the sign of birefringence.

So the idea of a specific component of a tanstuff which could reverse the birerengence sign and which is loosely bound to hide substance is not correct; it is the manner of combination that is responsible for this optical effect, the tannin being oriented in a particular manner with respect to the collagen fibres. It is not yet certain to what extent the special constitution of sumach tannin molecule and the form of the pores and capillaries present in leather fibres are responsible for the optical effect.

V. S. P.

EFFECT OF CHROME TANNING ON THE STRUCTURE AND WIDE-ANGLE X-RAY PATTERN OF COLLAGEN. M. S. Santhanam, *J.A.L.C.A.* **53**, 162 (1958). This paper deals with the changes produced in the X-ray diffraction pattern of collagen by chrome tannage. The various types of fixation of chromium complexes are discussed in relation to these observations which indicate that the type of fixation varies with the electrochemical nature of the chromium complex.

THE REACTION OF SOME THIOCYANATOCHROMIATE COMPLEXES WITH COLLAGEN, 1. THE REINECKE SALT. K. H. Gustavson, *J.A.L.C.A.* **53**, 192 (1958). The Reinecke salt, $[\text{Cr}(\text{CNS})_4(\text{NH}_3)_2]\text{NH}_4 \cdot \text{H}_2\text{O}$, reacts irreversibly with collagen, raising the shrinkage temperature by as much as 25°C. The reaction is, however, a very slow one, and the chromium complex that is fixed contains only two CNS groups per Cr. This indicates that tanning does not occur until part of the CNS groups have been displaced from the complex, probably by water molecules. Tanning experiments with different modified collagens indicate that the altered Reinecke salt is fixed chiefly by the guanidine group of the arginine residue.

THE ACTION OF ACRYLONITRILE AND ACRYLAMIDE UPON COLLAGEN. L. Seligsberger and H. Clayton, *J.A.L.C.A.* **53**, 90. (1958). Acrylonitrile and acrylamide are shown to react with limed hide in strongly alkaline solutions. The fixation of the monomers under such conditions results in a lowering of the shrinkage temperature and in a decreased chrome fixation. The dry fiber strength of the resulting leather is not materially affected. The ε-amino groups are considered to be the active sites in the cyano- and carbamoylethylation of collagen.

THE ACID MUCO POLYSACCHARIDES OF EMBRYONIC SKIN. Gerald Loewi and Karl Meyer, *Biochim et Biophys. Acta*, **27**, 453 (1958). The distribution of acid polysaccharides of skin of various stages of embryonic development were determined. It was found that embryo skin contained only very small amounts of chondroitin sulfate B in the presence of relatively large amounts of hyaluronic acid, while adult pig skin contained considerably more chondroitin sulfate B than hyaluronic acid. In addition embryo skin was found to contain a relatively large amount of chondroitin C.

-- K. T. J.

STUDY OF CHROME-GLUCONATE AND ALUMINO-GLUCONATE COMPLEXES. *L. Villa and S. Magarian, B.A.F.C.I.C. 20, 1. (1958).* In continuation of earlier work, the complexing ability of gluconate ion with respect to chrome and aluminium has been studied, using spectrophotometry for chrome and electrometric titration for aluminium. Perchlorates of the two metals were used in order to avoid interferences due to concurrent complexing.

The pH at which precipitation sets in, was studied with different proportions of gluconate ion and Cr^{+++} , with freshly prepared, boiled and 12 days aged chromium salt solutions. The pH values for 0, 0.25, 0.50, 0.75, 1, 2 and 3 moles of gluconate ion per mole of Cr were 5.5, 5.5, 5.6, 5.6, 5.65, 5.85 and 6.0 respectively with freshly prepared solution, 5.5, 5.5, 5.6, 5.6, 5.7, 5.8, and —, with boiled solution, 5.5, 5.5, 5.7, 5.7, 5.8, — and —, with the aged solution. The variation of absorbance with pH was studied for various wavelengths. These experiments would seem to indicate that while complexing makes its appearance even at pH 2 the masking is really significant only in alkaline medium. By the method of continuous variation, an attempt has been made to determine the molecular formula of the complex at pH 4.2. Since a maximum was obtained with equivalent quantities of Cr and gluconate ion (G), the formula $(\text{Cr G})^x$ has been proposed. After verifying the validity of Beer's Law in this case, the stability constant has been determined for this system in a very dilute solution with G/Cr ratio of 20 at 420 m μ .

The electrometric titration curves for the gluconate-aluminium salt system is composed of several portions corresponding to (1) neutralisation of free acid, (2) formation of $\text{Al}(\text{OH})$ and $\text{Al}(\text{OH})_2$ and (3) precipitation of the hydroxide. Determination of the precipitation point from the curves was difficult. The stability constant was calculated assuming the formula to be AlG_3 .

The stability constants for the different systems in moderately acid medium are: $[\text{FeG}] : K = 4.26 \times 10^{-6}$; $[\text{CrG}] : K = 2.62 \times 10^{-3}$; $[\text{AlG}_3] : K = 2.2 \times 10^{-6}$.

Gluconate ion can be considered, therefore, as a complexing agent for iron, chromium and aluminium—particularly for iron. Although perceptible in acid medium, this capacity is specially

enhanced in neutral or alkaline media. Though iron and chromium, appear to behave similarly towards gluconate, the iron complexes are much more stable. As for aluminium its ability to form complexes is clearly attenuated. AlG_3 could perhaps be considered as the normal salt derived from gluconic acid. The presence of OH groups, in this acid, capable of forming co-ordinate bonds, would explain the partial ionic character of this compound.

—V. S. P.

THE MASKING OF ZIRCONIUM SULPHATE SOLUTIONS. *T. S. Ranganathan and R. Reed. J.S.L.T.C. 42, 59. (1958).* The masking properties of various organic reagents in solutions of basic zirconium sulphate have been investigated. The sodium salts of hydroxy carboxylic acids e.g. citric, gluconic, glycollic and lactic appear to be the most effective.

TESTING

STITCH TEAR TESTS ON LIGHT LEATHER. *C. D. Pomeroy, J.S.L.T.C. 42, 90 (1958).* Stitch tearing loads have been measured with various distances between stitch holes, sizes of the wire forming the stitch hole, and directions in which the stitch was pulled through the leather. The tearing load is shown to increase as the distance between the holes increases, but the rate of increase with increasing distance is not constant. A thicker wire requires a greater load to pull the stitch through the leather, and a greater load is also required to pull the stitch through from grain to flesh than from flesh to grain. Further measurements show the relation between tearing load and the distance of the holes from the edges of the sample. The load increases as the distance from the edge increases, and a maximum is reached when the stitch passes completely through the leather and does not tear out through the edge of the sample. The distance from the edge when this maximum is reached is shown for various sizes of stitch. Wire used to form the stitch is shown to give results indistinguishable from those obtained using thread of the same diameter. Consequently results obtained using wire stitches apply equally to thread stitches.

COMPARISON OF VARIOUS METHODS FOR DETERMINING THE WATER VAPOR PERMEABILITY OF VEGETABLE-

RETANNED SIDE LEATHER. Mieth Maeser. *J.A.L.C.A.* 53, 132 (1958). The water vapor permeability of four lots of vegetable-retanned side leather varying only in grease content was measured by six different methods and by several modifications of two of the methods. The results show that all methods and modifications except the resistance method of Goodings, rate the four lots of leather in the same qualitative order. The quantitative variations in the results obtained by different methods are quite high.

POTENTIOMETRIC TITRATION STUDIES AND TANNING ACTION OF ALUMINIUM COMPLEXES. D. Williams-Wynn, *J.S.L.T.C.* 42, 22 (1958). The effect of the addition of formate, acetate, lactate, tartrate and citrate ions to solutions of sodium aluminate has been studied using the potentiometric titration technique. Even at ratios as high as 6 equiv./mol. aluminium, little useful masking action was found with formate and acetate. Tartrate, lactate and citrate stabilise aluminium solutions against precipitation at ratios of 2 equiv./mol. Al. Within the limits studied, it appears that 1 mol. tartrate combines with 1 mol. Al. to form a complex, while citrate and lactate form complexes which do not contain a constant ratio of masking agent to aluminium.

Experimental tannages were carried out using some of the complexes formed above, the samples being assessed for leatheriness, hydrothermal stability and resistance to wetting and drying. Formate, acetate, and lactate masking gives the most stable leather and highest shrinkage temperatures; citrate and tartrate complex too strongly to be of use in tanning.

RESOLUTION OF ACID MIXTURES IN NONAQUEOUS SOLVENTS: POTENTIOMETRIC TITRATION OF DIBASIC ACIDS WITH QUATERNARY AMMONIUM TITRANTS. Gerald A. Harlow and Garrard E.A. Wyld, *Anal. Chem.* 30, 69 (1958). The influence of the solvent on the resolution of acids by potentiometric titration has been studied. Curves are presented for the titration of sulphuric, oxalic and succinic acids in mixture of water and isopropyl alcohol, and for sulphuric acid in isopropyl alcohol, ethyl alcohol, methanol, ethyl ether and pyridine. The manner in which the properties of the solvent may be utilised in the analysis of acid mixtures is discussed. Mixtures containing acids of different charge types which cannot be resolved in water can be resolved in nonaqueous solvents.

DETECTION OF UREA, MELAMINE, ISOCYANATE AND URETHAN RESINS: RAPID GROUP TEST FOR NITROGEN, SILICON, PHOSPHOROUS & TITANIUM IN COATING MATERIALS. M. H. Swann and G. G. Esposito, *Anal. Chem.* 30, 107 (1958). Specific and simultaneous tests for urea-formaldehyde and melamineformaldehyde coating resins and a test for isocyanate and urethan are presented, that are applicable to coatings in general. A rapid acid-digestion is described for detection of nitrogen, phosphorous, silicon and titanium. These methods are useful for qualitative identification of synthetic resins in complex systems.

INFRARED ANALYSIS OF EMULSION POLISHES. John E. Murphy and Warren C. Schwemer. *Anal. Chem.* 30, 116 (1958). There has been a need for a rapid method for the qualitative determination of wax, resin, polymer and emulsifier in emulsion polishes. The techniques of infrared spectroscopy solve this problem in a satisfactory manner. The spectra of the common components of emulsion polishes and of a group of amine emulsifiers as their hydrochloride derivatives have been compiled. Complete and definite analyses of these formulated products can be obtained by first separating the product into its components and then determining the infrared spectra of these components.

HYDROXYPROLINE ANALYSIS AS A TOOL FOR TANNERY CHEMISTS. Robert M. Lollar, *J.A.L.C.A.* 53, 2 (1958). Data have been presented on the precision of the determination of hydroxyproline by a modified form of the Neuman-Logan Colorimetric procedure. The practical application of the method to the determination of "leather-making substance" in samples taken from skins and hides at process stages from flaying through curing and the tannery beamhouse processes is illustrated by an example. Suggestions are presented for studies designed to improve the method further, in the hope that its adoption by industry as a common control method will be hastened.

GENERAL

STUDIES OF LEATHER FUNGICIDES. Sverre Dahl and A. M. Kaplan, *J.A.L.C.A.* 53, 103 (1958). Two series of compounds structurally related to tetrachlorohydroquinone and to 4-nitrophenol have been investigated for effectiveness as leather fungicides. Among ten compounds, all having a 2, 3, 5, 6-tetra-

chlorobenzene ring in common but with different substituents on carbons 1 and 4, the most effective 1, 4-combination was OH, NO₂. Replacement of NO₂ in this combination with OH, Cl, and H decreased effectiveness in that order.

Among 11 aromatic nitro compounds without the tetrachloro structure, mildew-preventive activity was also associated with the 1,4 OH, NO₂ combination. Ethers of the phenols in both series of compounds were ineffective.

Bis (4-nitrophenyl) carbonate and bis (2-chloro-4-nitrophenyl) carbonate are effective mildew inhibitors apparently because of their decomposition into the corresponding free phenols under hot and humid conditions. They are of interest as leather fungicides because they are colorless and probably nontoxic, thus alleviating some of the disadvantages of 4-nitrophenol. Analytical methods for these two compounds and data on their performance in leaching tests are given.

NOTES & NEWS

LEATHER INDUSTRIES' PROBLEMS

Panel of Experts to Consider

The setting up of a panel of experts for leather and leather-goods industries, which play an important part in the national economy, has been suggested at a meeting convened here by the Ministry of Commerce and Industry, to consider the problems facing the industries and their development programmes. The Minister for Industry, Mr. Manubhai Shah, presided.

The panel will consider from time to time problems like shortage of raw hides in the country, survey of indigenous tanning materials, plantation of trees like wattle, and others, which provide tanning substances, and marketing of tanned hides and skins.

Standardization of leather goods for export markets and efficient functioning of existing units will also be examined by the proposed panel. It will make recommendations to Government for the development of the industry.

The meeting, which lasted two days, was attended by representatives of Central Government departments concerned, State Governments, industry and trade, research and technical institutions.

The meeting reviewed the present position of the industries and discussed ways and means of helping the existing units to improve the quality of their products and step up their output.

—*Hindustan Standard* 16-4-58

LEATHER MEN TACKLE DEAD SEA SCROLLS

Leeds University's Department of Leather Industries is the latest centre to be called in to give an estimation of the age of the Dead Sea Scrolls. The fragments of the Scrolls have been sent to the Department because the parchment used in making the Scrolls was prepared from animal skins.

The university investigators are sure that they did not originate in the Dead Sea area because the skin was treated differently from other specimens known definitely to have been prepared there. Dating the fragments is carried out by placing them in water and heating them slowly. At a certain stage in the process, they suddenly shrink. The older they are the lower the temperature at which this occurs. An accurate time scale is available. It has been drawn up by noting at what temperature this shrinkage takes place in the case of remains whose age is definitely known. Fragments sent there for testing have so far yielded two conclusions. In the first place it has been shown that although the Scrolls were found in the Dead Sea area, they did not originate there. The second finding is that they were certainly written before 600 A.D. It is likely to be the middle of next year before a definite statement can be made.

—*Morning Times*, 10-4-58

RESEARCH BEARS FRUIT

Practical Demonstration No. 2

(Second Series)

Process: Manufacture of upholstery leathers using Basic Aluminium Sulphate.

Period: 13th to 22nd May, 1958.

Place: Central Leather Research Institute-Tannery.

The next in the Second Series of practical demonstration will be on the 'Manufacture of upholstery leathers using Basic Aluminium Sulphate'.

The E. I. tanning industry produces leathers mainly intended for export. But due to various factors, the lower selection grades of this type of leather which form a considerable part of production do not fetch the same price as better quality leathers. However, these leathers could be upgraded to fetch better value by retanning and finishing for a variety of purposes including the manufacture of upholstery leathers. Earlier a process was demonstrated for the manufacture of lining leathers, sambhar leathers, by retanning inferior quality E.I. leathers with basic aluminium sulphate developed at this Institute. The process of manufacture of upholstery leather by retanning inferior grades of E.I. tanned kips with basic aluminium sulphate and finishing is proposed to be demonstrated this time.

Upholstery leather is now in demand for the upholstering of seats in Automobiles, Railways, Sofas, Chairs, etc., as well as for the manufacture of suit-cases, Air Travel bags, Document cases, Zip folio cases, fancy valets, etc. The upholstery leathers so prepared at Central Leather Research Institute, have been successfully used by the Industry for over a period of 1-2 years and proved to be good.

Though the process was originally patented, in the interest of the leather industry in India and in pursuance of the policy of the C.L.R.I. to bring the fruits of Research to the doors of the

industry, this process is being released to the trade and the process will be publicly demonstrated to the representatives of trade and industry. The demonstration will begin on May 13th, 1958 and will last for ten days. We shall much appreciate if you will intimate to us the names(s) of your representative(s) who would like to attend the demonstration and get all the pros and cons of the process clarified. After following the demonstration, we would request you to try out this process in your tannery and give us your valuable comments.

June: 1958—Practical Demonstration on the Manufacture of Full Chrome Glazed Kid.

July: 1958—Practical Demonstration on the Manufacture of Vegetable Tanned Sole Leather.

THE RAFI AHMED KIDWAI MEMORIAL PRIZES FOR AGRICULTURAL RESEARCH

The Indian Council of Agricultural Research has, with a view to creating an incentive for research workers and to recognising outstanding research work done by them in the fields of agriculture, animal husbandry and allied subjects, decided to institute 11 prizes of the value of Rs. 5,000 each. These prizes will be known as "The Rafi Ahmed Kidwai Memorial Prizes for Agricultural Research" and one prize will be awarded annually for outstanding research work done in India in each of the following subjects:—

1. Agronomy,
2. Agricultural Engineering,
3. Agricultural Chemistry,
4. Agricultural Botany,
5. Agricultural Zoology (including Fisheries),
6. Horticulture,
7. Animal Breeding,
8. Animal Nutrition (including Physiology and Biochemistry),
9. Animal Diseases,
10. Dairying, and
11. Agricultural and Animal Husbandry Economics and Statistics.

2. These prizes will be in the form of gold medals or cash or both and the first prize in each subject will be awarded for outstanding research work done in the country either individually or jointly, during the Calendar year 1957, i.e., 1st January to 31st December, 1957, and thereafter for such research work carried out during each subsequent calendar year.

3. The award of each of the prizes shall be based on significant advances in human knowledge in a particular subject as revealed by books, monographs or papers published in the name of the candidate or any other unpublished account of the outstanding research work done or discoveries and inventions made by him. The selection of a candidate for the award of a prize will be made on the recommendations of a Judging Committee consisting of eminent scientists appointed for the purpose by the Indian Council of Agricultural Research. The award of a prize may be made to more than one research workers and the prize money divided amongst them in such proportion and manner as may be decided by the Council.

4. All research workers, engaged in research work in India, in the fields of agriculture, animal husbandry and allied sciences are eligible for competing for these prizes. Applications should be submitted in quadruplicate (4 copies) in the prescribed form, which can be had, free of cost, from the Secretary, Indian Council of Agricultural Research, Queen Victoria Road, New Delhi. Candidates working in Research Institutes etc., should submit their applications through the Head of the Institute/Department etc. Others may submit their applications direct.

5. All applications should be sent in a sealed cover and addressed to the Secretary, Indian Council of Agricultural Research, Queen Victoria Road, New Delhi, so as to reach him not later than the 30th June, 1958. The cover of applications should be superscribed as under:

"Application for the award of 'The Rafi Ahmed Kidwai Memorial Prize for Research in'"

Applications received after the 30th June, 1958, will not be considered.

C. L. R. I. NEWS

Mr. T. S. Ranganathan, Senior Scientific Assistant, Central Leather Research Institute, has been awarded the degree of Doctor of Philosophy of the Leeds University, on the basis of his thesis 'Zirconium Tanned Leather and its retannage with vegetable tannin material'. Mr. Ranganathan resumed duty as Senior Scientific Assistant on return from U. K. after completing his training at the Leeds University under the Colombo Plan.

Shri P. S. Santhanam has been appointed as Senior Laboratory Assistant.

Dr. Y. Nayudamma has been nominated as a member of the Advisory Committee for the Madras Branch Office of the I.S.I. (with Mr. A. Ganesan as alternate).

Mr. S. N. Bose, Senior Scientific Assistant, was deputed to a Tannery at Vaniambadi to demonstrate the process of manufacture of Ficking Bands by the C.L.R.I. (Central Leather Research Institute) process.

Mr. A. Ganesan, Senior Scientific Officer and Mr. J. C. Deb, Senior Scientific Assistant rendered assistance to a tannery in Madras in the process of finishing.

Dr. S. K. Barat, Senior Scientific Officer attended the Symposium of the Defence Science Organisation held at Delhi.

Dr. Y. Nayudamma, Director, attended the meeting convened by the Ministry of Commerce and Industry for the development of the Leather Industry.

Dr. Y. Nayudamma visited the Tanneries in Bombay State in Connection with the Extension Service work to be taken up in Bombay State.

VISITORS

<i>Name</i>	<i>Date of visit</i>
F. A. Blasev, Illinois, U.S.A.	8-4-1958
M. B. Bhagvat, Tata Chemicals Ltd., Bombay	"
K. V. Itty, Tata Oil Mills Co., Ltd., Madras	"
V. Kondratiev, Soviet Economist, U.S.S.R.	9-4-1958
Dr. Ing. S. P. Raju, F. N. I., Hyderabad	15-4-1958
S. S. Kinny, Leather Industrial Co-operative Society Nagpur	19-4-1958
M. K. Billimoria, P. R. Tata & Co., Bombay	19-4-1958
B. Sreenivasan, Sarabhai Chemicals, Baroda	25-4-1958

